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BOTH D_1 AND D_2 RECEPTORS APPEAR TO BE INVOLVED IN THE INDUCTION OF PURPOSELESS CHEWING IN RATS

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Neuroleptic drugs can induce purposeless chewing in rats (Rupniak et al. 1986). The same behaviour can also be induced by acute administration of the D-1 agonist SKF 38393 (2,3,4,5-tetrahydro 7,8-dihydroxy-1-phenyl-1H benzazepine) and potentiated by administration of the D-2 antagonist sulpiride (Rosengarten et al. 1983). This led to the suggestion that purposeless chewing is a D-1 mediated behaviour. However, a full pharmacological analysis was not undertaken. We now report the effects on purposeless chewing of a range of selective D-1 and D-2 agonist and antagonist drugs alone and in combination.

Male Wistar rats (225-250 g) were acclimatised overnight in a quiet room. Rats were assessed individually in single cages for 5 minute periods at intervals for up to 3 hours following drug administration. Results are presented as the number of purposeless chewing movements occurring in a 5 minute period at the time of maximum drug effect. All drugs were administered by the i.p. route.

Acute administration of the D-1 agonist SKF 38393 (5-20 mg/kg), resulted in a dose dependent increase in the frequency of purposeless chewing. The D-2 antagonist sulpiride (10-100 mg/kg) also caused a dose dependent increase in the frequency of purposeless chewing. A low dose of SKF 38393 (5 mg/kg) in combination with sulpiride (100 mg/kg) increased the frequency of purposeless chewing above the level seen after either drug alone.

The D-2 agonist quinpirole (0.05-0.25 mg/kg) also induced purposeless chewing accompanied by an increase in yawning frequency. Quinpirole-induced purposeless chewing displayed a "bell shaped" dose response curve. The D-1 antagonist SCH 23390 (0.1-0.5 mg/kg) did not induce purposeless chewing except at the highest doses (1.0-5.0 mg/kg) employed.

Table 1 Effect of drug treatment on purposeless chewing in rats

Treatment	Control	Drug treated
SKF 38393 (20 mg/kg)	7.3 ± 2.1	36.7 ± 5.4*
Sulpiride (100 mg/kg)	6.2 ± 3.3	35.8 ± 10.5*
Quinpirole (0.1 mg/kg)	6.3 ± 3.0	30.0 ± 5.9*
SCH 23390 (0.1 mg/kg)	9.8 ± 2.3	8.8 ± 1.7

All results expressed as mean ± S.E.M.; * $p < 0.05$, Mann Whitney U test, compared to corresponding control.

SCH 23390 (0.05mg/kg) completely blocked the purposeless chewing induced by SKF 38393 (20 mg/kg), but had no effect on purposeless chewing induced by either sulpiride (100 mg/kg) or quinpirole (0.1 mg/kg). Co-administration of SKF 38393 (20 mg/kg) and quinpirole (0.1 mg/kg) did not induce purposeless chewing but produced stereotyped licking.

These results suggest that although purposeless chewing can be induced by acute administration of D-1 agonists, D-2 receptors can initiate and manipulate this behaviour.

Rupniak, N.M.J. et al. (1986) Psychopharmacology 88, 403-419.
Rosengarten, H. et al. (1983) Life Sci. 33, 2479-2482.

SUBSTANTIA NIGRA COMPLEX I DEFICIENCY IN PARKINSON'S DISEASE

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1-Methyl-4-phenylpyridinium (MPP^+), the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), destroys nigral dopamine containing cells by selective inhibition of NADH CoQ reductase (Complex I), the first enzyme protein complex of the mitochondrial respiratory chain (Nicklas et al. 1985; Vyas et al. 1986; Ramsay et al. 1986). In the present study, we have examined the activity of the mitochondrial respiratory chain in substantia nigra from patients dying with Parkinson's disease compared to control tissue.

Patients were matched for age and time between death and body refrigeration and to removal and freezing of brain. All parkinsonian patients received L-DOPA up to the time of death. The substantia nigra from 9 patients with Parkinson's disease and 9 controls were examined. There was no difference in the substantia nigra total protein concentration or citrate synthase activity between the two groups indicating that mitochondrial mass was similar per gram wet weight of tissue. Levels of succinate cytochrome c reductase, a marker for Complexes II and III, were also normal in tissues from patients with Parkinson's disease compared to controls but rotenone sensitive NADH cytochrome c reductase, a marker for Complexes I and III, was reduced (Table 1). The Complex I specific enzyme, NADH CoQ reductase, was reduced in substantia nigra from patients with Parkinson's disease compared to controls.

Table 1 Mitochondrial function in Parkinson's disease

	Control	Parkinson's disease
NADH cytochrome c reductase (rotenone sensitive) [a] n = 9	4.36 ± 1.41	2.68 ± 1.01*
Succinate cytochrome c reductase [b] n = 9	9.46 ± 3.01	9.59 ± 3.15
[a] : [b]	0.48 ± 0.16	0.30 ± 0.11*
NADH CoQ reductase (rotenone sensitive) n = 5	3.36 ± 0.44	2.34 ± 0.76*

* $p < 0.05$, Student's t test. Enzyme activities are expressed as nmol/min/mg total protein; mean ± 1 S.D.

These results indicate a selective reduction in the activity of Complex I in Parkinson's disease and parallel the effects produced by MPP^+. The Complex I deficiency may be primary, or secondary to some other metabolic defect, or due to the effects of L-DOPA. If causal, the deficiency could be due either to direct inhibition of Complex I, or to oxidative damage to mitochondrial DNA.

Nicklas, W.J., Vyas, I. & Heikkila, R.E. (1985) Life Sci. 36, 2503-2508.
Ramsay, R.R., Salach, J.I. & Singer, T.P. (1986) Biochem. Biophys. Res. Commun. 134, 743-748.
Vyas, I., Heikkila, R.E. & Nicklas, W.J. (1986) J. Neurochem. 46, 1501-1507.

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A COMPARISON OF LOCOMOTOR STIMULATION IN 6-HYDROXYDOPAMINE-LESIONED MICE INDUCED BY L-DOPA AND THE L-DOPA PRODRUG NB-355

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Recent evidence suggests that therapeutic response fluctuations to L-Dopa which emerge during chronic treatment in patients with Parkinson's disease, may to a large extent be explained by the pharmacokinetics of the drug (Nutt et al, 1984). Therefore, it has been proposed that such fluctuations could be reduced or eliminated by achieving improved steady-state levels of plasma L-Dopa. One method to improve bioavailability of L-Dopa is the use of prodrugs. In the present study we assessed the behavioural effects in mice of a novel L-Dopa prodrug L-3- (3-Hydroxy-4-pivaloyloxyphenyl) alanine (NB-355) which induces a longer duration and larger bioavailability of L-Dopa in plasma than L-Dopa itself (Ihara et al, 1989). The efficacy and duration of action of NB-355 plus carbidopa was compared to L-Dopa plus carbidopa in a rodent model of Parkinson's disease, whole brain dopamine depletion induced by 6-hydroxydopamine (6-OHDA).

Male BKTO mice (n=38) were anaesthetised with isoflurane (4%) and nitrous oxide (70%) and injected intracisternally with 100ug of 6-hydroxydopamine (25 ul volume in ascorbic acid/saline). One hour prior to anaesthesia all animals were given 25mg/kg desmethylimipramine to protect noradrenergic neurones. Twenty four hours later the entire procedure was repeated.

Three weeks later mice were divided into 2 groups (n=19 in each) and tested for their locomotor activity responses to 25mg/kg carbidopa plus L-Dopa or NB-355. Each animal received vehicle 125, 250 and 500umole/kg of L-Dopa or NB-355 according to a pseudo-latin square design. Treatments were separated by an interval of at least 3 days. Mice were injected i.p. with 25mg/kg Carbidopa and 30 min. later injected p.o. with vehicle, L-Dopa or NB-355. Locomotor activity was recorded in photocell cages for a period of five hours after the second injection. Data were analysed by multifactoral analysis of variance (ANOVA). At the end of the experiment brain dopamine levels of the lesioned mice and controls were measured by HPLC.

All lesioned animals had a dopamine depletion of 80% or greater and there was no difference in the extent of depletion between the L-Dopa and NB-355 treated animals. Both L-Dopa and NB-355 dose dependently increased locomotor activity in 6-OHDA lesioned mice. However, ANOVA revealed a significant difference in the motor stimulation induced by L-Dopa and NB-355 (main effect: $F(1,36) = 4.27$, $P < 0.05$). Thus, the maximal stimulatory effect of NB-355 was greater than that of L-Dopa at all doses tested. Furthermore, NB-355 had a longer duration of action than L-Dopa at doses of 250 and 500 umole/kg and this was indicated by ANOVA (drug x dose x time interaction : $F(27,972) = 2.35$, $p < 0.0001$) and multiple comparisions at each dose level using the Tukey test.

The results of these studies, together with data from primate studies (Rupniak et al, 1989) suggest that NB-355 may have an improved therapeutic profile compared to L-Dopa in the treatment of Parkinson's disease.

Ihara M., et al (1989) J. Pharm. Sci., in press
Nutt JG., et al (1984) N. Engl. J. Med., 310 : 483-488
Rupniak NMJ.,et al (1989) This meeting

NEUROTENSIN REVERSES THE EFFECTS OF AMPHETAMINE ON *IN VIVO* DOPAMINE METABOLISM IN THE ACCUMBENS BUT NOT THE STRIATUM

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The interaction between neurotensin (NT) and the mesolimbic dopamine system is well established. Supporting this assertion are reports showing that increases in extracellular DOPAC, measured by in vivo voltammetry are obtained in the nucleus accumbens after administration of NT into the ventral tegmental area (VTA) while no increase is seen in the striatum following intranigral injection of the peptide (Ford & Marsden, 1987). Moreover, the administration of NT in the nucleus accumbens reduces the hyperlocomotion induced by peripheral administration of d-amphetamine whereas the peptide fails to antagonise this effect when injected in the striatum (Ervin et al., 1981). Finally, the intracerebroventricular (ICV) administration of NT has been shown to antagonise the hyperactivity induced by amphetamine but not the stereotypy suggesting again a more selective effect of the peptide toward the mesolimbic system (Jolicoeur et al., 1983). The present study examines the effect of the central administration of NT in combination with amphetamine, on the extracellular level of DOPAC measured by in vivo differential pulse voltammetry, in both the nucleus accumbens and the striatum.

Male Wistar rats (250-280 g) were anaesthetised using chloral hydrate (400 mg/kg i.p.) and stereotaxically implanted with an indwelling cannula in the left lateral ventricle. Two electrically-pretreated 12 μm carbon fibre electrodes (Sharp et al., 1984) were implanted, one in the nucleus accumbens and the other in the striatum. Extracellular DOPAC was recorded every five minutes during a stabilisation period of 1 hour before drug administration. Saline (1 ml/kg s.c.) or amphetamine (2 mg/kg/ml s.c.) were administered and five minutes later saline (10 μl) or NT (10 μg/10 μl) were given i.c.v. Extracellular DOPAC was recorded for two hours after drug administration and results expressed as percentage differences compared with baseline value. Data were analysed by ANOVA.

Amphetamine produced similar decreases in extracellular DOPAC in the nucleus accumbens (52%) and the striatum (47%) 45 minutes after administration. NT (10 μg i.c.v.) produced a significant increase in the DOPAC peak in the nucleus accumbens which was maximal 50 mins after administration (30%). In the striatum NT produced a smaller but still significant increase (25% at 70 min). When NT was given 5 min after amphetamine, the peptide partially reversed the effect of amphetamine on DOPAC in the nucleus accumbens while there was no significant effect on the decrease in extracellular DOPAC produced by amphetamine in the striatum. Together, these results demonstrate that NT is able to reverse the decrease in extracellular DOPAC induced by amphetamine in the nucleus accumbens but not the striatum and support neurobehavioural studies indicating that neurotensin has a greater effect on mesolimbic rather than the nigrostriatal dopaminergic system .

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Ervin, G.N. et al. (1981) Nature 291, 73-76.
Ford, A.P.D.W. & Marsden, C.A. (1987) Br. J. Pharmac. 90, 237p.
Jolicoeur, F.B. et al. (1983) Neurosci. Biobehav. Rev. 7, 385-390.
Sharp, T. et al. (1984) Neuroscience 12, 1213-1221.

CHLORDIAZEPOXIDE CAUSES SPINALLY MEDIATED ANALGESIA IN THE RAT

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The use of intrathecal analgesics has become increasingly more important in the treatment of both acute and chronic pain. Opioids, especially mu agonists such as morphine and fentanyl, have been the main drugs of interest and a spinal action for these and delta and kappa agonists has been demonstrated in animals by Schmauss and Yaksh (1984). In addition to opioid receptors, benzodiazepine receptors have been demonstrated in the spinal cord (Mohler and Okada, 1977) and we have recently shown that midazolam produces spinal analgesia which is different from that caused by fentanyl (Serrao et al., 1989). We report here on the effects of intrathecal chlordiazepoxide, the archetypical benzodiazepine.

Male Wistar rats (140-240g) were anaesthetised with halothane and chronic lumbar intrathecal catheters were implanted as described previously (Serrao et al., 1989). After recovery from anaesthesia, and after each test session, an injection of 10 μl of 2% lignocaine was made via the catheter. Animals with intrathecal catheters showed paralysis of the hind limbs within 30 seconds; unless this occurred they were not included in the study. Electrical current threshold for pain (ECTP) was assessed by stimulation via electrodes on the tail and the skin of the neck. Drugs were injected intrathecally in a volume of 10 μl at a rate of 1 μl per second. ECTP measurements were made every 5 min for a suitable period before and after the injection.

Chlordiazepoxide was found to produce an increase in the tail threshold without affecting neck threshold, which indicated spinally mediated analgesia. This effect of intrathecal chlordiazepoxide was observed using doses ranging from 0.03 to 0.9 μmol and a sigmoid dose-response curve was obtained, with a maximum at 0.6 μmol. At doses higher than 0.9 μmol we found a significant increase in ETCP in the neck as well as the tail and thus a spinal site of action was not certain. The effects of antagonists were studied using chlordiazepoxide 0.6 μmol, and a dose of fentanyl, 0.74 nmol, which was equipotent in producing spinal analgesia and had previously been shown to be just maximal. Antagonist doses were also chosen on the basis of previous experiments (Serrao et al., 1989). Flumazenil (Ro 15-1788; 16.5 μmol kg^{-1} i.p.) was found to block the response to chlordiazepoxide but had no effect on the response to fentanyl. In contrast, naloxone (0.38 μmol kg^{-1} i.p.) blocked the response to fentanyl but had little effect on the spinal analgesia caused by chlordiazepoxide.

These results showed that intrathecal chlordiazepoxide caused analgesia by an action on the spinal cord, mediated through benzodiazepine receptors. The mechanism is different from that of mu opioid agonists such as fentanyl.

Schmauss, C. & Yaksh, T.L. (1984). J. Pharmacol. Exp. Ther. 228, 1-12.
Mohler, H. & Okada, T. (1977). Science 198, 849-851.
Serrao, J.M., Stubbs, S.C., Goodchild, C.S. & Gent, J.P. (1989). Anesthesiology 70, 780-786.

LITHIUM ENHANCES THE INHIBITORY EFFECTS OF RECEPTOR AGONISTS ON INOSITOL PHOSPHOLIPID HYDROLYSIS IN BRAIN SLICES

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Lithium (Li^+) is a potent therapeutic and prophylactic agent in the treatment of affective disorders, however, its underlying mode of action remains unknown. Avissar et al (1988) reported that Li^+ interfered with GTP binding in rat cortical membranes suggesting that G proteins may be a target for its action and we have recently shown that lithium amplifies the inhibitory effects of fluoroaluminate ions (Whitworth and Kendall, 1989) which might act as a g-protein negatively linked to phospholipase C catalysed phosphoinositide (PI) hydrolysis. This reasoning should, therefore, predict an enhancing effect by Li^+ on receptor mediated inhibitions of PI hydrolysis. We have now examined the effect of Li^+ on the modulation of PI hydrolysis due to excitatory and inhibitory receptor agonists.

Cross chopped slices (350μm x 350μm) of cerebral cortex from male or female Hartley guinea pigs and black C57 mice were prepared, preincubated and prelabelled with 24 KBq Myo-[2-^{3}H] inositol. Slices were then stimulated by the addition of agonists in combination with another agonist capable of inhibiting or enhancing the accumulation of total ^{3}H-InsPs due to the initial agonist. All incubations were performed in the presence and absence of LiCl (5mM) and terminated after 45 min. Separation of the total ^{3}H-InsPs was by anion exchange chromatography.

The effect of Li^+ on two receptor mediated inhibitions of inositol phospholipid hydrolysis are illustrated by the effect of glutamate (3mM) on the accumulation of total ^{3}H-InsPs due to muscarinic receptor stimulation with carbachol (1mM) in the guinea pig and the inhibition of the histamine (1mM) response due to adenosine (3×10^{-4} M) in the mouse. In the absence of Li^+ glutamate had no effect on the muscarinic response, however, in the presence of Li^+, glutamate significantly reduced the carbachol response by 24±4.8% ($p<0.05$, n=4). This inhibition was apparent over a range of carbachol concentrations (10^{-5}-10^{-3} M). Adenosine inhibited the accumulation of ^{3}H-InsPs due to histamine in the absence of Li^+ (41±7%). However, the reduction in the presence of Li^+ was significantly greater (62±4%, $p<0.05$, n=5).

Glutamate is capable of enhancing the ^{3}H-InsPs accumulation due to noradrenaline (3×10^{-4} M) in guinea pig by 228±32% but in contrast to the inhibitory effects Li^+ did not alter this enhancement (225±21%, n=3).

In contrast to the mouse, adenosine potentiates the histamine response in guinea pig (163±17% n=4), however, Li^+ does not enhance this potentiation (162±24%).

These findings provide further evidence that Li^+ enhances inhibitions of phosphoinositide hydrolysis, possibly via the activation of an inhibitory g-protein.

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Avissar, S. et al (1988) Nature 331, 440-442.
Whitworth, P. and Kendall, D.A. (1989) Br.J.Pharmac., in press.

Na^+ IONS DISTINGUISH BETWEEN THE BINDING OF A TERTIARY AND A QUATERNARY [^{3}H]-ANTAGONIST TO THE HISTAMINE H_1 RECEPTOR

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It has been reported that the binding of [^{3}H]-mepyramine to the histamine H_1-receptor in guinea-pig brain is unaffected by cations, whereas the inhibition of [^{3}H]-mepyramine binding by histamine is reduced by Na^+ and enhanced by Mg^{2+} and Mn^{2+} (Chang & Snyder, 1980). The same pattern is observed with [^{3}H]-doxepin as the H_1-receptor label (Toll & Snyder, 1982). This sensitivity of agonist binding to modulation by ions and relative insensitivity of antagonist binding seems to be a feature common to several neurotransmitter receptors. However, in the course of binding studies with a novel quaternary radioligand for the H_1-receptor, [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP), we observed that 100 mM NaCl inhibited both the specific and non-specific binding of [^{3}H]-QMDP (Treherne & Young, 1988). We report here the results of a comparative study of the effect of cations on the binding of [^{3}H]-mepyramine, a tertiary amine, and [^{3}H]-QMDP.

Guinea-pig (Dunkin-Hartley strain, males) cerebellar membranes were prepared by homogenisation in 50 mM Tris-HCl, pH 7.5, containing 1 mM EDTA, followed by centrifugation at 17,000 g for 30 min. The membranes were washed by resuspension in Tris buffer (no EDTA present) and recentrifugation. Measurement of the inhibition of the binding of 0.37-0.70 nM [^{3}H]-QMDP or 0.36-0.73 nM [^{3}H]-mepyramine was made essentially as described previously (Treherne & Young, 1988). Non-H_1-receptor binding was defined as binding insensitive to 1 uM temelastine. Cations were added as the chloride salts, with the exception of Zn, which was the sulphate.

Both the temelastine-sensitive and temelastine-insensitive binding of [^{3}H]-QMDP was inhibited by both mono- and di-valent cations. Of the monovalent ions studied Li and Na were the most effective as inhibitors of the binding to cerebellar H_1-receptors, IC_{50} circa 50 mM, followed by K, Rb and Cs (IC50 150 mM). Direct comparison of the chloride, sulphate and nitrate salts of Na failed to reveal any obvious differences in the inhibition of the binding of [^{3}H]-QMDP. Of the divalent ions Ca was somewhat more effective than Mg as an inhibitor of specific [^{3}H]-QMDP binding (IC_{50} 10 mM and 25 mM, respectively), but both were much weaker than Cd, the most potent ion tested (IC_{50} 10 uM). The order of potency of the divalent ions was Cd > Zn > Ni > Co > Mn > Ca > Mg. Curves of the inhibition of [^{3}H]-mepyramine binding by these ions were very similar to those for the inhibiton of [^{3}H]-QMDP. This was also true for Li, but Na was strikingly less effective in inhibiting the binding of [^{3}H]-mepyramine than that of [^{3}H]-QMDP (13 ± 2 (n=13) and 69 ± 1% (n=37) inhibition, respectively, by 100 mM NaCl). The basis for this selectivity of action of Na^+ on the binding of the quaternary antagonist remains unknown, as does the nature of the site or sites at which the other cations bind to inhibit the binding of both [^{3}H]-QMDP and [^{3}H]-mepyramine.

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Chang, R.S.L. & Snyder, S.H. (1980) J. Neurochem. 34, 916-922
Toll, L. & Snyder, S.H. (1982) J. Biol. Chem. 257, 13593-13601
Treherne, J.M. & Young, J.M. (1988) Br. J. Pharmacol. 94, 797-810

THE RELEVANCE OF THE DISTRIBUTION AND METABOLISM OF OCTANOATE TO ITS ANTICONVULSANT ACTIVITY IN THE MOUSE

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The medium chain triglyceride (MCT) diet provides relief from epileptic seizures in 60% of children who have failed to be controlled by conventional drug therapy (Schwartz et al., 1980). It has been previously reported (Bonser et al., 1989) that infusions of octanoate or decanoate, the major metabolites of this diet, can protect mice from seizures. A correlation between plasma levels of these metabolites and protection was also demonstrated. However, whether octanoate or decanoate themselves, or further metabolic products are responsible for the anticonvulsant effect of the acids in mice remains unclear. Therefore the distribution and metabolism of octanoate and the anticonvulsant action of its principle metabolites, beta-hydroxybutyrate and acetoacetate were investigated.

1-60 minutes after male mice (Tuck No. 1; 25-35g) had been injected with ^{14}C-sodium octanoate (0.1ml; 100mg ml^{-1} i.v.) or at the end of a 10-60 minute infusion (100mg ml^{-1}; 12.5µl min^{-1}), blood and urine were collected. The carcass was then frozen in liquid nitrogen for 15 minutes. Samples of brain, heart, liver, kidney, skeletal muscle and fat were removed from the mice and assayed for radioactivity. Octanoate was also measured using high performance liquid chromotography. Most of the radioactivity was found in the liver, but after 5 minutes very little of this radioactivity was in the form of octanoate. There was little accumulation of octanoate or radioactivity in extrahepatic tissues. Thus a few minutes after injection or infusion of octanoate most of it had been metabolised.

It seemed possible that octanoate's anticonvulsant activity may have resulted from the effect of its metabolic products, and so the anticonvulsant action of acetoacetate and beta-hydroxybutyrate was investigated after long infusions (30 minutes), when steady state levels had been achieved. Mice were given infusions of acetoacetate (60mg ml^{-1}; 14µl min^{-1} i.v.) or beta-hydroxybutyrate (120mg ml^{-1}; 14µl min^{-1} i.v.) for 30 minutes at which time they were subjected to one or two tests: the maximal electroshock test (platinum ball and clip ear electrodes, 15mA; 100Hz; 0.2sec.) or a slow infusion of pentylenetetrazole (15mg ml^{-1} in saline; 0.3ml min^{-1}) administered until a clonic seizure was evoked. After the test, the mice were anaesthetised and blood and brains removed. These were analysed for the presence of acetoacetate and beta-hydroxybutyrate using an enzymatic spectrophotometric method. Plasma levels of acetoacetate and beta-hydroxybutyrate were found to be 4.2mM and 6.3mM with corresponding brain levels of 0.05mM and 0.02mM respectively. No protection was shown against either of the convulsant stimuli.

Huttenlocher (1976) suggested that the anticonvulsant effect of the MCT diet in children was dependent upon maintenance of plasma levels of acetoacetate above 2mM and of beta-hydroxybutyrate above 0.6mM but our results suggest that the main metabolites of octanoate, acetoacetate and beta-hydroxybutyrate, are not anticonvulsant in these two animal models.

BONSER, J.C., DEAN, H.G. & GENT, J.P. (1989). Br. J. Pharmacol. 96: 362.
HUTTENLOCHER, P.R. (1976). Pediatr. Res., 10: 536-540.
SCHWARTZ, R.H., AYNESLEY-GREEN, A. & BOWER, B.D. (1980). Res. Clin. Forums, 2: 63-73.

EFFECTS OF CARBAMEZEPINE ON TRYPTOPHAN METABOLISM AND DISPOSITION IN THE RAT

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The anticonvulsant drug carbamazepine (CBZ) is structurally related to tricyclic antidepressants and its mood-stabilizing action has led to its use in prophylaxis and therapy of depression. The basis of these latter properties is, however, unclear. Pratt et al. (1984) found that chronic CBZ treatment of epileptic patients increases circulating tryptophan (Trp) concentrations, with the rise in the free, being relatively greater than that in the bound, fraction. This suggests that CBZ both displaces albumin-bound Trp and inhibits liver Trp pyrrolase activity. We have previously shown in rats that > 20 tricyclic, MAO-inhibitory and other antidepressants increase Trp availability to the brain, thereby enhancing 5-HT synthesis, by inhibiting liver Trp pyrrolase activity (Badawy & Evans, 1982; Badawy,1986; Badawy et al., 1987). It was therefore considered of interest to find out if pyrrolase activity could also be inhibited by CBZ and whether the drug effects on Trp metabolism and disposition are similar to, or could be distinguished from, those of standard antidepressants.

Male Wistar rats (150-170g) received CBZ either acutely [in single i.p. doses of 0.25-25mg/kg, dissolved in a dimethylformamide:saline mixture (1:3, v/v; 2ml/kg)] or chronically in drinking water (5mg/100ml; average daily dose: 5mg/kg).

Like antidepressants, CBZ inhibited rat liver Trp pyrrolase activity both in vitro and after acute administration. Unlike that by antidepressants, the CBZ inhibition was short-lived (maximum at 0.5h, vs 2h with antidepressants). Maximum inhibition was caused by a 5mg/kg CBZ dose (50% for total enzyme activity and 75% for that of the haem-free apoenzyme). Also unlike antidepressants, the CBZ inhibition was followed after treatment with doses of 10mg/kg and above by activation (increased haem saturation) of the enzyme, with the maximum increase (67%) occurring at 4h after a 20mg/kg dose. Chronic CBZ treatment at the above-mentioned dosage also inhibited pyrrolase activity. For example, after 1 week, the total enzyme and apoenzyme activities were decreased by 48% and 81% respectively.

Both acute and chronic CBZ administration increased Trp availability to the brain and thus enhanced 5-HT synthesis. For example, after 1 week of chronic treatment, brain Trp, 5-HT and 5-HIAA concentrations were increased by 87%, 46% and 26% respectively. The corresponding increases in these brain indoles observed at 2h after acute administration of doses of 1-25mg/kg were 20-59%, 17-48% and 22-43% respectively. Under these conditions, liver and serum Trp concentrations were also increased. With the 1mg/kg acute dose, the increases in free and total serum Trp concentrations were similar (21-22%), thus suggesting pyrrolase inhibition as the sole mechanism, whereas with larger acute doses and after chronic treatment, the rise in free, was relatively greater than that in total, Trp. The resultant decrease in Trp binding was associated with increases in serum non-esterified fatty acid concentration (of 42% at 1 week of chronic treatment and of 50-117% at 2h after acute administration of doses of 2.5-25mg/kg).

We conclude that CBZ enhances rat brain 5-HT synthesis by increasing Trp availability to the brain secondarily to inhibition of liver Trp pyrrolase activity, and suggest that the shorter duration of the pyrrolase inhibition and the subsequent activation of the enzyme caused by large doses, observed after acute treatment, may explain the clinical differences between CBZ and standard antidepressants.

Badawy, A. A.-B. (1986) Br. J. Pharmac. 89, 859P
Badawy, A. A.-B. et al. (1987) Br. J. Pharmac. 91, 345P
Badawy, A. A.-B. & Evans, M. (1982) Br. J. Pharmac. 77, 59-67
Pratt, J. A. et al. (1984) J. Neurol. Neurosurg. Psychiat. 47, 1131-1133

INCREASES IN INTRASYNAPTOSOMAL FREE $[Ca^{2+}]_i$ ELICITED BY GLUTAMATE ARE MEDIATED VIA A QUISQUALATE-TYPE RECEPTOR

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Synaptosomes were isolated from cerebral cortices of 2-4 male Wistar rats on discontinous percoll density gradients (Dunkley et. al., 1986) and $[Ca^{2+}]_i$ was measured using the Ca^{2+}-indicator fura-2 (Grunkiewicz et. al., 1985): basal $[Ca^{2+}]_i$ was found to be 161nM (median value) and the interquartile range was 133-225nM (n=108).

Dose response data was obtained for excitatory amino acid agonists and fitted to a standard curve of the form $y=R_{max}(X)/(EC_{50}+X)$ and the "goodness of fit" assessed using a correlation trend test (CTT).

Incubation of synaptosomes for 1 minute with L(+)-glutamate (10nM-100μM) produced a dose dependent increase in $[Ca^{2+}]_i$, the maximal response occuring at 10μM (R_{max}=36.6%, EC_{50}=0.38μM, p 0.003 CTT). This effect was stereospecific since D(-)-glutamate was less potent in increasing $[Ca^{2+}]_i$ (EC_{50}=0.74μM, p 0.001, CTT) but raised $[Ca^{2+}]_i$ by a comparable amount (R_{max}=31.2%).

Incubation with quisqualate (1nM-100μM) or ibotenate (0.1μM-600μM) for 1 minute also resulted in a dose dependent decrease in $[Ca^{2+}]_i$. (R_{max}: quisqualate = 37.9%, ibotenate = 57.0%). Quisqualate (EC_{50}=0.1μM) was more potent than ibotenate, or either L(+)- or D(-)-glutamate. However the amino acid agonists NMDA (1-100μM) and kainate (1-100μM) failed to increase basal $[Ca^{2+}]_i$. This data suggests that increases in $[Ca^{2+}]_i$ elicited by glutamate are via a quisqualate type receptor. The effects of 10μM concentrations of the above ligands were all blocked by L(+)-2-amino-4-phosphonobutyric acid (2APB, 10μM).

In addition the effect of ibotenate (10μM) and quisqualate (10μM) were not inhibited when extrasynaptosomal Ca^{2+} is removed or when synaptosomes were incubated with the ionophoric receptor blocker 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX). The effects of ibotenate and quisqualate were blocked however when synaptosomes were treated with compound L(+)-serine-o-phosphate (1mM). This data suggest that the quisqualate -type receptor which is involved in the effects of quisqualate, ibotenate and glutamate may be of the metabotrobic type.

Dunkley, P. R., Jarvie, P. E., Heath, J. W., Kidd, G. J., and Rostas J. A. P. (1986) Brain Res. 372, 115-129.

Grynkiewicz, G., Poenie, M. and Tsien, R. Y. (1985) J.Biol.Chem. 260, 3440-3450.

[^{3}H]-MK-801 LABELS MORE NMDA RECEPTOR SITES THAN [^{3}H]-TCP IN HUMAN CORTICAL MEMBRANES

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[^{3}H]-Thienylcyclohexylpiperidine (TCP) and [^{3}H]-MK-801 selectively bind to the N-methyl-D-aspartate (NMDA) receptor ion channel (Wong et al., 1988) and have been used to study this receptor in rat brain. In this study the binding of [^{3}H]-MK-801 and [^{3}H]-TCP have been compared under various conditions in human cortical membranes.

Grey matter from frozen blocks of tissue from 4 control subjects (age 70 ± 5y, post-mortem delay = 12 ± 5h, no protracted deaths), was homogenised and washed extensively (Procter et al., 1989). Assays were performed in 1ml Tris HCl (5mM, pH 7.4) containing approximately 200µg protein, [^{3}H]-TCP or [^{3}H]-MK-801 (5nM, except for Scatchard analysis), glutamate, glycine or glutamate plus glycine. Non-specific binding was determined with 100µM MK-801. Stimulated binding of both ligands had reached equilibrium after 2h at 25°C when the reaction was terminated by rapid filtration (Stirling et al., 1989).

Glutamate and glycine enhanced the binding of [^{3}H]-MK-801 and [^{3}H]-TCP in a concentration-dependent fashion (approximate ED_{50} 1-10µM). Glutamate and glycine together increased the binding of [^{3}H]-MK-801 to a much greater extent than that of [^{3}H]-TCP (Table 1).

Table 1 The effects of glutamate and glycine on [^{3}H]-MK-801 and [^{3}H]-TCP binding

Addition	Specific binding (fmol mgprotein^{-1}) [^{3}H]-MK-801	[^{3}H]-TCP
Zero	140 ± 20	108 ± 7
30µM glutamate	336 ± 31	171 ± 18
100µM glutamate	276 ± 11	233 ± 25
1000µM glycine	372 ± 5	276 ± 24
30µM glutamate and 1000µM glycine	725 ± 72*	333 ± 60

Values are mean ± s.e.mean, n = 3-4. *p <0.01, Student's 't' test compared with glycine or glutamate alone.

Saturation analysis under maximal amino acid stimulation (100µM glutamate + 1000µM glycine) demonstrated significantly ($p < 0.05$) more high affinity sites for [^{3}H]-MK-801 (mean ± s.e.mean, n = 3; B_{max} = 1.5 ± 0.3 pmol mgprotein^{-1}) than for [^{3}H]-TCP (B_{max} = 0.66 ± 0.17 pmol mgprotein^{-1}). The affinity of [^{3}H]-MK-801 for these sites (K_D= 5.0 ± 0.2nM) was also greater than the affinity of [^{3}H]-TCP (K_D= 16.8 ± 1.0nM). The potency of a number of inhibitors was identical for [^{3}H]-MK-801 and [^{3}H]-TCP binding, i.e. MK-801 >TCP >PCP >SKF10047 >dextromethorphan.

These data suggest that in human cortical membranes, in the presence of glutamate and glycine more binding sites for [^{3}H]-MK-801 are available than for [^{3}H]-TCP. These sites may differ in their modulation by glutamate and glycine.

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Procter, A.W., Wong, E.H.G., Stratmann, G.C., Lowe, S.L. & Bowen, D.M. (1988) J. Neurochem., (In press).
Stirling, J.M., Cross, A.J. & Green, A.R. (1989) Neuropharmacology, 28, 1-7.
Wong, E.H.F., Knight, A.R. & Woodruff, G.N. (1988) J. Neurochem. 50, 274-281.

DOES AN ENDOGENOUS NITROVASODILATOR MEDIATE NANC RELAXATIONS OF THE MOUSE ANOCOCCYGEUS?

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Relaxations of the mouse anococcygeus muscle to non-adrenergic, non-cholinergic (NANC) field stimulation and to nitrovasodilator drugs are selectively inhibited by N-methyl-hydroxylamine and selectively potentiated by M&B 22948 (Gibson & Mirzazadeh, 1989); this raises the possibility that the NANC nerves of the anococcygeus release an endogenous nitrovasodilator-like substance similar to that released by other cells, including vascular endothelium (Rees et al., 1989) and cerebellar neurones (Garthwaite et al., 1988). The experiments reported here form part of a study designed to test this possibility and to determine which of the several distinct types of nitrovasodilator drug (Waldman & Murad, 1987) most closely resembles the NANC transmitter.

Anococcygeus muscles from male mice were set up, in vitro, for the recording of isometric tension responses to drugs and field stimulation (Gibson & Mirzazadeh, 1989). Submaximal concentrations of 4 nitrovasodilator drugs were used: sodium nitroprusside (SNP, 0.02 - 0.5 μM), hydroxylamine (1 - 20 μM), sodium azide (1 - 20 μM), and nitric oxide (NO, 5 - 200 μM).

N(G)-monomethyl-L-arginine (LNMMA, 60 μM), which inhibits the synthesis of NO from arginine in endothelial cells (Rees et al., 1989), reduced NANC (10 Hz, 60 s) relaxations of the mouse anococcygeus by 50%; this effect was reversed by L-arginine (100 μM), but not by D-arginine (200 μM). Relaxations to NO, SNP, hydroxylamine, and azide were unaffected by LNMMA.

Hydroquinone (HQ, 50 μM), which inhibits EDRF- and NO- induced relaxations of aortic strips (Furchgott, 1984; Waldman & Murad, 1987), greatly reduced NO-induced relaxations of the mouse anococcygeus (by 90%). However, HQ did not affect relaxations to NANC stimulation, SNP, hydroxylamine, or azide.

Hypoxia (induced by bubbling the Krebs solution with 95% N_2 : 5% CO_2), which selectively inhibits NANC relaxations of the bovine retractor penis and rat anococcygeus (Bowman & McGrath, 1985), markedly reduced NANC relaxations of the mouse anococcygeus (by 65%); relaxations to azide were also reduced (by 60%), but those to NO, SNP, and hydroxylamine were either unaffected or slightly potentiated.

The selective inhibition of NANC relaxations by LNMMA, and its reversal by L-arginine but not D-arginine, suggests that the NANC transmitter may be formed from arginine, like EDRF in endothelial cells (Rees et al., 1989). However, while evidence suggests that EDRF is NO, this does not seem to be the case with the NANC transmitter of the anococcygeus since both HQ and hypoxia were able to distinguish between NANC relaxations and those to NO. Indeed, of the nitrovasodilators studied, azide most closely paralleled the NANC response; this may suggest that the NANC transmitter resembles the azide class of nitrovasodilator drug, which requires O_2-dependent activation in order to induce smooth muscle relaxation (Waldman & Murad, 1987).

Bowman, A. & McGrath, J.C. (1985) Br. J. Pharmacol. 85, 869 - 875.
Furchgott, R.F. (1984) Ann. Rev. Pharmacol. Toxicol. 24, 175 - 197.
Garthwaite, J., Charles, S.L. & Chess-Williams, R. (1988) Nature 336, 385 - 388.
Gibson, A. & Mirzazadeh, S. (1989) Br. J. Pharmacol. 96, 637 - 644.
Rees, D.D., Palmer, R.M.J., Hudson, H.F., & Moncada, S. (1989) Br. J. Pharmacol. 96, 418 - 424.
Waldman, S.A. & Murad, F. (1987) Pharmacol. Rev. 39, 163 - 196.

SURAMIN, A SELECTIVE INHIBITOR OF NANC TRANSMSSION IN RAT VAS DEFERENS: COMPARISON WITH NIFEDIPINE

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The trypanocide suramin has recently been suggested to be a specific antagonist of the P_{2x}-purinoceptor in mouse vas deferens (Dunn & Blakeley, 1988). Nifedipine has been used widely as a pharmacological tool to induce selective inhibition of the NANC component of the response of the rat vas deferens to single pulse field stimulation (French & Scott, 1981). We have compared the actions of suramin with those of nifedipine on responses of rat vas deferens to exogenous αβ-methylene ATP (MATP), NA and challenges of twin pulse field stimulation (TPFS).

Tension responses of isolated desheathed rat vasa deferentia were elicited at 5 minute intervals to challenges of TPFS (0.8msec width, 40V, 3s inter-pulse interval, via parallel platinum electrodes) and digitised at a sampling frequency of 100Hz and stored on floppy disc under the control of a BBC microcomputer (Marshall & Spriggs, 1985). Concentration-response curves were constructed to MATP and NA using 15 minute dose intervals and repeated 20 minutes after exposure to suramin or nifedipine.

Suramin alone (0.1μM - 1mM) failed to contract the vas deferens. However, suramin (3μM - 300μM) produced a reversible, concentration-related reduction in responses to MATP. At 30μM, suramin induced a rightward shift in the MATP concentration-response curve associated with an increased maximum response. At 300μM, suramin abolished contractions induced by MATP up to 30μM. No effect on NA concentration-response curves was apparent at either 30μM or 300μM suramin. In the presence of nifedipine (10μM) concentration-response curves to NA were abolished whilst those to MATP exhibited a small rightward shift accompanied by a depressed maximum.

The response of the rat vas deferens to the first pulse of a TPFS challenge shows a large first peak at 280msec followed by a second smaller peak 650msec after the stimulus. The response to the second pulse attains a greater first peak height with a diminished second phase (Marshall & Spriggs, 1984). Both nifedipine (10nM - 10μM) and suramin (100nM - 1mM) induced concentration-related inhibitions (up to 90%) of the NANC component of both responses to TPFS challenges leaving the noradrenergic components relatively intact and revealing a small residual component, peaking at 650msec, in the response to the second pulse.

These results demonstrate that suramin, like nifedipine selectively inhibits the NANC component of responses to TPFS challenges but differs in its effects on exogenous MATP and NA. In particular suramin antagonises responses to exogenous MATP without effect on NA, whilst nifedipine, at concentrations which have little effect on the NA component of the response to TPFS, antagonises the actions of exogenous NA in addition to those of exogenous MATP.

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Dunn, P.M. & Blakeley, A.G.H. (1988) Br.J.Pharmac. 93; 243-245.
French, A.M. & Scott, N.C. (1981) Br.J.Pharmac. 74; 321-323.
Marshall, R.W. & Spriggs, T.L.B. (1984) Br.J.Pharmac. 83; 383P.
Marshall, R.W. & Spriggs, T.L.B. (1985) Br.J.Pharmac. 85; 392P.

VASCULAR ENDOTHELIUM RELAXANT PROPERTIES IN A RAT MODEL OF CALCIUM OVERLOAD

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Fleckenstein *et al.*, (1985) have previously shown that treatment of rats with vitamin D_3 (a single injection of 300,000 IU, i.m.) plus nicotine (25 mg/kg, p.o., twice daily for 4 days) produces calcium overload in arteries. Using a similar model we showed that such treatment produces an increase in relaxation time following vasoconstriction in vitro (Thorin-Trescases *et al.*, 1989). Young, male Wistar rats (200 g), were given vitamin D_3 plus 1 day of treatment with nicotine, followed by 16 days of recovery. We then determined the degree of endothelium dependent (carbachol) or independent (papaverine) relaxation of noradrenergic vasoconstriction in isolated mesenteric arteries removed from these rats or from controls. The isolated mesenteric artery bed was perfused at a constant flow rate (4 ml/min) with Krebs bicarbonate. Noradrenaline was injected as a bolus (0.2 ml, 3×10^{-10} to 10^{-7} M) every 5 min. Vasoconstriction was estimated from the increase in perfusion pressure (Δ mm Hg) and relaxation from the time (s) from peak to 50% of this value. Following the construction of the noradrenaline concentration/response curve, noradrenaline was injected at a constant concentration (5×10^{-9} M) in a bolus every 5 min, in the presence or absence of a 5 min perfusion of either carbachol or papaverine (10^{-9} to 10^{-5} M for each). A similar protocol was applied to a separate series of mesenteric beds in which the endothelium was removed by passing air (0.2 ml/min) through the bed for 5 min.

n = 12/group	control		vitamin D_3 plus nicotine	
endothelium	present	absent	present	absent
Basal perfusion pressure				
mm Hg	18.3±0.9	23.3±2.1[b]	16.8±0.7	18.3±1.1[a]
Noradrenaline concentration/response curve.				
Δ (mm Hg): 10^{-7} M	131±4	145±8	148±6[a]	185±7[a,b]
relaxation (s): 10^{-7} M	52±5	109±12[b]	63±4[a]	115±5[b]
Δ (mm Hg): 3×10^{-9} M	23±3	60±9[a]	40±4[a]	79±7[a]
%relaxation by carbachol of vasoconstriction after 5×10^{-9} M noradrenaline				
Δ maximum	10±4	91±10[b]	39±5[a]	85±4[b]
% 10^{-7} M	43±5	98±14[b]	91±6[a]	116±7
%relaxation by papaverine of vasoconstriction after 5×10^{-9} M noradrenaline				
Δ maximum	4±1	4±1	22±5[a]	15±2[a]
% 10^{-7} M	44±4	56±6	101±3[a]	83±7[a,b]

Means ± SEM. [a] = $P < 0.05$ with corresponding control group mean, [b] = $P < 0.05$ with mean in presence of endothelium. The calcium content of the aorta in a parallel series was 554 ± 194 micromols/g dry weight, n = 10 for treated and 9 ± 1, n = 10 for control rats (Chillon *et al.*, unpublished results). In conclusion, vascular calcium overload produces a decrease in both endothelium-dependent and endothelium-independent relaxation.

Fleckenstein A., Frey M., Zorn J. and Fleckenstein-Grün G. (1985). Am J. Cardiol. 56, 3H-14H.
Thorin-Trescases N., Henrion D., Oster L., Thorin E., Hicks P.E. and Atkinson J. (1989). Br. J. Pharmacol. 96, 209P.

THE EFFECTS OF ACETYLCHOLINE, BRADYKININ, SODIUM NITROPRUSSIDE AND AP-II ON RENAL CORTICAL BLOOD FLOW IN THE RAT

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Thomas *et al.* (1988) used radiolabelled microspheres to measure regional blood flow and showed that the vasculature of the stomach and skeletal muscle were equally sensitive to the vasodilator actions of both acetylcholine (Ach) and bradykinin (BK), thought to be due to the release of endothelium-derived relaxing factor (EDRF). However, the kidneys showed selectivity towards the effects of Ach and the intestines towards those of BK. Here, using laser Doppler flowmetry, we show the time course of the Ach response on renal cortical flux (RCF), an index of cortical blood flow and the effects of inhibitors of muscarinic receptors (atropine) and of EDRF (gossypol and N^G-monomethyl-L-arginine (L-NMMA)).

Male Wistar rats (250-350g, Glaxo) anaesthetized with Inactin (Byk, 120mg/kg, i.p.) were used. After a tracheotomy and cannulation of: the right carotid artery for recording blood pressure (MAP) and heart rate; the left jugular and left femoral veins for administration of drugs; the left kidney was exposed through a mid-line laparotomy. A laser Doppler probe was positioned on the surface of the kidney and RCF was continuously measured. All animals were then treated with indomethacin (5mg/kg, i.v.). A 3 min infusion of either Ach (10µg/min), BK (15µg/min) or sodium nitroprusside (SNP; 4µg/min) or a single bolus dose of rat atriopeptin-II (AP-II; 15µg/kg) was then administered. Acetylcholine (0.5mg/50µl), BK (0.5mg/50µl) and SNP (0.5mg/50µl) were also applied topically onto the kidney.

Systemic Ach caused an initial fall in RCF and MAP. However, after 2 min RCF had returned to normal values, whereas the general hypotensive response to Ach was maintained. The cortex continued to dilate, giving an increase in RCF to a maximal response of 146±13% of control at 3.9±0.6 min. The RCF remained elevated for the duration of the recording period (20 min). A similar hypotensive response was observed after BK, although this waned due to a concomitant increase in heart rate. Bradykinin also caused a fall in the RCF which, unlike Ach, only returned to control levels after the cessation of the BK infusion. An intravenous infusion of SNP was associated with a fall in RCF and MAP. Upon cessation of the infusion, MAP returned to normal whilst the RCF showed a significant increase. This elevation in RCF was evident for the remainder of the experimental period. A bolus of AP-II caused an increase in RCF without any effect on heart rate or blood pressure. Topical application of Ach again increased RCF whilst BK and SNP elicited a decrease.

Atropine (0.3mg/kg, i.v.) abolished the increase in RCF seen after systemic or topical Ach. The increase in RCF after Ach was not mediated via the release of vasodilator eicosanoids since all animals were pretreated with indomethacin nor could it be wholly attributed to EDRF release for the response to topical administration of Ach was only partially inhibited by perfusion of the kidney with gossypol (100 µM) and unaffected by pretreatment of the rat with L-NMMA (100mg/kg), said to be an inhibitor of EDRF formation (Rees *et al.*, 1989).

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Rees, D.D., Palmer, R.M.J., Hodson, H.F. & Moncada, S. (1989) Br. J. Pharmacol. **96**, 418-424.
Thomas, G.R., Thiemermann, C., Walder, C. & Vane J.R. (1988) Br. J. Pharmacol. **95**, 986-992.

ENDOTHELIUM-DERIVED NITRIC OXIDE IS RESPONSIBLE FOR HYPERPOLARIZATION AND RELAXATION IN ARTERIAL SMOOTH MUSCLE

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In most arteries acetylcholine causes relaxation and hyperpolarization of the smooth muscle by an endothelium-dependent mechanism. The relaxation is due to release of endothelium-derived relaxing factor (EDRF) which is closely related to nitric oxide (NO). The cause of the hyperpolarization is not known, but it has been postulated that it results from the release of a factor that is distinct from EDRF (Taylor & Weston, 1988). Acetylcholine is also capable of hyperpolarizing cultured endothelial cells, and it is therefore possible that hyperpolarization recorded in the smooth muscle is a result of electrotonic spread from the endothelium via gap junctions between the two cell types (Olesen et al, 1987). We set out to determine whether the hyperpolarization was due to a humoral factor and, if so, whether this could be attributed to release of EDRF.

In order to examine whether the hyperpolarization in response to acetylcholine was humorally mediated spiral strips of uterine, mesenteric or coronary arteries were prepared from guinea-pigs. Membrane potential was recorded using conventional intracellular microelectrode techniques and isometric tension was measured simultaneously. The preparations were continuously superfused with physiological solution to which drugs were added as required. The preparations were depolarized and tension was increased with 0.1-1μM phenylephrine. In the presence of phenylephrine, acetylcholine (1μM) caused hyperpolarization and relaxation. Endothelium was removed from the strips by abrasion and acetylcholine no longer elicited hyperpolarization or relaxation. A piece of aorta, from either rat or guinea-pig, with endothelium intact, was pinned to the floor of the bath under strips that had been denuded of endothelium. Under these conditions acetylcholine again caused hyperpolarization and relaxation of the strips denuded of endothelium.

Since both hyperpolarization and relaxation are produced by an endothelium-derived humoral factor, we also examined the actions of NO. Ring segments of various arteries were mounted on a Mulvany-Halpern style myograph for simultaneous measurement of membrane potential and tension and superfused with phenylephrine as before. NO was infused directly into the perfusion line for 10s periods, with a delay of 1-2s before it reached the preparation. In uterine and mesenteric arteries of guinea-pigs NO (0.1-20μM) caused hyperpolarization and relaxation that mimicked those evoked by acetylcholine. NO also hyperpolarized and relaxed coronary and mammary arteries of guinea-pigs, tail arteries of rats, mesenteric arteries of dogs and femoral and saphenous arteries of rabbits. The nitrovasodilators, nitroprusside (10μM) and nitroglycerine (10μM) also hyperpolarized uterine and mesenteric arteries of guinea-pigs, tail arteries of rats and femoral arteries of rabbits. Hyperpolarization was abolished by exposure to solution containing 100mM potassium.

It has recently been shown that N-monomethyl-L-arginine (NMMA) interferes with the biosynthesis of EDRF (Rees et al, 1989). In the present study exposure to NMMA (17μM) for 5min reduced both hyperpolarization and relaxation in response to acetylcholine. The responses to acetylcholine were restored within 3min of removing L-NMMA.

We conclude that hyperpolarization of the smooth muscle induced by acetylcholine results from the generation of EDRF, and there is no evidence for a separate hyperpolarizing factor.

Olesen, S.P. et al (1987) Nature 331, 168-170
Rees, D.D. et al (1989) Br. J. Pharmacol. 96, 418-424
Taylor, S.G. & Weston, A.H. (1987) Trends in Pharmacol. Sci. 9, 272-274

THE INHIBITION OF ENDOTHELIUM-DERIVED NITRIC OXIDE (EDNO) BY NATIVE AND OXIDATIVELY MODIFIED LOW-DENSITY LIPOPROTEINS

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Atherosclerotic blood vessels from cholesterol-fed animals exhibit decreased responses to agents that elicit endothelium-dependent relaxation (Verbeuren et al.). This observation has been linked to low-density lipoproteins (LDL) which have been shown to inhibit responses to EDNO (Andrews et al. 1987). However, it is the LDL modified by oxidation which is present in atherosclerotic arterial intima that is thought to be atherogenic. We have therefore investigated the effect of native and oxidised LDL on EDNO responses.

LDL (density, 1.019-1.063 g/ml) were prepared from fresh human plasma by discontinuous density gradient ultracentrifugation in the presence of 0.3 mM EDTA, without the addition of thimerosal (Jacobs et al. 1989). The oxidative modification of LDL (oxLDL) was performed by Cu^{2+} catalysis for up to 24 hr (Parthasarathy et al. 1985). Oxidation was measured by fluorescence spectroscopy (Bruckdorfer et al. 1988). EDNO relaxations were assayed as previously described using intact aortic rings from 6 month-old New Zealand White rabbits (Andrews et al. 1987). Rings were pre-contracted with noradrenaline(NA), serotonin(5HT), adrenaline, $PGF_{2\alpha}$ or phenylephrine (PE) and relaxed by graded doses of acetylcholine (ACh). After washout, the tissues were pre-incubated with native LDL or oxLDL (0.1- 2 mg protein/ml) or Tyrode's buffer (control) for 0-60 min and then the cycle of contraction/relaxation repeated. Finally, after removal of the lipoproteins by washing, the EDNO responses of the tissues were again assessed to determine the reversibility of the effects.

In the presence of native LDL (1-2 mg protein/ml),a rapid reversible change in the sensitivity to ACh induced relaxation was observed in tissues pre-contracted with NA,Ad or 5HT compared with control tissues. This effect was demonstrated by a 10-fold rightward shift in their dose-response curves. No inhibition was found when tissues were pre-contracted with PE or $PGF_{2\alpha}$. In contrast, oxLDL (0.5-2 mg protein/ml) caused a time-dependent and often irreversible inhibition of relaxation, irrespective of the agonist used for pre-contraction. This inhibition resulted in both a rightward shift in the dose-response curve to ACh and sometimes an inhibition of maximal relaxation (70-100 %). The magnitude and nature of the inhibition depended on the LDL of individual donors in a reproducible way.

We have shown that pathophysiological concentrations of LDL have two effects, a rapid reversible inhibition which depends on the presence of the humoral agents,NA,Ad or 5HT and a time-dependent inhibition which correlates with the degree of oxidation of the LDL. We speculate that the effect of native LDL occur in hypercholesterolaemia whereas in atheromatous arteries, where oxLDL accumulates, an irreversible inhibition of EDNO results.

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Andrews, H.E., Bruckdorfer, K.R., Dunn, R.C. & Jacobs. (1987) Nature, 327, 237-239.
Bruckdorfer, K.R., Dunn, R.C., & Jacobs, M. (1988). Brit J. Pharmacol. 94, 410P.
Jacobs et al., (1989) This meeting.
Parthasarathy, S., Steinbrecher, U.P., Barnett, J., Witzum, J.L. & Steinberg, D. (1985) Proc. Natl. Acad. Sci. USA 82, 3000-3004.
Verbeuren, T.J., Jordaens, F.H., Zonnekeyn, L., Van Hove, C.E., Coene, M.C & Herman, A.G. (1986) Circ. Res 58, 552-564.

HAEMODYNAMIC EFFECTS OF N^G-MONOMETHYL-L-ARGININE (L-NMMA) IN CONSCIOUS LONG EVANS RATS

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Nitric oxide (NO), which mediates endothelium-dependent relaxation, is synthetized by vascular endothelial cells from L-arginine (Palmer et al., 1988a). The synthesis of NO is inhibited by L-NMMA (Palmer, et al., 1988b), which increases blood pressure in the anaesthetized rabbit (Rees et al., 1989). We have now investigated the regional haemodynamic effects of L-NMMA in conscious, male, Long Evans rats (320-360 g).

Animals were anaesthetized (sodium methohexitone, 60 mg kg^{-1}, i.p.) and one group had pulsed Doppler probes (Haywood et al., 1981) sutured around the left renal (R) and superior mesenteric (M) arteries and the distal abdominal aorta (H, to represent hind quarters). In another group of rats the left external carotid artery was tied off and a Doppler probe was sutured around the common carotid artery to monitor internal carotid (IC) flow. Seven to ten days later the animals were re-anaesthetized (sodium methohexitone 40 mg kg^{-1}) and intravascular catheters were implanted. The following day continuous measurements were made of mean blood pressure, instantaneous heart rate and Doppler shift signals (flow) for the R, M and H or IC probes. Changes in vascular resistance were calculated from mean blood pressure and mean Doppler shift (Haywood et al., 1981). L-NMMA (0.3-300 mg kg^{-1}, i.v.) was administered as bolus doses.

L-NMMA (3 and 30 mg kg^{-1}) caused a dose-dependent rise in blood pressure of 12 ± 1 and 45 ± 2 mm Hg (mean ± s.e.m., n=8) with a concomitant fall in heart rate of 18 ± 7 and 66 ± 5 bpm respectively in the RMH group. Corresponding values in the IC group were 11 ± 2 and 39 ± 2 mm Hg and 36 ± 10 and 66 ± 7 bpm respectively (n=8; all values were significantly different from baseline measurements). The table summarizes some of the haemodynamic results expressed as mean ± s.e.m. * $P < 0.05$ versus baseline (Friedman's test).

Table 1

Cardiovascular changes 2 min after L-NMMA administration (% change)

L-NMMA Dose (mg kg^{-1})		Group 1 R	Group 1 M	Group 1 H	Group 2 IC
3	Flow	-6 ± 1*	-13 ± 2*	2 ± 3	-12 ± 3*
	Resistance	19 ± 2*	29 ± 3*	10 ± 3*	28 ± 3*
30	Flow	-18 ± 1*	-29 ± 3*	-19 ± 4*	-34 ± 2*
	Resistance	78 ± 4*	106 ± 6*	83 ± 9*	110 ± 7*

These results indicate that the pressor responses to L-NMMA are not due to uniform vasoconstrictions in all vascular beds and therefore that endothelial-derived NO may contribute differentially to the control of tone in these vascular beds.

Palmer, R.M.J. et al., (1988a), Nature, 333, 664-666.
Palmer, R.M.J. et al., (1988b), Biochem. Biophys. Res. Commun., 153, 1251-1256.
Rees, D.D. et al., (1989), Proc. Natl. Acad. Sci., USA, 86, 3375-3378.
Haywood, J.R. et al., (1981), Am. J. Physiol., 241, H273-H278.

HAEMODYNAMIC EFFECTS OF ENDOTHELIN-1 AND ENDOTHELIN-3 IN CONSCIOUS LONG EVANS RATS

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Since there is evidence that endothelin-1 (Et1) and endothelin-3 (Et3) may exert different cardiovascular effects (Yanagisawa et al., 1988), we have investigated the regional haemodynamic actions of these peptides in vivo. Male, Long Evans rats (400-450 g) were anaesthetized (sodium methohexitone 60 mg kg^{-1}, i.p. supplemented as required) and had pulsed Doppler probes (Haywood et al., 1981) sutured around the left renal (R) and superior mesenteric (M) arteries and the distal abdominal aorta (H) to represent hindquarters. Animals were left at least 7 days and then briefly re-anaesthetized (sodium methohexitone, 40 mg kg^{-1} i.p.) for the implantation of intra-vascular catheters. Experiments were begun the following day when continuous measurements were made of mean arterial blood pressure (MAP), instantaneous heart rate (HR) and mean R, M and H Doppler Shift (DS) signals. Changes in regional blood flow (%) were derived from the mean DS signals, and changes in vascular resistances (VR) were calculated from MAP and mean DS signals (Haywood et al., 1981). On separate days animals received Et1 or Et3 (Peptide Insitute) in random order, dissolved in isotonic saline (0.1 ml) containing 1% bovine serum albumin. Doses of 0.004 and 0.04 nmol were given i.v., separated by at least 2 h. The table summarizes the results obtained with Et1 and Et3 at a dose of 0.04 nmol.

Table 1. Cardiovascular changes with Et1 or Et3 in conscious, Long Evans rats (n=8; mean (s.e.m.)).

Peptide	Et1		Et3	
Dose (nmol)	0.04		0.04	
Time (min)	0.5	5	0.5	5
HR ($bmin^{-1}$)	86(12)*	-48(10)*	50(11)*	-21 (6)*$^{+}$
MAP (mmHg)	-16 (3)*	22 (5)*	-12 (3)*	8 (3)*$^{+}$
RDS (%)	-14 (4)*	-36 (3)*	3 (4)	-16 (3)*$^{+}$
MDS (%)	-20 (5)*	-36 (3)*	-20 (4)*	-24 (3)*
HDS (%)	58(13)*	-12 (7)	48(10)*	2 (5)
RVR (%)	-1 (6)	95(14)*	-13 (6)*	30 (8)*$^{+}$
MVR (%)	11(13)	92(11)*	13 (8)	45(10)*$^{+}$
HVR (%)	-44 (5)*	46(13)*	-39 (5)*	8 (8)$^{+}$

*P<0.05 versus baseline (Friedman's test).
$^{+}$P<0.05, Et3 versus Et1 (Wilcoxon test).

These results corroborate the finding that Et3 is less pressor than Et1 (Yanagisawa et al., 1988), and indicate this difference is associated with a lesser vasoconstriction, particularly in the kidney. While such a difference could be due to more potent vasodilator effects of Et3 compared to Et1, opposing the vasoconstrictor action of the former (Warner et al., 1989), it is notable that the initial fall in HVR was similar with both peptides.

Haywood, J.R. et al. (1981). Am.J.Physiol. 241, H273-H278.
Warner, T. et al. (1989), Eur.J.Pharmacol. 159, 325-326.
Yanagisawa, M. et al. (1988) Proc.Nat.Acad.Sci. 85, 6964-6970.

ENDOTHELIN POTENTIATES VASOCONSTRICTION AND ANTAGONISES VASODILATATION SELECTIVELY IN THE PRESENCE OF A THROMBOXANE ANALOGUE

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Endothelin is a potent vasoconstrictor peptide produced by the vascular endothelium (Yanagisawa et al. 1988). We have previously found that native endothelium exerts an excitatory action on the vascular smooth muscle in isolated canine coronary arteries, and, in the presence of a thromboxane analogue (U46619), the endothelium abrogates the vasodilator actions of several agents (Dusting & Macdonald, 1988). We now show that endothelin potentiates selectively the vasoconstrictor action of U46619, and the vasodilator action of sodium nitroprusside is attenuated by endothelin and native endothelium in rat aorta.

Porcine endothelin (endothelin-1) constricted rat aortic rings (EC_{50} = 4.0± 0.6 nM) with a slow onset and long duration of action. Subthreshold concentrations of endothelin (0.3-1.0 nM) significantly potentiated the contractile responses to low concentrations of U46619. This synergy between endothelin and U46619 was abolished by nifedipine (0.1 μM). No synergy occurred between endothelin and phenylephrine.

In aortic rings precontracted with U46619, endothelin (1 nM) abrogated the vasodilator action of sodium nitroprusside (EC_{50} of nitroprusside increased 7-fold). Similarly, aortic rings with endothelium were 8-fold less sensitive to nitroprusside than rings without endothelium. This apparent antagonism of nitroprusside-induced vasodilatation by native endothelium was not affected by indomethacin (3 μM) or by haemoglobin (10 μM). The antagonism by native endothelium and by endothelin was abolished by nifedipine (0.1 μM).

Similar observations were made in rings of canine coronary arteries, in which native endothelium reduced the potency of the directly acting vasodilators nitroprusside, prostacyclin and isoprenaline. This antagonism of vasodilatation by the endothelium was observed in rings precontracted with U46619, but not in rings precontracted with $PGF_{2\alpha}$ or serotonin (Dusting & Macdonald, 1988). Vasodilatation attributable to endothelium-derived relaxing factor (induced by calimycin) was also weaker in rings contracted with U46619 than in rings contracted with serotonin.

These data suggest that endothelin generated by native endothelial cells exerts a physiological antagonism of endogenous vasodilators in the presence of a thromboxane analogue. If endogenous thromboxane A_2 acts similarly to U46619, the antagonism of vasodilator mechanisms, and the synergy between endothelin and thromboxane, may be important components of haemostatic function and vasospastic episodes.

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Dusting, G.J. & Macdonald, P.S. (1988). In: Vanhoutte PM ed. Vasodilatation: Vascular Smooth Muscle, Peptides, Autonomic Nerves and Endothelium. Raven Press , pp463-468.

Yanagisawa,M.,Kurihara,H.,Kimura,S., Tomobe Y., Kobayashi, M., Yazaki, Y., Goto, K. & Masaki, T. (1988). Nature 332, 411-415.

THE EFFECT OF N^G-MONOMETHYL-L-ARGININE (L-NMMA) ON THE HAEMODYNAMIC ACTIONS OF ENDOTHELIN-1 IN CONSCIOUS LONG EVANS RATS

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Endothelin-1 (Et-1; Yanagisawa et al., 1988) causes an initial hypotension and vasodilatation in the hindquarters of conscious rats (Gardiner et al, 1989). Since there is some evidence that Et-1 may stimulate EDRF release (Warner et al., 1988) and L-NMMA inhibits nitric oxide synthesis from L-arginine by vascular endothelial cells (Palmer et al., 1988), we assessed the effect of L-NMMA on the haemodynamic effects of Et-1 in conscious, Long Evans rats. Animals were anaesthetized (sodium methohexitone, 60 mg kg, i.p.) and had pulsed Doppler probes (Haywood et al., 1981) sutured around the left renal (R) and superior mesenteric (M) arteries and the distal abdominal aorta (H: to represent hindquarters). At least 7 days later, the rats were re-anaesthetized briefly (sodium methohexitone, 40 mg kg^{-1}, i.p.) and intravascular catheters were implanted. The following day continuous recordings were made of R, M and H Doppler shift signals (flow), instantaneous heart rate and mean arterial blood pressure (MAP). An estimate of vascular resistance was obtained by dividing blood pressure by Doppler shift. Vasodilator effects of Et-1 (Peptide Institute; 0.04 nmol in 0.1 ml 1% bovine serum albumin in isotonic saline) were measured 0.5 and 5 min after Et-1, either before or 10 min after bolus administration of L-NMMA acetate (300 mg kg^{-1}, i.v.).

The results are summarized in the Table (mean (s.e.m.), n=7).

Table 1

	Cardiovascular effects of Et-1 and L-NMMA					
	Baseline	Et-1 alone		L-NMMA Alone	L-NMMA + Et-1	
Time (min)		0.5	5.0		0.5	5.0
MAP (mm Hg)	108 (2)	99 (5)	135 (3)	149 (4)	119 (5)	163 (3)
R flow (kHz)	10.2 (0.6)	9.3 (0.9)	5.8 (0.6)	8.2 (0.7)	7.4 (0.8)	4.0 (0.5)
R resistance	11 (1)	11 (1)	25 (3)	19 (1)	18 (2)	44 (5)
M flow (kHz)	6.5 (0.8)	5.0 (0.5)	3.6 (0.3)	4.1 (0.5)	4.2 (0.5)	3.1 (0.4)
M resistance	18 (2)	22 (7)	40 (4)	40 (4)	31 (5)	56 (7)
H flow (kHz)	3.1 (0.4)	4.2 (0.4)	3.0 (0.4)	1.8 (0.3)	3.4 (0.5)	1.8 (0.4)
H resistance	39 (6)	26 (5)	52 (8)	97 (14)	42 (7)	116 (21)

These results provide no evidence that the hypotensive effects and the vasodilatation of the hindquarters caused by Et-1 are mediated by the release of endothelium-derived nitric oxide.

Gardiner, S.M., et al. (1989). J.Cardiovasc.Pharmacol. (in press).
Haywood, J.R. et al., (1981). Am.J.Physiol., 241, H273-H278.
Palmer, R.M.J., et al., (1988). Biochem.Biophys.Res.Commun., 153, 1251-1256.
Warner, T., et al., (1988). Br.J.Pharmacol., 95, 723P.
Yanagisawa, M., et al., (1988). Nature, 332, 411-415.

ENDOTHELIN-1 MODULATES THE INOTROPIC EFFECTS OF ISOPRENALINE IN RABBIT ISOLATED PAPILLARY MUSCLE

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Endothelin-1 (ET-1), in addition to its well documented vascular effects also has positive inotropic activity in isolated atrial and ventricular tissues from human, rat, guinea pig and rabbit together with positive chronotropic effects in guinea pig atria. In addition, ET-1 modulates sympathetic neurotransmission. We have studied the effects of ET-1 on the inotropic effects of isoprenaline and the involvement of Ca^{++} channels sensitive to dihydropyridines on this interaction.

Papillary muscles from male New Zealand White rabbits were suspended in Krebs'-Hensleit solution under 1g resting tension and electrically driven at 3Hz. Cumulative dose response curves to the inotropic effects of (-)isoprenaline (1 to 300nM) were constructed (a) in the absence of other drugs and then (b) in the presence of either ET-1, endothelin-3 (ET-3) or Bay K 8644 and (c) 1h after wash-out of the drug used. The involvement of dihydropyridine sensitive Ca^{++} channels in the actions of the ET-1 and Bay K 8644 was assessed by the addition of nicardipine to the bathing solution.

ET-1 caused a dose-dependent positive inotropic response, increasing the isometric twitch tension by 46% (6.7nM; n=6), 58% (21nM; n=7) and 66% (67nM; n=6) from a baseline tension of 250 $\pm$ 20mg (n=47). ET-1 (6.7nM) had no effect on the isoprenaline dose response curve but at higher doses (21nM and 67nM) the peptide caused 18% and 24% depressions of the maximum effect of isoprenaline respectively, without a shift in ED_{50}. ET-3 (67nM; n=5) increased contractile force by 40% but did not reduce the actions of isoprenaline. The intrinsic inotropic effects of ET-1 and the dose-dependent suppression of the isoprenaline response were reversible after washout. Nicardipine (300nM) did not modify the effects of ET-1, and if anything enhanced its interaction with isoprenaline.

Bay K 8644 (100nM; n=6) increased the basal contractility of the tissue by 40%, and its presence increased the tissue's responsiveness to low doses of isoprenaline, but at higher doses the combination of the two agonists caused spontaneous beats probably due to Ca^{++} overload. These effects of Bay K 8644 were antagonised by nicardipine (300nM).

We conclude that the intrinsic inotropic effect of ET-1 and its modulation of the effects of isoprenaline do not involve the dihyropyridine sensitive Ca^{++} channels stimulated by Bay K 8644. Furthermore, ET-3 is less potent than ET-1 as an inotropic agent and as a modulator of stimulation by isoprenaline suggesting that small structural variations within this family of peptides can reflect either varying potencies or diverse receptor populations.

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ENDOTOXIN DEPRESSES VASCULAR REACTIVITY TO PHENYLEPHRINE, CLONIDINE AND BAY K 8644 BUT NOT TO ENDOTHELIN

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Endotoxin depresses vascular responsiveness to noradrenaline (NA) and angiotensin (Gray et al. 1987). In contrast, vascular responsiveness to the Ca^{2+} channel activating drug BAY K 8644 was increased by endotoxin (Ives et al. 1986). We have now examined the effects of endotoxin on the vascular reactivity to phenylephrine, clonidine, BAY K 8644 and porcine endothelin. Rats were pithed under ether anaesthesia and ventilated artificially (100% O_2; 53 strokes/min, 1ml/100g). Rectal temperature was maintained at 37 ± 0.5 °C. Cannulae were placed in one common carotid artery for measurement of arterial blood pressure, and in both jugular veins for drug injection. After 20 min equilibration, E. coli endotoxin (Difco O55:B5, 1.0 mg/kg) or 0.9% saline were infused i.v. over 4h. At 1h, cumulative pressor dose-response curves were obtained to the above agents. These agents produced dose-dependent pressor responses, which, in the case of endothelin was always preceded by a dose-dependent depressor response. The responses were plotted as % maximum increase in mean arterial blood pressure against log dose. Each rat was used for one dose-response curve to one agent. Infusion of endotoxin produced a fall in mean arterial blood pressure which was maximal at 1h (control 59 ± 1.3 mm Hg (n = 34); endotoxin 50 ± 1.7 mm Hg, (n = 35); $P<0.01$). $ED_{max\ 50}$ values (μg/kg) were calculated for each agonist from the dose-response curves and are shown in the table (Mean ± S.E.M., n value in parentheses).

	Saline	Endotoxin	P (vs saline)
Phenylephrine	8.5 ± 1.6 (6)	28.4 ± 7.1 (6)	< 0.05
Clonidine	4.4 ± 1.2 (6)	20.5 ± 3.8 (6)	< 0.01
BAY K 8644	20.9 ± 4.7 (11)	47.5 ± 10.9 (9)	< 0.05
Endothelin	2.5 ± 0.3 (11)	3.0 ± 0.3 (14)	N.S.

The pressor dose-response curves to phenylephrine, clonidine and BAY K 8644 were shifted significantly to the right, as seen from the increase in the $ED_{max\ 50}$ values, without any change in the maximum responses. However, the responsiveness to endothelin was unaltered. Baseline blood pressure in endotoxin-treated rats receiving endothelin was not different from that in rats receiving BAY K 8644. As endothelin-mediated vasoconstriction, in contrast to the response to the other agents, appears to depend on intracellular calcium (Auguet et al. 1988), this may indicate that impairment of Ca^{2+} entry contributes to endotoxin-mediated impairment of vascular reactivity.

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Auguet M., Delaflotte, S. Chabrier, P. et al. (1988) Biochem. Biophys Res. Comm. 156: 186-192
Gray, G.A., Furman, B.L. and Parratt, J.R. (1987) Br. J. Pharmac. 92: 644P
Ives, N., King J.W.,Chernow B. and Roth B.L. (1986) Eur. J. Pharmacol. 130: 169-175

DIFFERENTIATION OF CENTRAL CCK RECEPTOR BINDING SITES USING THE NON-PEPTIDE ANTAGONISTS MK-329 AND L-365,260

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Cholecystokinin (CCK) receptor binding sites may be differentiated into two major classes depending on their affinity for desulphated CCK-8 (dCCK) or CCK-4 and the non-peptide antagonist MK-329 (L-364,718). CCK-A receptors show considerable selectivity for sulphated rather than desulphated peptide fragments and bind MK-329 with nanomolar affinity. In contrast, CCK-B receptors bind MK-329 weakly and show little discrimination between sulphated and desulphated peptides. Recently, Bock et al (1989) and Lotti and Chang (1989) have described the synthesis and pharmacological characterisation of a novel gastrin antagonist, L-365,260 ([3R(+)-N-(2,3-dihydro-1-methyl -2-oxo-5-phenyl -1H-1,4-benzodiazepin-3-yl)-N'-(3-methylphenyl urea]), which also has high affinity for CCK-B binding sites in the brain. Here, we describe the results of experiments which show selective inhibition of [125I]-Bolton Hunter CCK binding to CCK-B sites by L-365,260 in regions of rodent brain which contain both CCK receptor subtypes. We have also used L-365,260 to demonstrate differences in the nature of CCK binding sites in the spinal cord of several species.

Autoradiography of CCK binding sites was performed as previously described (Hill et al 1988) using 50pM [125I]-Bolton Hunter CCK-8 ([125I]-BHCCK). In sections of rat medulla containing the area postrema (AP) and nucleus tractus solitarius (NTS), L-365,260 (100nM) displaced [125I]-BHCCK binding to sites in the lateral NTS and spinal tract of the trigeminal nerve but not to medial NTS or AP: regions previously shown to contain CCK-A sites (Hill et al 1987). Measurements of the affinity of L-365,260 for CCK binding sites in the spinal cord of the rat, monkey and human confirmed previous data using MK-329, which indicated a species difference in the nature of the receptor present in this tissue (Table 1). Thus, in the monkey, MK-329 was a potent inhibitor of binding, whereas L-365,260 showed much lower affinity indicating a predominance of CCK-A binding sites. By contrast, in the rat where MK-329 bound with low affinity, L-365,260 was a more potent inhibitor of binding suggesting the binding sites are of the CCK-B subtype. Sulphated CCK-8 (SCCK-8) did not discriminate between species .

Table 1
pIC_{50} values for inhibition of CCK binding to sections of spinal cord

Compound	Rat	Monkey	Human
SCCK-8	9.17 ± 0.08	9.05 ± 0.04	9.19
dCCK-8	7.84 ± 0.02	6.61 ± 0.07	6.93
MK-329	7.15 ± 0.13	9.72 ± 0.15	9.56
L-365,260	7.89 ± 0.15	5.77 ± 0.32	6.35

data are mean ± s.e.m. from 3 or more separate experiments

These results support the notion that L-365,260 is a selective ligand for CCK-B receptors and may be used in conjunction with MK-329 to differentiate CCK binding sites within the CNS.

Bock, M.G., et al (1989) J. Med. Chem. 32,13-16.
Hill, D.R., et al(1988) Neurosci. Lett. 89.133-139.
Lotti, V.J. and Chang, R.S.L., (1989) Eur. J. Pharmacol. 162, 273-280.

ANTAGONISM OF CCK-INDUCED EXCITATION OF RAT VENTROMEDIAL HYPOTHALAMIC NEURONES BY A NEW SELECTIVE CCK-B RECEPTOR ANTAGONIST

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The rat ventromedial hypothalamic nucleus (VMH) contains high levels of cholecystokinin (CCK)-B receptor binding sites and CCK potently excites cells within this nucleus. It has previously been reported that MK-329 (L-364,718), a highly potent CCK-A receptor antagonist (Chang & Lotti, 1986), is relatively weakly active as an antagonist of CCK-induced excitations of rat VMH cells (Boden & Hill, 1988). Here we report the antagonism of CCK-induced excitations of cells in rat VMH slices in vitro by MK-329 and L-365,260, a new selective CCK-B receptor ligand (Lotti & Chang, 1989).

Coronal sections, 350μm thick, containing the VMH nucleus were cut using an Oxford vibratome. These were placed in a small perfusion chamber where they were continuously perfused with oxygenated artifical cerebrospinal (aCSF) fluid of the following composition (mM): NaCl 124; KCl 5; KH_2PO_4 1.25; $MgSO_4$ 2; $CaCl_2$ 2; $NaHCO_3$ 25: glucose 11; at 33°C. CCK dissolved in aCSF was applied for one minute periods. Extracellular recordings of single unit firing rate were made using conventional techniques and displayed as a continuous record on a chart recorder.

CCK (0.03 - 1.0μM) increased the firing rate of VMH cells in a concentration-dependent manner with an EC_{50} of approximately 30nM. Desulphated CCK-8 was only 5.2 $\pm$ 0.22 times less potent than CCK, in agreement with an action at CCK-B type receptors. MK-329 (0.3 & 1.0μM, equilibrated for 1h) produced parallel shifts to the right of the CCK concentration-response curve, which resulted in an extrapolated pA_2 = 6.6 $\pm$ 0.17 (n=8) for MK-329 as an antagonist of CCK-induced excitations of VMH cells. In this respect MK-329 is at least 1000 times less active than in blocking CCK-A receptors. L-365,260 (0.1 & 0.3 μM) also produced parallel shifts to the right of the CCK concentration-response curve which resulted in an extrapolated pA_2 = 7.48 $\pm$ 0.11 (n=6).

The potency of MK-329 as an antagonist of CCK responses in the VMH is in good agreement with its relatively low affinity for the brain, CCK-B type receptor. The results with L-365,260 indicate that it is the most potent antagonist of CCK-B type receptors yet described, in support of binding studies which show it to be a potent and selective inhibitor of I-125 CCK binding to guinea pig brain membranes. The potency and selectivity of L-365,260 suggest that it will be a useful tool to study the function of CCK-B type receptors in brain.

Chang, R S L & Lotti, V J (1986) Proc Natnl Acac Sci ISA 83 4923
Boden, P & Hill, R G, Br J Pharmacol, (1988), 94, 246-252
Lotti, V J & Chang, R S L (1989) Eur. J. Pharmac. 162, 273-280

ENHANCEMENT OF MORPHINE ANALGESIA AND PREVENTION OF MORPHINE TOLERANCE BY THE SELECTIVE CCK-B ANTAGONIST L-365-260

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Recent studies in our laboratory have shown that the selective Cholecystokinin (CCK) receptor antagonist MK-329 enhances morphine analgesia and prevents morphine tolerance (Dourish et al, 1988; O'Neill et al, 1989). CCK receptors have been classified into 2 subtypes termed CCK-A and CCK-B (Moran et al, 1986; Dourish and Hill, 1987). MK-329 has nanomolar affinity for CCK-A receptors and micromolar affinity for CCK-B receptors (Hill et al,1989). To assess whether CCK/opiate interactions are mediated by CCK-A or CCK-B receptors we compared the potency of the novel selective CCK-B antagonist L-365,260 (Lotti and Chang, 1989) to MK-329 in thermal and mechanical analgesic tests.

Male Sprague Dawley rats (250-300g) were used in all experiments. Pain thresholds for thermal stimuli were measured using a Socrel tail flick meter and for mechanical stimuli using an Analgesy-Meter (both from Ugo Basile). In all studies effects of drug treatments were expressed as % maximum possible effect which was calculated as: test threshold - baseline threshold/cutoff point (10S or 500g) x 100. Animals were injected s.c. with vehicle (20%DMSO/80% propylene glycol) or L-365,260 (3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1, 4 benzodiazepin-3-yl) -N'-(3-methyl-phenyl) urea) 10 min prior to i.p. injection of saline or morphine and pain thresholds determined at 20 min. intervals for 2h. L-365,260 (0.01-10.0mg/kg) alone had no effect on pain thresholds in the tail flick and paw pressure tests. However the drug enhanced analgesia induced by a subthreshold (4mg/kg) dose of morphine, in both tests. The effective dose ranges for morphine enhancement were 0.1-0.5mg/kg in the tail flick test and 0.05-0.2mg/kg in the paw pressure test.

Rats injected twice daily with incremental doses of morphine (8-48mg/kg i.p.) for 6 days became tolerant to the analgesic effects of the drug in the tail flick test. Twice daily s.c. injections of 0.2mg/kg L-365,260 prevented the developement of tolerance to morphine analgesia. In contrast, L-365,260 had no effect on the development of opiate dependence in these animals, as assessed by naloxone-precipitated withdrawal.

L-365,260 was approximately X10 more potent than MK-329 in enhancing morphine analgesia and preventing morphine tolerance. This order of potency correlates well with the potency of the two drugs as CCK-B receptor antagonists in rodent CNS (Hill et al, 1989; Kemp et al, 1989). Therefore it appears that CCK/opiate interactions in rodents are mediated by CCK-B receptors.

Dourish, C.T. and Hill, D.R. (1987) Trends Pharmacol. Sci. 8:207-208
Dourish, C.T., et al (1988) Europ. J. Pharmacol 147: 469-472
Hill, D.R. et al (1989) this meeting
Kemp, J.A. et al (1989) this meeting
Lotti, V.J. and Chang, R.S.L. (1989) Europ. J. Pharmacol 162: 273-280
Moran, T.H. et al (1986) Brain Res., 362: 175-179
O'Neill, M.F. et al (1989) Neuropharmacology, 28: 243-247

INTRAVENOUS ADMINISTRATION OF THE CHOLECYSTOKININ (CCK) ANTAGONIST MK-329 INCREASES OPERANT RESPONDING FOR FOOD IN PIGS

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Based on the observation that parenteral administration of CCK reduces food intake in the rat, Gibbs et al (1973) have suggested that CCK released from the small intestine during a meal acts in a negative feedback manner to induce post prandial satiety. It was argued that if this hypothesis was true, then CCK antagonists should block the effects of endogenously released CCK during feeding, and thereby increase meal size. Support for this hypothesis was recently provided by Hewson et al (1988) who demonstrated that the novel CCK antagonist MK-329 increased food intake in rats. The aim of the present study was to see if we could replicate these results in pigs trained to make operant responses for food.

Prepubertal large white pigs (6 male, 4 female, body weight 25-55 kg), were surgically prepared, under halothane anaesthesia, with an intravenous catheter in the external jugular vein. The animals lived separately in metal cages and were trained to press operant switch panels located in the cage with their snouts to obtain food and water reinforcements on a fixed ratio of 5. The pigs were put on the following feeding schedule: At 9.15 a.m. they received a small measured meal. Four hours later, a buzzer signalled that the feeder was activated, and that the pigs could press the food panel for reinforcement. The feeder was switched off after 2h. The occurrence of feeding was monitored by means of a computer-based data-logging system (BBC computer). Water was available ad lib.

EXPT. 1. The pigs were injected i.v. with either vehicle solution or MK-329 solution (17.5, 35, 70 and 140 μg/kg) 5 min before the feeder was activated. Each dose was tested at least twice on each animal. The total amount of food eaten by the pigs in the 2h after MK-329 injection was compared with that eaten after the corresponding vehicle injection using the paired t-test.
EXPT. 2. The pigs were injected i.v. with vehicle solution followed by saline, vehicle followed by CCK (1 μg/kg) or MK-329 (70 μg/kg) followed by CCK (1 μg/kg). The first injection was given 5 min before, and the second injection was given 5 min after, the feeder was activated. Each pig received all treatments. The results from the first experiment showed that MK-329 caused a dose-related increase in food intake. Doses of the CCK antagonist from 35-140 μg/kg produced significant increases ($P < 0.05$) in meal size. Maximal increase in food intake (approx. 50% above control) was observed at a dose of 70 μg/kg. In the second experiment, CCK (1 μg/kg) produced a short lasting decrease in feeding starting approximately 2-5 min after injection. The pigs stopped responding on the food panel for periods extending from 12 to 18 min. Pretreatment with MK-329 (70 μg/kg) completely abolished the inhibitory effects of CCK (1 μg/kg) on feeding.

In the present study, we have demonstrated that (i) i.v. administration of MK-329 increases operant food intake in pigs, and (ii) MK-329 pretreatment will completely abolish the inhibitory effect of exogenous CCK on operant feeding. These results confirm and extend those obtained by Hewson et al. (1988), and lend support to the hypothesis that endogenous CCK may play an important role in satiety.

We wish to thank Merck Sharpe and Dohme for providing MK-329.

Hewson, G. et al. (1988) Br. J. Pharmac. 93, 79-84.
Gibb, J. et al. (1973) J. Comp. Physiol. Psychol. 84, 488-494.

THE CHOLECYSTOKININ RECEPTOR ANTAGONIST MK 329 BLOCKS THE ANORECTIC EFFECT OF (+)-FENFLURAMINE IN THE RAT

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The selective cholecystokinin (CCK) receptor antagonist MK 329 (formerly L-364,718; 3S(-)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1-H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide) (Chang & Lotti, 1986) antagonizes the anorectic effect produced by exogenous CCK in rats (Dourish et al., 1988; Hewson et al., 1988). Several experiments were undertaken to address the following propositions: (i) that (+)-fenfluramine-induced anorexia depends not only on 5-hydroxytryptamine (5-HT) receptor activation (Neill & Cooper, 1989), but also on endogenous CCK activity at CCK receptors; (ii) that reversal of (+)-fenfluramine anorexia by MK 329 shows pharmacological specificity; (iii) that the reversal is not secondary to an intrinsic hyperphagic effect of MK 329.

In the main series of experiments, adult male rats were accustomed to 30 min daily access to a palatable, sweetened mash, until the levels of food consumption reached asymptote. They were neither food- nor water-deprived. (+)-Fenfluramine (3.0 $mg.kg^{-1}$, i.p., distilled water vehicle) reduced palatable food intake by 67% ($p<0.005$) from a baseline level of 18.3g/30 min. MK 329 (30 and 100 $\mu g.kg^{-1}$, s.c., 0.5% methylcellulose vehicle) almost completely reversed the anorectic effect of (+)-fenfluramine. In a smaller dose, MK 329 (10 $\mu g.kg^{-1}$, s.c.) did not significantly affect the anorectic effect. In tests of pharmacological specificity, MK 329 (10 - 100 $\mu g.kg^{-1}$, s.c.) did not block the anorectic effect of either the beta-carboline FG 7142 (10 $mg.kg^{-1}$, i.p.) or the selective dopamine D2 receptor agonist, quinpirole (0.3 $mg.kg^{-1}$, s.c). Furthermore, MK 329 (10 - 100 $\mu g.kg^{-1}$, s.c.) had no effect, when administered alone, on the level of palatable food consumption.

In further experiments, adult male rats were placed on a reversed lighting cycle, and their intake of powdered chow was routinely measured over the first 6h of the night period, when nocturnal feeding is maximal. The animals were neither food- nor water-deprived. (+)-Fenfluramine (3.0 $mg.kg^{-1}$) significantly reduced food intake by 67% (control level of intake was 13.5g over the 6h period) ($p < 0.005$). Its anorectic effect was significantly attenuated by MK 329 (30 and 100 $\mu g.kg^{-1}$). Administered alone, MK 329 (10- 100 $\mu g.kg^{-1}$) had no effect on nocturnal free feeding. Taken together, the results of these experiments imply that, at least in part, (+)-fenfluramine-induced anorexia depends on endogenous CCK activity at CCK receptors.

Chang, R.S.L. & Lotti, V.J. (1986). Proc. Natl. Acad. Sci. USA, 83, 4923-4926.
Dourish, C.T. et al. (1988). In Cholecystokinin Antagonists: Wang, R.Y. & Schoenfeld (eds) pp. 307-325, New York, Alan R. Liss.
Hewson, G. et al. (1988). Br. J. Pharmacol, 93, 79-84.
Neill, J.C. & Cooper, S.J. (1989). Psychopharmacology, 97, 213-218.

ANXIOLYTIC 5-HT ANTAGONIST, GR38032F, POTENTLY BLOCKS THE ANALGESIC CONSEQUENCES OF SOCIAL DEFEAT IN MALE MICE

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Non-opioid analgesia in defeated male mice is sensitive to a range of typical and atypical (e.g. buspirone) anxiolytics (Rodgers and Shepherd 1989). In the present study, the effects of the putative 5-HT_3 anxiolytic, GR38032F, on basal nociception and defeat analgesia were examined.

Subjects were group-housed male DBA/2 mice (Bantin & Kingman, Hull), housed under a reversed LD cycle in a temperature-controlled room (24±1°C). Testing was performed under dim red light during the dark phase of the cycle. Pain latencies (tail-flick assay) were determined both before and after defeat. For defeat experience, DBA/2 intruder mice were individually placed into the home cage of an aggressive resident conspecific and removed immediately upon display of species-typical defeat behaviour. GR38032F was dissolved in distilled water and administered i.p. 45 min before testing. Three successive studies were conducted to determine the minimum effective dose of the compound. Data were analysed by ANOVA and appropriate follow-up tests.

Analysis showed that, over the dose range tested (0.00001-1000µg/kg), GR38032F was without significant intrinsic activity on the mouse tail-flick assay. In the defeat model, and as summarised in Table I, GR38032F had highly potent effects over a very wide dose range. In each study, vehicle-treated mice evidenced a strong analgesia ($p<0.001$) in response to defeat experience. This effect was totally inhibited by 1.0-1000µg/kg GR38032F, with partial inhibition evident over the dose range 0.001-0.1µg/kg. A lower dose of 0.00001µg/kg was ineffective. These inhibitory effects on defeat analgesia were observed in the absence of any alteration in resident attack

TABLE I Effects of GR38032F (0.00001-1000µg/kg) on defeat analgesia in male mice. Data are presented as Mean ± (SE) tail-flick latencies (sec). Pre=baseline Post=post-defeat. *$p<0.05$ **$p<0.01$ ***$p<0.001$ versus baseline. §$p<0.05$ versus vehicle.

EXPT. 1	Vehicle	0.1	1.0	10.0	100.0	1000.0 µg/kg
Pre	2.8(0.2)	3.1(0.3)	3.2(0.2)	3.2(0.3)	2.7(0.1)	3.0(0.3)
Post	4.8(0.6)***	3.6(0.6)	3.4(0.5)	3.9(0.8)	3.4(0.4)	3.0(0.5)§

EXPT. 2	Vehicle	0.001	0.01	0.1 µg/kg
Pre	2.1(0.1)	2.1(0.1)	2.4(0.1)	2.1(0.2)
Post	4.9(0.9)***	2.9(0.3)*§	3.0(0.3)§	3.4(0.3)***§

EXPT. 3	Vehicle	0.00001	0.0001	0.001 µg/kg
Pre	2.3(0.2)	2.3(0.1)	2.2(0.1)	2.6(0.1)
Post	3.9(0.4)***	3.9(0.4)***	2.8(0.2)**§	3.0(0.3)**§

latency or intruder defeat latency. Together, these data imply a significant role for 5-HT_3 receptor mechanisms in the mediation of defeat analgesia and would not be inconsistent with the putative anxiolytic profile of this compound in rodents and primates (Jones et al. 1988).

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Jones B.J. et al. (1988) Br. J. Pharmac. 93: 985-993.
Rodgers R.J. and Shepherd J.K. (1989) Psychopharmacology 97: 163-165.

EVIDENCE THAT THE AMYGDALA AND NOT THE AREA POSTREMA IS INVOLVED IN THE PUTATIVE ANXIOLYTIC EFFECTS OF 5-HT_3 RECEPTOR ANTAGONISTS

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Various studies have demonstrated that systemically injected 5-HT_3 receptor antagonists (eg GR38032F, ICS205-930, BRL43694) will increase rat social interaction (SI) under conditions of maximal suppression (Tyers et al, 1987; Jones et al, 1988; Piper et al, 1988) Such a behavioural response is suggestive of an anti-anxiety effect. This finding is supported by the reported effectiveness of 5-HT_3 receptor antagonists in other animal models of anxiety (Jones et al, 1988; Piper et al, 1988). Recently 5-HT_3 binding sites have been identified in the CNS of various species including the rat (Kilpatrick et al, 1987). Subsequent autoradiographic studies (Kilpatrick et al, 1988) have shown that the area postrema (AP) contains the highest density of these 5-HT_3 sites with lower levels found in cortical and amygdaloid regions. The aim of this study was to investigate any role of the amygdala and the AP in the putative anxiolytic effects of 5-HT_3 antagonists.

Rats (220-250g) were implanted with chronic indwelling cannulae positioned approximately 5mm above the amygdala or AP, a recovery period of seven days was allowed between surgery and experimentation. A 30G stainless steel injection unit placed within the guide cannula only during injection allowed direct drug infusions to be made into either structure 5 min before testing. Only one rat of the pair was dosed and then assessed behaviourally. Under high light/unfamiliar test conditions, all the 5-HT_3 antagonists when injected into the amygdala significantly increased SI by comparison to vehicle pretreated controls, i.e. GR38032F (vehicle: 6.7 $\pm$ 1.9s, GR 100ng: 14.4 $\pm$ 2.0s, $p<0.05$), ICS205.930 (vehicle: 6.6 $\pm$ 1.2s, ICS 1ng: 18.2 $\pm$ 2.9s, 10ng: 14.9 $\pm$ 2.9s, $p<0.05$), BRL43694 (vehicle: 4.6 $\pm$ 1.2s, BRL 0.1ng: 14.8 $\pm$ 4.9s, 1ng: 15.5 $\pm$ 2.0s, $p<0.05$). Both GR65630A (1-10ng) and MDL72222 (100-1000ng) were similarly effective. No significant changes were seen following these treatments when testing was conducted under the low light/familiar procedure. GR38032F (100ng) failed to produce any significant increase in SI under either test condition after injection into the AP. At higher doses (1000-10000ng) SI was markedly reduced but so was locomotor activity and rearing thus indicating general behavioural hypoactivity.

Electrolytic lesions of the AP failed to block the increased SI induced by systemically administered GR38032F (0.1mgkg^{-1} po) or diazepam (1.5mgkg^{-1} po).

Thus the increased SI produced by the intra-amygdaloid injection of various 5-HT_3 receptor antagonists would imply an involvement of this structure in the putative anxiolytic effects of these compounds. However, despite evidence for an involvement of the AP in the antiemetic effects of 5-HT_3 antagonists (Higgins et al, 1988), the present data would exclude a role of this structure as a neuro-anatomical locus for their anti-anxiety effects.

Higgins, G.A. et al (1988) Br. J. Pharmac. 95, 504P
Jones, B.J. et al (1988) Br. J. Pharmac. 93, 985-993
Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1987) Nature (Lond.) 330, 746-748
Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1988) Neurosci. Lett. 94, 156-160
Piper, D.J. et al (1988) Br. J. Pharmac. 94, 314P
Tyers, M.B. et al (1987) Neurosci. Lett. 29, 568

SCOPOLAMINE-INDUCED DEFICITS IN A T-MAZE REINFORCED ALTERNATION TASK ARE ATTENUATED BY 5-HT_3 RECEPTOR ANTAGONISTS

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Central cholinergic function has traditionally been associated with the control of cognitive performance. This has been highlighted by biochemical, electrophysiological and pharmacological evidence (see review by Perry 1986). Scopolamine.HBr (a muscarinic receptor antagonist) has been widely used to produce experimental cognitive deficits in both animals (Flood & Cherkin 1986) and man (Sayer & Allen 1971). We have used this agent to produce deficits in performance of a task in which a rodent is required to retain spatial information, and have shown that such deficits can be reduced by treatment with selective 5-HT_3 receptor antagonists.

Male Lister Hooded rats (250-300g Bradford strain) were trained on a food reinforced alternation task using an elevated T-maze (modification of the protocol of Salamone et al. 1982). Food was withdrawn 2 days prior to the test, and throughout testing animals were deprived of food for 23h/day, but with water available ad libitum (85% normal body weight maintained). Rats were allowed 10 min habituation to the maze on day 1 (both arms baited, banana flavoured reward pellets) and were subject to a pretraining period of reinforced alternation on days 2-5 of the test, with training on days 6-9. All training consisted of paired trials (each pair constituting a "run"), the first being "forced" in that one arm was blocked whilst the other was baited. The second was a choice trial in which reward pellets were placed in the arm opposite to that reinforced in the first trial of the pair. A correct choice (i.e. alternation) was when the rat entered the arm containing the food on the choice trial. In addition to correct/incorrect choice, latency to reward was recorded for both forced and choice trials.

Scopolamine 0.25mg/kg i.p. b.d. produced a disruption in performance indicated as a reduction in correct responses (compared to control) over the 9 day test period [$F(6,42)=22.9$ $P<0.01$]. Four 5-HT_3 receptor antagonists were tested in this study: GR38032F (1.0ng/kg-100μg/kg), zacopride (1.0ng/kg-10.0μg/kg), ICS 205-930 (10ng/kg) and BRL 43694 (10ng/kg). Rats were treated i.p. twice daily with the 5-HT_3 antagonists concurrently with scopolamine treatment. All 5-HT_3 receptor antagonists inhibited the impaired performance caused by scopolamine. For example, the control, scopolamine and scopolamine + drug (10ng/kg) % correct responses were, respectively, on day 5 84.4, 28.1, 85.6 (GR38032F), 81.3, 46.4, 67.9 (zacopride), 75.0, 28.6, 71.4 (ICS 205-930), and 80.0, 40.0, 65.0 (BRL 43694) ($P<0.05$ Dunnett's t test for all drugs + scopolamine, compared to scopolamine). The inhibition of scopolamine impairment was dose-dependent using GR38032F and zacopride: maximum responses were maintained at the highest doses used. The 5-HT_3 receptor antagonists alone failed to improve performance in this type of T-maze task.

The present data indicate that impairments produced by scopolamine in a cognitive test in the rat can be inhibited by 5-HT_3 receptor antagonists. This would indicate a role for 5-HT in the modulation of cognitive processes via influence on cholinergic function, and a potential role for the 5-HT_3 receptor antagonists to improve cognitive deficits linked with a cholinergic dysfunction.

Perry, E.K. (1986) Br. Med. Bull. Vol.42, 63-69
Flood & Cherkin (1986) Behav. Neural. Biol. 45, 169-184
Sayer & Allen (1971) Biol. Psychiatry 3, 347-355
Salamone, J.D. (1984) Behav. Brain Res. 13, 63-70
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EFFECTS OF GR38032F ON PERFORMANCE OF COMMON MARMOSETS (CALLITHRIX JACCHUS) IN AN OBJECT DISCRIMINATION REVERSAL TASK

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One of the most common assessments of cognitive skills in primate is that of object discrimination reversal learning, where an animal is required to make a response in a manner opposite to a previously learned task. The following studies were designed to investigate the cognitive enhancing properties of the 5-HT_3 antagonist GR38032F (Butler et al 1988) in an object discrimination reversal task using the marmoset.

Adult marmosets (315-325g) were used. All animals had received extensive training in a Wisconsin General Test Apparatus (WGTA) and were experienced in object discrimination reversal learning. Throughout testing, animals were presented with a series of trials in the WGTA in which the screen was raised to reveal two plastic junk objects covering 2 food wells; the task was to select the food-rewarded object. The left/right position of the rewarded object was altered throughout each test session according to a pseudorandom schedule (Gellerman 1933). The inter-trial interval was approximately 15s and each trial lasted until the marmoset made a response. On completing 6 consecutive correct responses on the first food rewarded object (initial discrimination task) the reward paradigm was changed so that the marmoset was required to select the second, initially unrewarded object (reversal task) to the same criterion. On the first week of testing all marmosets received vehicle treatment to provide stable baseline data. Following five days of vehicle treatment animals were randomly assigned to treatment groups. Treatment was carried out using a blind, two-week crossover design.

Using the above testing procedure, the mean number (±S.E.) of trials to criterion for each day were found to be significantly decreased ($P<0.05$) by GR38032F at 1ng/kg s.c. and 10ng/kg s.c. given twice daily. Thus, the trials to criterion for the initial discrimination task were reduced from 19.6±1.3 to 7.4±0.93 and 13.8±1.1 to 7.3±0.7, respectively for vehicle and drug-treated animals (1 and 10ng/kg s.c.), and improvements for the reversal task were 27.5±1.6 to 11.1±1.2 and 20.2±2.1 to 9.2±1.0 respectively. Time/trial(s) for GR38032F were reduced from 14.8±1.4 to 5.8±0.7 and 17.1±1.1 to 8.9±1.0 respectively for vehicle and GR38032F treated animals (1 and 10ng/kg s.c.). GR38032F (10μg/kg s.c. b.d.) failed to modify performance in the initial discrimination task but significantly improved performance in the reversal task. As the dose of GR38032F was increased further to 100μg/kg s.c. b.d. some impairments in performance in the object discrimination task were recorded. In 3 out of 4 animals tested performance in the reversal task was impaired (from 12.1±0.7 to 19.0±1.8, $P<0.01$) with time(s)/trial delayed from 12.2±1.1 to 22.6±0.6 (vehicle/drug on day 2).

These data provide the first evidence that a 5-HT_3 receptor antagonist, over a range of doses, is able to significantly improve cognitive performance of marmosets in an object discrimination reversal task. Detriments in performance are only seen as the dose is raised 100,000 fold above the minimal effective dose.

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Butler, A. et al. (1986) Br. J. Pharmacol. 94, 397-412
Gellerman, J. (1933) Genet. Psychol. 42, 206-208

EFFECTS OF THE 5-HT_2 AGONIST DOI ON DORSAL RAPHE NUCLEUS 5-HT NEURONAL FIRING AND INTAKE OF FOOD IN FREELY FEEDING RATS

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2,5-dimethoxy-4-iodophenylisopropyl-amine (DOI) is a highly selective agonist at the 5-HT_2-receptor (Glennon *et al.*, 1986) but also with affinity for 5-HT_{1C} receptors. Furthermore initial binding and behavioural studies indicate that DOI may produce effects at a ketanserin insensitive site (McKenna *et al.*,1988; Pike *et al.*, 1989). Preliminary studies in our laboratory have demonstrated that DOI may alter neuronal firing in the dorsal raphe nucleus (DRN). The present study compares the effects of DOI and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT) an agonist at the 5-HT_{1A} somatodendritic autoreceptor on DRN 5-HT neuronal firing and food intake in satiated rats.

The electrophysiological recordings were carried out in anaesthetised (1.5-2% halothane) male Wistar rats. Single barrelled electrodes were implanted in the DRN. 5-HT neurones were identified by their characteristic slow regular firing pattern. In feeding experiments, rats were housed singly and had free access to food and water. All food measurements were made between 09.00 and 13.00h. An injection of saline (of equivalent volume and by the same route as the drug) was given the day before drug administration. One group of rats (n=10) received DOI (1mg/kg i.p.) and the other (n=10) 8-OHDPAT (0.32mg/kg s.c.).

DOI (50 g/kg i.v.) was found to produce a marked reduction of DRN 5-HT neuronal firing (60% of initial level (n=6)) which was sustained for 30 minutes. A similar reduction of firing was seen with 8-OHDPAT (10µg/kg i.v.). DOI (100µg/kg i.v.) caused a complete inhibiton of firing which last for over 90 minutes (n=6). Neuronal firing was also decreased by i.p. administration(1mg/kg) with this dose producing a comparable response to that obtained with 50 g/kg i.v. Rats injected with 8-OHDPAT doubled their food intake over the four hour period, while those given DOI showed no significant change from their control saline level.

These results show that peripherally administered DOI produces a decrease in DRN 5-HT neuronal firing without increasing food intake in satiated rats. The mechanism of action and receptor sub-type involved in the effect on DRN firing require further investigation. It has been proposed that the increase in feeding seen with 8-OHDPAT is due to the activation of the somatodendritic 5-HT_{1A} autoreceptor and the consequent inhibition of DRN 5-HT neuronal firing (Dourish *et al.*, 1986). If inhibition of DRN firing was associated with the hyperphagic response it would be anticipated that DOI would increase food intake. This did not occur suggesting that decreased 5-HT neuronal firing and hyperphagia are not necessarily related. It should however be noted here that a decrease in feeding has been seen with peripheral administration of DOI in food deprived rats (Schetchter and Simansky 1988). It is possible that this anorexic action of DOI may be able to mask changes in feeding due to inhibition of DRN neuronal firing.

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Dourish, C.T. , Hutson P.H., and Curzon, G. (1986). Appetite **7** (Suppl.), 127-140.

Glennon,R.A., McKenney, J.D.,Lyon, R.A. and Titler,M. (1986). J. Med. Chem. **29**, 194-199.

McKenna, D.J., Mathis, C.A. and Peroutka, S.J. (1988) Soc. Neurosci. Abstr., 18th Ann. Meeting, 247.12.

Pike, J., Kidd, E.J. and Marsden, C.A. (1989). Br. J. Pharmacol. (in press).

Schetchter, L.E. and Simansky, K.J. (1988). Psychopharmacology **94**, 342-346.

EVIDENCE TO SUGGEST THAT 8-OHDPAT, BUT NOT GR38032F, SPECIFICALLY ENHANCES FEEDING BEHAVIOUR IN MICE

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8-OHDPAT increases food intake in laboratory rodents, an effect believed to be mediated by agonist activity at the 5-HT_{1A} autoreceptor (Hutson et al., 1988). However, it has been suggested that this hyperphagic effect could be a consequence of non-specific arousal (Montgomery et al., 1988). The present study further investigated this hypothesis, by analysis of the effects of 8-OHDPAT on feeding in a situation in which other behavioural responses (e.g. defense) would be prepotent. As a positive result might purely be indicative of an anxiolytic action, the effects of a 5-HT_3 anxiolytic, GR38032F, were also assessed.

Subjects were non-deprived, group-housed male DBA/2 mice (Bantin & Kingman, Hull), housed under a reversed LD cycle in a temperature-controlled room (24±1°C). Testing was performed under dim red light during the dark phase of the cycle. Effects on basal feeding were assessed in a novel holding cage, with freely-available lab chow. More specific enhancement was studied by analysis of feeding behaviour within the home cage of an aggressive resident conspecific. In both paradigms, cumulative duration of feeding behaviour was recorded over a 300 sec test period. 8-OHDPAT (in saline), and GR38032F (in distilled water), were administered s.c. and i.p., 20 and 45 min before testing, respectively. Basal feeding data were analyzed by ANOVA and appropriate follow-up tests, while data from the social conflict paradigm were subjected to Kruskal-Wallis and Mann-Whitney analyses.

Data analysis (Table I) showed that 0.05-0.50mg/kg 8-OHDPAT significantly enhanced basal feeding. Furthermore, 0.10-0.50 mg/kg induced feeding during social conflict, a behaviour absent in all control animals. While 1.0 mg/kg of GR38032F, but not lower doses, significantly enhanced basal feeding, this compound did not induce feeding during social conflict.

TABLE I: Effects of 8-OHDPAT and GR38032F on feeding under basal, and social conflict conditions. Basal data are presented as Mean (±SE), and conflict data are presented as Median (LQ-UQ) durations (sec).
*p < 0.05 **p < 0.025 ***p < 0.002 versus vehicle.

8-OHDPAT:	VEH	0.05	0.10	0.50	1.0 mg/kg
Basal:	49(16)	109(14)*	119(20)**	105(23)*	51(20)
Conflict:	0(0-0)	0(0-4)	7.5(0-45)*	11.5(0-30.5)**	0(0-13.5)
GR38032F:	VEH	0.01	0.10	1.0 mg/kg	
Basal:	53(11)	54(11)	58(12)	95(13)**	
Conflict:	0(0-0)	0(0-0)	0(0-0)	0(0-0)	

Present data suggest that the enhancement of feeding by 8-OHDPAT is not simply an artifact of elevated arousal, but is directly related to motivational mechanisms of feeding. These results contrast with those obtained with GR38032F, and imply a rather less specific action of the latter compound.

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Hutson, P.H. et al. (1988) Eur. J. Pharmacol. 150, 361-366.
Montgomery, A.M.J. et al. (1988) J. Psychopharmacology 2(2)B.A.P. Abstracts.

FAILURE OF KETANSERIN TO SELECTIVELY BLOCK THE EXCITATORY ACTION OF 5-HT ON RAT SYMPATHETIC PREGANGLIONIC NEURONES

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Neuroanatomical studies have shown the existence of discrete groups of 5-hydroxytryptamine (5-HT) containing neurones in the brainstem, with extensive projections to the spinal cord. Many of these spinally projecting neurones make direct synaptic contacts with dendrites and somata of sympathetic preganglionic neurones (SPN) (Bacon & Smith, 1988). Data from indirect studies has indicated that 5-HT can have both excitatory and inhibitory actions on SPN; however, controversy exists as to the 5-HT receptor sub-types mediating these two actions (McCall, 1988; Yusof & Coote, 1988).

The present study was undertaken to determine the actions of 5-HT on identified SPN when it is applied directly in their vicinity by iontophoresis. SPN in the T1 and T2 segments of the spinal cord were identified antidromically by stimulation of the cervical sympathetic nerve of male Sprague-Dawley rats anaesthetised with chloralose (35 $mg.kg^{-1}$) and urethane (700 $mg.kg^{-1}$). Drugs were applied by iontophoresis from a 7-barrelled micropipette assembly.

The responses evoked by 5-HT, 5-carboxyamidotryptamine (5-CT), α-methyl-5-hydroxytryptamine (α-Me-5-HT) and 2-methyl-5-hydroxytryptamine (2-Me-5-HT), applied for 15-60 s, 0-100 nA, were observed in 136 SPN (Table 1).

Table 1

	Excitation	Inhibition	Biphasic	No Response	Total
5-HT	90	5	6	17	118
5-CT	44	1	0	7	52
α-Me-5-HT	18	0	0	9	27
2-Me-5-HT	0	10	7	9	26

Microiontophoretically applied ketanserin gradually abolished the excitatory responses evoked by 5-HT, 5-CT, α-Me-5-HT and noradrenaline (NA). No selective reduction in the responses evoked by 5-HT and α-Me-5-HT was observed. Brief ejections (1 min) of up to 15 nA or prolonged ejections (0-10 nA) in excess of 5 minutes of Ketanserin reduced the spontaneous or D,L-homocysteic acid-induced basal firing rates of all neurones tested. LY 53857 had similar actions to those described for Ketanserin.

In view of the inhibitory responses evoked by 2-Me-5-HT and occasionally 5-HT on SPN, the effect of the 5-HT_3 receptor antagonist, ICS 205-930, on these inhibitory responses was investigated. ICS 205-930 failed to reduce the 5-HT-evoked or NA-evoked inhibitory responses of SPN. However, a slight reduction in the basal firing rate was observed.

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Bacon, S. & Smith, D. (1988). J. Auton. Nerv. Syst. 24, 97-122.
McCall, R.B. (1988). Ann. Rev. Physiol. 50, 553-564.
Yusof, A.P.M. & Coote, J.H. (1988). J. Auton. Nerv. Syst. 22, 229-236.

THE EFFECTS OF CENTRAL ADMINISTRATION OF 8-OH-DPAT AND GR43175 ON AUTONOMIC OUTFLOW IN THE ANAESTHETISED CAT

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8-OH-DPAT and other 5-HT_{1A} agonists given i.v. produce a centrally mediated sympathoinhibition and an increase in cardiac vagal drive in anaesthetised cats (Ramage & Fozard, 1987; Ramage et al., 1988). The central site/s of action by which these agonists produce these effects remain to be determined. In the present experiments 8-OH-DPAT and the 5-HT_1-like agonist GR43175 (Humphrey et al., 1988) were administered into the IVth ventricle and their effects on different regional sympathetic outflows, vagal outflows to the heart, lungs and stomach and central respiratory drive were compared.

Cats, anaesthetised with α–chloralose (70 mg kg^{-1}) and pentobarbitone sodium (18 mg kg^{-1}), were artificially ventilated following paralysis with vecuronium bromide (200 μg kg^{-1}). Simultaneous recordings were made of renal (RNA), splanchnic (SNA), inferior cardiac (CNA) and phrenic nerve activity (PNA), plus blood pressure, heart rate, femoral arterial flow, tracheal pressure and intragastric pressure. The head was placed in a stereotaxic frame and a cannula was positioned so that its tip lay in the IVth ventricle (see Dey & Feldberg, 1976), through which cumulative doses of 8-OH-DPAT (2.5-40 nmol kg^{-1}) or GR43175 (10-160 nmol kg^{-1}) were given by microinjection (20 μl over 1 min). Atropine methonitrate (0.1 mg kg^{-1}) was given at the end of each experiment.

Cumulative doses of 8-OH-DPAT (n=4) caused dose related falls in mean BP and HR reaching maxima of -54 ± 8 mmHg (mean ± s.e. mean) and -62 ± 13 beats min^{-1} respectively. These falls were accompanied by falls in CNA, SNA and RNA which reached maxima of -63 ± 12%, -92 ± 3% and -93 ± 5% respectively by the highest dose. Cardiac differed from splanchnic and renal nerve activity in that sympathoinhibition only occurred at the higher doses, 20 and 40 nmol kg^{-1}. These changes were not associated with any changes in femoral arterial conductance, tracheal pressure or PNA. However, there was an increase in gastric motility. Administration of atropine after 8-OH-DPAT caused a mean increase in HR of 51 ± 17 beats min^{-1}. Cumulative doses of GR43175 (n=4) caused a fall in mean BP reaching a maximum of -22 ± 5 mmHg which was only associated with a decrease in RNA of -48 ± 10% by the highest dose. There were no overall changes in the other variables being recorded. Administration of atropine following GR43175 had no effect on HR.

These results confirm previous observations that 8-OH-DPAT produces a bradycardia and hypotension by a centrally mediated sympathoinhibition and an increase in vagal tone, and provide further evidence for a different sensitivity of different regional sympathetic outflows to 5-HT_{1A} agonists (Ramage and Wilkinson, 1988). They also indicate that the site of action of 8-OH-DPAT in producing these effects is within the pons/medulla. GR43175 produced only moderate hypotension which was accompanied by a reduction in RNA and not by an increase in cardiac vagal tone. Therefore it is suggested that the actions of GR43175 are mediated through receptors which do not mediate the characteristic effects of 8-OH-DPAT.

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Dey, P.K. & Feldberg, W. (1976) Br. J. Pharmac. 58, 383-393.
Humphrey, P.P.A., Feniuk, W., Perrin, M.J., Connor, H.E., Oxford, A.W., Coates, I.H. & Butina, D. (1988) Br. J. Pharmac. 94, 1123-1132.
Ramage, A.G. & Fozard, J.R. (1987) Eur. J. Pharmac. 138, 170-191.
Ramage, A.G. & Wilkinson, S.J. (1988) Br. J. Pharmac. 93, 121P
Ramage, A.G, Wouters, W. & Bevan, P. (1988) Eur. J. Pharmac. 151, 373-379.

MECHANISM OF 8-OH-DPAT-MEDIATED INHIBITION OF PLASMA INSULIN IN SPONTANEOUSLY HYPERTENSIVE RATS

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The involvement of 5-HT_{1A} receptors in the control of insulinaemia in normotensive rats has been described recently (Chaouloff & Jeanrenaud, 1987). The objectives of the present study were to investigate the mechanisms underlying 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT)-mediated inhibition of plasma insulin in spontaneously hypertensive (SH) rats.

Male SH rats (300-350 g) were implanted with catheters in the abdominal aorta and femoral vein. Arterial blood pressure (BP) and heart rate (HR) were recorded continously from fasted (18 h) conscious SH rats as described previously (Fozard et al., 1987). Effects of i.v. injections of 8-OH-DPAT or vehicle on basal and glucose-stimulated glycaemia, insulinaemia, BP and HR were determined simultaneously. Effects of 8-OH-DPAT on glucose-stimulated plasma insulin levels were also investigated in Inactin-anaesthetized and pithed SH rats. The effects on insulin secretion in vitro from isolated perfused rat pancreas were determined as described previously (Loubatieres et al., 1969). Insulin was determined using a radioimmunoassay based on the method of Herbert et al. (1965).

I.v. injections of 8-OH-DPAT, 150 µg/kg, into fasted conscious SH rats resulted in a significant increase (31 ± 6%, n = 4) in the basal glycaemia which was associated with small and variable increases in the basal insulin levels and marked decreases in BP (37 ± 6 mmHg) and HR (50 ± 7 beats/min). In contrast, in anaesthetized rats there were no changes in plasma glucose or insulin despite prominent cardiovascular changes in response to 8-OH-DPAT. Following treatment with 8-OH-DPAT, 150 µg/kg i.v., the peak increases in plasma insulin in response to a glucose load (0.5 g/kg i.v.) were significantly inhibited in both conscious (49 ± 9%, n = 5 - 8) and anaesthetized (33 ± 8%, n = 5) SH rats without alterations in glucose tolerance; in pithed SH rats, 8-OH-DPAT did not inhibit glucose-stimulated increases in plasma insulin and glucose tolerance was normal. In contrast to 8-OH-DPAT, clonidine (8 µg/kg i.v.) produced a marked and sustained inhibition (88 ± 4%, n = 4) of insulin levels associated with glucose intolerance in pithed rats. In the isolated perfused rat pancreas 8-OH-DPAT, 10^{-8} and 10^{-7} M, did not inhibit glucose (8.3 mM)-stimulated insulin secretion (8-OH-DPAT 33 ± 8; 39 ± 8 ng/min, respectively, control 38 ± 9 ng/min, n = 6); inhibition (39 ± 7%, n = 4, of basal) was seen only at 10^{-6} M.

The present data suggest that like the cardiovascular effects of 8-OH-DPAT (Fozard et al., 1987) the inhibition of glucose-stimulated insulin levels in the SH rat is mediated via an action on the central nervous system. Precisely how the presumed central 5-HT_{1A} receptor activation leads to hypoinsulinaemia without alterations in glucose tolerance remains to be elucidated.

Chaouloff, F. and Jeanrenaud, B. (1987) J. Pharmacol. Exp. Ther. 243, 1159 - 1166.
Fozard et al. (1987) J. Cardiovasc. Pharmacol. 9, 328 - 347.
Herbert, V. et al. (1965) J. Clin. Endocr. 25, 1375 - 1384.
Loubatieres, A.L. et al. (1969) Diabetologia 5, 1 - 10.

INVESTIGATION OF THE *IN VIVO* CARDIOVASCULAR EFFECTS OF ICI 169,369 IN ANAESTHETISED RATS

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It has previously been shown that the novel 5-HT_2 antagonist ICI 169,369 (Blackburn *et al.*, 1988) differed in its action in anaesthetised cats when compared to other 5-HT antagonists at high doses, in that it caused a large transient fall, followed by a rise, in blood pressure (Ramage, 1988). The present experiments were carried out in rats to investigate further the mechanism(s) of these effects.

Male Sprague Dawley rats (250-350 g) were anaesthetised with pentobarbitone sodium (90 mg kg^{-1}, i.p.) or pithed under halothane. Pithed rats were artificially ventilated with O_2-enriched room air, and blood gases and pH were maintained within the physiological range. In these rats diastolic BP was maintained around 100 mmHg by means of a continuous i.v. infusion of vasopressin (30 miu kg^{-1} min^{-1}). Cumulative doses of ICI 169,369 (0.1-3.0 mg kg^{-1}) were given i.v. and pretreatment with antagonists was carried out 10-15 min before giving ICI 169,369. Mean BP and HR were measured at the peak of the transient effects (10-15 s after injection) and immediately prior to the subsequent dose of ICI 169,369 or 7 min after the highest dose ('long term' effects). Means from each group were compared with the respective control group mean by a Dunnett test performed after a 2-way ANOVA, $p<0.05$ being considered significant. ICI 169,369 (10-80 μg kg^{-1}; n=3) was also given intracisternally in halothane-α–chloralose anaesthetised rats.

The effects of cumulative doses of ICI 169,369 are shown in Table 1. Pretreatment with atropine methonitrate and atenolol (1 mg kg^{-1}) was more effective in causing a significant reduction in the transient and long term bradycardias than atropine alone. Pretreatment with

Table 1

ICI 169,369 mg kg^{-1}		**0.1**	**0.3**	**1.0**	**3.0**
ΔHR beats min^{-1}	Transient:	-2±1	-14±3	-68±33	-157±25
(455±9; n=6)	'Long-Term':	-6±2	-16±2	-38±7	-75± 5
Δmean BP mmHg	Transient:	0	-2±1	-22±8	-53±9
(130±8; n=6)	'Long-Term':	-3±1	-11±2	-19±2	-35±7

MDL 72222 or ICS 205-930 (1 mg kg^{-1}) failed to affect either bradycardia. The transient bradycardia also failed to show cross tachyphylaxis with that induced by phenylbiguanide (100 μg kg^{-1}). However, in vasopressin-supported pithed rats the bradycardias were almost completely abolished along with the long term fall in BP. Only at high doses was a small transient fall in BP observed. ICI 169,369 given i.c. caused a rise in BP of 16 mmHg.

Taking into account the present observations with those of Ramage (1988), it is possible that ICI 169,369 is capable of stimulating vagal afferents by a non 5-HT_3 receptor mechanism. The ability of ICI 169,369 to lower 'long-term' BP and HR supports the view that 5-HT_2 antagonists cause hypotension by a central action in anaesthetised rats (Docherty, 1989). However, this did not seem to be true when ICI 169,369 was given centrally. The failure to observe a fall in BP upon i.c. administration may reflect other non-5-HT actions of ICI 169,369, such as an ability to release EDRF (Pires et al., 1989) and/or the ability to stimulate vagal afferents.

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Blackburn, T.P., Thornber, C.W., Pearce, R.J. & Cox, B. (1988) Eur. J. Pharmac. 150, 247-256.
Docherty, J.R. (1989) J. Pharmac. Exp. Ther. 248, 739-740
Pires, J.G.P., Jacobs, M. & Ramage, A.G. (1989) Br. J. Pharmac. 97, 515P
Ramage, A.G. (1988) Naunyn-Schmiedeberg's Arch. Pharmac. 338, 601-607.

[^{3}H]DIAZEPAM BINDS TO A PERIPHERAL-TYPE, GABA-MODULATED BENZODIAZEPINE BINDING SITE IN *E. COLI*

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In vertebrates two separate classes of benzodiazepine binding sites have been identified. The neuronal benzodiazepine binding sites, which bind clonazepam and Ro15-1788 with high affinity, are part of the $GABA_A$ receptor complex; in the presence of GABA, agonists will enhance the GABA response. The peripheral or mitochondrial benzodiazepine binding sites are not modulated by GABA and compounds active at this site, such as Ro 5-4864 and PK11195 , have been reported to effect a variety of cell functions (Anholt, 1986). Benzodiazepine binding sites have been reported as a late evolutionary development (Nielsen et al.,1977) but have more recently been identified in insect tissue (Abalis et al.,1983). In the present study we examine the binding of [^{3}H]diazepam in a prokaryotic species, Eschericia coli (E.coli).

[^{3}H]Diazepam binding was performed in 50mM Tris-HCl, pH 7.4 using well washed bacterial cultures (E.coli, NTCT 10814). Incubation was for 60 min at 0-4°C and was terminated by centrifugation. Non-specific binding was determined using 100μM diazepam. Specific binding was saturable and best fit by a two site model with K_{d1}=13.1 ± 5.4nM and K_{d2}=7300 ± 2100nM (mean ± S.E., n=10). The pharmacological specificity of the binding sites were examined using a range of benzodiazepines, and those most active in displacing specific binding from the vertebrate peripheral benzodiazepine binding site were the most effective displacers of E.coli [^{3}H]diazepam binding. The rank order of potency was Ro5-4864 > PK11195 > flunitrazepam > nitrazepam > clonazepam > Ro15-1788. Specific [^{3}H]diazepam binding could be enhanced by GABA over the concentration range 10^{-8} to 10^{-4} M, with maximal enhancement at 10^{-6}M.

In conclusion we have demonstrated specific saturable binding sites for [^{3}H]diazepam in bacteria. These binding sites have a pharmacological profile similar to the vertebrate peripheral benzodiazepine binding sites but resemble the vertebrate neuronal benzodiazepine binding sites in their modulation by GABA.

Abalis I.M., Eldefrawi M.E. & Eldefrawi A.T. (1983) Pestic. Biochem. Physiol. 20, 39-48.
Anholt R.R.H. (1986) Trends. Pharmacol. Sci. 12, 506-511.
Nielsen M., Braestrup C. & Squires R.F. (1978) Brain Res. 141, 342-346.

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A COMPARISON OF DRUG BINDING SITES ON HUMAN AND RAT ALBUMINS

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There are two major and structurally selective drug binding sites on human and bovine albumins (Birkett & Wanwimolruk, 1986). Warfarin and other weak acids, for example phenylbutazone, interact with site 1 whilst benzodiazepines such as diazepam bind to site 2. However, it is not known whether identical sites exist on albumins from other species and since the rat is frequently used in studies of drug disposition it was of interest to determine if sites 1 and 2 are present on rat albumin.

The binding of warfarin (2μM) and dansylsarcosine (2μM; site 2 probe) to both defatted human and rat albumins (20μM) was measured by fluorescence spectroscopy at 31°C. Displacement studies were done at pH 7.4 with: (1) phenylbutazone (20μM) and diazepam (20μM) and (2) fatty acids (20μM) with increasing carbon chain length (C5-C20). In addition, the effect of increasing the buffer pH from 6 to 9 on the fluoresence intensity of warfarin and dansylsarcosine was examined. Results are given as mean ± s.e. mean.

With human albumin, the fluorescence of warfarin decreased to 58 ± 0.8% (n = 3) of its control value when phenylbutazone was present. By contrast, there was no change in the fluorescence of dansylsarcosine. Diazepam, however, decreased the fluorescence intensity of dansylsarcosine to 64 ± 0.9% (n = 3) of its initial value, but the fluorescence of warfarin remained unchanged. Increasing the buffer pH from 6 to 9 resulted in an enhancement of warfarin's fluorescence such that at pH 9.0 fluorescence intensity was about 170 ± 1.8% (n = 6) of its value at pH 6.0. However, there was no significant alteration in the fluorescence of dansylsarcosine. Fatty acids with carbon chain lengths C5-C9 had no effect on the fluorescence of warfarin whereas C10-C20 fatty acids augmented fluorescence intensity. The C5-C9 acids decreased the fluorescence of dansylsarcosine with a maximum effect at C9. From C10-C20 this decrement of fluorescence became progressively less. With rat albumin, both phenylbutazone and diazepam had no marked effect on the fluorescence of site 1 or 2 probes. In addition, increasing pH did not alter the fluorescence of either warfarin or dansylsarcosine. The C5-C20 fatty acids did not influence warfarin's fluorescence but, a similar reduction in fluorescence to that seen with human albumin was found for dansylsarcosine. The maximum effect was also found with C9 and as the carbon chain length increased this reduction in fluorescence decreased.

The results obtained with human albumin are consistent with the existence of sites 1 and 2 (Birkett & Wanwimolruk, 1986). However, it is clear that the warfarin binding site on rat albumin displays different characteristics to site 1 on human albumin. Phenylbutazone failed to displace warfarin and there was no enhancement of fluorescence with either increasing pH or in the presence of C10-C20 fatty acids. A possible explanation for these differences between albumins is that warfarin has a much greater affinity for rat albumin than for the human protein. Although diazepam did not inhibit the binding of dansylsarcosine to rat albumin, the effect of pH and C5-C20 fatty acids on the fluorescence intensity of this ligand were similar with both proteins. It is concluded that there is a better equivalence between rat and human albumins with respect to site 2 than for the warfarin binding site.

Birkett, D.J. & Wanwimolruk, S. (1986) in 'Protein Binding and Drug Transport', Symposia Medica Hoechst 20, Tillement, J-P. & Lindenlaub E. eds. Schattauer Verlag, pp 11-23.

CONSEQUENCES OF A STEADY-STATE TERNARY COMPLEX MODEL

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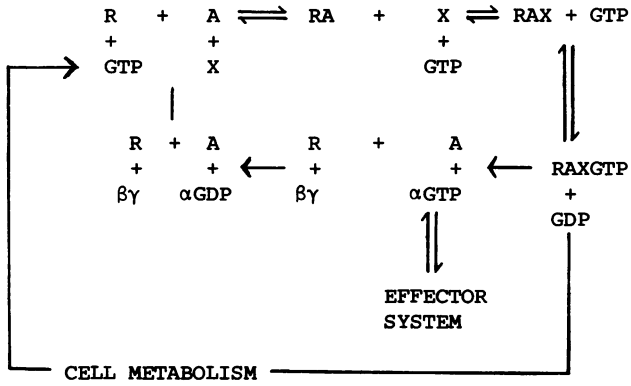
In the past, comparison of the properties of agonists and their use in receptor classification has been based mainly on ideas and equations developed using the occupancy model of drug action. It has become increasingly clear that interpretations of concentration-response curves in terms of this model cannot be regarded as satisfactory. Attempts have been made recently (Mackay,1987,1988) to develop null equations based on a version of the ternary complex model but this model too has serious limitations. Steady-state analysis has therefore been performed on the more realistic ternary complex model shown below, to derive null equations for the comparison of agonists and for estimating apparent affinity constants of agonists using irreversible antagonists.

Symbols

R = receptor

A = agonist

X = G-protein, which is a complex of α, β and γ sub units



The null equations developed in this way have been shown to have the same mathematical form as those developed previously using the occupancy model of drug action. However these new null equations predict that in general, for any one receptor-effector system, the apparent affinity constants of agonists (estimated from curve displacements produced by irreversible antagonists) may depend on the concentration of G-protein in the cell membranes and on the intracellular concentration of GTP. The same conclusion applies to estimates of relative potencies of agonists, though these are not likely to be quite so dependent on these tissue variables.
It is concluded that relative potencies, efficacies and apparent affinity constants of agonists, estimated from concentration-response curves, do not provide a totally dependable method for classifying receptor-effector systems.

Mackay,D. (1987) In Perspectives on Receptor Classification, (Black,J.W., Jenkinson,D.H., & Gerskowitch, V.P.eds.), pp.103-114, A.R.Liss.
Mackay,D. (1988) Trends Pharmacol. Sci.9,156-157

EVIDENCE IN FAVOUR OF THE STEADY-STATE TERNARY COMPLEX MODEL

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Competitive binding experiments have been carried out on rat atrial membranes. Suspensions of the washed membranes, ^{3}H-N-methyl-scopolamine (NMS) (3nM), and various concentrations of carbachol, oxotremorine or pentyl-trimethyl-ammonium (pentyl-TMA) were incubated in Krebs' solution for 30 min. at 27°C. The tube contents were then rapidly diluted and filtered. Bound NMS was estimated by liquid scintillation counting. Non-specific binding was taken to be that remaining when atropine (1 μM) was also present. The data were analysed in terms of the equilibrium ternary complex model (see e.g. Ehlert, 1985). The results are shown below. R_T and X_T are the total concentrations of receptor and of G-protein. K_{1A} is the affinity of agonist A for its receptor and K_{2A} is the affinity of the RA complex for X.

Agonist	K_{1A} x 10^{-4}M	$K_{2A} \cdot X_T$	X_T/R_T	n
Oxotremorine	509 ± 62	45.1 ± 5.7	0.48 ± 0.03	6
Carbachol	33.7 ± 4.2	56.2 ± 10.1	0.55 ± 0.03	6
Pentyl-TMA	5.7 ± 0.3	32.3 ± 13.3	0.47 ± 0.03	3

Each value is given as mean ± s.e.m. based on n results.

Apparent affinity constants of these agonists for the muscarinic receptors were estimated by comparing concentration-response curves determined at 27°C in the presence of 20 nM isoprenaline, on rat atria paced at 180 beats/min., before and after treatment of the tissue with propylbenzilyl-choline mustard. The bathing medium in these experiments was the same as that used in the ligand binding experiments. The values of these apparent affinity constants, K_{Aapp}, are shown below, together with values calculated from the null equation derived on the basis of the equilibrium ternary complex model (Mackay, 1988).

Agonist	K_{Aapp} x 10^{-4}M (Observed)	K_{Aapp} x 10^{-6}M(Calculated)
Oxotremorine	45.9 ± 3.6	235
Carbachol	4.7 ± 0.4	19.2
Pentyl-TMA	5.3 ± 1.3	1.9

Each observed value is given as mean ± s.e.m. based on 4 results.

The apparent affinity constants measured on the intact tissues are much less than those predicted by the equilibrium ternary complex model. The various discrepancies can be explained in terms of the steady-state ternary complex model (Mackay, 1989) since the concentration of guanosine-5'-triphosphate in rat atria has been found, by HPLC, to be approximately 150 μmole/kg surface-dried tissue.

Ehlert, F.J. (1985) Mol. Pharmacol., 28, 410-421.
Mackay, D. (1988) Trends in Pharmacol. Sci., 9, 156-157.
Mackay, D. (1989) Communication C49 at this Meeting.

POTENT INHIBITION OF RECEPTOR-LINKED PGI_2 SYNTHESIS BY NSAIDS IN THE RAT AORTA: EVIDENCE FOR EFFECTS ON CALCIUM MOBILISATION

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Although the role of prostanoids (PGs) in mediating vascular reactivity has been largely investigated using cyclooxygenase inhibitors (COIs) such as indomethacin, COIs exert effects on vascular reactivity that appear not to be PG-mediated (Altura & Altura, 1976; Hadhazy *et al.*, 1988). Studies have also indicated that COIs exert actions on intracellular systems such as G proteins, protein kinase C and calcium binding at the plasmalemma (Hadhazy *et al.*, 1988; Peplow, 1988). It has recently been established that receptor-linked prostacyclin (PGI_2) synthesis in blood vessels is mediated by G proteins, protein kinase C and cell surface calcium channels (Jeremy *et al.*, 1988). We therefore investigated the effect of three COIs (indomethacin, tiaprofenic acid and ibuprofen) on PGI_2 synthesis by the rat aorta when stimulated by noradrenaline (NA), phorbol ester dibutyrate (PDBU; a protein kinase Ca^{2+} activator), sodium fluoride (NaF; a G protein activator), calcium ionophore A23187 (A23187; a creator of artificial Ca^{2+} channels), arachidonate (AA; a PG precursor) and trauma (sonication: stimulates PGI_2 synthesis via extracellular Ca^{2+} activation of phospholipase A_2; Jeremy *et al.*, 1986).

Aortic tissue was processed for the assessment of PGI_2 stimulation/inhibition as previously described (Jeremy & Dandona, 1989). Tissue was preincubated with COIs for 1 h prior to the addition of PGI_2 stimulators ([final concentrations] NA[10 μM], NaF [10 mM], PDBU [1 μM], A23187 [20 μM], AA [20 μM]) or sonication, and further incubated at 37 °C for 1 h. PGI_2 (as 6-oxo-$PGF_{1\alpha}$) released into the supernatant were measured by specific radioimmunoassay.

Table 1 IC_{50}s (μM) of COIs on different stimulators of PGI synthesis.

	NA	NaF	PDBU	A23187	Trauma	AA
Indomethacin	0.009	0.01	0.01	0.01	0.33	0.30
Ibuprofen	0.05	0.05	0.05	0.05	1.21	1.25
Tiaprofenic acid	0.03	0.03	0.03	0.03	0.45	0.48

The present study demonstrates that although there was no difference in the IC_{50}s of the COIs when stimulated with NA, NaF, PDBU and A23187, there was a marked right shift in the IC_{50}s of the COIs when PGI_2 was stimulated by sonication (trauma) and AA. Since both trauma and AA-stimulated PGI_2 synthesis bypass Ca^{2+} channel-mediated stimulation of PG synthesis (Jeremy *et al.*, 1986), the present data suggest that the COIs are exerting inhibitory actions not only on cyclooxygenase but also (and more potently) on the mobilisation of Ca^{2+} linked to PGI_2 synthesis. Furthermore, since calcium is a universal second messenger, these data may not only be of importance to rationalising anomalous effects of COIs on vascular reactivity but also to the antiinflammatory efficacy and deleterious side effects of COIs.

Altura, B.M. & Altura, B.T. (1976) Fed. Proc. 35, 2360-2370.
Hadhazy, P., Malomvolgyi, B. & Magyar, K. (1988) Prost. Leuk. Ess. Fatty Acid Revs. 32, 175-186
Jeremy, J.Y., Mikhailidis, D.P. & Dandona, P. (1986) Naunyn Schmiedeberg's Arch. Pharmacol. 332, 70-73.
Jeremy, J.Y., Mikhailidis, D.P. & Dandona, P. (1988) Prost. Leuk. Ess. Fatty Acid Revs. 34, 215-218
Jeremy, J.Y. & Dandona, P. (1989) Br. J. Pharmac. 96, 243-250.
Peplow, P. (1988) Prost. Leuk. Ess. Fatty Acid Revs. 33, 239-252

PROSTANOID RECEPTORS MEDIATING CONTRACTION AND RELAXATION OF HUMAN ISOLATED UTERINE ARTERY

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We have shown previously that prostanoids can both relax and contract guinea pig uterine arterial smooth muscle and that relaxation is mediated via IP-receptors, and contraction by both TP- and EP_3-receptors (Baxter et al. 1988). Prostanoids can also constrict human uterine artery (Wilhelmsson et al., 1981; Vincent et al., 1983). However, the receptor population in this tissue has not yet been defined. In the present study we have examined the action of prostanoids on human isolated uterine artery and attempted to characterise the prostanoid receptors present.

Specimens of ascending uterine artery were obtained from non-pregnant, pre-menopausal patients at hysterectomy. Uterine arteries were cut into rings approximately 3mm in length and mounted under 1g tension in organ baths containing oxygenated (95% O_2/5% CO_2) Krebs solution at 37°C and indomethacin (2.8μM).

The stable thromboxane-A_2 mimetic U-46619 (11,9-epoxymethano PGH_2) was a potent contractile agonist (EC_{50} (95% C.L.) = 3.5 (1.8-6.7) nM, n = 6), whereas the natural prostanoids $PGF_{2\alpha}$, PGE_2 and PGD_2 were considerably weaker (equipotent concentration ratios, (U-46619 = 1); 743 (207-2667) n = 5, ≥3800 n = 5 and ≥900 n = 6, respectively). In a few preparations, PGI_2 caused small contractions at the highest concentration used (30μM). The TP-receptor blocking drug GR32191 caused concentration dependent rightward shifts of contractile concentration-effect curves to U-46619 (pA_2[range] = 8.96 [8.35-10.22] slope[range] = 0.84 [0.6-1.0], n = 3). At a concentration of 1μM GR32191 abolished contractile responses to all the natural prostanoids tested. GR32191 did not influence concentration effect curves to phenylephrine and was included in the Krebs solution throughout all subsequent experiments. Under these conditions the selective FP- and DP-receptor agonists ICI81008 and BW245C (Coleman, 1987) and the selective EP-receptor agonists sulprostone, rioprostil (Coleman et al., 1988) and butaprost (Gardiner, 1986) were inactive up to the highest concentration used (30μM). In preparations contracted with phenylephrine PGI_2 caused concentration-dependent relaxations of uterine arteries (EC_{50} = 12.7 (4.8-34.0)nM, n = 6). The potent IP-receptor agonists iloprost and cicaprost were more potent than PGI_2 (EPC = 0.09 (0.05-0.18), n = 4, and 0.08 (0.01-0.53), n = 4 respectively) whereas BW245C was considerably less potent (EPC>1000). Rioprostil and butaprost were inactive (EPC>30,000).

These data are consistent with the presence of only TP- and IP-receptors in human uterine artery mediating contractile and relaxant responses respectively. Although the naturally-occurring prostanoids, $PGF_{2\alpha}$, PGE_2 and PGD_2 produce constriction of the artery, it appears that these responses are mediated via TP-receptors.

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Baxter, G.S. et al. (1988) Br. J. Pharmac. 96, 71P.
Coleman, R.A. et al. (1988) Br. J. Pharmac. 95, 742P.
Coleman, R.A. (1987) in Prostaglandins and Related Substances, a Practical Approach, ed. C. Benedetto et al., IRL Press, Oxford: 267-303.
Gardiner, P.J. (1986) Br. J. Pharmac. 87: 45-56.
Vincent, J.E. et al. (1983) Advances in Prostaglandin and Leukotriene Research Vol. 12, edited by Samuelsson, Paoletti and Ramwell, Raven Press, N.Y.: 417-422
Wilhelmsson, L. et al. (1981) Prostaglandins, 22: 223-233.

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RENAL VASCULAR RESPONSES TO ARACHIDONIC ACID IN EXPERIMENTAL DIABETES

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Diabetes mellitus (DM) is accompanied by alterations in arachidonic acid (AA) metabolism and vascular responsiveness. We used isolated perfused kidneys of male Wistar rats, 4-6 weeks and 20-24 weeks after the induction of DM with streptozotocin (STZ, 70 mg/kg i.v.), to address renal vascular responses to AA and the endoperoxide analogue, U46619. Responses were compared to those obtained in kidneys from age-matched vehicle-treated rats. A minimum of 5 rats/group were used unless otherwise stated. Blood glucose levels in rats treated with STZ were greater than 350 mg/dl. In both control and diabetic rat kidneys AA (0.3-10 μg) and U46619 (10-1000 ng) produced dose-dependent increases in perfusion pressure. At 4-6 weeks and 20-24 weeks after STZ the vasoconstrictor responses to AA were enhanced compared to the corresponding control. Thus, 4-6 weeks after STZ the approximate ED_{50} for AA-induced vasoconstriction was 0.66 μg compared to 2.1 μg for the control. Similarly, 20-24 weeks after STZ or vehicle treatment, 3 μg AA elevated perfusion pressure by 143$\pm$27 mmHg and 40$\pm$10 mmHg, respectively ($p<0.01$). The enhanced vasoconstrictor response to AA in 4-6 week diabetic rats was associated with increased conversion of AA to immunoreactive prostanoids. Thus, in diabetic rat kidneys (n=3) 3 μg AA increased the efflux of PGE_2 and 6-keto-$PGF_{1\alpha}$ by 7.1$\pm$1.2 ng/min and 6.0$\pm$0.6 ng/min, respectively, compared to control values (n=4) of 3.9$\pm$0.5 ng/min ($p<0.05$) and 2.7$\pm$0.4 ng/min ($p<0.01$), respectively. In addition, vasoconstrictor responses to U46619 were increased in kidneys from diabetic rats. Thus, 4-6 weeks and 20-24 weeks after STZ, 100 ng U46619 increased perfusion pressure by 146$\pm$7 mmHg and 140$\pm$13 mmHg, respectively, compared to values in age-matched controls of 75$\pm$17 mmHg and 54$\pm$19 mmHg, respectively ($p<0.01$). Vasoconstrictor responses to AA and U46619 in diabetic and control rat kidneys were unaffected by inhibition of thromboxane synthase with CGS-13080 (5 μM) but were significantly reduced by the PGH_2/TxA_2 receptor antagonist, SQ 29548 (1 μM). This study demonstrates that the renal vasoconstrictor response to AA is increased in D.M. and may result from increased sensitivity of the renal vasculature to endoperoxides and, possibly, enhanced conversion of AA to endoperoxides (Quilley et al., 1989).

Quilley, J., McGiff, J.C. and Nasjletti, A. (1989) Brit. J. Pharmacol. 96:111-116.

A SINGLE α_1-ADRENOCEPTOR, DISTINCT FROM THE α_{1A} AND α_{1B} SUBTYPES, MAY MEDIATE CONTRACTION OF THE RAT AORTA TO PHENYLEPHRINE

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Two subtypes of α_1-adrenoceptor have recently been proposed (Morrow and Creese, 1986; Han et al., 1987). One, showing relatively high affinity for WB 4101 and linked to nifedipine-sensive Ca^{++} influx, is designated α_{1A}; the other, showing relatively low affinity for WB 4101 and linked to inositol phospholipid turnover, is designated α_{1B}. The α_{1B} site can be selectively inactivated by the alkylating agent, chloroethylclonidine. The rat aorta contracts to α_1-adrenoceptor agonists by both nifedipine sensitive and insensitive mechanisms (Chiu et al., 1987) suggesting mediation by both α_{1A} and α_{1B} sites. The present experiments were carried out to verify this hypothesis.

Spiral strips were cut from the thoracic aortae of male, Sprague-Dawley rats killed by cervical dislocation. The endothelium was removed and the tissues suspended under 1 g tension in a modified Tyrode solution (Fozard and Mobarok Ali, 1978). Concentration-response curves to phenylephrine were generated cumulatively in the absence and presence (20 min preincubation) of vehicle (no significant sensitivity change) or antagonists.

Consistent with the literature, incubation of rat aortae in nifedipine, 10^{-6} M, reduced both the EC50 (by 6.6-fold) and the maximum response (by 26%) to phenylephrine (n = 7). A summary of the effects of a number of putative antagonists obtained in the absence and presence of nifedipine 10^{-6} M is presented in Table 1.

Table 1 Effects of antagonists on the response to phenylephrine in the absence and presence of nifedipine

	No nifedipine			nifedipine 10^{-6} M		
	pA_2	Schild slope	n	pA_2	Schild slope	n
prazosin	9.69 ± 0.07	- 1.06 ± 0.09	4	10.03 ± 0.10	- 1.00 ± 0.13	4
yohimbine	6.54 ± 0.14	- 1.10 ± 0.18	4	6.54 ± 0.14	- 1.41 ± 0.14	4
phentolamine	8.24 ± 0.09	- 0.84 ± 0.11	4	8.50 ± 0.08	- 1.03 ± 0.10	4
WB 4101	9.14 ± 0.08	- 1.06 ± 0.10	7	9.57 ± 0.04	- 0.99 ± 0.05	6
5-methylurapidil	7.54 ± 0.15	- 1.31 ± 0.16	4	7.71 ± 0.11	- 1.13 ± 0.14	4
chloroethylclonidine	5.22 ± 0.01*		4	5.19 ± 0.13*		4

* pD'_2 values estimated after 30' incubation in chloroethylclonidine followed by repeated washing

The results confirm that the contractile response to phenylephrine is mediated through an α_1-adrenoceptor linked to both nifedipine sensitive and insensitive mechanisms. However, the fact that both surmountable (WB 4101 and 5-methylurapidil) and insurmountable (chloroethylclonidine) discriminatory antagonists yield similar data in the presence or absence of nifedipine is not consistent with the response being mediated by the putative α_{1A} and α_{1B} subtypes. A further α_1-adrenoceptor subtype with a unique pharmacology and linked to both nifedipine sensitive and insensitive mechanisms is implicated in the contractile response of the rat aorta to phenylephrine.

Chiu, A.T., Bozarth, J.M. and Timmermans, P.B.M.W.M. (1987) J. Pharmacol. exp. Ther. 240, 123 - 127.
Han, C., Abel, P.W. and Minneman, K.P. (1987) Nature 329. 333 - 335.
Morrow, A.L. and Creese, I. (1986) Mol. Pharmacol. 29, 321 - 330.
Fozard, J.R. and Mobarok Ali, A.T.M. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 301, 223 - 235.

HETEROGENEITY OF α_1-ADRENOCEPTOR BINDING SITES IN THE RAT HEART - SUBTYPE-SELECTIVE AGONISTS AND ANTAGONISTS

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Distinct α_1-adrenoceptor binding sites showing high (α_{1A}) and low affinity (α_{1B}) for WB-4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4 benzodioxane) and 5-methyl-urapidil can be distinguished in various tissues of the rat (Morrow & Creese, 1986, Minneman et al., 1988, Gross et al., 1988). In the present study we determined the distribution of these binding sites on membranes of the rat heart by using [^{3}H]prazosin as radioligand and several unlabelled adrenergic agents.

Table 1. Inhibition of [^{3}H]prazosin binding to rat heart membranes

	$pK_{I\,high}$	$pK_{I\,low}$	$\%_{high}$
(+)Niguldipine	10.7 ± 0.1	7.6 ± 0.1	19 ± 1
5-Methyl-urapidil	9.2 ± 0.3	7.4 ± 0.1	22 ± 5
YM-12617*	10.3 ± 0.2	8.9 ± 0.1	25 ± 1
WB-4101	10.3 ± 0.3	8.9 ± 0.1	22 ± 4
Oxymetazoline	8.9 ± 0.1	6.6 ± 0.1	16 ± 1
Amidephrine	6.5 ± 0.2	4.4 ± 0.1	13 ± 1

*5-(2-((2-(2-ethoxyphenoxy)ethyl)amino)propyl)-2-methoxybenzenesulfonamide HCl. Means ± s.e.mean of at least 3 separate experiments

Prazosin, rauwolscine and butanserine displaced [^{3}H]prazosin binding monophasically, whereas several other compounds gave biphasic inhibition curves which allowed calculation of the proportion of sites with high and low affinity (Fig. 1). (+)Niguldipine which was recently introduced by Boer et al. (1989) proved to be the most subtype-selective compound presently known. Incubation of membranes or right ventricle strips with chloroethylclonidine (CEC) according to Minneman et al. (1988) resulted in a 71 % loss of [^{3}H]prazosin binding sites. The majority of the remaining binding sites showed high affinity for niguldipine, 5-methyl-urapidil, YM-12617, oxymetazoline and amidephrine and apparently belonged to the α_{1A} subtype. In order to evaluate the functional role of α_{1A}- and α_{1B}-binding sites, the inotropic response to adrenaline (in the presence of propranolol) was recorded in right ventricle strips preincubated with or without CEC. This treatment increased rather than decreased the maximum response to α_1-adrenoceptor stimulation and resulted in a significant increase in the potency of 5-MU but not of prazosin.

These results demonstrate the existence of two distinct α_1-adrenoceptor binding sites in the rat heart and suggest that both sites may be functionally relevant and seem to interact in a complex manner which is not yet clearly understood.

Boer, R. et al. (1989) Naunyn-Schmiedeberg's Arch. Pharmacol.339,R91
Gross, G. et al. (1988) Eur. J. Pharmacol. 151, 333
Minneman, K.P. et al. (1988) Mol. Pharmacol. 33, 509
Morrow, A.L. & Creese, I. (1986) Mol. Pharmacol. 29, 321.

EFFECT OF DURATION OF DIABETES ON CARDIAC ADRENOCEPTOR-MEDIATED INOTROPIC RESPONSES

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There are many conflicting reports concerning the effect of experimental diabetes on cardiac adrenoceptor-mediated responses. We have examined rat left atrial and papillary muscle responses to isoprenaline and phenylephrine to determine whether the effects of diabetes are dependent on the cardiac tissue examined and/or the duration of diabetes.

Diabetes was induced in female wistar rats (200g) by a single intraperitoneal injection of streptozotocin (50 $mgKg^{-1}$) (STZ). Age matched controls were injected with citrate buffer vehicle. 2, 4 or 12 weeks later animals were killed and left atria (LA) and papillary muscles (PM) set up in aerated Krebs solution (11.7mmol l^{-1}) at 37°C. Tissues were paced at 1Hz and isometric developed tension recorded. Cumulative concentration-response curves to isoprenaline and phenylephrine (in the presence of 1 μM propranolol) were constructed in the presence of desipramine (1 μM) and metanephrine (10 μM). The remaining ventricular tissue was assayed for [^{3}H] dihydroalprenolol or [^{3}H] prazosin binding.

TABLE 1: EC_{50} values (nM) with 95% confidence limits for isoprenaline

Tissue	Duration of diabetes 2 weeks	4 weeks	12 weeks
Left Atria			
control	38.4 (21.9 - 67.3)	49.7 (8.5 - 291.5)	13.7 (7.2 - 26.0)
diabetic	5.1 (2.7 - 9.7)*	10.6 (8.8 - 12.7)*	76.8 (33.8 - 174.5)*
Papillary muscles			
control	57.6 (27.6 - 119)	77.5 (40.7 - 147.8)	85.0 (34.6 - 208.5)
diabetic	10.6 (3.5 - 32.6)*	8.0 (3.3 - 18.9)**	35.8 (10.6 - 120.8)

*$P < 0.05$ **$P < 0.01$

2 and 4 weeks after STZ-pretreatment, the responses of both LA and PM's to isoprenaline were enhanced and EC_{50} values were significantly reduced compared with controls (Table 1). The increase in responsiveness to isoprenaline at 2 weeks was accompanied by an increase in the density of ventricular [^{3}H] dihydroalprenolol binding sites from 40.6 ± 3.9 to 80.2 ± 12.8 fmol mg^{-1} protein ($P < 0.05$) while the dissociation constant for binding was unaffected. After 3 months of diabetes, the sensitivity of PM's to isoprenaline was similar to controls while responses of LA were significantly depressed with isoprenaline EC_{50} values being greater than in LA from age-matched controls (Table 1).

Responses of LA to phenylephrine were similar to controls at all time points examined, although responses of PM's to phenylephrine were enhanced 12 weeks after STZ-treatment. Phenyelphrine EC_{50} values at this time point were reduced although not significantly, the values being 15.2 (4.0 - 58.0) μM and 5.4 (0.2 - 151.2) μM in tissues from control and diabetic rats respectively, while the maximum response to phenylephrine relative to the isoprenaline maximum was increased from 45.1 ± 6.0% to 81.3 ± 6.7% in diabetic tissues ($P < 0.01$). The increase in α-adrenoceptor responsiveness at 12 weeks was not associated with a change in [^{3}H]prazosin binding. Neither the density of binding sites nor the dissociation constants were different between ventricular tissues from control (118.3 ± 11.1 fmol mg^{-1}, 0.10 ± 0.04 nM) and diabetic animals (126.8 ± 13.2 fmol mg^{-1} protein; 0.11 ± 0.03 nM).

These results indicate that the effect of diabetes on cardiac adrenergic responsiveness is dependent on the duration of diabetes and the tissue examined.

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SMOOTH MUSCLE PROLIFERATION FROM RABBIT AORTA IS INHIBITED BY 8-Br-cAMP BUT NOT 8-Br-cGMP

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Intimal proliferation of arterial smooth muscle cells leads to the development of atherosclerotic fibrous plaques and there are few agents known to inhibit it. To replicate, smooth muscle cells must transform from the 'contractile' phenotype to a 'synthetic' phenotype, and most previous studies of growth regulation have used synthetic phenotype cells. We have quantified proliferation of contractile smooth muscle cells from explants of rabbit aortic media and report that proliferation is inhibited by 8-Br-cAMP. The endothelium of segments of rabbit thoracic aorta was removed by abrasion and strips of underlying smooth muscle were pulled away from the adventitia. Smooth muscle strips were sliced into precise 1mm^2 explants using a tissue chopper and plated individually into 96-well microtitre plates containing 0.1 ml of DMEM tissue culture medium supplemented with 20% foetal calf serum. Explants were cultured at 37°C for up to 10 days ([^{3}H] thymidine (1μCi/ml) was added for the last 18 h) and were then extracted with 0.1 ml of ice cold 10% trichloroacetic acid (w/v). Extracts from 8 explants were pooled and centrifuged at 1500xg for 5 min at 4°C. The ATP concentration in the supernatants was measured as an assay of biomass and cell viability. The DNA concentration and radioactivity of the pellet were measured as assays of proliferation. Freshly isolated rabbit aorta had an ATP concentration (nmol/μg DNA) of 0.29±0.03 (SE, n=21) whereas explants before culturing had an ATP concn of 0.11±0.006 (n=42). In a second experiment (Table), the ATP concentration in explants increased 3 fold after 3 days in culture and 11 fold after 10 days. This was associated with a pronounced increase in thymidine incorporation, although DNA concentration rose nonsignificantly. The increases in ATP concentration and thymidine incorporation were attenuated dose-dependently by 8-Br-cAMP (10^{-5} to 10^{-3}M, some data not shown) and DNA concentration was decreased. No effect was observed with 8-Br-cGMP (10^{-5} to 10^{-3}M).

Days	Control	8-Br-cAMP (10^{-4}M)	8-Br-cGMP (10^{-4}M)
	ATP concentration (nmoles / 8 explants)		
0	0.17±0.02	N. D.	N. D.
3	0.53±0.09	0.37±0.09	0.58±0.09
10	2.1±0.4	0.75±0.07 *	2.4±0.2
	Thymidine incorporation (1000 dpm / 8 explants)		
1	1.2±0.2	N. D.	N. D.
3	55±4	4.2±0.2 *	67±17
10	139±9	20±1 *	164±21
	DNA concentration (μg / 8 explants)		
0	5±1	N. D.	N. D.
3	5±1	5±1	6±1
10	7±1	2.4±0.4 *	7±1

N. D., not determined; * $p<0.02$ vs corresponding control value (n=3 aortas)

Preparation of explants decreased cell viability by 60 to 90%. Despite this, culturing led to a reproducible increase in ATP and thymidine incorporation, thereby allowing cell proliferation to be quantified. The rise in DNA concentration appeared, however, to be obscured by a fall in DNA concentration in necrotic cells. Cell proliferation was inhibited by 8-Br-cAMP (and by forskolin, unpublished observations), suggesting a similar role for endogenous cAMP-elevating agents including adenosine, adrenaline and prostacyclin.

ANTAGONIST PROFILE OF SK&F104078 AT PRE- AND POST-JUNCTIONAL α_2-ADRENOCEPTORS

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Initial reports suggested that SK&F104078 (6-chloro-9-[(3-methyl-2-butenyl)oxy] -3-methyl-1H-2,3,4,5,-tetra-hydro-3-benzazepine) was an antagonist at post-junctional, but not pre-junctional, α_2-adrenoceptors (Hieble et al., 1986). Subsequent studies cast doubt on this proposal (Connaughton et al., 1988; Bertie et al., 1989). We have re-evaluated its antagonist profile against two well-established α_2-adrenoceptor agonists, B-HT933 and xylazine, at pre- and post-junctional α_2-adrenoceptors and compared it with that of yohimbine.

Antagonist potency at pre-junctional α_2-adrenoceptors in rat vas deferens (prostatic portion) and guinea-pig ileum was determined as described by Drew (1977, 1978 respectively). Its potency at post-junctional α_2-adrenoceptors in rabbit ear vein was established as described by Daly et al. (1988). All tissues were suspended in modified Krebs-Henseleit solution at 37°C, bubbled with 95% O_2/5% CO_2. Cumulative concentration-response curves to agonists were constructed in the absence of, and after 45 min exposure to, antagonist.

Table 1 Potency of SK&F104078 at α_2-adrenoceptors in vitro

Tissue	Antagonist Potency against			
	B-HT933		Xylazine	
	pA_2	slope	pA_2	slope
Rat Vas Deferens	6.48 (6.22-6.84)	0.96 (0.69-1.24)	nc	-
Guinea-Pig Ileum	<5.50	-	<5.50	-
Rabbit Ear Vein	6.67 (6.56-6.77)	1.04 (0.94-1.15)	6.70 (6.51-6.89)	1.09 (0.99-1.19)

Results are mean values (with 95% confidence intervals; n > 4).
nc - not calculated

SK&F104078 ($1x10^{-6}$-$1x10^{-5}$M) competitively antagonised B-HT933 at pre-junctional α_2-adrenoceptors in rat vas deferens but not guinea-pig ileum (up to 3 x 10^{-6}M). It was almost ineffective against xylazine in either tissue, but antagonised both agonists at post-junctional α_2-adrenoceptors in rabbit ear vein (Table 1). Yohimbine ($3x10^{-8}$-$1x10^{-6}$M) antagonised xylazine and B-HT933 with approximately the same potency in all preparations (pA_2 range 7.46-8.00). In pithed rats, SK&F104078 (10mg/kg i.v.) antagonised xylazine, but not B-HT933 at pre-junctional α_2-adrenoceptors in the heart. Yohimbine (1mg/kg i.v.) was equipotent against both agonists.

In summary, the antagonist activity of SK&F104078 was not confined to post-junctional α_2-adrenoceptors, but its effects at pre-junctional α_2-adrenoceptors were agonist and tissue dependent.

Bertie, K. et al., (1988). Br. J. Pharmac., 94, 433P.
Connaughton, S. et al., (1988). Br. J. Pharmac., 95, 702P.
Daly, C.J., et al., (1988). Br. J. Pharmac., 94, 1085-1090.
Drew, G.M., (1977). Eur. J. Pharmac., 42, 123-130.
Drew, G.M., (1978). Br. J. Pharmac., 64, 293-300.
Hieble, J.P. et al., (1986). J. Hypertens., 4, (suppl 6), S189-S192.

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DIFFERENTIAL EFFECT OF COCAINE ON ELECTRICALLY EVOKED TRITIUM OVERFLOW FROM MOUSE VAS DEFERENS AND RAT BRAIN CORTEX SLICES

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Blockade of neuronal uptake increases evoked noradrenaline (NA) overflow in rat brain cortex slices (Gothert et al., 1983) but not in mouse vas deferens (Hagan and Hughes, 1981). To investigate this discrepancy isolated mouse vasa deferentia (Tuck No. 1, 30-35g) and rat brain cortex slices (Wistar, 150-200g) were incubated with ^{3}H-NA and washed as detailed elsewhere (Hagan and Hughes, 1981). Electrical stimulation (vas deferens, 2.5 Hz, 2 ms width, 400 mA for 90 s every 14 min; brain slices, 3 Hz, 2 ms width, 20 mA for 120 s every 20 min) enhanced tritium overflow from both tissues. Drugs were added after a control period of stimulation and drug effects are expressed as the ratio of the fractional evoked tritium overflow in the presence of a drug to that in its absence. Results are given as mean ± s.e. mean.

Idazoxan (0.01-1μM) produced a concentration dependent increase in evoked tritium overflow from vas deferens (control 0.94±0.03; idazoxan 1μM 2.08±0.15; n=6; $P<0.001$; Student's t-test) and brain slices (control 0.94±0.04; idazoxan 1μM 2.03±0.16; n=6; $P<0.001$). Cocaine (0.1-10μM) failed to increase evoked tritium overflow from vas deferens (cocaine 10μM 0.95±0.08; n=6; $P>0.05$ compared to control) while in brain slices a concentration dependent increase was obtained (cocaine 10μM 1.81±0.14; n=6; $P<0.001$ compared to control). Idazoxan (1μM) + cocaine (10μM) greatly increased evoked tritium overflow (vas deferens 3.03±0.24; brain slices 3.21±0.36; n=5; $P<0.05$ compared to idazoxan alone values).

Increasing the frequency of stimulation (0.1, 0.5, 1, 3 and 6 Hz) resulted in a progressive increase in the evoked tritium overflow. In the presence of cocaine (10μM) fractional evoked tritium overflow from brain slices was significantly ($P<0.05$) increased at every frequency except 6Hz while in vas deferens no significant changes were produced by cocaine at any frequency tested ($P>0.05$). Bathing the vas deferens with the physiological saline used for brain slices resulted in about a 50% reduction in evoked tritium overflow but cocaine (10μM) still had no effect. Cocaine 10μM had no effect on evoked tritium overflow from vas deferens superfused in the apparatus normally used for brain slices (control 0.90±0.008; treated 0.93±0.03; n=4; $P>0.05$).

Unmetabolized ^{3}H-NA represented 54±8% and 63±1% (n=4) of the tritium in the evoked overflow from vas deferens and brain slices respectively as determined by column chromatography. Cocaine (10μM) increased markedly the amount of ^{3}H-NA in the evoked overflow from brain slices (dpm; control 2505±228; treated 4518±579; n=5; $P<0.01$) but not in vas deferens (control 3656±177; treated 3714±175; n=5; $P>0.05$). The overflow of endogenous NA, as determined by HPLC, was also increased significantly ($P<0.05$) by cocaine in brain slices (control 0.15±0.02ng; treated 0.28±0.03ng; n=6; $P<0.05$) but not in vas deferens (control 0.34±0.03ng; treated 0.31±0.06; n=4; $P>0.05$).

In conclusion, the different effect of cocaine in the two tissues is not due to any impairment in the function of the presynaptic α_2-adrenoceptors or neuronal uptake processes and is independent of the frequency of electrical stimulation and the experimental apparatus. The effect of cocaine is not due to a difference in the amount of NA contributing to the total tritum overflow and is still apparent when the overflow of endogenous NA is measured.

Gothert, M., Schlicker, E. and Kostermann, F. (1983). Arch Pharmacol., 332, 121-128.
Hagan, R.M. and Hughes, I.E. (1981). J. Auton. Pharmacol., 1, 385-390.

RESPONSES TO CATECHOLAMINES IN RAT DISTAL COLON MAY BE MEDIATED, IN PART, BY 'ATYPICAL' β–ADRENOCEPTORS.

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Responses to catecholamines which are resistant to blockade of classical α- and β-adrenoceptors have been reported in a number of isolated animal tissues. Several reports in the literature suggest that these responses to catecholamines are mediated via β-adrenoceptors which are 'atypical' in nature, like those mediating lipolysis in rat adipocytes (Stanton, 1972; Harms *et al*, 1977). Tissues in which 'atypical' β-adrenoceptors have been reported to exist include the guinea-pig ileum (Bond *et al*, 1986; Bond and Clarke, 1988) and the guinea-pig gastric fundus (Coleman *et al*, 1987). Evidence presented here supports the hypothesis that relaxatory responses to noradrenaline and isoprenaline in the rat distal colon (RDC) may be mediated, in part, via 'atypical' β-adrenoceptors.

Portions of the distal 6cm of the rat colon were suspended in Krebs' PSS at 37°C containing cocaine (3 μM), ascorbic acid (30 μM) and EDTA (30 μM), and were contracted by the hypertonic addition of KCl (30-40 mM) to the organ baths. Responses to noradrenaline and isoprenaline were measured in the presence of propranolol (1 μM) and phentolamine (1 μM), respectively.

Noradrenaline relaxed KCl-induced tone in the RDC in a concentration-dependent manner, with a pIC_{50} (mean ± se) of 6.3 ± 0.18 (n=4). These relaxations to noradrenaline were not antagonised by phentolamine (0.01-1 μM). This action of noradrenaline could not, therefore, be attributed to an action at α-adrenoceptors. Neither cirazoline (0.01-30 μM) nor UK 14,304 (0.01-30 μM) relaxed KCl-induced tone in the RDC. Isoprenaline relaxed KCl-induced tone in the RDC in a concentration-dependent manner, with a pIC_{50} (mean ± se) of 8.6 ± 0.19 (n=5). These relaxations to isoprenaline were antagonised in a non-competitive manner by propranolol (0.01-1 μM), the slope of the Schild plot being significantly less than unity (0.56 ± 0.38, 95% C.L., n = 17). In the presence of propranolol (1 μM) and phentolamine (1 μM), the selective 'atypical' β-adrenoceptor agonist BRL 37344 (Arch *et al*, 1984) relaxed KCl-induced tone in the RDC in a concentration-dependent manner, with a pIC_{50} (mean ± se) of 6.97 ± 0.16 (n=6).

In the presence of phentolamine (1 μM) and propranolol (1 μM), BRL 37344 (1 μM) desensitised responses to noradrenaline and isoprenaline, causing mean rightward 18- and 19-fold shifts of the concentration-response curves (CRC's) to noradrenaline and isoprenaline respectively ($p < 0.01$, n = 4). However, BRL 37344 had no effect on the relaxatory response to papaverine in the RDC, suggesting that its effect was not simply a non-specific effect on all relaxatory responses in the RDC. In the presence of phentolamine (1 μM), tachyphylaxis of responses to BRL 37344 itself was also evident, with a second CRC shifted to the right approximately 14-fold.

The weak, non-competitive antagonism displayed by propranolol and phentolamine against relaxatory responses to catecholamines in the RDC, together with the clear tachyphylactic action of BRL 37344 on both its own response and on the responses to noradrenaline and isoprenaline under conditions of α- and β-adrenoceptor blockade, support the hypothesis that responses to noradrenaline and isoprenaline in the RDC may be mediated, in part, by 'atypical' β-adrenoceptors similar to those present in rat adipocytes and other tissues.

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Arch, J.R.S., Ainsworth, A.T., Cawthorne, M.A., Piercy, V., Sennitt, M.V., Thody, V.E., Wilson, C. & Wilson, S. (1984) Nature 309, 163-165.
Bond, R.A., Charlton, K.G. & Clarke, D.E. (1986) J. Pharmacol. Exp. Ther. 236, 408-415.
Bond, R.A. & Clarke, D.E. (1988) Br. J. Pharmac. 95, 723-734.
Coleman, R.A., Denyer, L.H. & Sheldrick, K.E. (1987) Br. J. Pharmac. 90, Proc. Suppl., 40P.
Harms, H.H., Zaagsma, J. & de Vente, J. (1977) Life Sci. 21, 123-128.
Stanton, H.C. (1972) Arch. Int. Pharmacodyn. 196, 246-258.

COMPARATIVE INHIBITORY EFFECTS OF FPL 63547 AND ENALAPRIL ON RAT TISSUE CONVERTING ENZYME

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FPL 63547 is a novel inhibitor of angiotensin converting enzyme (ACE) which has antihypertensive effects of long duration in spontaneously hypertensive rat (Carr et al., 1988). It is currently thought that local renin angiotensin systems exist in many tissues (Dzau, 1987) and there is evidence that inhibition of tissue ACE and hence local production of angiotensin II may contribute to the cardiovascular actions of ACE inhibitors (Unger et al., 1984). We have compared the inhibitory effect of FPL 63547 and enalapril on tissue ACE in various sites in the rat after oral dosing.

In preliminary studies FPL 63547 (15 and 50 µg/kg p.o.) and enalapril (0.5 and 1.5 mg/kg p.o.) were administered to conscious normotensive rats (male Sprague-Dawley, 250-350g). At each of the fixed time points after dosing (1, 3, 5, 15, 24 and 48h), rats (n = 3) were killed, dissected rapidly, and the relevant tissues examined for the extent of inhibition of ACE. In the heart, kidney, lung, skeletal muscle and plasma FPL 63547 was more potent and of longer duration of effect compared with enalapril. Both compounds lacked inhibitory activity in the brain.

Further studies were performed to compare a range of doses of FPL 63547 (3-50 µg/kg p.o.) and enalapril (5-2000 µg/kg p.o.) at the time of maximum effect (3h). Drug effects were compared with vehicle treated controls in groups of 5 rats. Dose versus inhibition curves were constructed for FPL 63547 and enalapril for the heart, kidney and lung. Regression analysis showed no significant deviation from parallelism for the kidney and lung. The potency ratios (FPL 63547: enalapril) were 3.1:1 (95% confidence intervals, (c.i.)=2.1-4.5) and 6.6:1 (95% c.i.=5.3-8.4) respectively. However, for the heart in three independent experiments, the dose response curves were non-parallel; enalapril having the shallower slope. The difference between FPL 63547 and enalapril was greater for the heart than either of the other organs as exemplified by the ratio of ID_{50} values of 20.3:1 (95% c.i.= 10.7-38.3).

Relative to enalapril, FPL 63547 may show selectivity for the heart compartment of ACE. The possible pathophysiological role of the cardiac renin angiotensin system and implications of local inhibition of ACE have been reported (Dzau, 1987). Further studies will be necessary to determine whether these differences in activity between FPL 63547 and enalapril have functional significance.

Carr, R.D. et al., (1988) Br. J. Pharmacol. 95 487P.
Dzau, V.J. (1987) Amer. J. Cardiol. 59 59A-65A.
Unger, T. et al. (1984) J. Cardiovasc. Pharmacol. 6 872-880

THE EFFECTS OF PINACIDIL AND FELODIPINE ON RAT PULMONARY ARTERY ARE ALTERED IN MONOCROTALINE-INDUCED PULMONARY HYPERTENSION

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The responsiveness of isolated pulmonary blood vessels to spasmogens is known to be altered in rats with monocrotaline (MCT)-induced pulmonary hypertension (Altiere *et al.*, 1986; Wanstall & O'Donnell, 1989). In this study the calcium entry blocking drug, felodipine (FEL), and the potassium channel opening drug, pinacidil (PIN), have been compared as vasorelaxants on isolated preparations of pulmonary artery from rats with MCT-induced pulmonary hypertension.

MCT-treated rats (105 mg/kg s.c. 4 weeks before the experiment) had pulmonary artery pressures of 30±2.7 mm Hg, n=20, compared with saline-treated rats (17±0.8 mm Hg, n=27), and had right ventricular hypertrophy. Single ring preparations (3mm long) of main pulmonary artery (endothelium not removed) were set up in physiological salt solution (37°C, 95%O_2/5%CO_2) at resting forces (MCT, 20 mN; saline, 10 mN) corresponding to the respective pulmonary artery pressures. Cumulative concentration-response curves were obtained to noradrenaline (NA), 5-hydroxytryptamine (5-HT) or endothelin (ET) in the absence and presence of 10 nM FEL or 3 μM PIN (30 min contact).

MCT-induced pulmonary hypertension caused significant ($P<0.05$, Student's *t* test) increases in the potency (neg log EC50) of NA (saline 7.98±0.07, n=9; MCT 8.48±0.08, n=8) and 5-HT (saline 5.19±0.05, n=8; MCT 6.53±0.11, n=7), and significant decreases in the maximum responses (mN/mm²) to NA (saline 15.1±1.5, n=9; MCT 10.7±1.04, n=8) and ET (saline 29.1±1.8, n=5; MCT 10.6±1.7, n=4). In both groups of rats FEL and PIN each caused non parallel shifts in the concentration-response curves to NA and 5-HT with depressions in maximum response. PIN was more effective than FEL. The depression in NA maximum by FEL was more pronounced in MCT-treated rats (68% reduction) than in saline-treated rats (28% reduction), and both FEL and PIN caused a decrease in NA potency in MCT-treated rats (FEL 0.60±0.08 log units, n=3; PIN 0.68±0.06 log units n=4) which was not seen (FEL 0.04±0.03 log units, n=3) or was significantly ($P<0.05$) less (PIN 0.37±0.06 log units, n=4) in saline-treated rats. Similarly, when 5-HT was the spasmogen, the effects of FEL and PIN were enhanced in pulmonary hypertension. FEL and PIN had no effect on the concentration-response curves to ET except for a small (37%) depression in ET maximum by PIN in MCT-treated rats.

In conclusion, in contrast to the vasodilators acetylcholine and isoprenaline, which are reported to be less effective in MCT-induced pulmonary hypertension (Altiere *et al.*, 1986), FEL and PIN showed increased vasorelaxant activity, at least against contractions induced by NA or 5-HT. The potency of NA and 5-HT as spasmogens was also increased in MCT-induced pulmonary hypertension. If these two effects of MCT-treatment are related, it could indicate an increase in the involvement of voltage-dependent calcium channels in the spasmogenic effects of NA and 5-HT in MCT-induced pulmonary hypertension.

Altiere, R.J., Olson, J.W. & Gillespie, M.N. (1986) *J. Pharmac exp.Ther.*, 236, 390-395.
Wanstall, J.C. & O'Donnell, S.R. (1989) *Clin. exp. Pharmac. Physiol.* (in press)

EFFECTS OF K^+-CHANNEL OPENERS ON BOVINE TRACHEAL SMOOTH MUSCLE

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The novel class of compounds which initiate relaxation of smooth muscle by opening K^+-channels in the cell membrane have therapeutic potential as bronchodilators (Arch et al 1988). One means of studying the properties of these compounds is by measuring their effect on ^{86}Rb efflux (as a marker for changes in K^+-permeability). While such an approach has been successful for vascular smooth muscle, where large changes in the rate of Rb efflux occur, the effect of cromakalim (CRK) on Rb efflux from guinea-pig tracheal muscle is relatively small (Allen et al 1986, Foster 1989). We have examined the actions of CRK, pinacidil (PIN) and diazoxide (DZ) on Rb efflux and mechanical activity in bovine trachea - a richer source of muscle than guinea-pig trachea.

Small strips of tracheal muscle (approx 15mg wet weight) were prepared. For tissue bath studies these were suspended under an initial tension of 1g in Krebs at 37°C. The tissues were contracted with KCl (25 or 80mM) and, once tension had stabilised, compounds were added cumulatively at 10-fold concentration increments. For efflux studies, the muscle strips were attached to a gassing manifold and incubated for 60min in Krebs at 37°C before transfer to Krebs containing ^{86}Rb (1μC_i/ml) for a further 120min. Tissues were then transferred at 4 min intervals through a series of tubes containing Krebs, with compound under test added to three tubes in the middle of the run. The rate of Rb efflux was calculated as a fractional rate constant (KRb) for each sample.

CRK, PIN and DZ caused relaxation of bovine trachea contracted by 25mM KCl with IC_{50} values of 0.4μM, 1.5μM and 45μM respectively (n=5). In contrast, none of the compounds at concentrations up to 100μM (300μM for DZ) relaxed tissues which had been contracted by 80mM KCl. Following 45min incubation with the K^+-channel blocker glibenclamide (GBC, 1μM) the IC_{50}'s for CRK, PIN and DZ were 12μM, 18μM and 270μM respectively (n=5). CRK (1-30μM) and PIN (3-30μM) markedly increased the rate of Rb efflux. Thus, for example, CRK (10μM, n=8) and PIN (30μM, n=5) produced 2.8 and 2.5 fold increases in KRb respectively. Stimulation of Rb efflux by CRK and PIN was blocked by GBC (0.3-1μM). DZ (1mM), isoprenaline (1μM) and aminophylline (1mM) each had no effect on Rb efflux.

K^+-channel openers stimulate Rb efflux from bovine trachea to a much greater extent than from guinea-pig trachea, despite similar potency as muscle relaxants in the two tissues. Bovine tracheal smooth muscle therefore offers considerable advantages over guinea-pig tissue for studies of Rb efflux. DZ is a considerably weaker relaxant of this tissue than either CRK or PIN although the effects of all three compounds were inhibited by GBC. The failure of DZ to stimulate Rb efflux suggests that a part of its relaxant activity may be due to actions unconnected with the opening of Rb-permeable K^+ channels. We conclude that CRK and PIN relax bovine trachea by opening a Rb-permeable, glibenclamide-sensitive K^+-channel.

Allen SL, Boyle JP, Cortijo J, Foster GP, Small RC. (1986) Brit.J.Pharmac. 89 395-405
Arch JRS, Buckle DR, Bumstead J, Clarke GD, Taylor JF, Taylor SG. (1988) Brit.J.Pharmac. 95 763-770
Foster KA. (1989) Brit.J.Pharmac. 96 233P

COMPARISON OF THE EFFECTS OF GASTRIN/CHOLECYSTOKININ-RELATED PEPTIDES IN RAT GASTRIC MUCOSA AND GUINEA-PIG ILEUM

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Gastrin (G-17) and cholecystokinin (CCK-8) have diverse actions in the gastrointestinal tract including stimulation of gastric acid secretion and contraction of smooth muscle. Binding studies using gastrin/CCK related peptides have suggested that these peptides mediate their effects via three receptor types: CCK_A, CCK_B (Innis and Snyder, 1980) and gastrin receptors (Praissman et al., 1983). Comparable studies of gastrin/CCK receptors have not been made in functional studies: In the present study the rank order of agonist potencies of gastrin/CCK related peptides have been investigated in rat isolated gastric mucosa (RGM) and guinea-pig isolated ileum longitudinal muscle myenteric plexus (GPI-LMMP) to provide such a functional comparison.

RGM was prepared for measurement of acid secretion as described previously (Reeves and Stables, 1985). Strips of GPI-LMMP were suspended in organ baths (resting tension, 0.8g) and contractions recorded isometrically. All tissues were bathed in Krebs-Henseleit solution, containing phosphoramidon (1μM) and bestatin (100μM), gassed with 95% O_2:5% CO_2 and maintained at 37°C. Concentration-response curves were constructed for agonists (cumulatively for RGM and sequentially for GPI-LMMP) and could be repeated after washing.

Table 1 shows rank order of agonist potencies for the peptides in both preparations. In RGM, the gastrins (G-17, non-sulphated G-17) and pentagastrin (PTG) were potent full agonists. CCK-8 was a partial agonist, producing only 29% of the PTG maximum response at a concentration of 10nM. In addition, CCK-8 (100nM) inhibited subsequent responses to PTG (100nM) by 71±13% (n=4). The activities of the peptides in GPI-LMMP differed from those observed in RGM. In GPI-LMMP, CCK-8 was greater than 1000 times more potent than the other peptides (Table 1). Non-sulphated G-17 was virtually inactive and at a concentration of 1μM produced a small inhibition of CCK-8 responses (concentration ratio = 2.6, n=3).

Table 1 Rank orders of agonist potency of gastrin/CCK related peptides in RGM and GPI-LMMP

RGM	G-17	(=CCK-8)	nsG-17	PTG	nsCCK-8
	0.21	p.a.	0.61	1	11
GPI-LMMP	CCK-8	G-17	nsCCK-8	PTG	nsG-17
	1	1220	2000	78000	10000

Values are equipotent molar ratios (geometric mean, n=4 to 6) relative to pentagastrin in RGM (EC_{50}=26[15-45]nM) and CCK-8 in GPI-LMMP (EC_{50}=0.7 [0.3-1.4]nM). pa = partial agonist, ns = non-sulphated.

Comparison of agonist rank orders of potencies in these isolated preparations is consistent with the view that the effects of gastrin/CCK peptides in RGM to stimulate acid secretion are mediated by gastrin or CCK_B receptors whilst their contractile effects in GPI-LMMP are mediated by CCK_A receptors. These functional preparations may be useful systems with which to determine the activities of gastrin/CCK agonists and antagonists.

Innis, R.B. and Snyder, S.H. (1980), Proc. Natl. Acad. Sci., 77, 6917-6921.
Praissman, M. et al. (1984), J. Receptor Res., 3, 647-665.
Reeves, J.J. and Stables, R. (1985), Br. J. Pharmac., 86, 677-684.

INTERACTION OF PARAFLUOROHEXAHYDROSILADIFENIDOL (PFHHSID) AT MUSCARINIC RECEPTORS *IN VITRO*

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Muscarinic receptors exist in multiple subtypes, 5 of which have been identified from genomic cloning studies (m1, m2, m3, m4 and m5; see Bonner et al. 1988 for references) and 3 of which have been postulated from radioligand binding and functional studies (M_1, M_2 and M_3; Doods et al. 1987). Pirenzepine and methoctramine are M_1 and M_2 selective antagonists respectively (see Eglen et al. 1988 for references), whilst pFHHSiD has been postulated (Lambrecht et al. 1988) to be the first selective M_3 antagonist.

We have characterised pFHHSiD in guinea-pig ileum and left, paced atria (Eglen et al. 1988). Endothelial dependent relaxations were elicited in rings of rat aorta, rabbit jugular vein and dog femoral artery when precontracted with 0.1 μM phenylephrine (Eglen et al., 1988). Rings of dog femoral vein, in which contractile responses only are observed to muscarinic agonists, were also prepared (O'Rourke and Vanhoutte, 1987). Carbachol was used as the agonist and pA_2 values (Arunlakshana and Schild, 1959) were calculated using 60 min equilibration at each of the 4 antagonist concentrations employed.

pFHHSiD dextrally shifted the concentration-response curves in a parallel fashion in all preparations with no significant depression in the maxima. The mean (± s.e. mean) pA_2 values for pFHHSiD are shown in table 1.

Table 1 pA_2 VALUES AND SCHILD SLOPES IN VARIOUS IN VITRO PREPARATIONS

Preparation	Subtype	pA_2	Schild Slope	n
Femoral Vein	M_1	7.10 ± 0.09	1.1	10
Left Atria	M_2	6.14 ± 0.08	1.2	6
Ileum	M_3	7.86 ± 0.04	1.0	6
Aorta	M_3	7.68 ± 0.07	1.0	8
Jugular Vein	M_3	7.74 ± 0.11	1.0	8
Femoral Artery	M_3	7.90 ± 0.10	1.1	7

The pA_2 values were similar in ileal and vascular preparations indicating the presence of M_3 receptors. The pA_2 value in the left atria was in accordance with the presence of an M_2 receptor, whilst the intermediate value in the dog femoral vein was indicative of an M_1 receptor. pA_2 values in this latter tissue for pirenzepine, 7.85 ± 0.10 (1.0) and methoctramine, 6.1 ± 0.05 (1.2), were also indicative of M_1 stimulation. pFHHSiD may represent a useful probe in the functional and molecular biological classifications of muscarinic receptor subtypes.

Arunlakshana, O and Schild, H.O. (1959) Br. J. Pharmac. 14, 48-58.
Bonner, T.I. et al (1988) Neuron 1, 403-410.
Doods, H.N. et al (1987) J. Pharm. exp. Ther. 242, 257-262.
Eglen, R.M. et al (1988) Br. J. Pharmac. 95 1031-1038.
Lambrecht, G. et al (1988) Eur. J. Pharmac. 152, 193-194.
O'Rourke, S.T. and Vanhoutte, P.M. (1987) J. Pharm. exp. Ther. 241, 64-67.

ANTAGONISM BY METOCLOPRAMIDE AND BRL 43694 OF 5-HT-INDUCED RESPONSES IN RABBIT ISOLATED VAGUS NERVE

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There is uncertainty as to the mechanism of action of 5-HT_3 receptor antagonists on vagus nerve (see Ireland & Tyers, 1987; Richardson et al.,1985). One possible reason is that two different 5-HT-induced responses have been measured, i.e. 5-HT-induced depolarization and inhibition of C-spike amplitude, respectively. In these experiments we have compared the action of metoclopramide, reported to be a competitive antagonist at 5-HT_3 receptors (Ireland & Tyers, 1987), and BRL 43694, a potent 5-HT_3 receptor antagonist (Fake et al., 1987) on both of these 5-HT-induced responses recorded simultaneously. Extracellular recordings were made from rabbit vagus nerve using the method described by Marsh et al. (1987). The action of the antagonists (metoclopramide 0.3-10 μM, pre-incubation one hour; BRL 43694 0.3-10 nM, pre-incubation three hours) was assessed from cumulative concentration-response curves (CCRC) to 5-HT (0.1-300 μM), using the method of Schild (Arunlakshana & Schild, 1959). The results are shown in the table.

5-HT action.	Depolarization	Vagal C-spike inhibition
METOCLOPRAMIDE.		
Slope.	0.70 (0.9-0.69)	0.74 (0.93-0.54)
pA_2.	7.41 (7.57-7.25)	7.63 (7.97-7.28)
pA_2*.	7.04 ± 0.04	7.13 ± 0.06
	(20 dose-ratio determinations from 5 preparations).	
BRL 43694.		
Slope.	0.91 (1.24-0.58)	0.74 (1.08-0.39)
pA_2.	10.16 (10.58-9.74)	10.86 (11.49-10.24)
pA_2*.	10.03 ± 0.09	10.31 ± 0.1
	(20 dose-ratio determinations from 16 preparations).	

Slopes and apparent pA_2 values from Schild plots, mean values (95% confidence levels). pA_2* from Schild equation, means ± s.e.mean.

Although the antagonists appeared to be more potent on 5-HT-induced C-spike inhibition than on depolarization, differences were not significant. Metoclopramide did not seem to act competitively in that the slope of the Schild plot was significantly less than unity and there was a marked increase in the maximal response of the 5-HT CCRC. These features remain unexplained, but the shallow Schild plot was not due to an uptake mechanism, as citalopram (0.1-10 μM, n=4) had no effect on the 5-HT CCRC. BRL 43694, on both 5-HT-induced responses, caused parallel rightward shifts with no change in maximal response, except at 10 nM which reduced the maximal 5-HT-induced depolarization by about 40%. The slope of the Schild plots were not significantly different from unity. Thus, BRL 43694 at concentrations below 10 nM appears to behave competitively.

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Arunlakshana,O. & Schild,H.O. (1959) Br.J.Pharmac.14:48-58.
Fake,C.S. et al. (1987) Br.J.Pharmac.91:335P.
Ireland,S.J. & Tyers,M.B. (1987) Br.J.Pharmac.90:229-238.
Marsh,S.J. et al. (1987) Neuroscience 23:275-289.
Richardson,B.P. et al. (1985) Nature 316:126-131.

A RECEPTOR FOR 5-HT IN HUMAN ATRIUM

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Little is known about the nature of the myocardial receptors that mediate the positive inotropic effects of 5-hydroxytryptamine (5-HT). 5-HT and (+)lysergic acid diethylamide (LSD) increase force of mollusc hearts (Greenberg,1960), presumably through an increase in the concentration of cyclic AMP (Sawada et al., 1984). In cat heart, methysergide blocks the positive inotropic effects of 5-HT on atria (K_B=20 nM) but not on papillary muscles, indicating that atrial and ventricular 5-HT receptors are distinct (Kaumann,1985). Does 5-HT augment contractile force of human heart and if so, through what receptors ? To answer these questions, we carried out experiments on right atrial appendages obtained from patients with coronary artery disease undergoing bypass surgery.

Atrial strips were paced at 0.5 Hz in Krebs' solution (containing 0.2 mM ascorbate) as described (Gille et al.,1985). Usually, 4 strips were prepared from each atrial appendage. Two strips were used for force measurements, one as control and the other incubated with a blocking agent for a 5-HT receptor type. A cumulative concentration-effect curve for 5-HT was determined for each and the experiment terminated with 200 µM (-)isoprenaline (ISO). The other 2 strips were incubated with 0.4 µM (±)propranolol. One strip served as control. The other was exposed to a single concentration of 5-HT for 5 min. which caused an equilibrium inotropic response. All four strips were then freeze-clamped and extracts assayed for cyclic AMP (radioimmunoassay) and for cyclic AMP-dependent protein kinase (cA-PrK) activity ratio (Murray et al.1988).

5-HT increased contractile force in a concentration-dependent manner. The effects of 5-HT were not blocked by ketanserin (0.4 µM), methysergide (1 µM), LSD (0.4 µM), methiothepin (0.4 µM), yohimbine (4 µM), (±)propranolol (0.4 µM), (-)pindolol (0.4 µM) or MDL 72222 (2 µM), excluding an involvement of 5-HT receptors of type 1,2 or 3 (Bradley et al.,1986). However, ICS 205930 (2 µM) surmountably antagonised the positive inotropic effect of 5-HT, increasing the log (EC_{50}) for 5-HT from -6.94±0.09 to -5.82±0.16 (mean ± s.e.m., n=5 ß-blocker-treated patients) without altering the maximal response to 5-HT (57±5% and 51±6% of ISO response in the absence and presence of ICS 205930 respectively). 5-HT (1-30 µM) increased cyclic AMP from 11.0±1.5 to 19.7±2.7 pmol.(mg protein)$^{-1}$ ($p<0.05$) and the cA-PrK activity ratio from 0.27±0.02 to 0.35±0.03 ($p<0.01$). These are 21% and 40% of the corresponding effects of (-)isoprenaline.

Conclusions: The observed blocking potency of ICS 205930 (K_B = 0.2 µM) is lower than would be expected from its affinity for $5\text{-}HT_3$ receptors (Bradley et al.,1986) but similar to that observed by Dumuis et al.(1988) against 5-HT-induced stimulation of adenylate cyclase in brain (K_B = 0.5 µM). The 5-HT receptors of human atrium may therefore resemble the cerebral 5-HT receptors, designated $5\text{-}HT_4$ by Dumuis et al. Our results are consistent with the pathway:
5-HT → $5\text{-}HT_4$ → ↑ cyclic AMP → ↑cA-PrK activity → ↑ force.

We thank the surgical staff of Papworth Hospital for supply of human atrial tissues.

Bradley, P.B. et al. (1986). Neuropharmacology 25, 563-576.
Dumuis, A. et al. (1988). Mol. Pharmacol. 34, 880-887.
Gille, E. et al. (1985). Naunyn-Schmiedeberg's Arch. Pharmacol 331, 60-70.
Greenberg, M.J. (1960). Br. J. Pharmac. 15, 375-388.
Kaumann, A.J. (1985). J.Cardiovasc. Pharmacol. 7(Suppl. 7), S76-S78.
Murray, K.J. et al. (1988). Biochem. Soc. Trans. 16, 355.
Sawada, P.R. et al. (1984). J. Neurophysiol. 51, 361-374.

CONTRIBUTION OF AMINE RE-UPTAKE TO OBSERVED DIFFERENCES IN HISTAMINE AND 5-HT SECRETION IN RAT MAST CELLS

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We have shown (Cohen *et al*, 1987; Purcell *et al*, 1988a) that compound 48/80 induces a greater percentage release of histamine than 5-HT, from rat mast cells isolated from different anatomical locations, despite differences in the ratio of histamine:5-HT within these cells. This suggests differential secretory mechanisms for the two amines, although this does not accord with convincing evidence that they share similar granular storage sites (Uvnas, 1972). Mast cells can take up exogenous histamine and 5-HT (Purcell *et al*, 1988b), and we have addressed the question of whether post-secretory uptake of amines may explain observed differences in their evoked secretion.

Mixed peritoneal cells were obtained by lavage from female Wistar rats, and the mast cells purified (>90%) over bovine serum albumin (38%). Purified cells were incubated (37ºC, 15 min) with varying concentrations of clomipramine (10^{-8}-10^{-5} M, 5 min preincubation), or fluoxetine (10^{-6}-10^{-4} M, 5 min preincubation) in HEPES buffered Locke's solution (pH 7), and either compound 48/80 (1.0 μg/ml) in secretion experiments, a mixture of histamine (1.0 μg/ml) and 5-HT (0.2 μg/ml) in uptake experiments, or buffer alone. The supernatant was assayed fluorimetrically for the presence of histamine and 5-HT (Cohen *et al*, 1987). Results are corrected for the respective spontaneous amine release, with or without the drugs.

Similar gravimetric amounts of amine were removed from the supernatant, despite the fact that histamine was in a five-fold excess of 5-HT: thus 66 ± 14 ng of histamine (6 nmol) and 58 ± 7 ng of 5-HT (3 nmol) was subject to uptake by the cells (n=6). Clomipramine (10^{-7}-10^{-5} M) and fluoxetine (10^{-6}-10^{-4} M), significantly inhibited 5-HT uptake and were without effect upon histamine uptake. Compound 48/80 (1.0 μg/ml) induced a greater percentage release of histamine (63%, n=6) than 5-HT (33%, n=6). Both clomipramine and fluoxetine increased the observed percentage release of 5-HT, at concentrations which inhibited uptake of this amine: histamine secretion was unaffected by incubation with either drug. To determine whether this phenomenon was dependent upon the extent of amine release, purified mast cells were preincubated with clomipramine (10^{-5} M) or fluoxetine (10^{-4} M), concentrations which produced marked reduction in 5-HT uptake, prior to the addition of compound 48/80 (0.1-30.0 μg/ml). In the presence of these drugs the concentration effect curves of histamine and 5-HT were collinear, such that there was no difference between the percentage output of 5-HT and that of histamine.

These results suggest measured differences in amine release reflect selective 5-HT reuptake rather than differential amine output, and imply a single secretory mechanism for histamine and 5-HT.

Cohen, DL *et al* (1987) Br. J. Pharmacol. **90**, 101P
Purcell, WM *et al* (1988a) Br. J. Pharmacol. **95**, 673P
Purcell, WM *et al* (1988b) Br. J. Pharmacol. **94**, 370P
Uvnas, B (1972) Proc. Int. Congr. Pharm. **4**, 306-326

THE STIMULATORY EFFECT OF 5-HT ON HUMAN OSTEOBLASTS IS ANTAGONISED BY KETANSERIN TARTRATE AND IMIPRAMINE

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Osteoblasts are the principal cells involved in the formation of new bone, and play a crucial role in the reparative process following a bone fracture. Serotonin (5-HT) is contained in platelet granules and is released in large amounts by blood platelets at sites of injury. It has also been shown to increase cell proliferation in fibroblast and endothelial cells and may act as a growth factor (D'Amore & Shepro, 1977). We therefore carried out a study to assess the effect of 5-HT and anti-5-HT drugs on two markers of osteoblast function: alkaline phosphatase (ALP) production and [^{3}H]thymidine incorporation.

Isolation and culture of bone cells from trabecular fragments of human bone was carried out as described by Beresford *et al.* (1984), and standard cell culture techniques were used as previously described by us (Khokher & Dandona, 1988). The effect of drugs was assessed by incubating cells, $5x10^4$ per well, with and without test substances, for 24 h. ALP activity was measured in supernatants using an Encore™ Centrifugichem® and a kit from Baker Instruments Ltd (Egham, Surrey, UK). [^{3}H]thymidine incorporation was assessed by detaching the cells with trypsin centrifuging and liquid scintillation counting at the end of the incubation.

5-HT stimulated ALP production and [^{3}H]thymidine incorporation in human osteoblasts in a dose-dependent manner (ALP $EC_{50} \simeq 0.75$ μM). This effect could not be demonstrated with tryptophan, 5-OH-tryptophan or 5-hydroxyindole acetic acid at similar concentrations. The 5-HT-induced stimulatory effect ([5-HT] = 1 μM) on ALP production and [^{3}H]thymidine incorporation were blocked in a dose-dependent manner by ketanserin tartrate and imipramine with similar IC_{50}s (ALP $IC_{50} \simeq 1$ μM, [^{3}H]thymidine incorporation $IC_{50} \simeq 0.1$ μM). Both drugs were without effect on their own. Pargyline and the 5-HT antagonist BRL-43694 were also unable to block the 5-HT-induced stimulatory effects consistently and were without effect on their own.

These results suggest that 5-HT stimulates secretion and mitosis by osteoblasts and that these effects may be mediated by 5-HT receptors; and/or that the effect of 5-HT on osteoblast function involve captation of this amine. Further studies with more selective 5-HT antagonists and other 5-HT uptake inhibitors are in progress.

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D'Amore, F. & Shepro, D. (1977) J. Cell. Physiol. 92, 177-184
Beresford, J.N., Gallagher, J.A., Poser, J.W. & Russell, R.G.G. (1984) Metab. Bone Dis. Rel. Res. 5, 229-234.
Khokher, M.A. & Dandona, P. (1988) Br. J. Rheum. 27, 291-294.

HETEROGENEITY OF THE REGULATION OF PHOSPHOLIPASE C IN T LYMPHOCYTES

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Protein kinase C (PK-C) activation by phorbol esters potentiates, whilst PK-C inhibition by staurosporine inhibits UCHT1 (anti-CD3 antibody; O'Flynn et al, 1985) - induced elevation of cytosolic free calcium levels ($[Ca^{2+}]_i$) in human peripheral blood derived T lymphoblasts (Ward et al, 1988). However, in the human leukaemic T cell line Jurkat, activation of PK-C has been shown to inhibit CD3-induced elevation of $[Ca^{2+}]_i$ (Cantrell et al 1989). In the present study, we have examined the consequences of PK-C activation on CD3-mediated phosphatidylinositol (PI) metabolism in both human T lymphoblasts and the Jurkat cell line to see whether the differences between the cells in PK-C regulation of receptor-operated $[Ca^{2+}]_i$ levels are reflected in the regulation of phospholipase C-mediated PI metabolism.

Inositol labelling of the cells (10^8 cells/ml) and inositol (poly)phosphate(s) extraction and determination was essentially as described previously (Cockcroft et al, 1987). The cells were washed and resuspended in Hanks balanced salt solution (whole cells) or permeabilisation buffer (for composition see Stutchfield and Cockcroft, 1988) prior to use. Four minute pre-treatment with the PK-C activator phorbol dibutyrate (PDBU 100 nM) caused a marked inhibition ($p<0.05$) of UCHT1 (0.25-10µg/ml)-induced elevation of total [^{3}H]-inositol phosphates in Jurkat. The inhibition by 100nM PDBU of 4 and 10µg/ml UCHT1 responses was 40±11% and 64±8% inhibition (n-8) respectively. The effect of PDBU was dose-dependent (1-100nM) but could not be mimicked by the inactive phorbol ester 4 α phorbol (100nM,n=4). In marked contrast, PK-C stimulation had no inhibitory effect on UCHT1 (0.25-10µg/ml) induced elevation of total [^{3}H] inositol phosphates in human T lymphoblasts. In Streptolysin-O permeabilised Jurkat, but not T lymphoblasts, significant ($p<0.05$) total [^{3}H] inositol phosphate generation (5-7 fold enhancement above basal levels) was induced by physiological levels of Ca^{2+} (50-150nM). Calcium induced (50, 100, 150nM) phospholipase C activity in Jurkat was inhibited by 4 minute pre-treatment with 100nM PDBU (38±9, 39±2, 49±10% inhibition (n-4) respectively), whereas 4α phorbol (100nM) had no effect.

The different calcium sensitivity of PI metabolism and the different effect of PK-C stimulation on calcium and receptor-operated PI metabolism in Jurkat and T lymphoblasts indicates heterogeneity of phospholipase C regulation in T lymphocytes. Multiple isozymes of phospholipase C have been decribed (Pann-Ghill et al, 1988) and it remains to be determined whether the differential regulation of phospholipase C reflects different phospholipase C isozyme coupling to CD3 in Jurkat and T lymphoblasts.

Cantrell DA et al (1989) J. Immunol. (In Press)
Cockcroft S et al (1987) Immunology 60:209-212
O'Flynn K et al (1984) Biochem.J. 219:661-666
Pann-Ghill S et al (1988) Cell 54: 161-169
Stutchfield J & Cockcroft S (1988) Biochem.J. 250:375-382
Ward SG et al (1988) FEBS Letts. 239:363-366

DISSOCIATION OF 5-LIPOXYGENASE INHIBITION AND METHAEMOGLOBINAEMIA INDUCTION BY INDAZOLINONES

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We have previously reported selective 5-lipoxygenase inhibition by ICI207968 (2-(3-pyridylmethyl)-indazolinone) and related compounds (McMillan et al, 1989). In common with other lipoxygenase inhibitors, 2-substituted indazolinones have the potential to participate in redox reactions and this property may contribute to their mechanism of action. Other redox inhibitors have been reported to induce methaemoglobinaemia (MHb)(Fort et al,1984). We report here on induction of MHb with ICI207968 and on structural modifications which dissociate MHb from lipoxygenase inhibition.

Inhibition of 5-lipoxygenase in vitro and ex vivo were investigated in anti-coagulated blood stimulated with calcium ionophore A23187 (McMillan et al, 1986). MHb in whole blood was monitored spectrophotometrically and was expressed as a percentage of total haemoglobin.

Oral administration of ICI207968 to rats produced inhibition of ex vivo LTB_4 release by whole blood with ED_{50} values of 2.5, 10 and 25 mg/Kg p.o.respectively at 1, 3 and 5 hours after dosing. MHb was not detected following oral dosing in the rat even in a sub acute study with twice daily dosing at 100mg/Kg p.o.for 10 days. Oral administration of ICI207968 (5mg/Kg) to dogs produced significant inhibition of ex vivo LTB_4 release: mean inhibitory values of 95, 90 and 75% were obtained at 1, 3 and 6 hours respectively ($P<0.01$ in each case). At this dose, ICI207968 induced mean MHb of 25 and 20% at 1 and 3 hours respectively but MHb was not detected at 6 hours. At 1mg/Kg p.o., ICI207968 produced 80% inhibition of ex vivo LTB_4 release ($P<0.01$) 1 hour after dosing and this was not accompanied by induction of MHb. At this dose ex vivo LTB_4 release had returned to control values within 3 hours. In vitro studies demonstrated that ICI207968 had comparable inhibitory potency against LTB_4 release by dog, human and rat blood (IC_{50} values 0.5-1.5uM) but there were marked species differences in the sensitivity to MHb induction with EC_{25} values in dog, human and rat blood of 7.5, 80 and 250uM respectively. The 4-(N-n-pentyl carboxamido) analogue of ICI207968 had similar potency against LTB_4 release in dog blood (IC_{50}= 1.5uM) but did not induce significant MHb at concentrations up to 250uM. Disssociation of LTB_4 inhibition and MHb induction was also observed with the 4-(N-n-pentyl carboxamido) analogues of the 2-methyl and 2-unsubstituted indazolinones.

These data demonstrate that ICI207968 induces species-dependent MHb induction and, in view of the sensitivity of dog blood to MHb induction with ICI207968, in vivo studies in this species are not recommended. The demonstration of amidic analogues that retain LTB_4 inhibitory potency but do not induce MHb indicates that different structural features are critical for LTB_4 inhibition and MHb induction by 2-substituted indazolinones.

Fort, F.L., Pratt, M.C., Carter, G.W., Lewkowski, J.P., Heyman, I.A., Cusick, P.K. and Kestertson, J.W. (1984). Fund. & Appl. Toxicol. 4,216.
McMillan, R.M., Millest, A.J., Proudman, K.E. and Taylor, K.B. (1986). Br.J.Pharmacol. 87, 53P.
McMillan, R.M., Masters, D.J., Vickers, V.C., Proudman, K.E., Foster, S.J., Pilgrim, W.P. and Bruneau, P. (1989). Br. J. Pharmacol. 96, 36P.

EFFECT OF NABUMETONE AND ITS PRINCIPAL METABOLITE ON RAT AND HUMAN GASTRIC MUCOSAL PROSTANOID SYNTHESIS AND PLATELET FUNCTION

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Non-steroidal antiinflammatory drugs (NSAIDs) cause gastric erosion and ulcerogenesis through inhibition of the gastroprotective prostaglandins (PGs) I_2 and E_2. In order to obviate these deleterious side effects, "prodrugs" such as nabumetone have been developed. Although weak inhibitors of cyclooxygenase, prodrugs are converted by the liver to metabolites which are more potent inhibitors of cyclooxygenase. Nabumetone may therefore obviate the occurrence of gastric erosion while maintaining efficacy as an antiinflammatory drug systemically. To further investigate prodrugs and their metabolites, we compared the effects of nabumetone and 6-methoxy-2-naphthylacetic acid (6MNA; the principle metabolite of nabumetone) with naproxen and indomethacin on *in vitro* synthesis of PGI_2 and PGE_2 by rat and human gastric mucosa and rat gastric mucosa *ex vivo*. To study the effect of 6MNA on peripheral target tissues, the effect of this metabolite on human platelet aggregation and thromboxane A_2 (TXA_2) synthesis was also investigated.

Gastric mucosa (GM) was obtained from male Sprague Dawley rats and from patients undergoing partial gastrectomy. GM discs were then prepared using a 5 mm diameter punch, and incubated with NSAIDs in Kreb's Ringer bicarbonate buffer (KRB) for 1 h at 37 °C. Supernatants were collected for estimation of PGs by specific radioimmunoassay (Jeremy *et al.*, 1985). In the *ex vivo* rat studies, NSAIDs (indomethacin, naproxen and nabumetone) were administered by gavage and the animals killed by a blow to the hea d at 30 min or 2 h. GM discs were prepared, incubated, and PG output assessed as described above. Platelet aggregation was carried out using standard aggregometry methods (Mikhailidis *et al.*, 1983). Platelet TXA_2 synthesis by platelet pellets was assessed as previously described (Jeremy *et al.*, 1988).

In vitro PGI_2 and PGE_2 synthesis by rat and human GM was inhibited by indomethacin, naproxen and 6MNA (in this order of potency), whereas nabumetone was completely without effect. In the *ex vivo* studies, indomethacin and naproxen significantly inhibited GM PGI_2 and PGE_2 synthesis at 30 min post gavage at a dose of 1 $mg.kg^{-1}$, whereas nabumetone was without significant effect even at the highest dose of 80 $mg.kg^{-1}$ at 2 h post gavage. Platelet aggregation and TXA_2 synthesis were similarly inhibited by the NSAIDs (indomethacin > naproxen > 6MNA >>> nabumetone).

These results support the view that nabumetone does not inhibit gastroprotective prostanoids, whereas its active metabolite 6MNA is an effective inhibitor of prostanoid synthesis in peripheral target tissues.

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Jeremy, J.Y., Mikhailidis, D.P. & Dandona, P. (1985) Agents & Actions 17, 205-208.
Jeremy, J.Y., Mikhailidis, D.P., Thompson, C.S. & Dandona, P. (1988) Diab. Res. 8, 177-181
Mikhailidis, D.P., Jeremy, J.Y., Barradas. M.A., Green, N & Dandona, P. (1983) Br. Med. J. 287, 1495-1498

THE EFFECT OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON IL-1 SECRETION FROM MURINE MACROPHAGES

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Non-steroidal anti-inflammatory drugs (NSAIDs) eg. aspirin, indomethacin etc., are widely used to treat the symptoms of rheumatoid arthritis (RA) but may increase pathogenic mechanisms (Kunkel *et al.*, 1986; Dale & Penfield, 1987). The former found that NSAIDs increased interleukin-1 (IL-1) secretion from macrophages (MØ). They and others have suggested that prostaglandin E_2 (PGE_2) is a down-regulator of IL-1 secretion. However, Otterness *et al.* (1988) were unable to show any significant potentiation of IL-1 secretion by NSAIDs. The above authors used the thymocyte co-stimulator bioassay to quantitate IL-1. The assay is strongly inhibited by PGE_2 and also responds to other lymphokines such as IL-6, IL-2, IL-4 and TNF_α. Thus increases in proliferation of the thymocytes may not necessarily represent increased IL-1 secretion since cyclooxygenase inhibition will increase thymocyte proliferation to IL-1.

We have re-evaluated the effect of NSAIDs on IL-1 secretion using a sensitive and selective bioassay designed by Gearing *et al.* (1987). CTLL proliferation was measured as outlined by Tada *et al.* (1986). MØ monolayers were produced by adherence of 1×10^6 peritoneal exudate cells, from 8-12 week BKW mice, to a 24 well flat-bottomed tissue culture plate and rinsing. The monolayer was overlaid with 1ml of serum-free RPMI-1640 containing L-glutamine 2mM; gentamicin 50$\mu g ml^{-1}$ and lipopolysacchride 1$\mu g ml^{-1}$. Varying concentrations of NSAIDs were added and the cell-free supernatants were collected after 20 h incubation at 37°, 5% CO_2 95% air and 100% humidity. The PGE_2 concentration in the supernatants was quantitated using a radioimmunoassay. All values for IL-1 secretion are expressed as percentage of the secretion observed with LPS 1$\mu g ml^{-1}$ in the table below.

ASP (μM)	IL-1	PGE_2 $ng ml^{-1}$	IND (μM)	IL-1	PGE_2 $ng ml^{-1}$	PIR (μM)	IL-1	PGE_2 $ng ml^{-1}$	NaMec (μM)	IL-1	PGE_2 $ng ml^{-1}$
100	113±12	0.17	100	305±42*	0.018	100	170±10*	0.03	—	—	—
10	110±9	2.7	10	176±6*	0.012	10	130±22	0.04	10	280±33*	0.053
1	99±8	2.3	1	150±9*	0.012	1	116±30	0.033	1	217±13*	0.089

*Significant difference from LPS control $P<0.05$ (Students t-test).
IL-1 mean ± sem (n=3); PGE_2 mean (n=2).

The results show that indomethacin (IND) and sodium meclofenamate (NaMec) produce dose-dependent increases in IL-1 secretion which are only partially related to PGE_2 inhibition. Piroxicam (PIR) 10μM although producing similar levels of PGE_2 inhibition to NaMec 10μM is far less potent at increasing IL-1 secretion. Aspirin (ASP) had no significant effect on IL-1 secretion. Addition of PGE_2 10ng to the NSAID-treated monolayers inhibits potentiated IL-1 secretion induced by PIR 100 μM and diminishes but does not ablate increased IL-1 secretion by IND 100 μM and NaMec 10 μM.

Dale, M.M., Penfield, A. (1987) *Br. J. Pharmac.*, 92:63-68.
Gearing, A.J.H. *et al.* (1987) *J. Immunol. Methods* 99:7-11.
Kunkel, S.L. *et al.* (1986) *J. Immunol.* 136:186-192.
Otterness, I.G. *et al.* (1988) *Cell Immunol.* 114:385-397.
Tada, H. *et al.* (1986) *J. Immunol. Methods* 93:157-165.

THE POTENTIATION OF HUMAN NEUTROPHIL SUPEROXIDE GENERATION BY SODIUM MECLOFENAMATE

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The non-steroidal anti-inflammatory drugs (NSAIDs) are thought to mediate their anti-inflammatory effects by inhibition of cyclooxygenase, but a number of anomalous results suggest that there may be additional effects which could increase inflammatory mechanisms. We have shown that the NSAID, indomethacin, increases superoxide production from neutrophils activated with post-receptor stimuli (Dale & Penfield, 1985). Here we present another example, sodium meclofenamate, which increases both post-receptor and receptor-mediated superoxide generation.

Superoxide generation by human neutrophils was measured spectrophotometrically using the superoxide-dismutase inhibitable reduction of ferricytochrome *c* to ferro-cytochrome *c*. Neutrophils were prepared from human blood by Ficoll-Isopaque separation with subsequent hypotonic lysis to remove the red blood cells. Neutrophil viability was greater than 99% and purity greater than 97%. Cells were pre incubated with sodium meclofenamate for 20 minutes at 37°C before stimulation.

Sodium meclofenamate (10-100μM) produced a marked left shift of the dose-response curve to the post-receptor stimuli: diC_8, a diacylglycerol analogue, and fluoride, a G-protein activator. A similar pattern of results was seen with the receptor stimuli: platelet activating factor, opsonized zymosan, IgG and heat-aggregated IgG. Although sodium meclofenamate produces a significant potentiation of O_2^- generation with these stimuli, it has no effect on unstimulated cells.

It has been reported that another NSAID, benoxaprofen, which produces enhanced superoxide generation from neutrophils, mediates its effects by an action on protein kinase C (Lukey *et al.*, 1988). However, when tested with a sensitive Triton mixed micellar assay (Hannun et al., 1985), we could not show protein kinase C activation with either sodium meclofenamate or benoxaprofen. We propose that sodium meclofenamate and possibly benoxaprofen work by inhibiting the diacylglycerol (DAG) metabolising enzyme, DAG kinase, thereby increasing DAG levels and promoting protein kinase C activation. Similar results to sodium meclofenamate were found with the specific DAG kinase inhibitor, R59022, in that both potentiated all these stimuli (Muid *et al.*, 1987).

Toxic oxygen metabolites generated by active phagocytic cells have been implicated in tissue damage. Superoxide produced by neutrophils can modify IgG molecules causing them to form aggregates which, in turn, can act as stimuli for superoxide generation (Lunec *et al.*, 1985). In addition, these complexes could function as auto-antigens, thus triggering a self-perpetuating cycle of auto-immune reactions thought to underlie rheumatoid arthritis. Non-steroidal anti-inflammatory drugs which increase receptor-mediated O_2^- release could possibly exacerbate tissue damage.

Dale, M.M. & Penfield, A. (1985) FEBS Letts. 185, 213-217.
Hannun,Y.A., Loomis,C.R. & Bell,R.E. (1985) J. Biol. Chem. 260, 10039-10043.
Lukey, P.T., Anderson, R. & Dippenaar, U.H. (1988) Br. J. Pharmac. 93, 289-294.
Lunec, J., Blake, D.R., McCleary, S.J., Brailsford, S. & Bacon, P.A. (1985) J. Clin. Invest. 76, 2084-2090.
Muid, R.E., Penfield, A. & Dale, M.M. (1987) Biochem. Biophys. Res. Commun. 143, 630-637.

OPERATION OF A NA^+/H^+-EXCHANGE MECHANISM IS NOT A PREREQUISITE FOR HUMAN PLATELET ACTIVATION

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It has been known for some years that activation of human platelets results in an uptake of Na^+ and an increase in cytosolic pH. Recently, Siffert and Akkerman (1987) have suggested that these effects result from the operation of a Na^+/H^+ antiporter in the platelet, the activation of which is necessary to trigger an elevation of cytosolic Ca^{2+} ($[Ca^{2+}]_i$) and thereby induce cell activation. However, this hypothesis appears largely to rely upon studies performed with thrombin as the platelet stimulant. We have therefore extended these studies by examining the ability of a range of agents to elevate $[Ca^{2+}]_i$ and cause platelet aggregation in human Fura-2-loaded gel-filtered platelets (GFP) under conditions where a Na^+/H^+ exchange mechanism would be expected to be operative (external Na^+ concentration ($[Na^+]_o$) = 150mM) or inoperative ($[Na^+]_o$ <1mM).

Human GFP were loaded with Fura-2 as described elsewhere (Watts et al., 1987) and suspended in a physiological salt solution containing either 150mM Na^+ or one in which all Na^+ salts were replaced with either choline or N-methyl-D-glucamine (NMDG) salts. The elevation of platelet $[Ca^{2+}]_i$ induced by thrombin, adenosine diphosphate (ADP), platelet activating factor (PAF) and U-46619 was monitored in each of the three media (Watts et al., 1987). Platelet aggregation to the same agonists as well as to adrenaline was also monitored in parallel utilising an electronic platelet counting technique. In GFP in which Na^+ was replaced by choline or NMDG, thrombin-induced elevation of $[Ca^{2+}]_i$ was inhibited with an 8-fold rightward displacement of its concentration-effect curve. In contrast elevation of $[Ca^{2+}]_i$ by ADP, PAF and U-41669 was unaffected. Platelet aggregation to thrombin and adrenaline was also inhibited in both low Na^+ media (e.g. mean concentration-ratio values of 9 and 38 for the two agonists respectively in the choline-containing GFP) whereas that to ADP, PAF and U-46619 was unaffected. Further evidence for a possible involvement of a Na^+/H^+ exchange process being involved in thrombin-induced $[Ca^{2+}]_i$ mobilisation and platelet aggregation was sought using the claimed selective inhibitor of this process, ethylisopropylamiloride (EIPA)(Vigne et al., 1983). The effect of the compound upon elevations in $[Ca^{2+}]_i$ could not be assessed since, over the concentration range 20-100μM, it markedly quenched fluorescence in Fura-2-loaded GFP. However, EIPA (100μM) was without effect upon thrombin-induced platelet aggregation, a finding inconsistent with results utilising the low $[Na^+]_o$ media. In contrast, over the range 10-100μM, EIPA inhibited adrenaline-induced platelet aggregation. This latter effect may, however, be explained by the reported α_2-adrenoceptor blocking activity of EIPA (Howard et al., 1987).

Thus, under conditions of low $[Na^+]_o$, when a Na^+/H^+ exchange system should have been inoperative, elevation of $[Ca^{2+}]_i$ and platelet aggregation induced by thrombin was inhibited whereas these same effects induced by ADP, PAF and U-46619 were unaffected. The use of EIPA failed, however, to confirm the involvement of a Na^+/H^+ exchange mechanism in platelet activation induced by thrombin. Whilst not ruling out the operation of a Na^+/H^+ exchange system being involved in platelet activation to some agonists such as thrombin, the present results indicate that other agonists can induce activation independently of such a process.

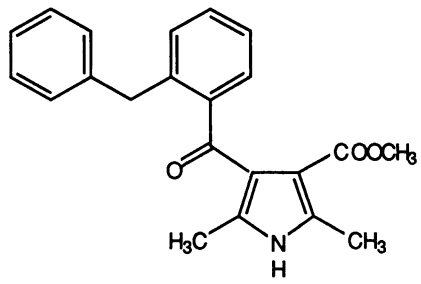
Howard, M.J. et al (1987), Am. J. Physiol., 253, F21-F25
Siffert, W. and Akkerman, J.W.N. (1987), Nature (Lond.), 325, 456-458
Vigne, P. et al. (1983), Biochem, Biophys. Res. Comm., 116, 86-90
Watts, I.S. et al (1987), Thromb. Haemostasis, 58, 466

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CALCIUM CHANNEL ACTIVATOR PROPERTIES IN A NOVEL NON-DIHYDROPYRIDINE - FPL 64176

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Bay k8644 is the prototype of a small group of structurally-related dihydropyridine calcium activators. These agents increase calcium influx through voltage-operated channels (VOC) by interacting with specific dihydropyridine binding sites on the channels (Schramm et al., 1983, Bellemann, 1984). Here we report some of the pharmacological properties of FPL 64176, a benzoyl pyrrole, which are consistent with it being the first member of a new class of calcium channel activator. In this study FPL 64176 has been compared directly with Bay k8644.



FPL 64176

In rat pituitary GH3 cells depolarised with 50mM K^+ both FPL 64176 and Bay k8644 were potent enhancers of ^{45}Ca uptake (pD_2, 7.79 ± 0.05 and 7.83 ± 0.14 respectively, n = 6), whereas uptake at physiological K^+ (5mM) was unaffected by FPL 64176 and Bay k8644 at concentrations up to 10^{-6}M. Neither FPL 64176 nor Bay k8644 contracted quiescent (5.4 mM K^+) guinea pig aortic rings up to 10^{-6}M, but both produced concentration related increases in tension in tissues pre-contracted with 30 mM K^+ (pD_2: FPL 64176 6.90 ± 0.02, n = 8; Bay k8644 - 7.8 ± 0.02M, n = 8).

In paced guinea pig atria (1 Hz, 1.5 x threshold v, 5 ms) FPL 64176 was a potent inotropic agent producing changes in developed tension equivalent to 50% of the isoprenaline maximum at 7.31 ± 0.06 (n = 7), compared with isoprenaline (7.74 ± 0.21, n = 7), ouabain (7.06 ± 0.15, n = 4), Bay k8644 (5.71 ± 0.25, n = 8) and milrinone (<3, n = 4). [^{3}H]-Isradipine (4.6 x 10^{-11}M) binding in cardiac sarcolemmal membranes was displaced in a concentration dependent manner by isradipine (IC_{50} 2.6 x 10^{-10}M, n = 3), and Bay k8644 (IC_{50} 1.1 x 10^{-8}M, n = 3). FPL 64176 produced only weak displacement at concentrations above 10^{-6}M (IC_{50} 3.9 x 10^{-6}M, n = 3).

We have demonstrated that FPL 64176 enhances calcium uptake and contraction in depolarised, but not quiescent tissues, and is a positive inotropic agent, properties which are compatible with activation of VOC. In these respects it was qualitatively similar to Bay k8644. However, the relative potencies of FPL 64176 and Bay k8644 varied between the different tissues studied and, unlike Bay k8644, FPL 64176 did not displace [^{3}H]-dihydropyridine binding at pharmacologically relevant concentrations. These observations suggest a novel mode of action on the channel for FPL 64176.

Schramm, M., Thomas, G., Towart, R. and Franckowiak, G. (1983) Nature, 303, 535-537.
Bellemann, P. (1984) FEBS Lett., 167, 88-92.

SK&F 96365, A NOVEL INHIBITOR OF RECEPTOR-MEDIATED CALCIUM ENTRY AND AGGREGATION IN QUIN2-LOADED HUMAN PLATELETS

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Ca^{2+} can enter the cytosol by discharge of intracellular stores and by various forms of receptor-mediated Ca^{2+} entry (e.g. Hallam & Rink, 1989). Compounds that inhibit Ca^{2+} mobilisation by either means might be expected to reduce Ca^{2+}-dependent cellular responses such as platelet aggregation. Such compounds might therefore have potential as antithrombotics that would be effective against all agonists that work through an elevation in $[Ca^{2+}]_i$. SK&F 96365 (1-{β-[3-(p-Methoxyphenyl)-propyloxy]-p-methoxyphenethyl} -1H-imidazole hydrochloride) selectively inhibits Ca^{2+} entry into platelets (as well as other cell-types) in response to various agonists. Aggregation of quin2-loaded platelets stimulated by the same agonists is also inhibited by similar concentrations of SK&F 96365.

Freshly isolated human platelets were loaded with quin2 to a cytosolic concentration of 1mM in order to buffer heavily changes in $[Ca^{2+}]_i$. Under these conditions ADP (20uM) or thrombin (1U/ml) stimulate a small rise in $[Ca^{2+}]_i$ (from c.100nM to c.200nM) in the absence of extracellular Ca^{2+} and a larger rise (from c.100nM to c.1000nM) in the presence of extracellular Ca^{2+} (Rink et al, 1982; Hallam & Rink, 1985a). The additional response in the presence of extracellular Ca^{2+} is attributed to Ca^{2+} entry. SK&F 96365 inhibited ADP-stimulated Ca^{2+} entry with an IC_{50} of 8.5uM and the response to thrombin was inhibited with an IC_{50} of 11.7uM. Release of Ca^{2+} from intracellular stores (i.e. the rise in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}) in response to both ADP and thrombin was only inhibited by very high concentrations of SK&F 96365 (IC_{50} = 120uM). A more direct measure of agonist-stimulated divalent cation influx across the plasma membrane involves the use of extracellular Mn^{2+}, which quenches cytosolic fura-2 upon entry into the cell. ADP, thrombin and U46619 stimulate Mn^{2+} entry into fura-2 (or quin2)-loaded platelets (Hallam & Rink, 1985b), and Mn^{2+} entry in response to each of these agonists was inhibited by SK&F 96365 with IC_{50} values of 27uM, 23uM and 18uM respectively.

The above results show that SK&F 96365 inhibits Ca^{2+} entry into platelets in response to various agonists. Platelet aggregation is known to be associated with elevations in $[Ca^{2+}]_i$ (Rink et al, 1982). When platelets are loaded with quin2, aggregation is largely dependent on Ca^{2+} entry (because rises due to internal release are heavily buffered). Under these conditions, SK&F 96365 inhibited aggregation stimulated by U46619 with an IC_{50} of 19uM.

Hallam, T.J. & Rink, T.J. (1985a) J. Physiol. 368, 131-146.
Hallam, T.J. & Rink, T.J. (1985b) FEBS Lett. 186, 175-179.
Hallam T.J. & Rink, T.J. (1989) Trends in Pharm. Sci. 10, 8-10.
Rink, T.J., Smith, S.W. & Tsien, R.Y. (1982) FEBS Lett. 148, 21-26.

[^{3}H] IMIPRAMINE AND [^{3}H] PAROXETINE BINDING TO FRESH AND FROZEN HUMAN PLATELET MEMBRANES

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[^{3}H]Imipramine ([^{3}H]IMIP) labels sites associated with 5-HT uptake in brain and platelets. Although numerous studies have investigated platelet [^{3}H]IMIP binding as a putative peripheral marker of depression, the findings are inconsistent (Mellerup & Plenge, 1988). These inconsistencies and the wide variations in binding parameters reported for control subjects may in part be related to methodological differences such as platelet preparation and storage. In this study we have compared [^{3}H]IMIP binding to human platelet membranes prepared from freshly isolated platelets and platelets from the same subject stored frozen prior to assay. In parallel we studied the binding of the selective 5-HT uptake inhibitor [^{3}H]paroxetine ([^{3}H]PAR), which is thought to label a different subunit to [^{3}H]IMIP within the 5-HT transport complex (Mellerup et al, 1985).

Blood samples (60 ml) were obtained from 20 (10 for each ligand) healthy drug-free volunteers. Samples were divided into two equal portions and platelets isolated. One portion was stored at -80^{o}C for 30 days, the other was immediately washed, platelet membranes prepared by hypotonic lysis/homogenisation and assayed. The frozen samples were subsequently processed in a manner identical to that used for fresh platelets. Aliquots of platelets were incubated with [^{3}H]IMIP (0.2-8.7 nM) or [^{3}H] PAR (0.01-1 nM) in 50 mM Tris-HCl pH 7.5 containing 120 mM NaCl and 5 mM KCl for 75 and 90 min respectively. Specific binding was defined with 100 μM desipramine ([^{3}H]IMIP) or 1 μM citalopram ([^{3}H]PAR). Bmax and Kd values were determined by computerised non-linear analysis of saturation plots.

Table 1 The binding of [^{3}H]IMIP and [^{3}H]PAR to fresh and frozen platelets

	[^{3}H]IMIP			[^{3}H]PAR		
	Bmax	Kd	Protein	Bmax	Kd	Protein
Fresh	920 ± 52	0.54 ± 0.02	104 ± 7	870 ± 59	0.035 ± 0.005	93 ± 13
Frozen	832 ± 63	0.52 ± 0.05	80 ± 5*	815 ± 29	0.021 ± 0.005	47 ± 8*

Values are means ± SEM, n = 10 for each group. Kd = nM, Bmax = fmol/mg protein, protein = μg protein/assay. * $p < 0.05$ by paired t-test.

Both ligands labelled similar numbers of sites. The mean number of [^{3}H]IMIP and [^{3}H]PAR binding sites did not differ significantly between fresh and frozen platelets (Table 1). The Kd of [^{3}H]PAR was an order of magnitude lower than that of [^{3}H]IMIP and was lower ($p = 0.055$) in frozen than fresh platelets. In contrast the Kd of [^{3}H]IMIP was not influenced by freezing. Freezing caused a significant reduction in total protein content.

The number of 5-HT uptake sites in blood platelets are not significantly altered by freezing/storage for 30 days and thus this is unlikely to be an important factor in explaining discrepancies in values reported for controls or depressed subjects.

We thank Beecham Pharmaceuticals for generous supplies of [^{3}H]paroxetine.

Mellerup, E.T. et al (1985) Eur. J. Pharmacol. 106, 411-413.
Mellerup, E.T. & Plenge, P. (1988) Acta. Psychiat. Scand. Suppl 345, 61-68.

APPARENT SEPARATION OF BENZODIAZEPINE TOLERANCE AND DEPENDENCE: FLUMAZENIL DOES NOT ABOLISH THE WITHDRAWAL EFFECT

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We recently demonstrated that the intravenous pentylenetetrazole (PTZ) test, with which benzodiazepine tolerance can be demonstrated, could also be used to study benzodiazepine dependence in mice (Feely et al., 1989). Tolerance was represented by a reduced anticonvulsant effect after repeated dosing: dependence was represented by a proconvulsant effect after withdrawal. In this report, we describe the results of a comparison between lorazepam, nitrazepam and clonazepam and the effects of the benzodiazepine antagonist flumazenil (Ro 15-1788) on the withdrawal response.

Convulsive threshold was measured using a protocol described previously (Gent et al., 1986). Adult male mice (Tuck No. 1, 25-35 g) were used throughout the study. To assess tolerance, 5 animals per group were used, but to measure withdrawal effects, we used groups of 10 in order to assess more accurately the significance of the inevitably small differences from control values. Preliminary experiments were carried out with all drugs to establish suitable doses and to study the time courses of action. All three benzodiazepines were given s.c. 30 min before testing with PTZ. When flumazenil was used, it was given i.p. 5 min before the test. Lorazepam and clonazepam were given in saline, nitrazepam and flumazenil in a propylene-glycol based vehicle. Pretreatment with the three benzodiazepines consisted of twice daily dosing i.p. for 3 days.

The results are expressed as the minimum convulsant dose (MCD) of PTZ in mg kg^{-1} (mean $\pm$ s.e. mean) which elicits a clonic seizure; significance was assessed with analysis of variance.

Drug (dose)	1st dose	7th dose	Controls	12h Withdrawn
Lorazepam ($0.2mg\ kg^{-1}$)	108.0 $\pm$ 2.5	93.3 $\pm$ 2.0	41.65 $\pm$ 1.1	35.1 $\pm$ 0.8
Nitrazepam ($0.6mg\ kg^{-1}$)	89.6 $\pm$ 2.1	80.3 $\pm$ 0.8	41.65 $\pm$ 1.1	37.1 $\pm$ 1.0
Clonazepam ($0.2mg\ kg^{-1}$)	88.5 $\pm$ 1.3	92.2 $\pm$ 1.7	40.59 $\pm$ 1.0	34.1 $\pm$ 1.1

Significant ($P<0.05$) tolerance occurred to the effects of lorazepam and nitrazepam but not to clonazepam. However, a significant proconvulsant effect after withdrawal ($P<0.01$) occurred in all cases. Since a marked fall in convulsive threshold occurred with lorazepam, we measured the MCD of PTZ in lorazepam withdrawn animals after injection of flumazenil (4 mg kg^{-1} i.p.; a dose which completely blocked the acute effects of lorazepam but which had no effect on its own). The MCD of PTZ in the withdrawn goup given flumazenil (37.2 $\pm$ 1.1) was not significantly different from that in the withdrawn group not given flumazenil; both were significantly lower than that in controls.

Thus lorazepam and nitrazepam both induced tolerance, and withdrawal of all 3 drugs led to a proconvulsant effect which may be taken as an indication of dependence. The lack of significant tolerance to clonazepam was unexpected, but not surprising since we have previously shown that tolerance develops only slowly to this drug (Gent et al., 1985). That a withdrawal reponse should occur after clonazepam was an interesting finding, since it suggests not only that tolerance and dependence can be separated, but that dependence may occur without obvious tolerance. Since flumazenil did not significantly alter the withdrawal response we measured after lorazepam, it seems unlikely that an endogenous ligand is involved in this case.

Feely, M., Peaker, S., Gent, J.P. & Boyland, P. (1989) Br. J. Pharmacol. (Proceedings, in press)
Gent, J.P., Bentley, M. Feely, M. & Haigh, J.R.M. (1986) Eur. J. Pharmacol. 128, 9-15
Gent, J.P., Feely, M. & Haigh, J.R.M. (1985) Life Sciences 37, 849-856

CHRONIC BENZODIAZEPINE AGONIST TREATMENT INCREASES THE ACTIONS OF THE INVERSE AGONIST FG7142 ON THE HIPPOCAMPAL SLICE

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The mechanisms of the adaptations that occur on chronic treatment with benzodiazepine agonists are not well understood. Receptor down-regulation occurs only after high doses of agonists. Chronic benzodiazepine agonist treatment increased effects of benzodiazepine inverse agonists, such as FG7142 (Little et al, 1987, 1988). Evidence suggested that this was an increase in the intrinsic properties of the inverse agonist rather than precipitated withdrawal. We have now studied the effects of the partial inverse agonist, FG7142, on field potentials in the hippocampal slice, *in vitro*, following chronic treatment of mice *in vivo* with the benzodiazepine agonist flurazepam. The treatment schedule was the same as that used in our earlier behavioural studies.

Male mice, CD1 strain (30-35g) were given seven daily i.p. injections of flurazepam, 40 mg/kg i.p. Controls received saline injections. Hippocampal slices were prepared 48h after the last chronic treatment injection, a time when the actions of FG7142 *in vivo* were greatly increased and our evidence showed that there was no benzodiazepine agonist in the CNS (Little et al, 1987). Extracellular recordings were made from stratum pyramidale, CA1 area, with orthodromic paired pulse stimulation (50 msec interval) of Schaffer collateral/commissural fibres, by an observer blind to the chronic treatment. Input/output curves were constructed in normal medium, then in successively increasing concentrations of FG7142 (10 nM to 25μM) perfused for 20 mins. In order that comparisons could be made between preparations from control and flurazepam-treated mice, the areas of the population (pop.) spikes for each preparation, and any secondary discharges, were calculated as percentages of the maximum population spike in normal medium (first reponse of the pair). The effects of FG7142 were then expressed as the differences between the responses in normal and in FG7142-containing medium. The effects of FG7142 were compared by nonparametric analysis of variance for slices prepared from control and flurazepam-treated mice, n = 6 per group (Meddis, 1984)

Effects of FG7142, 50 nM Results as mean ± s.e.m. for changes in % responses, **P<0.001

Stimulation as multiple of threshold:-		*1.0*	*1.5*	*2.0*	*3.0*
Control slices:	First pop.spike:	4 ± 9	6 ± 11	8 ± 19	-5 ± 20
	secondary discharges:	-8 ± 8	-1 ± 11	28 ± 15	61 ± 37
	Second pop.spike:	11 ± 20	-20 ± 13	-4 ± 13	24 ± 14
	secondary discharges:	-17 ± 11	34 ± 29	6 ± 28	-7 ± 19
Flurazepam:	First pop.spike:	14 ± 13	40 ± 27	13 ± 20	16 ± 26
treatment	secondary discharges:	0 ± 0	7± 5	37 ± 16	48 ± 15
	Second pop.spike:	6 ± 7	4 ± 20	7 ± 11	11 ± 17
	secondary discharges**:	11 ± 1	104 ± 56	73 ± 53	115 ± 72

Chronic flurazepam treatment increased the production of secondary discharges by FG7142, particularly following the second response of the slices to the paired pulse stimulation. This reflects the ability of cells to repolarise after stimulation. and may also indicate decreased inhibition. GABA-mediated inhibition would normally affect the second response to the paired stimulation. In our earlier behavioural studies this chronic flurazepam treatment schedule changed the actions of FG7142 *in vivo* from proconvulsant to convulsant. It is therefore possible that alterations in repolarisation and/or in inhibition may be involved in the increased action of FG7142 *in vivo* seen after chronic benzodiazepine agonist administration.

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Little,H.J.,Nutt,D.J.,Gale,R.,Sellars,N. & Taylor,S.C.1988 Neuropharmacology,27,383-389
Little, H. J., Nutt, D. J. & Taylor, S. C. 1987, Brain Res. Bull., 19, 371 - 378
Meddis, R. 1984, "Statistics Using Ranks" Basil Blackwell Ltd

THE CENTRAL ADMINISTRATION OF THE GABA-B RECEPTOR AGONIST, BACLOFEN, INDUCES FEEDING IN THE SATIATED PIG.

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GABA exerts its effects by acting at two pharmacologically distinct receptor subtypes, viz. GABA-A and GABA-B receptors (see Meldrum, 1987). In recent years, considerable evidence has accumulated to support the view that a central GABAergic mechanism is involved in the control of food intake and pharmacological studies have revealed that this action is mediated via central GABA-A receptors (Morley, 1987). However, very little attention has been paid to the GABA-B receptor subtype, and as far as we are aware, no attempt has been made to examine the effects of GABA-B agonists and antagonists on feeding. In the present study, we investigated the effects of the GABA-B agonist baclofen on food and water intake in satiated pigs.

Prepubertal large white pigs (10 male, 8 female; body weights: 30-60kg) were housed individually in metabolic cages. The animals were trained to press operant switch panels located in the cage with their snouts on a fixed ratio of 5 for food and water reinforcements. Each pig was implanted, under halothane anaesthesia, with a stainless steel guide tube aimed at the lateral cerebral ventricle for subsequent intracerebroventricular (ICV) injections of drugs (see Baldwin and Thornton, 1986 for details). In addition, 2 pigs were also prepared with intravenous catheters in the external jugular vein for the i.v. administration of drugs. Food and water were available ad lib., and consummatory behaviour was continously monitored on a data logger. ICV injection of baclofen (25, 50 and 100 nmol) or vehicle (physiological saline) were carried out between 1000h and 1130h each weekday. The criterion for drug administration was that the pig had not eaten for at least 30 min. prior to injection. Drug sessions were conducted twice a week, and control sessions bracketed each drug session. The effects of drug treatment on food and water intake for each pig was compared with the saline control values recorded on the preceding day using the paired t-test.

Baclofen (25-100 nmol) caused a dose-related increase in food intake in the satiated pigs. The 25 nmol dose had no significant effect on feeding compared with control data. In contrast, 50 nmol baclofen increased feeding ($P<0.01$) during the first 15 min. after icv injection, while the 100nmol dose increased feeding ($P<0.01$) during the first 30 min. Eating usually began 2-5 min after injection but there was no significant effect on water intake. Baclofen (50 and 100 nmol) caused the pigs to display signs of ataxia about 10-20 min after injection and they eventually went into a deep sleep for between 2-3h. When they awoke they displayed no signs of illness and usually began eating and drinking again. The effects of baclofen (50 nmol) on feeding could be completely abolished by pretreating the animals with the novel GABA-B receptor antagonist phaclofen (500 nmol icv). In addition, the observation that i.v. administration of baclofen (100 nmol) to 2 pigs had no effect on food intake, eliminated the possibility that baclofen may have a peripheral mode of action. The results of this study have indicated that baclofen can induce feeding in sated pigs by acting on central GABA-B receptors. It remains to be determined whether this action has a physiological role in the regulation of food intake.

Baldwin, B.A. and Thornton, S.N. (1986) Physiol. Behav. 36, 325-328
Morley, J.E. (1987) Endoc. Rev. 8, 256-287
Meldrum, B. (1987) J. Psychopharm. 1, 1-5

ACTIONS OF 5-HT$_{1A}$ LIGANDS AND STANDARD ANXIOLYTICS ON MOUSE EXPLORATORY BEHAVIOUR IN A TWO-COMPARTMENT LIGHT:DARK ARENA

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Although buspirone is a clinically effective anxiolytic (eg: Rickels et al., 1982) it, and other 5-HT$_{1A}$ ligands are not consistently active in a range of animal anxiety models (Gardner, 1988). Using a model of anxiety based on the exploratory behaviour of mice in a two-compartment light:dark arena (Costall et al., 1988) we have examined the activity of several 5-HT$_{1A}$ ligands, ranging from the agonists/partial agonists 8-OH-DPAT, buspirone, gepirone and ipsapirone, to the putative antagonists BMY 7378 (Yocca et al., 1987) and MDL 73005 (Hibert et al., 1988).

The test apparatus (based on the design of Costall et al., 1988) consisted of an open-topped box (81 x 36 x 27 cm high), one third of which was painted black, illuminated under dim red light and partitioned from the remainder of the box, which was painted white and brightly lit with a 60W spotlight located 40cm above the floor. An opening (7.5 x 7.5 cm) was located in the centre of the partition at floor level. The floor of the box was lined into a grid pattern of 9 cm squares. Female albino Tuck (T/O strain) mice (22-30g; n=8 per treatment), which were housed in the darkened experimental room for 18h prior to experimentation, were dosed s.c. with either saline or the test compounds (0.01-5.0 mg/kg) 30 min prior to being placed in the centre of the light compartment. The number of line crossings, rears, the time spent in each section of the box and the number of transitions between the two compartments were recorded in a 5 min test period.

Vehicle control mice were markedly less active in the light compartment relative to the dark, spending between 34-44% of the total time in the light. The ranges of control values (mean ± s.e.m. per 5 min) in these experiments were:- light (rears 8.8 ± 2.1 - 14.0 ± 1.8; line crossings 47.4 ± 7.7 - 70.6 ± 11.6), dark (rears 31.9 ± 2.4 - 44.6 ± 3.2; line crossings 103.8 ± 15.0 - 125.8 ± 4.5). The most consistent and reproducible effects of standard anxiolytics were to markedly and significantly ($p < 0.05$, ANOVA/Dunnett's test) increase rearing and motor activity in the light, at doses which did not modify dark compartment activity. Effective dose ranges (mg/kg s.c.) were:- chlordiazepoxide (0.5-5.0), diazepam (0.2-3.0 p.o.), meprobamate (50). In all experiments involving 5-HT$_{1A}$ ligands a chlordiazepoxide or diazepam treatment group was included as a positive control. With the exception of gepirone, 5-HT$_{1A}$ ligands also significantly ($p < 0.05$) increased light compartment rearing and motor activity. The doses which induced maximal effects (with percentage increases, relative to vehicle controls, in light compartment rearing/line crossings) were:- buspirone 1.0 (86/54); ipsapirone 1.5 (123/83); BMY 7378 1.0 (121/55); MDL 73005 2.0 (58/36); 8-OH-DPAT 0.1 (134/97). Higher doses of these compounds (0.3 mg/kg or greater for 8-OH-DPAT, 3.0-5.0 mg/kg for all other compounds) were sedative, in that frequencies of rearings and line crossings were decreased in both compartments (though more markedly in the dark).

Our data on buspirone in this model are in reasonable agreement with those obtained by Costall et al., 1988. The mechanism by which buspirone and other 5-HT$_{1A}$ ligands induce these effects may involve either a presynaptic agonist action (to attenuate 5-HT release) and/or a postsynaptic antagonist action. Both 5-HT$_{1A}$ agonist and antagonist properties of these ligands have been demonstrated in various functional models of the 5-HT$_{1A}$ receptor.

Costall, B. et al., (1988). J. Pharm. Pharmacol., 40, 494-500.
Gardner, C.R. (1988). Gen. Pharmacol., 19, 347-356.
Hibert, M. et al., (1988). Br. J. Pharmacol., 93, 2P.
Rickels, K. et al., (1982). J. Clin. Psychiat., 43, 81-86.
Yocca, F.D. et al., (1987). Eur. J. Pharmacol., 137, 293-294.

THE DIFFERENTIAL EFFECTS OF RU24969 UNDER HALOTHANE COMPARED TO CHLORAL HYDRATE ANAESTHESIA

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Previously it has been shown that RU24969 (5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl) IH indole succinate, 5-HT_1 agonist) at a dose of 10 mg/kg i.p. decreased both release and metabolism of 5-hydroxytryptamine (5HT) (Brazell et al., 1985) in chloral hydrate (600 mg/kg) anaesthetized rats. In a study of the effects of RU24969 on dorsal raphe neuronal firing we observed that doses of RU24969 administered without problems under chloral hydrate were lethal to animals anaesthetized with halothane (1.5-2%). This observation led us to compare the effects of RU24969 given intravenously (i.v.) and intraperitoneally (i.p.) under each anaesthetic.

Male Wistar rats in the weight range 350-400 g were anaesthetized with either halothane (1.5-2%) or chloral hydrate (600 mg/kg), venous cannulae were implanted in all sixteen rats together with a glass recording electrode (4-6 MΩ) implanted in the dorsal raphe nucleus for extraneuronal electrophysiological recordings. 5HT dorsal raphe neurones were characterized by their slow (0.5 - 2 spikes/sec) regular firing pattern. Rats under halothane and given RU24969 (40 ug/kg i.v.) died within the following 10 minutes. When RU24969 (20 ug/kg i.v.) was administered unit activity in the dorsal raphe nucleus was successfully recorded. Rats anaesthetized with chloral hydrate and given RU24969 at a dose of 0.5 mg/kg i.v. survived for several hours and were used for unit activity recordings with RU24969 again producing no change in 5HT neuronal firing, though a dose of 1 mg/kg i.v. proved fatal within 15 min. A further group of rats were anaesthetized with halothane (1.5-2%) and were given RU24969 (10 mg/kg i.p. or 5 mg/kg i.p.). Those with the larger dose died within 15 minutes of the injection whereas those given 5 mg/kg lasted several hours. In contrast 10 mg/kg i.p. could be safely administered to rats anaesthetized with chloral hydrate as shown in previous studies (Brazell et al., 1985; Martin & Marsden, 1986). RU24969 following i.p. administration failed to alter 5HT neuronal firing either under halothane or chloral hydrate anaesthesia.

Thus, it would seem that a twenty-five times greater dose of RU24969 can be intravenously administered to rats anaesthetized with chloral hydrate compared to those under halothane while double the dose of RU24969 can be administered intraperitoneally to rats anaesthetized with chloral hydrate compared to halothane. As both chloral hydrate and halothane suppress respiration, it would seem unlikely that the addition of RU24969 exacerbates this phenomenon in one but not the other. As yet the reason for the different effects of RU24969 under these two anaesthetics is unclear and warrants further investigation.

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Brazell, M.P., Marsden, C.A., Nisbet, A.P. and Routledge, C. (1985). Br. J. Pharmac. 86, 209-216.
Martin, K.F. and Marsden C.A. (1986). Eur. J. Pharmac. 121, 135-139.

RS-30199, AN AZEPINE WITH AFFINITY FOR THE 5-HT$_{1A}$ SITE

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Adrenoceptors show structural similarities to 5HT$_{1A}$ receptors and we have shown that agents with high affinity for 5HT$_{1A}$ receptors can distinguish between α_2-adrenoceptor subtypes (Brown et al., 1989). We now show that slight structural modifications of an α_2-adrenoceptor antagonist can confer affinity for 5HT$_{1A}$ sites. Radioligand binding assays were performed essentially as described by Hoyer et al. (1985) using the appropriate ligands and tissues as shown in Table 1. RS-30199-193 (5-chloro-2methyl-1,2,3,4,8,9, 10,10a-octahydronaphth-[1,8-cd]-azepine) is an analogue of SKF-86466 (DeMarinis et al., 1983); (pKi: α_2 7.0; 5HT$_{1A}$ 7.9) with an additional 6-membered ring. The analogue with a 5-membered ring (RS-10869-193; 5-chloro-2 methyl-1,3,4,8,9,9a-hexahydro 2H-indeno [1,7-cd] azepine), although potent had less selectivity (pKi α_2 8.2; 5HT$_{1A}$ 7.6). RS-30199-193 has high affinity for the 5HT$_{1A}$ site.

Table 1. Affinity values of RS-30199-193 (pKi mean±s.e.mean)

5HT$_{1A}$	[^{3}H]-8-OH-DPAT		rat cortex	7.91±0.08
5HT$_{1B}$	[^{125}I]-cyanopindolol		rat striatum	<5
5HT$_{1C}$	[^{3}H]-mesulergine	pig	choroid plexus	<5
5HT$_{1D}$	[^{3}H]-5HT		baboon cortex	<5
5HT$_2$	[^{3}H]-ketanserin	rat	frontal cortex	5.9±0.1
α_1	[^{3}H]-prazosin		rat cortex	6.2±0.1
α_2	[^{3}H]-yohimbine		rat cortex	7.0±0.1
β_1	[^{3}H]-dihydroalprenolol		guinea-pig heart	6.2±0.2
β_2	[^{3}H]-dihydroalprenolol		guinea-pig lung	5.7±0.2
D_2	[^{3}H]-spiperone		rat striatum	6.0±0.3
CEB	[^{3}H]-nitrendipine		rat striatum	<5

In guinea pig hippocampal slices RS-30199-193 (3-100nM) behaved as a partial agonist in that it increased submaximal forskolin-induced cyclic AMP changes, but with a lower maximal effect than that induced by 8-hydroxy-n-dipropylaminotetralin or 5HT. In an in vivo binding assay, RS-30199-193 (3 & 10mg/kg, s.c.) was as potent as buspirone in causing inhibition of [^{3}H]-8-OH-DPAT (administered i.v. by the tail vein 30 min post dose) binding in the frontal cortex of the rat. However, RS-30199-193 was 3-fold less potent than buspirone in causing antagonism of flat body posture, fore-paw treading, head-weaving and increased locomotion induced by 8-OH-DPAT (300μg/kg, s.c.) in the rat; the compound did not induce these behaviours per se at doses up to 30mg/kg s.c.. The weaker potency against the 8-OH-DPAT-induced behaviours may be a consequence of effects at a different subtype of receptor (see Small et al., Redfern et al., this meeting).

Brown, C.M. et al.,(1989) Br.J.Pharmacol. (in press)
DeMarinis, R.M. et al., (1983). J.Med.Chem., 26, 1213-1219.
Hoyer, D. et al., (1985). Eur.J.Pharmacol., 118, 13-23.

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ANXIOGENIC-LIKE PROPERTIES OF RS-30199 IN THE RAT

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RS-30199 (Brown *et al*, 1989) is an azepine derivative with a similar affinity to buspirone for the 5-HT_{1A} binding site in the rat brain, but which does not have the same agonistic effects in the guinea pig ileum preparation as buspirone, ipsapirone or 8-hydroxy-di-n- propyl-aminotetralin (8-OH-DPAT; Small & Spedding, 1989). The compound displaces [^{3}H]-8-OH-DPAT from cerebral cortex in *in vivo* binding assays following intravenous administration to the rat, implying good penetration of the central nervous system (Brown *et al.*, 1989). We now describe its effects on the activity of rats in tests designed to distinguish between anxiety-related and other central effects.

In male Sprague-Dawley rats, RS-30199 (10mg/kg s.c.; n=10) reduced spontaneous locomotor activity, measured over a 9 min period 40 min after injection, by 60% compared to the saline-treated control group (n=10; $p < 0.001$). This was not due to motor impairment as there was no significant effect on the performance of these rats in an accelerating rotarod test (saline group: 97 $\pm$ 16 s; RS-30199 group: 75 $\pm$ 13 s) . In order to study the effect on exploratory behaviour in more detail, rats which had been housed singly for 4-8 days were placed sequentially in an elevated X-maze and then in a holeboard containing a shaded corner (Redfern & Williams, 1989). There were 11-14 rats in each group. RS-30199 (0.3-30 mg/kg i.p.) produced a dose-related suppression of open arm (X-maze) and open field (holeboard) activity. In the X-maze (5 min), a dose of 30mg/kg i.p. produced a >50% reduction in the total number of arm entries ($p < 0.002$), in the proportion of open:enclosed arm entries ($p < 0.002$) and in the time spent on the open arms ($p < 0.02$). In the holeboard (12 min), the initial emergence latency from the shaded corner was elevated after a 3 mg/kg dose of RS-30199 ($p = 0.05$). At a dose of 30 mg/kg i.p there was a >50 % reduction in line crossings ($p < 0.05$). Forays, head dips, rears and time in the open area were similarly reduced, but not significantly. There were no signs of drowsiness or hypotonia in the RS-30199 treatment groups during handling.

Taken together, these data indicate that, in the rat, RS-30199 is anxiogenic rather than sedative, and has effects opposite to those of buspirone in the holeboard (Redfern & Williams, 1989). Further investigation of the actions of this drug in other behavioural tests is required in order to clarify this.

Brown, C. *et al* (1989) Proc. Brit. Pharmac. Soc. (this meeting).
Redfern, W.S. & Williams, A. (1989) Proc. Brit. Pharmac. Soc. (this meeting).
Small, C. & Spedding, M. (1989) Proc. Brit. Pharmac. Soc. (this meeting).

ACUTE EFFECTS OF CENTRALLY-ACTING DRUGS ON THE BEHAVIOUR OF RATS IN AN ELEVATED X-MAZE AND A PARTIALLY-SHADED HOLEBOARD

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Exploratory behaviour in a novel environment is disinhibited by non-sedative doses of anxiolytic drugs (File, 1985). One test of such behaviour is the elevated X-maze (Handley & Mithani, 1984). As it is unwise to rely on one test alone, we first tested rats in an X-maze and then placed them in a modified holeboard which combines an emergence latency test with a holeboard activity test.

Male Sprague-Dawley rats (140-230g) were housed singly for 4-8 days before testing. The rats were transferred to the testing room on the day before the experiments and were handled by the experimenter on the day before and 0.5h before the test session (09.00-12.30h). Rats (6-18 per group) were injected (i.p.) with either drug or vehicle, and 0.5h later each rat was placed on the elevated X-maze comprising 2 open arms (length 50 cm, width 10 cm) facing each other, and 2 enclosed arms (length 50 cm, width 10 cm, height 20 cm) with an open roof, and was elevated 50 cm from the floor. Observations were made over 5 min., then the rat was transferred to the holeboard (length 75 cm, width 46 cm, height 30 cm) containing a small roof (12 x 15 cm) fixed 10 cm above the floor in one corner. The floor was delineated into 8 sectors and contained 8 holes (diameter 3.5 cm). The rat was placed in the shaded corner and observations were made of the initial emergence latency, forays from the shaded corner, lines crossed, rears, head dips and time in the open area, over 12 min.

X-maze: Buspirone (1mg/kg) reduced the time on the open arms ($p < 0.02$; U-test) and a dose of 10 mg/kg reduced the total number of arm entries ($p < 0.002$). Lower doses (0.1 & 0.3 mg/kg) had no effect. Diphenhydramine (30 mg/kg) decreased the total number of arm entries ($p < 0.002$) without affecting the proportion of open:enclosed arm activity. Picrotoxin (5 mg/kg) reduced the total number of arm entries ($p < 0.002$), the ratio of open:total arm entries ($p < 0.02$) and the time on the open arms ($p < 0.002$). Chlordiazepoxide (2.5 mg/kg), picrotoxin (2 mg/kg) and amphetamine (1 mg/kg) had no significant effects in the X-maze.
Emergence latency: Buspirone (10 mg/kg) and picrotoxin (5 mg/kg) increased ($p < 0.02$) the emergence latency from the shaded corner; other drugs had no effect.
Holeboard: Buspirone (1 mg/kg) increased the number of forays ($p < 0.05$) and line crossings ($p < 0.05$). Amphetamine (1 mg/kg) increased all activities in the holeboard ($p < 0.05$). Picrotoxin (2 & 5 mg/kg) reduced head dips ($p < 0.05$) and line crossings ($p < 0.05$). Chlordiazepoxide (2.5 mg/kg; $p = 0.05$) and diphenhydramine (30 mg/kg; $p < 0.05$) reduced head dipping.

In conclusion, use of the modified holeboard in conjunction with the X-maze allows analysis of the effects of drugs on exploratory activity, and may help to distinguish between effects on anxiety and arousal, and other central effects which influence activity in an unfamiliar environment.

File, S.E. (1985) Neurosci. Biobehav. Rev. 9, 45-54.
Handley, S.L. & Mithani, S. (1984) N-S Arch. Pharmacol. 327, 1-5.

WHICH 5-HT RECEPTORS ARE INVOLVED IN THE INHIBITION OF OBJECT BURYING BEHAVIOUR BY 5-HT UPTAKE INHIBITORS?

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5-HT reuptake inhibitors selectively inhibit object burying behaviour at doses which do not affect immersion-induced grooming in mice, according to Broekkamp and Jenck (1987). We have therefore started to investigate which 5-HT receptor(s) might be involved.

Female MF1 mice (25-35 g) were placed individually in polypropylene cages (42 x 24 x 12 cm) containing 20 glass marbles evenly spaced on 5 cm deep sawdust and the number of marbles at least 2/3 buried counted 30 min later. Motor activity was assessed separately by 5 min exposure to a circular runway placed on an Animex activity recorder. All drugs were injected ip 30 min before test except 8-OHDPAT (10 min) and 5-MeODMT (15 min). Antagonists were injected 10 min before the test drug. ED50s, calculated as the dose in mg/kg, to reduce marble burying to 50% of that of the saline controls are given below with 95% confidence limits where log-dose/response curves were shown to be linear.

Zimeldine (7.14 [1.5-33.5]), citalopram (12.5), fluvoxamine (3.96), indalpine (4.89), fenfluramine (3.15 [.22-42]), DOI (.18 [.004-7.9]), mCPP (.58 [.13-2.6]) and TFMPP (.46 [.35-5.9]) all reduced marble burying at doses which did not affect locomotion. 5-HTP (7.2), 5-MeODMT (2.47), 8-OHDPAT (2.13) and gepirone (10.06) reduced both marble burying and locomotion at all effective doses. RU24969 (.60 [.21-1.5]) reduced marble burying while increasing motor activity. Buspirone was ineffective below 20 mg/kg, a dose which also reduced locomotion. Ipsapirone (1-20 mg/kg) was without effect on marble burying.

Ritanserin (10 mg/kg), and ICI 169369 (10 mg/kg) prevented DOI-induced inhibition of burying, but together with ICS 205-930 (10 mg/kg) or pindolol (5 mg/kg) these agents not only failed to abolish the response to zimeldine, but actually increased zimeldine inhibition of burying (eg marbles buried: saline 5.3 (s.e.m. 1.2), zimeldine 3 mg/kg 4.0 (1.4), ritanserin 5 mg/kg 8.7 (2.3), zimeldine 3 mg/kg + ritanserin 5 mg/kg 1.3 (0.4) ($p<0.01$). The drug combinations did not affect locomotion.

The potent activity of DOI, mCPP and TFMPP in selectively inhibiting burying suggests the possible involvement of 5-HT_{1C} receptors (Hoyer, 1988) in this effect, without eliminating the possibility that 5-HT_2 and 5-HT_{1B} receptors are also involved. Multiple receptor involvement may explain the potentiating effects of the selective antagonists on zimeldine inhibition of burying.

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Broekkamp C L & Jenck F (1987). Behavioural Pharmacology of Serotonin, International Congress, Amsterdam, S12.
Hoyer D (1988). Trends Pharmac. Sci., 9, 89-94.

THE EFFECT OF 5-HT ON RAT PIAL ARTERIOLES *IN VIVO*.

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In vitro studies have shown 5-hydroxytryptamine (5-HT) to be a potent constrictor of large cerebral arteries in a number of animal species including man (Edvinsson et al., 1983). However, there is evidence from studies using direct and indirect methods of assessment which indicate that 5-HT in vivo may cause only dilatation of smaller cerebral arteries and arterioles (Harper and MacKenzie, 1977, Saxena & Verdouw, 1985). We have therefore compared the effect in vivo of topical application of 5-HT with a number of 5-HT agonists on rat pial arterioles using a cortical cup technique.

Male Wistar rats (300-400g) were anaesthetised with thiopentone (120mg/kg i.p.). A tracheostomy was performed and the left femoral artery and vein cannulated for blood pressure measurement and drug injection respectively. The heart rate was measured from the E.C.G. A craniotomy was performed over the left parietal cortex and dura incised to expose the underlying cortex. Pial vessels on the surface of the cortex were irrigated with artificial CSF (pH = 7.3) and visualised using a microscope and closed-circuit television system. Individual pictures were sequentially digitised and computer enhanced on-line and the diameter of the selected vessel automatically measured at predetermined intervals by the appropriate software. 5-HT or one of the following agonists 5-carboxamidotryptamine (5-CT), (+)α-methyl-5-HT (α-Me-5-HT), GR43175 (prepared at W.R.L.) was then applied directly on to the vessel in incremental doses (1.25-20nmol) from five-barrelled micropipettes (overall tip size of 20 µm). One barrel always contained artificial CSF. This method of application produced no significant effect on either systemic blood pressure or heart rate.

		% change of initial diameter (mean + s.e.m.)				
	n	Control	2.5 nmol	5nmol	10nmol	20nmol
5-HT	5	-0.3±0.7	4.7±0.4**	7.5±0.7**	12.7±0.9**	17.6±0.7**
α-Me-5-HT	3	-0.6±1.4	4.0±0.8	6.2±0.6**	12.7±0.7**	15.3±2.1**
5-CT	4	-0.6±0.5	-0.7±1.3	-2.4±0.7	0.3±1.2	6.6±1.3 **
GR43175	3	-1.5±0.9	-8.1±2.6*	-11.2±2.3**	-14.4±3.5*	

*p<0.05, **p<0.01 Average initial arteriole diameter = 49.5± 1.2 µm.

Preliminary studies showed that on small extraparenchymal arterioles (diam.<20µm) 5-HT had little or no effect. Similarly, on the large vessels (diam.>90µm) 5-HT produced variable and inconsistent responses in that small dilatations and constrictions were noted in the same preparation. However, on vessels with a diameter between 20 - 90µm, 5-HT always produced dose-related dilatation. This effect was produced in a dose range higher than that usually associated with in vitro bioassay experiments and always followed a slight delay (12-20 sec). Similarly, α-Me-5-HT produced only dilatation of arterioles 20 - 90 µm in diam., being approximately equipotent with 5-HT. 5-CT also induced dilatation but only at doses 5-7 times higher than those of 5-HT. In contrast, the 'atypical' 5-HT$_1$-like agonist GR43175 (Humphrey et al, 1988) caused the constriction of those vessels which respond to 5-HT with a dilatation. The relative order of potency of the compounds studied suggests that the dilatatory effect of 5-HT is mediated through a receptor similar to that described by Ieff et al, (1987) and Martin et al, (1987) which induces endothelium dependent relaxation of rabbit jugular vein.

Edvinsson, L. et al. (1983) In Vascular Neuroeffector Mechanisms Eds. J.A.Bevan p251-258.
Harper, A.M. & MacKenzie, E.T. (1977) J. Physiol. (Lond) 271, 735-746.
Saxena, P.R. & Verdouw P.D. (1985) Br. J. Pharmac. 84, 533-544.
Humphrey, P.P.A. et al. (1988) Br. J. Pharmac. 94, 1123-1132.
Ieff P. et al (1987) Br. J. Pharmac. 91, 321-331.
Martin G.R. et al (1987) N.S. Arch. Pharmac. 336, 365-373.

ACTIVATION OF THE 5-HT$_2$ RECEPTOR SUBTYPE INCREASES RAT PLASMA ACTH CONCENTRATION

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1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI) is a potent hallucinogen which is reported to function as a selective agonist at the 5-HT$_2$ receptor (Glennon, 1986). Because of the implication of this receptor subtype in the mediation of the effects of psychotomimetic drugs, as well as its association with a number of psychiatric illnesses, the search for physiological markers of 5-HT$_2$ receptor subtype activation is of interest. McCall and Harris(1988) have examined the effects of DOI on the frequency of sympathetic nerve discharge (SND) in anaesthetized cats, and concluded that 5-HT$_2$ activation was responsible for measured increases in SND, since DOI shows high affinity at 5-HT$_2$ sites and the effects were reversed by the 5-HT$_2$ antagonists ketanserin and LY 53857. However, Hoyer and Karpf (1988) have recently shown that DOI has a high affinity for 5-HT$_{1C}$ receptors as it is a potent displacer of both [^{125}I]-SCH 23982 and [^{3}H]-mesulergine; compounds with high 5-HT$_{1C}$ affinity. Taken together, these data suggest the possibility of an important role for the 5-HT$_{1C}$ receptor subtype in mediating some effects of DOI which heretofore have been ascribed to the 5-HT$_2$ receptor subtype. We have thus investigated the plasma ACTH response to DOI in the rat, with the aim of distinguishing the role of 5-HT$_2$ from 5-HT$_{1C}$ receptors in the mediation of measured changes.

Male Sprague-Dawley rats (200$\pm$50g.) were sacrificed by cardiac puncture under halothane anesthesia 30 minutes after s.c. injection with DOI or vehicle (n=5-7 per dose). DOI significantly increased plasma ACTH in a dose-dependent manner (ED$_{80}$=0.8 mg/kg). The ED$_{80}$ dose was used in subsequent experiments which consisted of the s.c. administration of antagonist or vehicle at time zero, followed by s.c. administration of DOI at 30 minutes, and cardiac puncture at 60 minutes. Rat plasma ACTH was measured by two-site immunoradiometric assay (Euro-Diagnostics BV, Apeldoorn, Holland) (See Gilbert, et al., 1988).

The ACTH response to DOI was blocked by the 5-HT$_2$ receptor antagonists ICI 170,809, mesulergine, spiperone, and ketanserin, whilst the 5-HT$_{1A}$/5-HT$_{1B}$ receptor antagonist, (-)-pindolol, had no effect (Table 1).

Table 1: Effects of 5-HT Antagonists on the Plasma ACTH Response to DOI in the Rat.

Drug	*ED$_{50}$*	*Dose Range (mg/kg)*	*N (per dose)*
Spiperone	.009	0, .01, .05, .25, 1.25	7
Mesulergine	.014	0, 0.01, .05, 0.1, 1.00	4-9
Ketanserin	.064	0, .01, .05, .25, 1.25	7
ICI 170,809	.29	0, .01, .05, .25, 1.25	9
(-)-Pindolol	>5	0, 0.50, 1.00, 2.00, 5.00	9

The lack of demonstrable inhibition by (-)-pindolol, a drug with high affinity at 5-HT$_{1A}$/5-HT$_{1B}$ sites but not 5-HT$_{1C}$/5-HT$_2$ sites effectively minimizes the former receptor subtypes' importance, as well as beta-receptor involvement in the mediation of the DOI effect on ACTH. Additionally, spiperone has approximately 600-fold greater affinity for the 5-HT$_2$ than for the 5-HT$_{1C}$ site (Hoyer, 1988), and ketanserin, which shares nanomolar affinity at the 5-HT$_2$ site but not at dopaminergic sites, also inhibited the DOI-mediated ACTH increase. Thus it appears that 5-HT$_2$ receptor stimulation is more important than that of the 5-HT$_{1C}$ receptor in the production of the DOI-induced ACTH response.

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Gilbert, F., Dourish, C.T., Brazell, C., McClue, S., & Stahl, S.M.(1988) *Psychoneuroendocrinology*, 13, 471-478.
Glennon, R.A.(1986) *Life Sciences*, 39, 825-830.
Hoyer, D.(1988) *Journal of Receptor Research*, 8(1-4), 59-81.
Hoyer, D., & Karpf, A.(1988) *European Journal of Pharmacology*, 150, 181-184.
McCall, R.B., & Harris, L.T.(1988) *European Journal of Pharmacology*, 151, 113-116.

COMPARISON OF DOI-INDUCED SCRATCHING AND HEAD-TWITCH BEHAVIOURS: DIFFERENTIAL ACTION OF 8-OH-DPAT

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We have previously shown that DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane), a selective 5-HT_2/5-HT_{1C} agonist (Hoyer, 1988) produces scratching as well as head-twitching behaviour in mice (Heaton et al., 1988). Head-twitching is thought to be mediated by 5-HT_2 receptors and is powerfully modulated by a variety of other transmitters (Handley & Singh, 1986). We have therefore begun to investigate whether DOI-induced scratching resembles (head-) twitching in its pharmacology.

Male MF1 mice (20-30g) received DOI ip and were videotaped in groups of 3 over the next 10 minutes (data is for at least 6 animals/dose group). Twitch frequency was counted as previously (Handley & Singh, 1986), scratching was recorded as number of bouts in 10 min. Each bout lasted for 1-3 sec and consisted of continous rapid scratching of the flank or abdomen by the ipsilateral hindlimb. Other drugs were injected ip 30 min before DOI unless otherwise stated.

Dose-related scratching occurred between 1-10 mg/kg DOI compared with 0.1-1.0 mg/kg for head-twitching. 2.5 mg/kg DOI was therefore used to induce scratching and 0.5mg/kg for twitching. Ritanserin antagonised both twitching (ID_{50} 0.24 [.05-.86] mg/kg) and scratching (ID_{50} 0.03 [.0001-2.93] mg/kg).

Twitching and scratching were similarly affected by FLA63 40 mg/kg (-4h) (62% and 95% inhibition respectively) and clonidine 0.05 mg/kg (97% and 61% inhibition), although idazoxan (0.5 mg/kg) potentiated twitching (to 134%) but not scratching (90%). The chief difference was found in the response to the 5-HT_{1A} agonist 8-OHDPAT (0.1-1.0 mg/kg) which potentiated scratching but inhibited twitching (eg 8-OHDPAT 1.0 mg/kg increased scratching to 188% control and reduced twitching to 25% control).

In view of the low dose of ritanserin required to antagonise it, the scratching response to DOI is likely to be mediated by 5-HT_2 receptors. Scratching appears to resemble head-twitching in its modulation by noradrenergic mechanisms but the two behaviours contrasted sharply in their response to the 5-HT_{1A} agonist 8-OHDPAT. Inhibition of DOI head-twitching by 8-OHDPAT has previously been reported by Arnt & Hyttel (1989). Since the same dose of 8-OHDPAT had opposite effects on twitching and scratching, it is likely that simple behavioural interference by the '5-HT syndrome' can be eliminated as a cause of these interactions. This effect of 8-OHDPAT also suggests that 5-HT_2 receptor effects are not all modulated in the same way by 5-HT_{1A} receptor activation.

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Handley, S. L. & Singh, L. (1986) Trends Pharmac. Sci. 7, 324-8
Heaton, J. C. P. Njung'e, K. & Handley, S. L. (1988) Br. J. Pharmac. 94, 388P
Hoyer, D. (1988) Trends Pharmac Sci. 9, 89-94
Arnt, J. & Hyttel, J. (1989) Eur. J. Pharmac. 161, 45-51

CHRONIC ADMINISTRATION OF THE 5-HT$_2$ ANTAGONIST RITANSERIN PRODUCES ELEVATION OF 5-HT-MEDIATED PI HYDROLYSIS

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Chronic administration of 5HT$_2$ antagonists eg, ritanserin, causes "down-regulation" of 5HT$_2$ receptors in rat cerebral cortex (Leysen et al, 1986; Twist et al, 1988). This antagonist induced "down-regulation" is unusual and the mechanisms involved are unclear. We hypothesised that chronic administration of an antagonist would cause "up-regulation" of the receptor-linked 2nd messenger system and that the reported receptor "down-regulation" is a direct effect on the receptor rather than one mediated by the 2nd messenger system. As 5HT$_2$ receptors are linked to phosphoinositide hydrolysis (Conn and Sanders-Bush, 1984), this 2nd messenger system was examined by measuring inositol monophosphate (IP$_1$) accumulation in brain slices.

Wistar rats (350 g) received ritanserin (10 mg/kg/day) or vehicle (tartaric acid, 50 mM, pH 5) for 28 days (Twist et al, 1989). After a 3 day drug free period, animals were sacrificed and frontal cortices were cross-chopped into 300 x 300 µM prisms using a McIllwain chopper. Slices were preincubated with [^{3}H]myo-inositol (50 µCi) in oxygenated Tyrodes buffer for 1 hour before incubation in Tyrodes buffer containing LiCl (10 mM) +/- 5HT (1 µM, 100 µM or 1 mM) for 1 hour at 37°C. Reactions were stopped with trichloroacetic acid (15%). Samples were washed with diethylether (x 3) and [^{3}H]IP$_1$ was extracted from the aqueous phase by ion exchange chromatography (Berridge, 1983). Free inositol was eluted using 12 x 1 ml H_2O and [^{3}H]IP$_1$ was eluted using 12 x 1 ml ammonium formate (200 mM) in formic acid (100 mM). Samples were counted in a scintillation counter using Optiphase Hisafe III (12 ml). Data on IP$_1$ accumulation is shown below.

	5HT (1 µM)	5 HT (100 µM)	5HT (1 mM)
Control	118.44 ± 5.77(9)	124.21 ± 3.59(14)	114.80 ± 10.47(5)
Ritanserin	124.33 ± 8.22(9)	139.79 ± 5.30(14)*	175.80 ± 41.97(5)

Values are expressed as % change from corresponding basal levels (100%) ± SEM (paired t-test - *$p<0.05$)

There was no difference in the basal levels of [^{3}H]IP$_1$ accumulation in the controls compared to the ritanserin treated animals. In the presence of 5HT however, the accumulation of [^{3}H]IP$_1$ was greater in the ritanserin group compared to controls and at 100 µM 5HT, this difference was statistically significant ($p<0.05$).

The results support our hypothesis that the ritanserin mediated "down-regulation" of receptors is a direct effect but that the overall effect of chronic administration of a receptor antagonist is a supersensitivity in the system.

Leysen, J.E., Van Gompel, P., Gommeren, W., Wostenborghs, R. and Janssen, P.A.J. (1986) Psychopharmacol. 88: 434-444.
Twist, E.C., Hirst, A.D., Brazell, C., Stahl, S.M. and Campbell, I.C. (1989) Brit. J. Pharmacol. 96: 301p.
Berridge, M.J. (1983) Biochem. J. 212: 849-858.
Conn, P.J. and Sanders-Bush, E. (1984) Neuropharmacol. 23: 993-996.

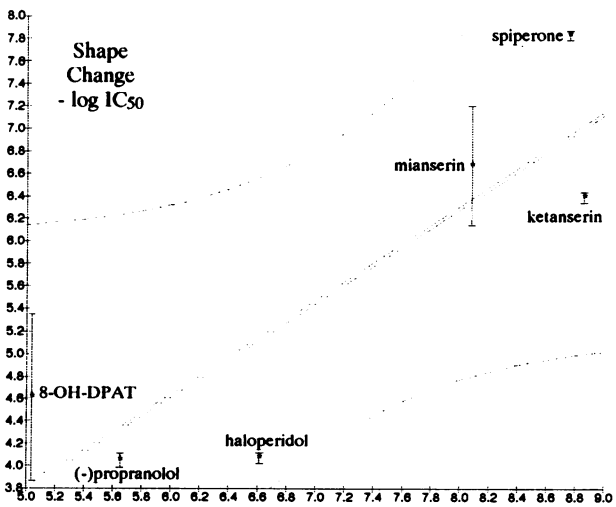
5-HT-INDUCED PLATELET SHAPE CHANGE IN THE RAT: POTENCY OF INHIBITION CORRELATES WITH 5-HT$_2$ BINDING AFFINITY

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The platelet has been proposed as a model for the serotonergic neuron because, like the neuron, there exists a mechanism for active transport of 5-HT, the subcellular vesicular storage of 5-HT, and the presence of a 5-HT transporter which is imipramine sensitive (Pletscher and Laubscher, 1980). Importantly, a receptor of the 5-HT$_2$ subtype is also demonstrable in neurons and platelets both in man and in animals including the cat and rabbit (see De Clerck and Herman, 1983 (review)). Stimulation of these receptors in vitro leads to specific, measureable physiological events like platelet shape change which may be followed by platelet aggregation (Graf and Pletscher, 1979; Peters and Grahame-Smith, 1980). Despite the abundance of existant data regarding the binding affinity of 5-HT antagonists to rat membrane preparations, the functional utility of 5-HT-induced rat platelet shape change has heretofore not been characterized.

Male Sprague-Dawley rats (250-300g.) were sacrificed by cardiac puncture under halothane anaesthesia yielding a volume of 12 ml whole blood. Blood was collected via a 16 g needle into a plastic syringe preloaded with 1 ml acid citrate dextrose anticoagulant, and then split into equal volumes in capped plastic tubes for centrifugation at 40 x g. for 15 minutes. The platelet-rich plasma supernatant (PRP) was collected into a plastic tube, covered and incubated at 37°C pending the completion of a second 15 min. centrifugation of the red cell fraction at 400 x g. which yielded platelet-poor plasma supernatant (PPP). A sample of PRP was counted using a Scquoia-Turner Cell-Dyn 900 semi-automated counter, and then diluted with autologous PPP to 200ul aliquots with platelet concentration of 350,000/ml in the micro-volume siliconized glass cuvettes of a PAP-4 platelet aggregometer (Bio-Data Corp.). The aggregometer was connected via the analogue output to an IBM PC, and voltages generated by the aggregometer light transducer sampled as previously described (Brazell, et al., 1988).

FIGURE 1: 5-HT-Induced Shape Change in Rat Platelets v. Binding Affinity at the 5-HT$_2$ Receptor



5-HT$_2$ antagonists were added in volumes of 2.0 ul, 90 seconds prior to addition of 10^{-5} M 5-HT, a previously determined EC$_{100}$ (2.0 ul). Measured platelet shape change was expressed as a percentage of control values (5-HT alone), and dose-response curves from at least 3 animals were pooled for the determination of drug IC$_{50}$ values.

The 5-HT antagonists tested appear in Figure 1. A significant correlation exists between the potency of inhibition of 5-HT-induced platelet shape change and these antagonists' binding affinity at the cortical 5-HT$_2$ ($r = .75$, $p < .03$), but not at 5-HT$_{1A}$, 5-HT$_{1B}$, or 5-HT$_{1C}$ binding sites (Hoyer, 1988).

Thus, it appears that 5-HT-induced platelet shape change is a useful model for the assessment of 5-HT$_2$ mediated events in the rat.

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Brazell, C., McClue, S., & Stahl, S.M.(1988) *Br. J. Pharmac.* 93, 189P.
De Clerck F.F., & Herman, A.G.(1983) *Fed. Proc.* 42, 228-232.
Graf, M., & Pletscher, A.(1979) *Br. J. Pharmac.* 65, 601-608.
Hoyer, D.(1988) *J. Receptor Res.*, 8(1-4), 59-81.
Peters, J.R. & Grahame-Smith, D.G.(1980) *Eur. J. Pharmac.* 68, 243-256.
Pletscher, A., & Laubscher, A.(1980) *J. Neural Transm., Suppl.* 16, 7-16.

α_2-ADRENOCEPTORS AND K-OPIATE RECEPTORS DECREASE ^{45}Ca ENTRY INTO RAT CORTICAL SYNAPTOSOMES BY CLOSURE OF N-TYPE CA^{2+} CHANNELS

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In adult rat brain cortical synaptosomes prepared by the method of Dunkley et al (1988), clonidine (0.1-100μM) and the selective K-opiate agonist U50488H(1-100μM) signigficantly reduced ^{45}Ca uptake in a time- and dose-dependent manner. In physiological medium, the maximum inhibition (50% and 55% at 100μM clonidine and U50488H) occurred after 2 min: IC_{50} values were 80nM and 90nM, respectively.

The L-type Ca^{2+} channel antagonist nifedipine(1μM) had no significant effect on ^{45}Ca influx in physiological medium (containing 5mM K^+), though there was an approximately 20% decrease in the presence of high concentrations of the drug(100μM). Nifedipine, however, caused a significant blockade of ^{45}Ca entry in medium containing 10-15mM K^+, demonstrating that L-type Ca^{2+} channels on synaptosomes are operational when the membrane is depolarised: under these conditions, there were additive inhibitory effects on ^{45}Ca entry into synaptosomes when clonidine(1μM) and nifedipine (1μM) or U50488H(1μM) and nifedipine(1μM) were co-incubated.

Treatment of synaptosomes in physiological medium with the N- and L-type Ca^{2+} channel blocker w-conotoxin fraction GVIA(w-CgTx,0.5μM) (Olivera 1984), resulted in a 35% reduction in ^{45}Ca uptake. w-CgTx(0.5μM) or w-CgTx(0.5μM) plus the α_2-adrenergic antagonist idazoxan(1μM) or plus the low affinity K-opiate antagonist naloxone(20μM) abolished the inhibitory effects of clonidine or U50488H on ^{45}Ca uptake, although idazoxan or naloxone alone did not alter the blockade of L-type Ca^{2+} channels caused by nifedipine.

The data supports our earlier studies (Adamson et al 1987; Xiang et al 1988) and demonstrates that under depolarising but not resting conditions, there are functional L-type Ca^{2+} channels on CNS nerve endings. Secondly, the results show that clonidine and U50488H decrease $[Ca^{2+}]_i$ by preventing Ca^{2+} entry into nerve terminals and suggest that these inhibitory effects occur by closure of N- but not L-type Ca^{2+} channels. The data provide a biochemical rationale for the effects of α_2-adrenergic agonists and K-opiate agonists on neurotransmitter release and possibly for their mode of action as sedative as well as hypotensive and antinociceptive drugs, respectively.

Adamson, P. et al. (1987) Eur.J.Pharmac. 142:261-266.
Dunkley, P. R. et al. (1988) Brain Res. 441:59-80.
Olivera, B. M. et al. (1984) Biochem. 23:5087-5092.
Xiang, J-Z. et al. (1988) Br.J.Pharmac. 95:597p.

PRESYNAPTIC MUSCARINIC CHOLINOCEPTORS INCREASE $[CA^{2+}]_i$ BY PROTEIN KINASE C-DEPENDENT OPENING OF L-TYPE CA^{2+} CHANNELS

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Stimulation of muscarinic acetylcholine receptors (mAChR) may increase intracellular free calcium ($[Ca^{2+}]_i$) in neuroblastoma cell lines (Lambert et al., 1989) and inhibit calcium currents in certain neurones (Wanke et al., 1987). Some subtypes of mAChRs are coupled to inositolphospholipid hydrolysis, while other inhibit adenylate cyclase (Bonner, 1989, for review).

As studies have demonstrated that phorbol esters (by mimicking the action of diacylglycerol on protein kinase C) increase $[Ca^{2+}]_i$ in synaptosomes via opening of an L-type calcium channel (Adamson et al., 1989) and that presynaptic muscarinic receptors hydrolyse phosphoinositides (Brammer et al., 1988), we examined the effect of carbachol mediated stimulation of presynaptic mAChR on $[Ca^{2+}]_i$.

Using fura-2 loaded synaptosomes from rat cerebral cortex (Adamson et al., 1989), we observed that carbachol (5 min incubation) produced a dose-dependent (1-100µM) increase in synaptosomal $[Ca^{2+}]_i$ with a maximum response of 22% and an EC_{50} of 58µM. The effect of carbachol (100µM) on $[Ca^{2+}]_i$ was antagonised by atropine with an IC_{50} of 200nM.

The rise in $[Ca^{2+}]_i$ produced by carbachol (100µM) was effectively blocked by the L-type calcium channel blocker nifedipine: the IC_{50} for nifedipine was 13nM.

Carbachol (100µM) induced increases in $[Ca^{2+}]_i$ was reduced if the synaptosomes were preincubated for 30 minutes with the protein kinase inhibitor H7 (Hidaka et al., 1984), (50µM): 4.0±1.4% increase (n=4) as opposed to 17.3+2.8% (n=4;solvent chloroform) in the control or with the more selective protein kinase C inhibitor staurosporine (Tamaoki et al., 1986), (1µM): 3.7+3.7% increase (n=3), control 20.0+1.5% (n=12; solvent DMSO). Values are ± SEM

These results support the conclusion that (in a manner analogous to the effect of phorbol esters) stimulation of muscarinic acetylcholine receptors in synaptosomes from rat cerebral cortex increases $[Ca^{2+}]_i$ by protein kinase C dependent activation of L-type calcium channels.

Adamson, P., Hajimohamadreza, I., Brammer, M. J., & Campbell, I. C. (1989) Eur.J.Pharmacol., 162, 59-66.
Bonner, T. I. (1989) Trends Neurosci. 12, 148-151.
Brammer, M. J., Hajimohammadreza, I., Sardiwal, S., Weaver, K. (1988) J.Neurochem. 51, 514-521.
Hidaka, H., Inagaki, M., Kawamoto, S., Sasaki, Y. (1984) Biochemistry, 23, 5036-4041.
Lambert, D. G. et al. (1989) Br.J.Pharmacol. 96, 132P.
Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. & Tomita, F. (1986) Biochem.Biophys.Res.Commun. 135, 397-402.
Wanke, E., Ferroni, A., Malagaroli, A., Ambrosini, A., Pozzan, T. & Meldolesi, J. (1987) Proc.Natl.Acad.Sci.USA 84, 4313-4317.

BILATERAL ABDOMINAL VAGOTOMY ABOLISHES BINDING OF [^{3}H]-BRL 43694 IN FERRET DORSOVAGAL COMPLEX

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5-HT_3 receptor antagonists are effective in treating vomiting due to radiation and cytotoxic drugs. The early descriptions of 5-HT_3 receptors were in peripheral tissues but recent attention has focussed on the existence of displaceable binding sites in the dorsal vagal complex and on the role of such "central receptors" in the vomiting response. The precise distribution of 5-HT_3 binding sites within the dorsal vagal complex is unclear. Pratt and Bowery have provided evidence that such binding sites may be located on terminals of vagal afferents in the medulla of the rat (a non-vomiting species). We have investigated the effects of bilateral abdominal vagotomy on the binding of ^{3}(H)-BRL 43694 in the dorsal vagal complex of the ferret, an animal with well characterized emetic behaviour.

Adult male ferrets with bilateral abdominal vagotomy, bilateral abdominal splanchnectomy and sham operated controls were anaesthetized 18 days after the initial surgery and perfused with saline. Brains were removed and frozen immediately. Cryostat sections were incubated in 10nM (^{3}H)-BRL 43694 (s.a. 21.2 Ci/mmol) in HEPES buffer at room temperature for 45 minutes. Adjacent sections were similarly incubated with the addition of 100μM GR 38032F. Sections were air dried and exposed to Amersham Hyperfilm for 6 weeks along with tritium standards to allow quantitative densitometric analysis.

High levels of specific binding were seen in the NTS of the 3 control animals (118.2,130.5 and 135.0 fmol/mg tissue) and in the 2 splanchnectomy-treated animals (128.4 and 121.6 fmol/mg tissue). Specific binding in the AP of controls (82.0,73.0 and 58.4 fmol/mg tissue) was similar to the splanchnectomy group (49.8 and 63.8 fmol/mg tissue). No specific binding was detectable in the NTS or AP in any of the 4 ferrets which had undergone bilateral subdiaphragmatic vagotomy.

Grant and Arvidsson have shown that lesions of peripheral branches of primary sensory neurones result in transganglionic degeneration of the central afferent projections. Since degeneration of medullary vagal afferent terminals most probably followed the vagal lesions described in this study, we hypothesise that the loss of 5-HT_3 binding sites observed was due to their location on these terminals.

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Pratt G.D. and Bowery N.G. (1989) Br. J. Pharmac.,97,414P.
Grant G. and Arvidsson J. (1975) Brain Res.,95,265-279.

GR38032F IMPROVES PERFORMANCE IN A MOUSE HABITUATION TEST, AND INHIBITS CHOLINERGIC-LINKED DEFICITS

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Studies investigating the role of 5-hydroxytryptamine (5-HT) in cognition using compounds with a non-selective action on 5-HT receptors have produced contradictory results. 5-HT receptor agonists and antagonists have either failed to influence or cause an enhancement or impairment of performance in animal tests (see Ogren et al 1981). In the present work we used the selective 5-HT_3 receptor antagonist GR38032F (Butler et al 1988) to investigate the role of 5-HT_3 receptors in moderating behaviour in a mouse habituation test.

Young adult (6 to 8 weeks old) and aged (8 to 10 months old) male albino BKW mice were used. Behavioural testing was carried out daily when mice were taken from a dark environment and placed into the centre of the white brightly illuminated area of a two compartment white and black test box. The latency of the initial movement from the aversive white area to the black section was determined from video recording. Mice received treatment with scopolamine (0.25 mg/kg i.p. b.d.), or electrolytic or ibotenic acid (IB) lesions of the nucleus basalis (NB) (the latter reduced cortical ChAT activity by 34-57%)

Table 1. The effect of GR38032F (10ng/kg i.p. b.d.) on the habituation response of young adult and aged mice and antagonism of the impairment induced by scopolamine and NB lesions

Treatment Groups	Latency to enter the dark(s)					
	day 1		day 3		day 6	
	Vehicle	GR38032F	Vehicle	GR38032F	Vehicle	GR38032F
Young adult mice	12.0±1.3	10.1±1.1	11.6±1.7	<1.0**	2.2±0.3	<1.0**
+ Scopolamine	13.1±1.6	10.2±0.8	16.6±2.1	2.4±0.4**	18.0±1.9	<1.0**
+ Electrolesion NB	12.5±1.4	9.0±0.8	10.1±1.3	3.8±0.4**	12.9±1.3	<1.0**
+ IB lesion NB	11.2±1.3	7.8±0.9	19.3±2.0	4.0±0.4**	17.1±1.8	<1.0**
Aged mice	9.9±1.2	4.5±0.6*	9.6±1.3	1.7±0.2**	9.4±1.0	1.1±0.2**

n = 10. <1.0s = latency less than time taken to operate remote video system. *P<0.05, **P<0.001 (decreased latency compared to vehicle, Dunnett's t test).

Mice normally habituate to the test situation over a 4 to 6 day period, and this is measured as decreased latency to enter the dark. Mice treated with GR38032F (10ng/kg i.p. b.d.) habituate more rapidly (see data for day 3). Scopolamine treatment or NB lesions impair habituation (see data for day 6). This impairment is inhibited by GR38032F, and the improved habituation can be seen on days 3 and 6 (Table 1). Aged mice similarly fail to habituate except when treated with GR38032F and improved habituation can be seen on days 1, 3 and 6 (Table 1). It is concluded that GR38032F can improve basal performance of young and aged mice in the habituation test and inhibit the impairments caused by a cholinergic deficit. This may correlate with the ability of the 5-HT_3 receptor antagonists to prevent the inhibitory action of 5-HT on acetylcholine release (Barnes et al 1989).

Barnes, J.M. et al (1989) Nature in press
Butler, A. et al (1988) Br. J. Pharmac. 94: 397-412
Ogren, S. et al (1981) Adv. Expl. Med. Biol. 133: 681-705
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SURFACE N-WAVE INHIBITION BY OXOTREMORINE-M IS MEDIATED BY PRE-SYNAPTIC M1 RECEPTOR STIMULATION IN GUINEA-PIG OLFACTORY CORTEX

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Radioligand binding studies have demonstrated the existence of more than one type of muscarinic binding site. According to these results, high affinity sites for pirenzepine were shown in the higher structures of the brain, superior cervical ganglia and in the enteric nervous system and were called M_1 receptors whereas low affinity sites were identified in atrial and ileal tissues, and named M_2 receptors (Hammer et al, 1980). These data were confirmed by autoradiographic studies (Wamsley et al, 1984; Spencer et al, 1986). Recently, using an extracellular recording technique, Williams & Constanti (1988) described the inhibitory effects of muscarinic agonists on the surface-negative field potential (N-wave) evoked by lateral olfactory tract (LOT) stimulation in guinea-pig olfactory cortex slices maintained in vitro. These inhibitory effects appeared to be mediated via presynaptyc M_1 muscarinic receptor stimulation since pirenzepine, but not gallamine, antagonized them in a potent and competitive fashion. Moreover, oxotremorine, was found to be a weak competitive antagonist on this system. Oxotremorine-M, a quaternary analogue of oxotremorine, has been reported to be a potent muscarinic agonist (Bebbington et al, 1966) and stimulant of brain PI hydrolysis, an effect also induced by carbachol (CCh) (Fisher et al, 1984). The aim of the present study was to test the effects of OXO-M on the evoked N-wave amplitude, recorded in guinea-pig olfactory cortex slices.

Bath superfusion (2 min) with OXO-M (5-80 uM; n=8 slices) produced a dose-dependent reduction of the N-wave amplitude, without affecting the LOT compound action potential. The peak effect was observed approx. 7 min after the drug application and complete recovery was usually obtained 45 min after. In comparison with CCh (5-300 uM; n=12 slices), OXO-M was 4.6 times more potent in reducing the N-wave amplitude (IC_{50}=87.9±2.4 and 19.2±1.5 uM, respectively) (means±s.e.m.). Telenzepine (5-100 nM), a new selective M_1 antagonist, competitively antagonized the effects of OXO-M (pA_2=7.91; n=9 slices) and of CCh (pA_2=8.19; n=12 slices), whereas methoctramine (300 nM) and AF-DX 116 (up to 500 nM), two cardio-selective M_2 antagonists, were ineffective in this respect.

In conclusion, our experiments suggest that OXO-M inhibits the surface-negative field potential by stimulating presynaptic M_1 muscarinic receptors in the olfactory cortex slice.

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Bebbington, A. et al (1966) Br. J. Pharmacol. 26, 56-67.
Fisher, S.K. et al (1984) J. Neurochem. 43, 1171-1179.
Hammer, R. et al (1980) Nature 283, 90-92.
Spencer, D.G. et al (1986) Brain Res. 380, 59-68.
Wamsley, J.K. et al (1984) Life Sci. 34, 1395-1402.
Williams, S.H. & Constanti, A. (1988) Br.J.Pharmacol. 93, 855-862.

ANTI-CHOLINERGIC AND ANTI-HISTAMINIC PROPERTIES OF CHLORPROMAZINE AND FIVE METABOLITES DETERMINED ON GUINEA-PIG ILEUM

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Chlorpromazine (CPZ) has numerous pharmacological properties, including antagonism at muscarinic and H-1 receptors (Courvoisier et al 1953). The compound is extensively metabolised and some of the metabolites are pharmacologically active. To see to what extent metabolites might contribute to the anti-cholinergic and anti-histaminic properties, and to investigate any structure activity relationships that might exist, pA_2 values were determined using guinea-pig ileum.

7-Hydroxychlorpromazine (7OHCPZ), monodesmethylCPZ (MDCPZ), didesmethylCPZ (DDCPZ), CPZ amine oxide (CPZNO) and CPZ sulphoxide (CPZSO) were provided by Dr A.A.Mainian, NIH, Bethesda, USA. Octanol partition coefficients (P) were obtained using standard methods (Leo et al 1963). Ionisation constants of CPZ, MDCPZ and DDCPZ were determined from their limiting solubilities and the pKa for CPZSO determined spectrophotometrically (Albert & Serjeant, 1962). pH-Partitioning into heptane was used to derive the pKa for 7OHCPZ (Whelpton, 1989).

Table 1.: Physicochemical properties and pA_2 values for CPZ and 5 metabolites.

Compound	Log P	pKa	pA_2 (s.d.)*	
			Acetylcholine	Histamine
CPZ	5.30	9.3	6.29 (0.08)	7.75 (0.05)
7OHCPZ	4.63	9.2	5.91 (0.08)	7.71 (0.14)
MDCPZ	4.94	10.2	5.79 (0.11)	6.61 (0.18)
DDCPZ	4.47	9.9	5.45 (0.08)	5.61 (0.22)
CPZNO	2.73	4.7	5.19 (0.04)	6.59 (0.09)
CPZSO	2.32	9.0	4.91 (0.10)	6.61 (0.07)

* n=5

None of the metabolites was more potent than the parent compound. The anti-cholinergic properties and octanol partition coefficients correlated ($r = 0.92$). Analysis of variance (Goodford, 1973) showed this to be highly significant, $F(1,4) = 22$, $p<0.01$. No such relationship existed for the anti-histaminic properties. However, demethylation had a marked effect, the potency fell by a factor of approximately 10 for each N-methyl group removed. This does not appear to be due to differences in pKa. The more polar CPZNO and CPZSO were also less potent than the parent compound.

Albert, A. & Serjeant, E.P. (1962) Ionisation Constants of Acids and Bases, Methuen & Co. Ltd., London.
Courvoisier, S., et al (1953) Arch. Int. Pharmacodyn. 92, 305-361.
Goodford, P.J. (1973) Adv. Pharmacol. Chemother. 11, 51-97.
Leo, A., Hansch, C. & Elkins, D. (1971) Chem. Rev. 71, 525-553.
Whelpton, R. (1989) Trends Pharmacol. Sci. 10, 182-183

REGIONALLY SELECTIVE EFFECTS OF CHRONIC NICOTINE ON RAT BRAIN TYROSINE HYDROXYLASE ACTIVITY: A TIME-COURSE STUDY

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The cholinergic agonist nicotine (NIC) stimulates the release of catecholamines from in vitro preparations of various areas of rat brain (e.g. Westfall et al. 1974). Given in vivo acutely, it stimulates dopamine release and synthesis in the nucleus accumbens (n.acc), and catecholamine synthesis in the hypothalamus and hippocampus (Mitchell et al. 1987, Brazell et al. 1989). Chronic pretreatment potentiates this effect selectively in the hippocampus (Mitchell et al. submitted). 28 days treatment also increases the activity of tyrosine hydroxylase (TH) selectively in this area, although acute nicotine is without additional effect on TH (Joseph et al. submitted). A time course study of the effects of chronic NIC on TH activity in this area, and various related catecholaminergic areas was therefore undertaken.

Separate groups (n=7) of male Sprague-Dawley rats (Haarlan-Olac), initial weight 250-300g, received daily injections of nicotine bitartrate (0.8 mg/kg s.c. as free base) neutralised in saline, or the vehicle alone for 3, 7, 14, 21 or 28 days. Sacrifice was 24h later; brain regions were dissected from 1.5-2.0 mm coronal slices on ice and immediately frozen pending analysis. TH activity was assayed with saturating concentrations of co-factor (6MPH4) and ascorbate as reductant (Lerner et al. 1978), and alumina separation of DOPA produced (Hendry and Iversen, 1971).

The activity in dopaminergically innervated areas (bed nucleus, caudate, n.acc) was unaffected by NIC at any time point studied. In noradrenergically innervated areas, TH was significantly increased ($p<0.02$ or better). The increase was maximal at 7 days (207% of control) in the cerebellum, at 21 days (147%) in the hypothalamus and reached 205% and 131% of control at 28 days in the hippocampus and frontal cortex respectively. It was also significantly increased (223% of control), but only at 7 days, in the locus coeruleus. This pattern is very similar to that reported after single dose reserpine (Zigmond, 1979) or repeated stress, and is consistent with increased enzyme synthesis in the cell bodies, followed by transport to the terminals. Although the site of action of this effect of NIC remains to be determined, it may be important in the behavioural adaptation that occurs to repeated NIC administration in rats (Stolerman et al, 1973), and indeed in man.

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Brazell, M.P. et al (1989) Br.J.Pharmac. 96, 339P
Hendry, I.A. and Iversen, L.L. (1971) Brain Res. 29, 159-162
Lerner, P. et al (1978) Neurochem.Res. 3, 641-651
Mitchell, S.N. et al (1987) Br.J.Pharmac. 91, 430P
Stolerman, I.P. et al (1973) Psychopharmac. 30,329-342
Westfall, T.C. (1974) Neuropharmac. 13, 693-700
Zigmond, R.E. (1979) J.Neurochem. 32, 23-29

IDENTIFICATION OF THE HYPOTHALAMIC RECEPTORS INVOLVED IN THE INCREASE IN BLOOD PRESSURE DURING STIMULATION OF THE C_1 AREA.

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Immunohistochemical techniques have demonstrated a population of neurones containing the adrenaline synthesizing enzyme in a restricted zone (C1 cell group) of the medulla oblongata (Hokfelt et al., 1974). Several studies have demonstrated that the adrenergic neurones in C1 are an important central vasomotor pathway (Ross et al., 1983; Goodchild et al., 1984). It is established that neurones in the C1 region project both to the brain (including the posterior hypothalamus (PH)) and to the intermediolateral region of the thoracolumbar spinal cord. It has been demonstrated that stimulation of C1 results in an increase in blood pressure (BP) which is attenuated by the central injection of a B_2 antagonist but enhanced by the central injection of an α_2 antagonist, suggesting that the increase in BP might involve B2 and α2 adrenoceptors (Ward-Routledge et al., 1988). However, these observations do not rule out the possibility that other neurotransmitters present in the C1 neurones or present in the PH could be affected by C1 stimulation and participate in the pressor effect. The present study further examines the hypothalamic neurotransmitters involved in the C1 induced increase in BP.

Anaesthetized, male Wistar rats were placed in a stereotaxic frame and an injection cannula was unilaterally implanted in the PH (rostral-caudal -3.8, lateral + 0.5, vertical -8.2 relative to bregma). A concentric needle electrode (SNE 100) was implanted into the C1 region (rostral-caudal -11.8, lateral +1.6, vertical -10.5 relative to bregma) ipsilateral to the cannula. The C1 region was electrically stimulated for 5 min (2 V, square wave pulses 1 ms, 40 Hz for 10 s every 60 s) following a 30 min stabilisation period. BP was monitored via a catheter previously implanted in the abdominal aorta through the caudal artery. 15 min after the first C1 stimulation, saline or drugs were injected i.v. or into the PH (1 μl over a period of 1 min) then 15 min later the C1 region again was stimulated. Differences between the increase in BP produced by C1 stimulation before and after saline or drug injection were examined using Student's paired t-test.

The increase in BP produced by C1 stimulation was significantly attenuated by pentolinium (1 mg/kg i.v.), propranolol (20 μg into the PH) and clonidine (8 μg into the PH). The C1 stimulation induced rise in BP was not affected by d-propranolol (20 μg), clonidine (3 μg) or idazoxan (66 μg); however the inhibition produced by clonidine (8 or 27 μg) was significantly reduced by prior administration of idazoxan (66 μg). These results confirm that stimulation of the C1 area provides excitatory drive to the preganglionic sympathetic neurones of the spinal cord and to the posterior hypothalamus with increased BP which is in part centrally modulated by α_2 and B adrenoceptors. The effect of C1 stimulation on BP was also significantly attenuated by the administration into the PH of atropine (8 μg) and methysergide (10 μg) suggesting a cholinergic and serotoninergic involvement in the pressor response. On the other hand the C1 response was not altered by cimetidine (11 μg), chlorphenydramine (12 μg) or naloxone (10 μg) excluding the participation of endogenous histamine and opioids in the pressor effect.

Goodchild, A.K. et al. (1984) Neurosci. Lett. 45, 267-272.
Hokfelt, T. et al. (1974) Brain Res. 66, 235-251.
Ross, C.A. et al. (1983) Brain Res. 273, 356-361.
Ward-Routledge, C. et al. (1988) Br. J. Pharmacol. 94, 609-619.

SUBTYPES OF α_1-ADRENOCEPTOR BINDING SITES IN HUMAN BRAIN: RADIOLIGAND BINDING AND RECEPTOR AUTORADIOGRAPHY

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Radioligand binding studies suggest that α_1-adrenoceptor recognition sites labelled by [^{3}H]prazosin are heterogeneous. Several adrenergic agents, e.g. WB-4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane), phentolamine and 5-methyl-urapidil discriminate between two α_1-adrenoceptor binding sites in various tissues of the rat (Morrow & Creese, 1986; Minneman et al., 1988; Gross et al., 1988). The sites with high and low affinity for these compounds were designated α_{1A} and α_{1B}, respectively (Morrow & Creese, 1986). Chloroethylclonidine was shown to irreversibly inactivate α_{1B}-binding sites (Minneman et al., 1988). Thus far, α_1-adrenoceptor subtypes have only been reported in experiments using rat tissues and it remained unknown whether different α_1-adrenoceptor recognition sites can also be distinguished in tissues of other species including man.

Therefore, we investigated radioligand binding to α_1-adrenoceptor recognition sites of rat and guinea pig tissues and studied α_1-adrenoceptors of human brain tissue obtained during surgery or post mortem by conventional radioligand binding techniques and receptor autoradiography. α_1-Adrenoceptors were labelled by 0.2 nM [^{3}H]prazosin, non-specific binding was defined by 10 µM phentolamine. 5-Methyl-urapidil which has a 80- to 90-fold higher affinity for α_{1A}- as compared to α_{1B}-adrenoceptor binding sites displaced [^{3}H]prazosin biphasically in rat and guinea pig cerebral cortex, hippocampus and vas deferens indicating two distinct binding sites (α_{1A} and α_{1B}) but monophasically in rat and guinea pig spleen (α_{1B}). In membranes of human cerebral neocortex, 5-methyl-urapidil also discriminated between two distinct α_1-recognition sites. In order to visualize α_{1A}- and α_{1B}-binding sites autoradiographically, [^{3}H]prazosin binding to human brain slices was determined after preincubation with chloroethylclonidine or in the presence of unlabelled 5-methyl-urapidil in a concentration which occupies about 90 % of α_{1A}-binding sites but hardly affects α_{1B}-sites. It is shown that in human hippocampus the distribution of chloroethylclonidine-resistant [^{3}H]prazosin binding sites (α_{1A}) differs from that of binding sites with low affinity for 5-methyl-urapidil (α_{1B}).

Our results demonstrate the existence of two distinct populations of [^{3}H]prazosin binding sites in brain tissue of different species including man which may represent separate α_1-adrenoceptor subtypes.

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Gross, G. et al.(1988) Eur. J. Pharmacol. 151: 333
Minneman, K.P. et al. (1988) Mol. Pharmacol. 33: 509
Morrow, A.L. & Creese, I. (1986) Mol. Pharmacol. 29: 321

CHRONIC GUANABENZ TREATMENT DOWN-REGULATES α_2-ADRENOCEPTORS IN RABBIT BRAIN

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Clonidine and guanabenz have been reported to act by stimulation of central alpha$_2$-adrenoceptors leading to a reduction in catecholamine turnover and reduced sympathetic outflow (Kobinger 1978). Prolonged activation of alpha$_2$ receptors may lead to desensitisation (Brodde et al 1982) and it has been proposed that the sympathetic hyperactivity following withdrawal of chronic clonidine treatment may be mediated by down-regulation of postsynaptic alpha$_2$-adrenoceptors in the CNS (Finberg & Kopin 1987). However decreases in alpha$_2$-adrenoceptor binding in the brain after chronic agonist treatment have been hard to demonstrate (Armstrong et al 1983), although changes in response have been observed by several groups (Finberg & Kopin 1987).

We have examined some effects of chronic guanabenz treatment on alpha$_2$-adrenoceptor number and function in the rabbit brain and periphery. Guanabenz (20 umoles/kg/day) or vehicle was adminstered for 6 days to groups of rabbits (n=6-8). Pressor responses to intravenous alphamethylnoradrenaline (alpha MeNA)(5-100 ug) and depressor responses to intracisternal clonidine (1 ug/kg) (Yakubu et al 1988) were used to study peripheral vascular and central alpha$_2$-adrenoceptor-mediated functions respectively. [^{3}H] yohimbine (1.25-37 nM) binding to tissue homogenates from rabbit forebrain, hindbrain and kidney were performed (Yakubu et al 1988) to study effects of treatment on alpha$_2$-adrenoceptor number.

Both pressor responses to alpha-MeNA and the depressor response to intracisternal clonidine were significantly attenuated by chronic guanabenz treatment. The dose of alpha-MeNA required to increase mean arterial pressure (MAP) by 30 mmHg was increased from 20±5 for controls to 65±10 ug for the treated group. The maximum fall in MAP after clonidine injection was observed at 5 mins for the treated group (-14±10 mmHg) and at 15 mins for controls (-26±15 mmHg). The falls in MAP were significantly attenuated at 15, 30, and 45 mins after injection (ANOVA). Chronic guanabenz infusion significantly reduced ($p<0.05$) [^{3}H] yohimbine binding to forebrain and hindbrain (Bmax forebrain 118±22 control and 53±18 guanabenz treated; hindbrain: 109±14 control and 60±29 fmoles/mg protein treated)(unpaired t-test). There was no change in the number of kidney binding sites nor was there any significant treatment effects on K_D in any tissues.

Thus, chronic guanabenz infusion significantly attenuated both peripheral and central alpha$_2$-adrenoceptor mediated functions. The different effects of guanabenz treatment on [^{3}H]yohimbine binding in brain and kidney could be related to the different subtypes of alpha$_2$-adrenoceptor predominating in brain and kidney or to differences in drug distribution. In addition the changes in receptor density observed in the brain could contribute to withdrawal reactions observed after abrupt cessation of treatment with centrally acting drugs.

Armstrong J.M., Atkinson J., Langer S.Z., Pimoule C. (1983). Br. J. Pharmacol. 78: 26p.
Brodde O.E, Anlauf M, Graben N, Bock K.D. (1982). Eur. J. Clin. Pharmacol. 23: 403-409.
Finberg J.P.M. & Kopin I.J. (1987). Eur. J. Pharmacol. 138: 95-100.
Kobinger W (1978) Rev. Physiol. Biochem. Pharmacol.81: 31-39.
Yakubu M.A., Hamilton C.A., Reid J.L. (1988). Brain Res. 463: 289-295.

α- AND β–ADRENOCEPTOR REGULATION OF CYCLIC AMP ACCUMULATION IN A MIXED PRIMARY ASTROGLIAL CELL CULTURE

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Two morphologically distinct cell types, which appear to represent different stages of astroglial differentiation, can be detected in primary astrocyte cultures from neonatal rat brain (Pollenz & McCarthy, 1986; Trimmer & McCarthy, 1986). The predominant cell type consists of flat polygonally shaped protoplasmic astroglial cells while the second cell type (fibrous) has short processes and grows on top of a layer of protoplasmic cells. Previous studies have indicated that rat astroglia express both α- and β-adrenoceptors which respectively inhibit and stimulate cyclic AMP accumulation (McCarthy & De Vellis, 1979; Evans et al., 1984). Here we report the effects of noradrenaline (NA) and isoprenaline (ISO) on cyclic AMP accumulation in primary cultures containing (a) only protoplasmic astroglia or (b) both protoplasmic and fibrous astroglia.

Primary cultures of neonatal brain astroglia were prepared essentially as described by (Rumsby et al., 1984) from either 1 day (for mixed protoplasmic/fibrous cultures) or 5 day (for protoplasmic cultures) old rat pups and maintained in culture for 14 days in DMEM medium supplemented with 2mM glutamine and 10 % foetal calf serum at 37°C. Cyclic AMP accumulation was measured in cells prelabelled with [^{3}H]adenine as described by Donaldson et al. (1988).

NA and ISO stimulated cyclic AMP accumulation in both protoplasmic (EC_{50}[NA] = 0.7 ± 0.2 μM, n=3; EC_{50}[ISO] = 0.5 ± 0.2 μM, n=3) and mixed fibrous/protoplasmic (EC_{50}[NA] = 0.10 ± 0.02 μM, n=4; EC_{50}[ISO] = 0.29 ± 0.07 μM, n=5) cell cultures. In the mixed fibrous/protoplasmic cultures a combination of ISO (1μM) and NA (1μM) produced a cyclic AMP response which was only 29.7 ± 4.0 % of that produced by ISO alone (n=4). This attenuation of the response to ISO was antagonised by the α_2-adrenoceptor antagonist yohimbine (1μM). Similarly, the response to 1μM NA alone in these cells was augmented by 1 μM yohimbine (from 22 ± 5 % to 70 ± 3 % of the response to 1μM ISO, $p < 0.05$, n=3) but not by prazosin (1μM). In contrast, no consistent attenuation of the response to ISO (1μM) was produced by either 1μM NA (104.2 ± 22.7 % of ISO response, n=6) or 10 μM NA (103.2 ± 17.5% of ISO response, n=4) in astroglial cultures containing only protoplasmic cells.

These data indicate that there are differences in the extent of α_2-receptor-mediated inhibition of cyclic AMP accumulation between the two primary astroglial preparations. It remains to be established, however, whether this is due to a preferential expression of α_2-receptors in the fibrous cells or to an interaction between the two different cell classes.

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Donaldson, J. et al., (1988) Biochem. Pharmacol. 37, 715-723.
McCarthy, K.D. & De Vellis, J. (1979) Life Sci. 24, 639-650.
Pollenz, R.S. & McCarthy, K.D. (1986) J. Neurochem. 47, 9-17.
Trimmer, P.A. & McCarthy, K.D. (1986) Dev. Brain Res. 27, 151-165.
Rumsby, M. et al. (1984) J. Neuroimmunol. 7, 1-20.

BRAIN β-ADRENOCEPTOR LOSS AFTER A PHENELZINE INJECTION DOES NOT INVOLVE RECEPTOR INTERNALISATION

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Studies of cells in culture have shown that exposure to β-adrenergic agonists can promote rapid receptor internalisation (Toews & Perkins, 1984). The existence of this phenomenon in brain, has however, not been established. We have investigated the rapid β-adrenoceptor down-regulation which results from acute administration of a single acute high dose of phenelzine, with the object of determining whether a rapid desensitisation/internalisation period precedes the down regulation.

Male Wistar rats (200-230g) were sacrificed 1,3,6 and 20 hours after a single injection of phenelzine (50mg/kg/ip). Specific binding to β-adrenoceptors was measured using [^{3}H]dihydroalprenolol (0.1-3nM) and non specific binding was assessed in the presence of propranolol (10μM). B_{max} and Kd values were assessed from six point saturation curves. Internalised receptors have been reported to appear in light density fractions following sucrose density centrifugation and this experimental procedure was used (Koshikawa et al., 1987). Cortical homogenates were applied to sucrose gradients (0.5-1.5M) and centrifuged to equilibrium (110,000g/16h/4°C). Two ml fractions were pumped out and analysed for specific [^{3}H]DHA binding (Burgisser & Lefkowitz, 1984).

A significant reduction (17%) (P 0.01) in β-adrenoceptor number was found in homogenates (P_2) after the acute phenelzine injection: the Kd was unchanged. In vitro studies showed that phenelzine (0.2nM-2μM) had no direct effect on [^{3}H]DHA binding.

The analysis of the density gradient data showed that there was no significant migration of specific binding sites for [^{3}H]DHA at any of the times examined ie 1,3, 6 and 20 hours after the phenelzine administration, although at 3,6 and 20 hours there was a significant decrease in specific binding in the plasma membrane fraction (ie around 0.9M sucrose).

From these studies, we conclude that down regulation is promoted by a process other than receptor internalisation.

Burgisser, E & Lefkowitz, R. J. in Brain Receptor Methodologies, Marangos, P. J. et al, Eds. Acad. Press, Florida, 1984, pp 230-252.
Koshikawa, N., Durcan, M. J., Weaver, K., Lawrence, K., & Campbell, I. C. (1987) Neuropharmacol., 26, 1346-1349.
Toews, M. & Perkins, J. P. (1984) J.Biol.Chem., 259, 11844-11850.

EFFECT OF DOPAMINE ON NEUROTENSIN-STIMULATED INOSITOL PHOSPHATE ACCUMULATION IN RAT STRIATAL SLICES

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Neurotensin (NT) receptor stimulation is coupled via phospholipase C to inositol 1,4,5-trisphosphate formation in a variety of rat brain regions, including the striatum (Goedert et al., 1984). We have previously reported (Ford et al., 1989) that NT fails to influence dopamine (DA)-stimulated adenylate cyclase activity in the same brain region; in this study, we have examined the converse situation, whether DA itself has any effect on the second messenger response to NT-receptor stimulation. Other workers have demonstrated that DA-receptor stimulation may inhibit inositol phosphate production in rat striatal slices (Pizzi et al., 1987), although this was not confirmed by Kelly and co-workers (1988). Elucidation of any such DA-NT interaction may facilitate our understanding of the mechanism by which NT inhibits cerebral DA function.

Inositol phosphate (IP) production was measured using the myo-[2-^{3}H] inositol prelabelling method of Brown et al. (1984), in cross-chopped slices (0.35mm) of striata from male Sprague Dawley rats (250-350g), in the presence of 5mM lithium chloride. Agonists (NT or carbachol) were present in the incubation for a 45 min stimulation period. Antagonists were present from the commencement of the incorporation period (40 min). Dopamine was added 10 min prior to NT or carbachol. All experiments with NT included bacitracin (28 mg/l) in the Krebs-Henseleit incubation buffer.

NT (1μM) increased basal IP accumulation to 142.7±8.4% (mean ± s.e.m., n=6; $p<0.01$) which was significantly lower ($p<0.05$) than that produced by NT in the presence of DA (100μM; 177±13.9%). The α_1-adrenoceptor antagonist prazosin (1μM), which was reported to decrease the IP response to DA in the presence of elevated KCl (Kelly et al., 1988) did not affect the response to DA plus NT. Inclusion of the D_2 receptor antagonist sulpiride (50μM) in the incubation medium was also without effect on the DA-NT synergy. To assess the specificity of this response, the effect of DA (100μM) on carbachol-induced IP accumulation was examined. It was observed that carbachol (100μM) produced an IP level of 490.5±20% of basal ($p<0.01$, n=4), and that DA (100μM) significantly reduced this to 420±24% ($p<0.05$). In the presence of sulpiride (50μM) this was further lowered to 391±14% ($p<0.01$).

We conclude that DA can potentiate NT-induced phosphoinositide hydrolysis in the rat striatum in vitro, although the mechanisms involved, apparently unrelated to α_1 or D_2 receptors, remain unclear. It would appear that this response is specific for NT-receptor activation, as DA inhibits the IP production in response to muscarinic receptor activation.

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Goedert, M. et al. (1984) Brain Research, 323: 193-197.
Ford, A.P.D.W. et al. (1989) Br.J.Pharmacol. Proceedings of Bristol Meeting.
Pizzi, M. et al. (1987) Eur.J.Pharmacol., 136: 263-264.
Kelly, E. et al. (1988) J.Neurochem., 51: 918-924.
Brown, E. et al. (1984) J.Neurochem., 42: 1379-1387.

EFFECT OF N-ETHYLMALEIMIDE ON DOPAMINE D_2 RECEPTOR REGULATION OF STRIATAL ADENYLATE CYCLASE ACTIVITY AND [^{3}H]-ACETYLCHOLINE RELEASE

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Many functions of dopamine D-2 receptors are thought to be mediated by their coupling, via G_i or other pertussis toxin-sensitive G proteins, to inhibition of adenylate cyclase or to other effector systems such as K^+ channels or intracellular Ca^{2+} (Vallar & Meldolesi, 1989). The striatum contains several functionally-differing populations of D-2 receptors, including presynaptic autoreceptors regulating dopamine release and heteroreceptors regulating acetylcholine release, as well as receptors regulating adenylate cyclase activity. The alkylating agent N-ethylmaleimide (NEM) is thought to inactivate the same G proteins as pertussis toxin and, unlike the latter, is effective for short term in vitro treatment. However, recent studies have shown that NEM treatment of striatal slices inactivates D-2 receptor regulation of adenylate cyclase but not autoreceptor regulation of dopamine release (Herdon, 1988 a,b), suggesting that the latter could be occurring via a different type of G protein. The present studies have continued these investigations by comparing the effects of NEM treatment on D-2 receptor regulation of adenylate cyclase activity and ^{3}H-acetylcholine release.

D-2 receptor regulation of D-1 receptor-stimulated adenylate cyclase activity in cross-chopped rat striatal slices was measured using a ^{3}H-adenine prelabelling method as described previously (Herdon, 1988b). D-2 receptor regulation of electrically-stimulated (3Hz, 2msec, 30mA) ^{3}H-acetylcholine release from superfused slices prelabelled with ^{3}H-choline (20μCi) was assessed using a minor modification of published methods (Herdon & Nahorski, 1987). Two 4 min periods of stimulation were applied 40 min apart (S_1 & S_2); spontaneous ^{3}H release was measured before each stimulation and stimulated ^{3}H release above this level was taken as representing ^{3}H-acetylcholine release. The choline uptake blocker hemicholinium-3 (10μM) was present throughout the superfusion period. Results are presented as means ± SEM of 4-8 experiments.

Preincubation of slices for 30 min with 30μM NEM greatly reduces the enhancement of dopamine (30μM)-stimulated adenylate cyclase activity by the D-2 antagonist sulpiride (30μM) (Herdon, 1988b); in the present studies, preincubation with 20μM NEM was equally effective (% increase with sulpiride: control 76 ± 6%; NEM 11 ± 3%, $p < 0.05$), whereas 10μM NEM was much less effective. Preincubation of ^{3}H-choline-prelabelled slices with 30μM NEM produced very large increases in spontaneous ^{3}H release and greatly decreased S_2/S_1 ratios for electrically-evoked release, making it difficult to assess D-2 receptor activity (data not shown). Preincubation with 20μM NEM also produced a 4-6 fold increase in spontaneous release as well as decreasing S_2/S_1 ratios to 61 ± 4% of control values. The D-2 agonist pergolide (1μM), added 20 min before S_2, reduced electrically-evoked ^{3}H-acetylcholine release from control slices by 62 ± 4%; the reduction by pergolide of release from NEM-treated slices was slightly though significantly less (43 ± 7%, $p < 0.05$).

These results suggest that treatment with even a fairly low concentration of NEM can largely inactivate the G_i proteins coupling D-2 receptors to adenylate cyclase. The same NEM treatment produced substantial non-specific effects on ^{3}H-acetylcholine release, but did not seem to prevent the regulation of release by D-2 receptors. One possibility, therefore, is that neither presynaptic D-2 autoreceptor regulation of dopamine release (Herdon, 1988 a,b) nor heteroreceptor regulation of acetylcholine release (present studies) involve an NEM-sensitive G protein. A more likely alternative, however, is that these receptor systems may possess a substantial "G protein reserve" so that most of their available G protein can be inactivated by NEM without blocking receptor function.

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Herdon, H. & Nahorski, S. (1987). N. S. Arch. Pharmacol. 335, 238-242.
Herdon, H. (1988a). Br. J. Pharmacol. 94, 351P.
Herdon, H. (1988b). Eur. J. Pharmacol. 154, 115-116.
Vallar, L. & Meldolesi, J. (1989). Trends Pharmacol. Sci. 10, 74-77.

AGONIST-INDUCED DESENSITIZATION OF THE D_1 DOPAMINE RECEPTOR EXPRESSED BY A HUMAN ASTROCYTOMA CELL LINE

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Little is known about the regulation of the D1-dopamine receptor. In contrast, considerable advances have been made using cell culture, in our understanding of the regulation of the β-adrenoceptor, which is similarly linked to adenylate cyclase. We recently reported that the human astrocytoma cell line D384 expressed D1-dopamine receptors (Balmforth et al., 1988). In the present study we have examined whether persistent occupation of the D-1 dopamine receptor desensitizes dopamine-stimulated cyclic AMP formation in intact D384 cells. In addition we have compared the effect of potentiating agonist stimulation of cyclic AMP, by simultaneous addition of a low concentration of forskolin, on the level of desensitization attained.

Intracellular cyclic AMP formation in D384 cells was assayed using a ^{3}H-adenine prelabelling method as described previously (Balmforth et al., 1988). The cells were preincubated (1h) with agents prior to the assay of intracellular cyclic AMP formation stimulated by a maximally-effective concentration of dopamine (1mM).

Preincubation of D384 cells with dopamine resulted in a time dependent attenuation of cyclic AMP responsiveness to subsequent dopamine stimulation. This effect was agonist specific since the PGE1 stimulation of cyclic AMP of similarly treated cells remained unchanged (table 1).

Table 1. D-1 dopamine receptor desensitization time course

Time of preincubation with 1mM DA (min)	0	2	5	15	30	60
DA % response	100	87 ± 3	82 ± 2	75 ± 3	52 ± 3	37 ± 2
PGE1 % response	100	95 ± 1	102 ± 2	102 ± 4	99 ± 2	102 ± 3

Each value is the mean ± SEM (n = 3)

The attenuation by dopamine was concentration dependent (EC50=1uM), with a maximal observed at 100uM. A comparison of dopamine concentration-response curves of control and dopamine preincubated cells revealed no change in the EC50, but a marked attenuation of the maximal response to 36 ± 4% of the control. Preincubation of cells with dopamine in the presence of D-1, but not D-2 selective antagonists, partially prevented the observed loss of responsiveness to subsequent dopamine stimulation. Attenuations in dopamine responsiveness were also obtained when D384 cells were preincubated with D-1, but not D-2 receptor agonists. The level of attenuation attained appeared to be related to agonist efficiency in stimulating cyclic AMP: SKF38393 < 3,4-dihydroxynomifensine < fenoldopam < 6,7-ADTN = dopamine. However, increasing the efficiency of 3,4-dihydroxynomifensine stimulation of cyclic AMP (from 13% to 160% of dopamine maximum), via the synergistic effect of adding a low concentration of forskolin, produced no further change in the attenuation of the subsequent response to dopamine.

These results suggest that the D1-dopamine receptors expressed by D384 cells undergo homologous desensitization. Uncoupling of the D-1 dopamine receptor appears to be independent of cyclic AMP formation, analogous to the mechanism proposed for the β-adrenoceptor (Lefkowitz et al., 1986).

A.J.B. is a Wellcome Trust Research Fellow in Mental Health

Balmforth, A.J. et al. (1988) J. Neurochem. 51, 1510-1515
Lefkowitz, R.J. et al. (1986) Trends Pharmacol. Sci. 7, 444-448

REGIONAL CHANGES IN [^{3}H]-SCH 23390 BINDING TO STRIATUM FOLLOWING L-DOPA TREATMENT OF 6-OHDA LESIONED RATS

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Alteration in striatal D-1 receptors may occur in Parkinson's disease or in response to L-DOPA therapy. In rats with a unilateral 6-OHDA lesion of the nigrostriatal pathway autoradiographic studies showed no change in ^{3}H-SCH 23390 binding to rat striatal slices (Savasta et al. 1987, 1988). However, the effect of L-DOPA treatment has not been studied using this technique. We now report the effect of a unilateral 6-OHDA lesion and chronic L-DOPA treatment on ^{3}H-SCH 23390 binding to rat striatal slices.

Male Wistar rats received a unilateral 6-OHDA (8 µg) lesion of the medial forebrain bundle. Other animals underwent a sham surgical procedure using injection of 0.9% saline. Those animals showing marked contralateral rotation to apomorphine (0.5 mg/kg s.c.) 3 weeks post surgery were selected for further study. Animals were dosed for 24 weeks with either L-DOPA (173-205 mg/kg/day) plus carbidopa (21.6-25.6 mg/kg) or carbidopa alone via the drinking water. After a drug washout period of 4 days animals were decapitated and brains frozen. Serial sections (20 µM) of rat striata (level +7.5 Paxinos & Watson, 1982) were incubated with ^{3}H-SCH 23390 (0.2 nM). Non-specific binding was determined by the inclusion of 10^{-7}M cis-flupenthixol.

There was no difference observed in specific ^{3}H-SCH 23390 binding between sham-operated and 6-OHDA lesioned animals. Similarly the 6-OHDA lesion did not alter ^{3}H-SCH 23390 binding in the ipsilateral striatum compared to the intact contralateral striatum. However, in animals treated with L-DOPA for 6 months there was a selective increase in ^{3}H-SCH 23390 binding in the lateral area of the ipsilateral striatum compared to same region in the contralateral striatum (Table 1). There was no difference in ^{3}H-SCH 23390 binding in the medial area between the ipsilateral and contralateral striatum.

Table 1 Effect of 6-OHDA lesion and L-DOPA treatment on ^{3}H-SCH 23390 binding to lateral striatum

	Ipsilateral striatum (fmol/mg wet weight tissue)	Contralateral striatum (fmol/mg wet weight tissue)
6-OHDA + carbidopa (n = 5)	116.4 ± 10.0	102.1 ± 12.6
6-OHDA + L-DOPA/carbidopa (n = 5)	147.7 ± 12.8*	103.6 ± 9.4

Results were analysed using a Student's t test. * $p < 0.05$ vs contralateral striatum.

Denervation of the striatum alone does not alter the regional binding of ^{3}H-SCH 23390. However, chronic L-DOPA treatment can cause a selective regional increase in ^{3}H-SCH 23390 binding. This change may contribute to the long term complications of L-DOPA therapy in Parkinson's disease.

Savasta, M., Dubois, A., Feuerstein, C., Benavides, J., Scatton, G. (1987) Biogenic Amines 4, 419-429.
Savasta, M., Dubois, A., Benavides, J., Scatton, B. (1988) Neurosci. Lett. 85, 119-124.
Paxinos, G., Watson, C. (1982) The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

NB-355: A PRODRUG FOR L-DOPA WITH REDUCED RISK FOR DYSKINESIAS IN MPTP-TREATED PRIMATES

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Poor bioavailability, short half-life and unpredictable absorption contribute to neurological complications experienced after chronic therapy with L-DOPA ("wearing-off", "on-off" effects and dyskinesias). A prodrug with improved absorption and slow conversion to L-DOPA might overcome these problems.

NB-355 (L-3(3-hydroxy-4-pivaloxyloxyphenyl)alanine) is a catechol mono ester of L-DOPA (Ihara et al, 1989). We have compared the ability of NB-355 and L-DOPA to stimulate locomotor activity and induce dyskinesias in parkinsonian squirrel monkeys.

Locomotor activity was recorded for 4.5h in 10 min time bins in individual cages with infra-red photocell units. Dyskinesias (chorea and dystonia) in the limbs or the rest of the body were each rated on a scale of 0-4 every 15 min for 4h (max possible score = 128). Carbidopa was given as a 1h pretreatment in a fixed dopa equivalent dose ratio of 1:4 mg/kg p.o. as is used clinically. NB-355 and L-DOPA were examined in a dopa equivalent dose range of 10-80 mg/kg p.o.

Both NB-355 (20-80 mg/kg) and L-DOPA (10-40 mg/kg) increased locomotor activity. Approximately double the dopa equivalent dose of NB-355 was required to increase activity to the same level seen after L-DOPA treatment. For doses of NB-355 and L-DOPA which stimulated activity to similar levels over 4.5h, the time course indicated a more gradual increase and lower peak and longer duration of action for NB-355 than for L-DOPA. Dyskinesias were markedly less severe for 40 mg/kg of NB-355 than for 20 mg/kg of L-DOPA despite a similar efficacy in stimulating locomotion. Dyskinesias were less severe at the highest dose of L-DOPA due to the induction of stereotypic climbing which was generally not observed with NB-355.

Treatment	Dopa Equivalent Dose (mg/kg p.o.)	Activity Counts/4.5h	Dyskinesia score/4h
Control	-	1500 ± 594	ND
L-DOPA	10	3243 ± 894	11.5
	20	5057 ± 1652	57
	40	7350 ± 1472	36
NB-355	20	1980 ± 959	11.4
	40	4735 ± 1503	13.5*
	80	6394 ± 1435	38

* $p < 0.05$ compared to L-DOPA 20 mg/kg, Mann Whitney U-test.

Our findings indicate that NB-355 is capable of equivalent stimulation of locomotor activity to L-DOPA but with a longer duration of action and substantially reduced risk for induction of dyskinesias in primates.

Ihara M et al (1989). J. Pharm. Sci. in press.

EVIDENCE TO SUGGEST A DIFFERENTIAL INVOLVEMENT OF CORTICOSTEROIDS IN THE EXPRESSION OF OPIATE AND OPIOID ANALGESIA

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Several lines of evidence now suggest that the ACTH/corticosteroid axis may be involved in the expression of opiate/opioid analgesia. Of particular relevance in this context are data indicating that adrenalectomy and corticosteroids enhance opioid/opiate analgesia (Miczek et al 1986). Whilst these findings are strongly suggestive of a prime role for corticosteroids in the expression of opiate analgesia it is necessary to examine the influence of the absence of corticosteroids per se before firm conclusions can be drawn.

For these studies 25-30g DBA/2 mice were injected with the 11 B-hydroxylase inhibitor Metyrapone, which blocks the synthesis of corticosterone from 11-Deoxycorticosterone. In the absence of data concerning the effects of Metyrapone on baseline pain responding a series of titration studies were conducted to examine this. 30 mins following drug administration (0 - 160 mg/kg Metyrapone) animals were assayed for tail-flick latency (TFL) at 0,15,30,45 and 60 mins post-injection. Data were analysed by ANOVA which revealed a bi-phasic effect, with 160 mg/kg inducing significant analgesia and 1 - 20 mg/kg producing only weak and inconsistently reproduceable analgesia. In the second series of studies the influence of Metyrapone on analgesia induced by 5 mg/kg morphine was studied. ANOVA revealed that 20 - 80 mg/kg potently and 0.1 - 1 mg/kg partially blocked this form of antinociception. Finally, although the analgesia induced by exposure to 35 bite attacks in a standard Resident-Intruder Paradigm is known to be opioid-mediated, Metyrapone was without effect. Data from these studies are summarised below.

mg/kg Metyrapone

	0.05	0.1	0.5	1	5	10	20	40	80	160
Basal	-	-	-	*	*	*	*	-	-	***
Opioid							-	-	-	
Morphine	-	Partial Block(?)	Partial Block	Partial Block	-	-	Block	Block	Block	

- = no effect * = weak analgesia *** = potent analgesia

Current data demonstrate that Metyrapone has (i) biphasic effects on baseline pain sensitivity (ii) no influence on opioid analgesia at the doses thus far examined, yet (iii) fully blocks morphine analgesia at high and partially blocks this response at low doses. Thus, these data possibly suggest a difference in the mechanisms involved in the expression of opioid and opiate analgesia.

Miczek KA et al Annals of NY Acad Sci 467: 14-29 (1986).

INTERLEUKIN 1β AND LIPOPOLYSACCHARIDE EFFECTS ON PROSTAGLANDIN E_2 RELEASE FROM RAT BRAIN SLICES

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Bacterial lipopolysaccharide (LPS) produces fever by causing the synthesis and release of endogenous pyrogenic cytokines such as interleukin 1 (IL-1). The febrile responses to exogenous and endogenous pyrogens are both mediated in the brain by prostanoids, notably PGE_2, and are both blocked by inhibitors of cyclo-oxygenase (Dascombe, 1985). The effects of LPS and recombinant IL-1β on PGE_2 release from rat brain tissue have been studied to determine the mechanism by which pyrogens stimulate PGE_2 production centrally.

Male Sprague Dawley rats were killed by concussion and cervical dislocation. Cerebral hemispheres were removed, cross chopped (0.5 mm x 0.5 mm) and suspended (10 mg wet weight/ml) in sterile, pyrogen-free Krebs solution, gassed with 95% O_2/5% CO_2, containing the drugs. Following incubation at 37°C for the times indicated below, the supernatant was removed and stored at -20°C until assayed in duplicate for PGE_2 content by radioimmunoassay.

LPS from E.coli (10 μg/ml, contact time 30 min) stimulated PGE_2 release from brain slices ($P<0.05$, n=7 brains), but other concentrations (1 and 100 μg/ml) had no significant effect. Human IL-1β (DuPont, 1-100 ng/ml contact time 20 min, n=5) had no significant effect on PGE_2 release. Murine IL-1β (DuPont, contact time 20 min, n=8) caused a dose related increase in PGE_2 release (Table 1), which was inhibited by flurbiprofen (1 μg/ml, n=5).

Table 1 Effect of murine IL-1β on PGE_2 release from rat brain slices

	IL-1β (ng/ml)			
	control	1	10	100
Normal Krebs	224 ± 52	244 ± 59	314 ± 76*	413 ± 104*
Flurbiprofen	147 ± 44	95 ± 47⁺	202 ± 65	48 ± 26⁺

Values are mean ± s.e.mean PGE_2 concentrations (pg/ml), * $P<0.05$ compared to paired control, ⁺ $P<0.05$ compared to response to IL-1β in normal Krebs.

Quinacrine (1mM) had no effect on basal release of PGE_2, but reduced the responses to murine IL-1β (10 ng/ml:181 ± 28 pg PGE_2/ml, 100 ng/ml:167 ± 15 pg PGE_2/ml, n=5). In calcium-free Krebs containing EGTA (2mM), basal release of PGE_2 was increased (446 ± 41 pg/ml, n=6, $P<0.05$) and the responses to murine IL-1β (10 and 100 ng/ml, n=6) were abolished.

These results show that murine IL-1β produces a dose dependent increase in PGE_2 release from rat brain slices. The absence of an effect by human IL-1β in this study may be due to its lack of homology with rat IL-1β. Recombinant IL-1β induced release of PGE_2 from brain tissue appears to involve the activation of a calcium dependent phospholipase, possibly phospholipase A_2, as, for example, in synovial cells (Godfrey et al, 1987) and fibroblasts (Solito & Parente, 1989).

EJR is an ONO Pharmaceuticals Research Student.

Dascombe, M.J. (1985) Prog. Neurobiol. 25, 327-373
Godfrey, R.W. et al (1987) Biochem. Biophys. Res. Comm. 142, 235-241
Solito, E. & Parente, L. (1989) Br. J. Pharmac. 96, 656-660

HAEMODYNAMIC RESPONSES TO ENDOTHELIN AFTER PERIPHERAL AND CENTRAL ADMINISTRATION IN THE CONSCIOUS RAT

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Endothelin (ET-1), a recently discovered endothelial-derived peptide, is a potent vasoconstrictor in vitro and elicits a biphasic cardiovascular response in anaesthetised, ganglion-blocked rats following intravenous administration (Yanagisawa et al 1988; Wright et al 1989). The present study investigated the haemodynamic actions of ET-1 in conscious freely moving male WKY-rats (270-300 g), after intravenous (i.v.) and intracisternal (i.c.) administration.

Intracisternal guide cannulae were implanted under brietal sodium anaesthesia (60 mg kg^{-1}; i.p.) and the rats given 7 days to recover from surgery. The femoral artery and vein were cannulated on the study day for blood pressure measurement and drug administration respectively. Three doses of ET-1 (0.1,0.3, & 1.0 nmol) were given i.v. in a randomised order to each rat (n=5) in a volume of 0.1 ml over a period of 1 minute. In separate animals injections of ET-1 (0.01-0.03 nmol) were made i.c.in a volume of 2.5 ul over the same time period,one dose per rat (n=4-6).

ET-1 (0.1-1 nmol) i.v. evoked a biphasic cardiovascular response.The initial effect was a transient (⩽ 30 sec) dose-related hypotension with a corresponding tachycardia (Table 1). The initial depressor response was followed by a marked dose-related increase in MAP which was prolonged (⩽ 60 mins). No effect was observed on HR (Table 1).

Table 1: Maximum hypo (a) and hypertensive (b) responses and HR after ET-1 (i.v.).

ET-1	MAP (mmHg) + SEM			HR (b/min + SEM)		
nmol	Basal	(a)	(b)	Basal	(a)	(b)
0.1	125.8±4.5	123±6	135*±4.7	322±21.5	304*±19.7	298.5±20.2
0.3	126±5.3	107*±7.8	146*±4.3	320±23.02	377*±31.3	292±29.6
1.0	128±2.5	86*±3.3	156*±1.9	337±27.18	438*±17.4	310±7.8

ET-1 (0.01-0.03 nmol) i.c. increased MAP but had variable effects on HR (Table 2). In some cases administration of ET-1 elicited a marked increase in MAP, a fall in HR, convulsions and death.

Table 2: Maximum hypertensive response (a) & corresponding HR (b) after ET-1 (i.c.)

ET-1	MAP (mmHg + SEM)		HR (b/min + SEM)	
nmol	Basal	(a)	Basal	(b)
0.01	122.1±1.90	162.8±14.5	302.7±11.2	292±16.9
0.02	119.4±3.4	180±23.2	315±32.1	390±32.4
0.03	128.8±3.1	184.5*±15.2	322.5±9.2	305±15

Thus, increases in MAP are observed after both peripheral and central administration of ET-1. However it is not clear at present whether the central response is a direct effect on parenchymal or neuronal Endothelin receptors or secondary to cerebral vasoconstriction.

* $p < 0.05$ different from basal values, Repeated Measures ANOVA.

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Yanagisawa, M. et al (1988) Nature, 332, 411-415.
Wright, C.E. and Fozard, J.R. (1989) Proceedings of the British Pharmacological Society, April 1989.

PHARMACOLOGICAL ACTIONS OF ENDOTHELIN (ET-1) ON CARDIAC TISSUE *IN VITRO*

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Porcine endothelin (ET-1) has potent vasoconstrictor effects in vivo and in vitro (Yanagisawa *et al.*, 1988). ET-1 also has positive inotropic and chronotropic effects in guinea pig, rat and human isolated tissues (Ishikawa *et al.*, 1988; Moravec *et al.*, 1989). We have studied the action of ET-1 on cardiac tissue using functional, autoradiographical and electrophysiological techniques.

Left (paced) and right (unpaced) atria (rat, male Sprague-Dawley, 250g) were prepared for measurement of inotropic and chronotropic responses respectively (Ishikawa *et al.*, 1988). ET-1 was added in a cumulative fashion (0.1 nM-1 μM). Transverse slices (20 μm) of rat heart were prepared for autoradiographical studies (Sharif *et al.*, 1988) using 20 pM [^{125}I] ET-1 in 20 mM HEPES buffer containing 5 mM $MgCl_2$ and 0.1% BSA. Non-specific binding was defined using 0.5 μM ET-1.All values quoted are the means ± s.e.mean, n=4-10. Recordings of calcium channel currents in embryonic chick myocytes were made as described by Anderson *et al.*, (1988).

ET-1 elicited concentration-dependent positive inotropic and chronotropic responses (-log EC_{50} values of 8.0 ± 0.05 and 8.1 ± 0.10 respectively). The maximum positive chronotropic and inotropic responses were 28 ± 4 and 35 ± 5 % respectively of that attained by 0.1 μM isoprenaline. The time taken to attain an equilibrium response to ET-1 were significantly ($p<0.05$) longer than that for isoprenaline (0.1 μM ET-1 : 25 ± 2 min; 0.1 μM isoprenaline 8 ± 3 min). High density specific binding of labelled ET-1 was located on the atrial appendages and the aortic arch (6.3 ± 0.16 and 2.2 ± 0.6 amol.mm^{-2} respectively. ET-1 (25-100 nM) did not enhance the calcium channel currents evoked by depolarisation to 0 mV from a holding potential of -80 mV. Instead ET-1 (100 nM) reduced the amplitude of the calcium channel current by around 40%.

The positive chronotropic and inotropic responses are in good agreement with the data of Ishikawa *et al.* (1988) but in contrast to Moravec *et al.*(1989) who observed no positive chronotropic response in the rat. ET-1 binds to specific sites in the myocardium, through which it may exert its physiological effects. ET-1 is not a direct activator of calcium channel currents in embryonic chick myocytes suggesting the mechanism of action may be due to release of calcium from intracellular stores. ET-1 elicits a stimulation of inositol phospholipid hydrolysis in rat atria (Kloog *et al.*, 1988) which may account for the above responses.

Anderson, A. J. *et al.*, (1988) J. Physiol., **407**, 130P
Ishikawa, T. *et al.*, (1988) Am. J. Physiol., **255**, H970-973
Kloog, Y. *et al.*, (1988) Science, **242**, 268-270.
Moravec, C. M. *et al.*, (1989) Biochem. Biophys. Res. Comm., **159**, 14-18
Sharif, N. A. *et al.*, (1988) Neurosci. Lett., **86**, 272-278
Yanagisawa, M. *et al.*, (1988) Nature, **332**, 411-415

INFLUENCE OF ENDOTHELIUM ON THE CONTRACTILE RESPONSE OF ISOLATED RAT AORTA TO SYNTHETIC TETRADECAPEPTIDE RENIN SUBSTRATE

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Recently, evidence for the existence of a vascular renin-angiotensin system (RAS) has been shown (see review, Dzau, 1988). The purpose of the present work was to determine the pathway(s) of the formation of angiotensin II (ANGII) from synthetic tetradecapeptide renin substrate (TDRS) in the isolated rat aorta and to study the possible role of the endothelium in the formation of ANG II. Therefore, the contraction of aortic rings with and without endothelium to TDRS, angiotensin I (ANG I) or ANGII was studied in the presence or absence of inhibitors of the RAS.

Aorta rings were taken from male Sprague Dawley rat (300-350 g). The rings were left intact or mechanically rubbed to remove endothelial cells and mounted in 20 ml organ baths filled with a physiological salt solution at 37°C gassed with 95% O_2 : 5% CO_2. Tissues were contracted with a depolarizing solution containing 100 mM KCl, which induced a maximal contraction (1079 ± 30 mg, n=23), and challenged with ACh (1 µM) which induced a relaxation (36.8 ± 1.1%, n=23) in intact rings and no relaxation in rubbed rings. Responses to single concentrations of ANGII, ANGI and TDRS were obtained by adding the appropriate concentration into the bath, in the presence or absence of a pharmacological inhibitor of the RAS.

In the presence of a functional endothelium, TDRS induced an increase in tension which was concentration-dependent. The maximal response induced by TDRS (429 ± 49 mg, n=4) was similar to that produced by ANGI and ANGII (496 ± 46 mg and 530 ± 73 mg, n=5). The sensitivity of the aorta to TDRS (EC_{50} = 0.25 ± 0.09 µM, n=4) was about 2 times less than that of ANGI and ANGII (0.14 ± 0.05 µM and 0.13 ± 0.03 µM, n=5). However, the differences didn't reach statistical significance. ANGII receptor blockade (^{1}Sar-^{8}Ile 18 nM) completely inhibited the responses to ANGII, ANGI and TDRS (1 µM). Converting enzyme inhibition (enalaprilat 6.1 µM) had no effect on the response to ANGII (1 µM) but completely blocked the responses to ANGI and TDRS (1 µM). Renin inhibition (CGP 44099A 4 µM) had no effect on the responses to ANGII and ANGI (1 µM) but inhibited those to TDRS (1 µM). In the absence of the endothelium, the maximal response to TDRS, ANGI and ANGII were increased by about 2 fold. Also, the sensitivity of the aorta to TDRS, ANGI and ANGII was increased (EC_{50} values were decreased by 4 to 15 fold). This effect may be due to release of Endothelium-Derived Relaxing Factor since in intact preparations, methylene blue (3 µM) (Al Osachie & Godfraind, 1986) increased the contractile response to TDRS and ANGII (1 µM) to a similar level as endothelium removal. In the absence of endothelium, ANGII receptor blockade, converting enzyme inhibition and renin inhibition still inhibited the contractile response induced by TDRS (0.1 µM).

In conclusion, the formation of ANGII from TDRS in the rat aortic preparations show that converting enzyme and a renin-like aspartic proteinase are present in the aortic wall. Furthermore we show that endothelium is not essential for the conversion of TDRS to ANGII but modulates the response to the endogenously produced ANGII.

Al Osachie I. & Godfraind, T. (1986). Br. J. Pharmacol., 89, 525-532.
Dzau, V. (1988). Circ. Res., 77 (suppl.I), I-4-I-13.

LDL INHIBITS RELAXATIONS EVOKED BY THIMEROSAL IN RABBIT AORTA

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Endothelium-dependent relaxations in the rabbit aorta are accounted for by the release of endothelium-derived nitric oxide (ENDO) (Palmer et al. 1987). This response is inhibited by low-density lipoproteins (LDL) in aorta pre-contracted with noradrenaline (NA) or serotonin (5HT) (Andrews et al. 1987). Here, we investigate the inhibition of ENDO-mediated relaxations evoked by thimerosal, an agent which acts by a post-receptor mechanism (Forstermann et al. 1986).

LDL (density, 1.019-1.063 g/ml) were prepared from fresh human plasma by discontinuous density gradient ultracentrifugation in the presence of 0.3 mM EDTA but in the absence of thimerosal (Chung et al. 1980). ENDO relaxations were assayed as previously described using isolated intact rings of thoracic aorta from 6 month-old New Zealand White rabbits (Andrews et al. 1987). Tissues were pre-contracted with noradrenaline (NA) or serotonin (5HT) and relaxed by acetylcholine (ACh). After washout and equilibration for 30 min, LDL (2 mg protein/ml) or Tyrode's buffer (control) was added to the organ bath and the pre-contracted tissues were relaxed by graded doses of thimerosal. After maximal relaxation was achieved, the tissues were incubated for 60 min before a further challenge with 0.5μM ACh, washout and equilibration. A further relaxation to ACh was then performed on the pre-contracted tissues to determine the reversibility of the effects.

ACh (0.1μM) and thimerosal (0.7μM) caused a relaxation in control tissues of 90-100%. After maximal relaxation by thimerosal was achieved, the tissues slowly regained their initial tension. On challenge with ACh, both LDL-treated and control tissues were unable to relax. The dose-response curves obtained for the relaxations to thimerosal in the presence of LDL showed a 10-fold rightward shift compared with control tissues.

Thimerosal as described here, like mellitin and polyarginine exhibits a biphasic response in aortic rings (Rapoport et al. 1989). Namely, a rapid relaxations followed by slow contraction which results in desensitization to ACh. LDL inhibits the relaxation induced by thimerosal but not the desensitization. The inhibition by LDL of relaxation evoked by A23187 (Andrews et al. 1987) and thimerosal, serves to confirm a post-receptor mechanism for the effect. Furthermore, we have discovered that thimerosal, which was used previously as a anti-bacterial agent in the preparation of LDL (Andrews et al. 1987), is not readily dialysed from the LDL. Consequently, the true nature of the inhibition of EDNO responses by LDL was previously masked by desensitization (Plane et al. 1989).

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Andrews, H.E., Bruckdorfer, K.R., Dunn, R.C. and Jacobs, M. (1987). Nature 327, 237-239.
Chung, B.Y., Wilkinson, T., Geer, J.C. and Segrest, J.P. (1980). J. Lipid Res. 21, 284-291.
Forstermann, U., Goppelt-Strube, M., Frolich, J.C. and Busse, R. (1986). J. Exp. Ther. 238, 352-359.
Palmer, R.J.M., Ferrige, A.G. & Moncada, S. (1987). Nature 327, 524-526.
Plane, F. et al. (1989). This Meeting.
Rapoport, R.M., Ashraf, M. and Murad, F. (1989). Circulation Research 64, 463-473.

COMPARATIVE EFFECTS OF LIGNOCAINE AND ELECTROMAGNETIC INDUCTION ON IMPULSE CONDUCTION IN FROG ISOLATED NERVE-MUSCLE PREPARATION

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The local anaesthetic drug,lignocaine,is known to inhibit impulse conduction by a well-known mechanism,i.e.,by blocking the Na^+ channel,in nerve or muscle fibres. Recently,Brain & Wali (1988) have shown that electromagnetic induction(EMI) also can block conduction (by unknown mechanism) in the frog isolated sciatic nerve-gastrocnemius muscle preparation. The aim of the present investigation was to evaluate and compare the nerve blockade produced by these two methods in the same preparation of the frog nerve-muscle system.

The frog sciatic nerve-gastrocnemius muscle was dissected out and set up in an organ bath containing 100 ml of Ringer solution.The nerve trunk was passed through an induction coil(copper coil,20-100 turn,0.5 mm diameter and 0.6 ohm).The induction coil was wound on a P.V.C. tube,internal diameter 2 mm. The nerve trunk was moistened with Ringer solution, and the gastrocnemius muscle was laid in the organ bath,with one end pinned while the other was attached,via a thread,to a force-displacement transducer and a pen recorder. The temperature of the bath was 22-25° C. The nerve trunk was electrically stimulated at 0.5 Hz with 0.25-0.6 V(supramaximal) and 1 ms pulse duration.The pattern of electrical stimulation was interrupted by a period of EMI (from a d.c. source 1.5-4 V at a frequency of 100 min^{-1}) for 2-4 min, operated via a make-and-break switch. The contractile responses were recorded isometrically.

Electrical stimulation(ES) produced repeatable,reliable and fairly constant pattern of twitch contractions in the frog gastrocnemius muscle (control 2.4±0.1 g, tension,mean±s.e.,n=8 experiments-frogs). EMI, after a latency of 15 s, reduced and then blocked these responses in about 2 min.The inhibitory effect was reversed after the cessation of the EMI,and washing out the preparation in Ringer solution. Recovery of the blocked twitches occurred within 5-6 min. The time taken to produce 50% maximum inhibition of twitch tension by EMI was 80±5 s.

Lignocaine(0.1-100 μM) produced concentration-dependent inhibition of the twitch contractions in the frog muscle(control 3.6±0.2 g).The mean EC_{50} values(i.e., concentration to produce 50% maximum inhibition) of lignocaine-induced blockade was 0.8±0.1 μM, and 95% blockade was obtained with 8 μM lignocaine in 3±0.2 min. When compared to EMI inhibition,95% blockade by EMI was obtained in 1±0.1 min, thus blockade with EMI was faster than that with lignocaine in the same muscle.

The mechanism of lignocaine-induced inhibition of twitch tension is different from that produced by EMI,in that the latter may interfere with the ionic fluxes across the nerve membrane,probably by causing damage to the membrane,e.g.,the myeline sheath and Schwann cells.This has been found to be the case in the present study. Other workers (Kolin,1968;Oberg,1973;Polson,Barker & Freeston,1982) have considered EMI advantageous over ES. Moreover,EMI can be made to produce nerve blockade similar to that produced by an effective dose(or concentration) of a local anaesthetic drug(Scurlock,Meyamaris & Gregus,1978).

Brain,A.I.J.& Wali,F.A.(1988). Br.J.Pharmacol. 95, 843 P
Kolin,A.(1966). Physics Today, 21, 39-50
Oberg,P.A.(1973). Med.& Biol. Eng., 11, 55-64
Polson,M.J.R.,Barker,A.T.& Freeston, I.L.(1982). Med.Biol.Eng.& Comput., 20, 242-244
Scurlock,J.E.,Meyamaris,E.& Gregus,J.(1978). Acta Anaesthesiol.Scand., 22, 601-608

NORADRENALINE-INDUCED DEPOLARIZATION AND CONTRACTION IN THE RAT ISOLATED SMOOTH MUSCLE OF SEMINAL VESICLE

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The rat seminal vesicle is mainly innervated by adrenergic neurones (Bouquet & Noach, 1971), although cholinergic and peptidergic innervations have also been reported by other workers (Fedan,Besse,Carpenter & Teague,1977; Meldrum & Burnstock,1985). The aim of the present investigation, was to further analyse the mechanism of action of noradrenaline (NA), in the rat seminal vesicle, by measuring its effects on membrane potential changes (depolarization) and the subsequent muscle contraction, recorded simultaneously.

Adult, male, Sprague-Dawley rats, weighing 200-300 g, were killed by a blow to the head and bled. The seminal vesicle was dissected out and was cut spirally into strips, 2-3 cm long and 2 mm wide, for the recordings of the mechanical responses, and 8-10 cm long and 2 mm wide, for the recordings of the electrical responses. The mechanical responses were recorded isometrically, using a force-displacement transducer and a pen recorder, whereas the electrical responses were recorded by the sucrose-gap method (Keatinge,1978; Wali,1982).

Noradrenaline (1-1000 μM) produced concentration-dependent depolarization and contraction in the smooth muscle of the rat isolated seminal vesicle. A mean maximum depolarization and contraction of 4.6 ± 0.2 mV and 2.5 ± 0.1 g tension, were produced by NA (500 μM) (means$\pm$s.e.,n=8 experiments-rats). The ratio of the contraction (C) to the depolarization (D) was 6.7:1.0, in the control situation, i.e., in the absence of any antagonists, e.g.,phentolamine (1 μM). Phentolamine (1 μM) reduced both types of NA -induced responses by 3.3 -5.0 fold, and the ratio of the C/D was increased to 10:1.0 (control 6.7:1.0). The mean concentration of NA which produced 50% maximum responses (depolarization or contraction), in the rat seminal vesicle, in the absence and presence of phentolamine (1 μM) were 15 ± 1.0 μM (depolarization, control), 50 ± 6.0 μM (depolarization in phentolamine), and 100 ± 8 μM (contraction, control), 500 ± 21 μM (contraction in phentolamine), respectively. The differences between the control responses and the responses in the presence of phentolamine were significant ($P < 0.001$).

It was concluded that noradrenaline produced both depolarization and contraction in the rat isolated seminal vesicle. The differential effect of the action of noradrenaline (on depolarization and contraction responses) is not clear in the present study, but it may in part explain the mode of action of noradrenaline (and its ionic basis) in the smooth muscle.

Bouquet, J.& Noach,E.L.(1971). Arch.Int.Pharmacodyn.Ther. 189, 397-398
Fedan, J.S., Besse, J.C., Carpenter, F.G. & Teague, R.S.(1977). J.Pharmacol. Exp.Ther. 201, 285-297
Keatinge, W.R.(1978). J.Physiol.Lond. 279, 275-289
Meldrum, L.A.& Burnstock, G.(1985). Eur.J.Pharmacol. 110, 363-366
Wali, F.A.(1982). Br.J.Pharmacol. 77, 565 P

INTERACTIONS OF GALANIN WITH VASOACTIVE INTESTINAL PEPTIDE ON HUMAN COLONIC MOTILITY *IN VITRO.*

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Galanin (GAL) is a 29a.a. peptide found in the enteric nerves of a number of species including man (Rokaeus, 1987). In some instances GAL appears to co-exist with vasoactive intestinal peptide (VIP, Melander et al, 1985; Furness et al, 1987).

In the present study the presence of galanin-like immunoreactivity (GAL-IR) was confirmed in nerve fibres supplying the myenteric plexus and both the longitudinal (LM) and circular (CM) muscle layers of the human colon. Co-existence of GAL-IR with VIP-IR could be seen in nerve fibres supplying both muscle layers; in LM over 70% of GAL-IR fibres contained VIP-IR.

GAL (0.01-6.21μM) produced dose dependent, TTX (1.57μM) resistant, contractions of both LM and CM. For LM the highest dose tested (1.24μM) caused 86±6% (n=6) of a maximum contraction to acetylcholine while for CM the highest dose tested (6.21μM) caused 20±4% (n=3) of the maximum. To investigate the possibility that GAL may interact with VIP, responses of muscle strips to VIP only and VIP plus GAL were compared. GAL (0.01μM) reduced submaximal relaxations to VIP (0.1μM, LM; 0.02μM, CM) by 68±24% on LM (n=6, $P<0.05$) and by 18±21% on CM (n=4, $P>0.05$). Submaximal relaxations to noradrenaline (0.45μM) were not reduced by GAL (0.01μM).

In summary GAL-IR is co-localised with VIP-IR in nerve fibres innervating human colonic muscle. GAL has a direct excitatory motor effect on both muscle layers, in contrast to VIP which causes relaxation, GAL reduces the responsiveness of LM to VIP.

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Furness, J.B., Costa, M., Rokaeus, A., McDonald, T.J. & Brooks, B. (1987). Cell Tiss. Res. 250, 607-615.

Melander, T., Hokfelt, T., Rokaeus, A., Fahrenkrug, J., Tatmedo, K. & Mott, V. (1985). Cell. Tiss. Res. 239, 253-270.

Rokaeus, A. (1987). TINS 10, 158-164.

EFFECT OF GANGLIOSIDE TREATMENT ON SUBSTANCE P-LIKE IMMUNOREACTIVITY AXONAL TRANSPORT IN RATS WITH EXPERIMENTAL DIABETES

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Ganglioside treatment prevents some neurological defects in diabetic rats which may be progenetive for diabetic neuropathy (Calcutt, Tomlinson & Willars, 1988). This study examined the effect of ganglioside treatment on deficient axonal transport of substance P-like immunoreactivity (SPLI) in sciatic nerves of diabetic rats. Control and diabetic (50 mg/kg streptozotocin i.p.) male Wistar rats were maintained for 4 weeks, with or without mixed bovine brain ganglioside treatment (10 mg/kg/day i.p.). At the end of the protocol, under halothane anaesthesia, two tight ligatures were placed 1.5 cm apart on the mid-femoral region of the left sciatic nerve to arrest anterograde and retrograde axonal transport. After 12 h the accumulation and redistribution of SPLI (mean$\pm$SEM; measured by radioimmunoassay) at and between the ligatures was measured, along with the SPLI content of the unconstricted contralateral nerve and L_4 and L_5 dorsal root ganglia. Background SPLI in unconstricted nerves of untreated controls was 90 ± 10 pg/mm and in untreated diabetics it was 72 ± 11. SPLI in the L_4 dorsal root ganglion was 671 ± 78 pg in controls and 436 ± 68 pg in diabetics ($p<0.05$). The accumulation of SPLI proximal to the upper ligature (anterograde axonal transport) and distal to the lower ligature (retrograde transport) were respectively 3331 ± 573 pg and 225 ± 49 pg in controls and 1956 ± 161 pg and 77 ± 33 pg in diabetics (both $p<0.05$). However, the mobile fraction of SPLI and its accumulation rates, as calculated using the SPLI redistribution profile between the ligatures, were similar in controls and diabetics. A similar pattern of changes was observed when the ganglioside-treated diabetics were compared with the ganglioside treated controls. Proximal accumulations were 3265 ± 648 pg (ganglioside-treated controls) and 1950 ± 182 pg (ganglioside-treated diabetics). Distal accumulations (respectively) were 260 ± 72 pg (treated controls) and 145 ± 45 pg (treated diabetics). Thus, ganglioside treatment was without effect on the observed deficits in SPLI in constricted nerves and dorsal root ganglia of diabetic rats.

Calcutt,N.A., Tomlinson,D.R. & Willars,G.B. (1988) J. Neurochem. 50, 1478-1483.

NOVEL, POTENT AND SELECTIVE AGONISTS AT NK-1 AND NK-2 RECEPTORS

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The lack of selectivity of the endogenous neurokinins substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) has created a need for potent and selective neurokinin receptor agonists to aid characterisation of neurokinin receptor-mediated actions, both *in vitro* and *in vivo*. Furthermore, in view of the susceptibility of neurokinins to inactivation by tissue peptidases (e.g. in guinea-pig isolated trachea; Ireland et al., 1988), it would be advantageous if selective agonists were also peptidase-resistant. Here, we describe the actions of two neurokinin agonists, δ-aminovaleryl[L-Pro^9,N-MeLeu^{10}]SP(7-11) (GR73632) and [Lys^3,Gly^8-R-ϒ-lactam-Leu^9]NKA(3-10) (GR64349) at NK-1 receptors in guinea-pig ileum longitudinal muscle (GPI), NK-2 receptors in rat colon muscularis mucosae (RC) (see Brown et al., 1985) and NK-3 receptors in rat everted portal vein (RPV) (see Mastrangelo et al., 1986) (Table 1).

Table 1: Mean EC_{50} values (nM) ± s.e. mean, ($n \geq 3$) for contractions induced by neurokinin agonists.

Agonist	GPI (NK-1)	RC (NK-2)	RPV (NK-3)
SP	5.6±0.7	167±20	7500±1200
GR73632	4.0±0.5	961±175	>15,000
NKA	13.7±2.1	1.7±0.3	563±99
GR64349	4237±1295	3.7±0.6	1177±446
NKB	68.0±28.0	27.3±11.9	6.3±1.3

In order to test the stability of these selective agonists to peptidases, we have utilised spiral strips of guinea-pig isolated trachea (GPT). In this preparation, the contractile response to naturally-occurring neurokinins is markedly potentiated by peptidase inhibitors (Stephens-Smith et al., 1988). Therefore, we examined the potency of the agonists to contract GPT in the absence and presence of the endopeptidase inhibitor phosphoramidon (1μM) and the aminopeptidase inhibitor bestatin (100μM). Whereas the concentration-response curves for SP and NKA were displaced markedly to the left (>50-fold shift in EC_{50}), the EC_{50} values for GR73632 (4.7±0.3nM) and GR64349 (9.9±2.2nM) were little affected (<5-fold shift).

These results suggest that GR73632 and GR64349 are potent, selective and metabolically stable agonists at NK-1 and NK-2 receptors respectively. Moreover, the potencies of these selective agonists in the GPT are consistent with the existence of both NK-1 and NK-2 receptors mediating the contractile response to neurokinins in this preparation (cf Mcknight et al., 1988, and Ireland et al., 1988).

Brown, J.R. et al. (1985) in 'Tachykinin Antagonists': Hakanson, R. & Sundler, F. (ed.), pp. 305-312. Amsterdam, Elsevier.
Ireland, S.J. et al. (1988) Regulatory Peptides 22, 93.
McKnight, A.T. et al. (1988) Regulatory Peptides 22, 126.
Mastrangelo, D. et al. (1986) Eur. J. Pharmac. 134, 321-326.
Stephens-Smith, M. et al. (1988) Regulatory Peptides 22, 177.

THE MUSCARINIC RECEPTOR SUB-TYPE MEDIATING AIRWAY RESPONSES TO ACETYLCHOLINE IN THE GUINEA-PIG

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Binding studies show that approximately 50% of muscarinic receptors in guinea-pig lung are of the M1 sub-type, having high affinity for pirenzepine (Mak & Barnes, 1988). We have investigated whether M1 receptors have a functional role in mediating changes in lung resistance (R_L) and dynamic compliance (C_{dyn}) in response to exogenous acetylcholine (ACh) in the anaesthetised guinea-pig.

The method used is an adaptation of that previously described (Gater et al, 1988). A sub-maximal dose of ACh (normally 10-20ug/Kg i.v.) causing an approximate 2-fold increase in R_L and a 50% fall in C_{dyn} and HR was established and then repeated at 10 minute intervals until responses were consistent. Cumulatively increasing i.v. doses of muscarinic antagonists were given and the ACh responses re-determined after each dose. The doses of antagonists (ug/Kg ± s.e.mean) which inhibited the ACh response by 50% (IC_{50}) are shown in the table.

IC_{50} values for muscarinic antagonists against acetylcholine induced changes in R_L, C_{dyn}, and HR

Antagonist	R_L	C_{dyn}	HR	n
Atropine	0.78 ± 0.2	0.96 ± 0.2	2.0 ± 0.08	7
Ipratropium Bromide	1.7 ± 0.4	1.5 ± 0.3	2.0 ± 0.1	3
4-DAMP	2.26 ± 0.7	2.79 ± 0.9	119.6 ± 22	7
Pirenzepine	90.4 ± 26.5	83.8 ± 23.4	244.0 ± 80	5
Methoctramine	⩾300	⩾300	21.2 ± 3.5	6

In agreement with others (Maclagan et al, 1989) we have found that pirenzepine is approximately 100 fold weaker than atropine against the ACh-induced changes in R_L, C_{dyn} and HR. Thus M1 (high pirenzepine affinity) receptors are not involved in these responses to ACh. The muscarinic receptors which have low affinity for pirenzepine include M2 (cardiac) and M3 (smooth muscle) sub-types. These can be differentiated in vitro by 4-DAMP (M3-selective) and methoctramine (M2-selective), (Mihm & Wetzel, 1987). We have shown here that 4-DAMP is markedly lung selective, whilst methoctramine has the converse selectivity. We therefore conclude that M3 receptors, but not M1 receptors, mediate the functional responses to acetylcholine in the airways of the anaesthetised guinea-pig.

Gater P.R., Moore B.A. & Alabaster V.A. (1988) Brit.J.Pharm. 93 242P
Maclagan J., Fryer A.D. & Faulkner D. (1989) Brit.J.Pharm. In press.
Mak J.C.W. & Barnes P.J. (1988) Brit.J.Pharm. 95 777P
Mihm G. & Wetzel B. (1987) Drugs of the Future 12 1127

COMPARISON OF THE *IN VIVO* POTENCY OF METHOCTRAMINE FOR PRE- AND POST-JUNCTIONAL MUSCARINIC RECEPTORS IN GUINEA-PIG LUNG.

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Stimulation of pulmonary parasympathetic nerves causes bronchoconstriction via release of acetylcholine onto M_3 muscarinic receptors on airway smooth muscle. Transmitter release is inhibited by activation of pre-junctional M_2 receptors and blockade of these autoreceptors by the muscarinic antagonist gallamine causes facilitation of vagally-induced bronchoconstriction (Fryer and Maclagan, 1987). This drug however, is also a weak antagonist for post junctional M_3 receptors, causing an under-estimate of its potency for the neuronal M_2 receptors. Recently a new muscarinic antagonist, methoctramine, has been described which has more than 100 times greater affinity for M_2 receptors in the heart than for M_3 receptors on tracheal smooth muscle *in vitro* (Giraldo et al., 1988).

In the present experiments, the effects of methoctramine have been studied in the lung to compare its potency on pre- and post-junctional muscarinic receptors. Guinea-pigs (350-450g), anaesthetised with urethane, were vagotomised and respired by positive pressure, constant volume ventilation from a Harvard rodent respirator. Bronchoconstriction was induced alternately by stimulation of the peripheral end of the right vagus nerve (30 Hz, 0.2ms, 10-40 V, 5s) or by i.v. acetylcholine (ACh 5-10ug.kg^{-1}) and recorded as an increase in tracheal pressure above the normal excursions generated by the ventilator (Fryer and Maclagan, 1987) before and during cumulative administration of methoctramine.

Methoctramine caused inhibition of the bradycardia induced by vagal nerve stimulation (ED_{50} 38 = 5 nmol.kg^{-1}, n=6). In contrast, in the lung facilitation of vagally-induced bronchoconstriction occurred at all doses (ED_{50} 58 = 7 nmol.kg^{-1}, n=6). ACh-induced bronchoconstriction, however , was inhibited at doses exceeding 60 nmol.kg^{-1} (ED_{50} 81 = 11 nmol.kg^{-1}, n=6). Methoctramine did not affect ACh-induced hypotensive responses at doses upto 240nmol.kg^{-1}.

These results suggest that in the guinea-pig *in vivo* methoctramine shows approximately a two fold greater potency for the M_2 receptors in the heart than for M_3 receptors on airway smooth muscle. Therefore the M_2/M_3 selectivity previously reported for methoctramine *in vitro* is not demonstrable *in vivo* in this model. In the lung, methoctramine also has greater potency for the pre-junctional M_2 receptors on pulmonary parasympathetic nerves compared to the post-junctional M_3 receptors of airway smooth muscle. Thus, *in vivo*, this antagonist can be used to demonstrate pre-junctional, pulmonary, neuronal muscarinic receptors, showing a potency ratio for M_2/M_3 receptors in the guinea-pig lung comparable to that previously described for gallamine (Fryer and Maclagan 1987), but methoctramine has the experimental advantage of a shorter duration of action.

Fryer, A. & Maclagan, J. (1987) Naunyn-Schmied Arch. Pharmac. *335*, 367-371.
Giraldo, E., Micheletti, R., Montagna, E. et al (1988) J. Pharmac. Exp. Ther. *244* (3) 1016-1020.

ACTIONS OF Ro 31-6930, A NOVEL POTASSIUM CHANNEL OPENER, ON GUINEA-PIG TRACHEAL SMOOTH MUSCLE PREPARATIONS

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Ro 31-6930 2-(6-cyano-2,2-dimethyl-2H-1-benzopyran-4-yl)pyridine-1-oxide is a novel potassium channel opener possessing smooth muscle relaxant and antihypertensive properties (Paciorek et al., 1989). The aim of this study was to evaluate the actions of Ro 31-6930 on guinea pig tracheal smooth muscle preparations both in vitro and in vivo.

Tracheal rings were opened out and suspended in Krebs (37°C, 95% O_2/ 5% CO_2) under 1 g tension for the isometric recording of tone. Tracheae were incubated with indomethacin (3 µM) prior to being exposed to ≈EC_{75} concentrations of agonists. Test agents were administered cumulatively and relaxation was expressed as a percentage of the maximal relaxation to 1 mM aminophylline. Ro 31-6930 (0.001-1.0 µM), cromakalim (0.03-3.0 µM), salbutamol (0.001-1.0 µM) and theophylline (0.3-100 µM) evoked dose related reductions in spontaneous tracheal tone with IC_{50} values of 0.044 ± 0.019, 0.2 ± 0.006, 0.021 ± 0.001 and 21 ± 2.0 µM respectively (mean ± s.e.mean n = 4). Over a similar dose range all four bronchodilators evoked dose related reductions in leukotriene D_4 (0.02 µM) induced tone with IC_{50} values of 0.043 ± 0.009, 1.19 ± 0.34, 0.012 ± 0.004 and 7.2 ± 3.0 µM respectively. Tone induced by 0.1 µM U46619, a thromboxane A_2 mimetic (Coleman et al., 1981) was reduced in a dose related fashion by all four agents with IC_{50} values of 0.091 ± 0.023, 0.61 ± 0.13, 0.016 ± 0.001 and 53.5 ± 7.8 µM respectively. Betahistine (10 µM) induced tracheal tone (Brown et al., 1986) was also reduced in a dose related fashion with IC_{50} values of 0.053 ± 0.013, 0.35 ± 0.12, 0.0059 ± 0.0014 and 7.6 ± 2.4 µM respectively.

Bronchoconstriction was assessed in urethane (1.75 gkg^{-1} i.p.) anaesthetised, pithed, vagotomised guinea pigs by the air overflow method (Konzett & Rössler, 1940). ED_{65} doses of 5-HT (1.5-8.0 µg kg^{-1} i.v.) were administered every 5 min until reproducible bronchospasm was established. Ro 31-6930 (0.01-0.1 $mgkg^{-1}$ i.v.), cromakalim (0.03-1.0 $mgkg^{-1}$ i.v.), salbutamol (0.001-0.01 $mgkg^{-1}$ i.v.) and theophylline (1.0-30.0 $mgkg^{-1}$ i.v.) were then administered cumulatively 10 min prior to each 5-HT challenge. Agents evoked dose related reductions in bronchoconstriction with ID_{50} values of 0.016 ± 0.003, 0.123 ± 0.028, 0.004 ± 0.001 and 15.6 ± 2.3 $mgkg^{-1}$ respectively.

These results demonstrate that Ro 31-6930 compares favourably with salbutamol as a bronchodilator, and provides further evidence to suggest that potassium channel openers may be useful in the treatment of asthma (Buckle et al., 1987).

Buckle, D.R. et al (1987) Br.J.Pharmacol. 92, Proc.Suppl. 744P.
Brown, E.A. et al (1986) Br.J.Pharmacol. 87, 569-578.
Coleman, R.A. et al (1981) Br.J.Pharmacol. 73, 773-778.
Konzett, H. & Rössler, R. (1940) Arch.Exp.Path.Pharmak. 195, 71-74.
Paciorek, P.M. et al (1989) This meeting.

COMPARISON OF THE EFFECTS OF CROMAKALIM (BRL 34915) ON RABBIT ISOLATED BASILAR ARTERY AND THORACIC AORTA.

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Cromakalim (CROM) is a novel vasodilator which appears to act by opening K^+ channels in smooth muscle cells (Hamilton et al, 1986). CROM has been reported to relax potently a variety of precontracted, isolated peripheral blood vessels and to inhibit spontaneous mechanical activity in the portal vein from a variety of species. However, relatively little is known about the effects of CROM in isolated cerebral blood vessels. The aim of the present study was to characterise the relaxant properties of CROM in a cerebral artery, the basilar artery, and to compare this with effects in thoracic aorta, a peripheral artery.

Rings of rabbit isolated basilar artery or thoracic aorta were mounted for isometric tension recording in Krebs' bicarbonate buffer bubbled with 95%O_2–5%CO_2 at 37°C. The tissues were precontracted with EC_{50} concentrations of noradrenaline (NA), 5–hydroxytryptamine (5–HT), neuropeptide Y (NPY) or KCl and then challenged with CROM (0.01–10μM) applied cumulatively.

CROM (0.01–10μM) produced dose–related relaxations of basilar arteries precontracted with 5–HT (0.06μM), KCl (20mM) and NPY (0.05μM). The IC_{50} values and maximum % relaxations induced by CROM are shown in table 1. NA produced small, transient contractions of basilar arteries and so was not studied further in this tissue.

In rabbit thoracic aortae precontracted with NA (0.3μM), KCl (30mM) or 5–HT (1μM), CROM (0.01–10μM) produced small, dose–dependent relaxations which did not reach 50% relaxation even at CROM (10μM) (see table 1). NPY (up to 3μM) had no contractile effect on aorta.

Table 1 IC_{50}(M) for CROM against the following agonists:

Tissue	5HT	KCl	NA	NPY
Basilar	$1.8(\pm0.5)\times10^{-7}$	$2.5(\pm0.8)\times10^{-7}$	–	$(2.1\pm0.1)\times10^{-7}$
	(131±13.2%)	(100±0%)	–	(123±3.3%)
Aorta	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$	–
	(27±9.1%)	(44±4.8%)	(49±12.0%)	–

(Values in parentheses below IC_{50} values represent max. % relaxations obtained).

The results show that CROM produces potent, dose–related relaxations of the precontracted rabbit basilar artery irrespective of the spasmogen used to induce tone. In contrast, CROM produced relatively small relaxations of precontracted rabbit aorta. The effects of CROM in the cerebral vessel are interesting and require further investigation.

Hamilton, T.C. et al (1986). Br. J. Pharmacol., 88, 103–111.

Ro 31-6930, A NOVEL POTASSIUM CHANNEL OPENER WITH SMOOTH MUSCLE RELAXANT AND ANTIHYPERTENSIVE PROPERTIES

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Ro 31-6930, 2-(6-cyano-2,2-dimethyl-2H-1-benzopyran-4-yl)pyridine-1-oxide is a novel pyridine N-oxide derivative with potassium channel opening properties. The aim of this study was to evaluate some of the in vivo and in vitro pharmacology of this compound.

Portal veins were removed from male Sprague Dawley rats (≈250 g) and suspended in normal Krebs (37°C, 95% O_2/5% CO_2) under 0.5 g tension for the isometric recording of tone. Tissues were contracted with 20 mM KCl and inhibitory dose response curves constructed to cumulative administration of Ro 31-6930 or cromakalim (Hamilton, Weir & Weston, 1986). When activity was abolished the organ bath concentration of KCl was increased to 80 mM and the increase in tone recorded. Ro 31-6930 0.01-0.3 µM and cromakalim 0.1-3.0 µM evoked dose related inhibition of 20 mM KCl responses (IC_{50}: 0.038 ± 0.002 µM and 0.24 ± 0.03 µM) respectively, and had no effect on responses evoked by 80 mM KCl (mean ± s.e.mean n = 4).

When portal veins were loaded with ^{86}Rb, 1.5 µCi ml^{-1} for 90 min and the ^{86}Rb allowed to efflux into ^{86}Rb free MOPS PSS, the efflux rate coefficient (3.81 ± 0.51% 3 min^{-1}) became constant after approximately 20 min. Tissues were then challenged with Ro 31-6930 (5 µM) and a marked increase in the ^{86}Rb efflux rate coefficient (6.52 ± 0.92% 3 min^{-1}, peak effect) was detected (mean ± s.e.mean n = 5), $p<0.05$, unpaired t-test.

Male spontaneously hypertensive rats (300-350 g) were anaesthetised with methohexitone (50 $mgkg^{-1}$ i.p.). The right carotid artery was cannulated with a polyethylene catheter which was exteriorised at the back of the neck. Rats were allowed to recover and used the following day for measurements of mean arterial pressure (MAP) and heart rate. Ro 31-6930 0.01, 0.03 and 0.1 $mgkg^{-1}$ p.o. evoked significant reductions in MAP of 61.3 ± 5.0, 82.3 ± 3.3 and 98.2 ± 4.5 mmHg, 15 min after dosing. MAP remained below control values for 8-24 h following dosing. The reductions in MAP were accompanied by reflex tachycardia of 81.7 ± 7.9, 61.7 ± 15 and 103 ± 21 beats min respectively. Cromakalim 0.1, 0.3 and 1.0 $mgkg^{-1}$ p.o. evoked significant reductions in MAP of 44.3 ± 4.7, 97.5 ± 8.2 and 97.3 ± 8.2 mmHg, 15 min after dosing. MAP returned to control values between 4-8 h later. The reductions in MAP were accompanied by reflex tachycardia of 43.3 ± 6.1, 100 ± 5.8 and 90.0 ± 15 beats min^{-1} respectively (mean ± s.e.mean n = 6), $p<0.05$, split plot ANOVA.

These results show that Ro 31-6930, a novel potassium channel opener, possesses a similar profile of pharmacological activity to cromakalim but has a greater potency, and possesses a longer duration of action as an antihypertensive agent in the spontaneously hypertensive rat.

Hamilton, T.C., Weir, S.W. & Weston, A.H. (1986) Br.J.Pharmacol. 88, 103-111.

ANTAGONISM OF VASORELAXATION TO CROMAKALIM AND BRL 38227 BY NON-SULPHONYLUREA HYPOGLYCAEMIC AGENTS

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Relaxation of noradrenaline(NA)-contracted rabbit isolated mesenteric artery (RIMA) by the K^+ channel activator, cromakalim, can be antagonised by sulphonylurea hypoglycaemic agents such as glibenclamide (Wilson 1989). Two non-sulphonylurea hypoglycaemic drugs, meglitidine (Garrino et al, 1985) and pirogliride (Tutweiler et al, 1978), and glibenclamide, have now been examined as antagonists of vasorelaxation induced by cromakalim or its active (-)-enantiomer, BRL 38227 (Buckingham et al., 1986).

Isometric tension was recorded from rings of RIMA (n=5-6 per treatment) suspended in Krebs-Henseleit solution at 37°C (Clapham & Wilson, 1987). Tissues were contracted with NA, (0.1 mM) then relaxed by the cumulative addition of either cromakalim or BRL 38227. After a 30 min washout period, the tissues were again contracted with NA then incubated for a further 15 min with either pirogliride, meglitidine, glibenclamide or vehicle. The concentration- response curve to cromakalim or BRL 38227 was then repeated. IC_{50} values for cromakalim and BRL 38227 were obtained pre- and post-treatment and, after correcting for the increase in sensitivity observed in the controls, concentration ratios were calculated. Where parallel shifts of the curves to the right were obtained, pA2 values were calculated. Otherwise the reduction in maximum response was noted.

Meglitidine produced concentration-related parallel rightward shifts of the cromakalim concentration-response curve to yield a pA2 of 5.66 (Table 1); glibenclamide produced a similar effect against BRL 38227 with a pA2 of 6.86. Pirogliride produced non-parallel concentration-related rightward shifts of the BRL 38227 curve, the maximum responses being reduced by 2.4, 11.4 and 37.5% by pirogliride 1, 3 and 10 µM respectively when compared with the vehicle controls.

Table 1. Antagonism of cromakalim- or BRL 38227-induced vasorelaxation.

Antagonist	Concentration	Concentration ratio	Mean pA2
Meglitidine	1 µM	1.59	
(Vs cromakalim)	10 µM	5.08	5.66 ± 0.06
	100 µM	39.7	
Glibenclamide	0.3 µM	3.14	
(Vs BRL 38227)	1 µM	6.62	6.86 ± 0.07
	3 µM	29.9	
Pirogliride	1 µM	1.35	-
(Vs BRL 38227)	3 µM	5.83	
	10 µM	27.7	

Glibenclamide exhibits apparently competitive antagonism of BRL 38227-induced vasorelaxation with similar potency to that observed previously against cromakalim (Wilson, 1989). This result suggests a common site of action for cromakalim and its (-)-enantiomer, BRL 38227. Meglitidine, a structural analogue of glibenclamide but lacking the sulphonylurea moiety, also demonstrated apparently competitive antagonism. Pirogliride, a structurally unrelated hypoglycaemic agent, was a potent antagonist against BRL 38227, but inhibition appeared non-competitive. It remains to be determined whether this qualitative difference is a function of the antagonist drug or is related to a different site of inhibition.

Buckingham, R.E. et al (1986) Br. J. Pharmac. 87, 78P.
Clapham, J.C. and Wilson, C. (1987) J. Autonom. Pharmac. 7, 233-242.
Garrino, M.G. et al (1985) Diabetologia 28, 697
Tutweiler, G.R. et al (1978) Diabetes 27, 856.
Wilson, C. (1989) J. Autonom. Pharmac. 9, 71-78

POTASSIUM CHANNELS OF THE T-LYMPHOCYTE HYBRIDOMA CELL LINE DO-11.10.

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T-cell hybridomas such as the DO-11.10 cell line can be activated by mitogens to produce interleukin-2 (IL-2) (Shimonkevitz et. al. 1983) and thus can be used as a model for studying the mechanisms of action of drugs that promote or block T-cell mitogenesis. Mature T-cells require functional K channels to respond to mitogenic stimuli (Lewis & Cahalan 1988a). In this communication we present data to show the presence of such channels in DO-11.10 cells and show some of the basic properties of these channels.

Cells were held at a potential of -70 or -80mV using the whole-cell patch-clamp recording technique and depolarising steps were applied for 150-500ms every 15s to various test potentials. This protocol induced an outward current which inactivated slowly and which became detectable when the test potential was at values more positive than -50mV. The maximum whole-cell conductance obtained was 4.91±0.71nS (mean±s.e.m., n=8 cells). This outward current showed use-dependent inactivation. If test potentials were applied for 500ms every 1s to a potential of +20mV the peak current progressively declined until it was completely inactivated. This observation suggests that the current is carried by the "n" or normal-type K channels present in developing and activated T cells of the mouse but virtually absent in adult mouse quiescent T cells (Lewis & Cahalan 1988b). The current could be blocked by replacing internal K ions with Cs ions and was virtually abolished by external application of 100μM quinine.

The single channels that make up the whole-cell K current were observed using outside-out and cell-attached patches. These channels had a main conductance value of 14-16pS, however, rare but distinct subconductance states were also observed. Quinine (50μM) produced a flickery block of these channels without any reduction in the observed single channel conductance. This can be contrasted with TEA which has been shown to reduce the observed single channel conductance of the K channels of mouse T cells (Lewis & Cahalan 1988a).

While many drugs that promote (PHA) or block (TEA, 4-AP, quinine) mitogenesis act on these K channels (Chandy et. al. 1984), D-penicillamine (1-3mM) and auranofin (10nM) which promote IL-2 production and auranofin (10μM-1mM) which blocks it (Oben et. al. 1988) had no detectable effect on the whole-cell K current or its gating.

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Chandy, K.G., DeCoursey, T.E., Cahalan, M.D., McLaughlin, C. & Gupta, S. (1984) J. Exp. Med. 160 369-385.
Lewis, R.S. & Cahalan, M.D. (1988a) Science 239 771-775.
Lewis, R.S. & Cahalan, M.D. (1988b) Trends in Neurosci. 11 214-218.
Oben, J.A., Wallace, G.R., Chain, B.M. & Foreman, J.C. (1988) Br. J. Pharmac. 95 534P.
Shimonkevitz, R., Kappler, J., Marrack, P. & Grey, H. (1983) J. Exp. Med. 158 306-316.

LOCALIZATION OF β-ADRENOCEPTOR SUBTYPES IN THE CORONARY ARTERIES AND GRACILIS ARTERIES OF THE DOG AND PIG

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Whether large coronary arteries contain β_1 or β_2-adrenoceptors has been the subject of considerable controversy. In vitro studies have generally concluded that β_1-adrenergic receptors exert primary control over large coronary arteries. However Schwartz and Velly (1983), using radioligand binding studies, have reported a ratio of β_1:β_2-adrenoceptors of approximately 2:1 in large coronary arteries of the pig. In the present study we report the autoradiographic localization of beta-adrenoceptor subtypes in the large coronary arteries of both dog and pig. Measurements were also made in dog gracilis artery, where β_2-adrenoceptors are thought to predominate.

Hearts and gracilis muscles were excised rapidly from three dogs and three pigs anaesthetized with α-chloralose (0.1g/kg). The tissues were dissected, transferred to 0.1M phosphate buffer (pH 7.4) containing sucrose (0.32M) and rapidly frozen in Arcton 12 previously cooled to -196°C. Tissue was stored at -80°C prior to use. Frozen tissue sections (20μm thick) were cut on a cryostat and thaw-mounted on to poly-1-lysine-coated slides. Tissue sections were incubated with 25pM [^{125}I]iodocyanopindolol (ICYP) for 2 h at 37°C. Alternate sections were incubated with radioligand in the presence of either 10^{-7}M ICI 118,551 (a β_2-selective antagonist) or 10^{-7}M betaxolol (a β_1-selective antagonist) to discriminate between the beta-adrenoceptor subtypes. Non-specific binding was determined in the presence of 10^{-7}M-ICI 118,551 plus 10^{-7}M betaxolol. Autoradiograms were generated as described by Carstairs et al (1985).

In both the dog and pig there was intense labelling of large coronary arteries by ICYP. The labelling was predominantly associated with vascular smooth muscle, but not, apparently, with the endothelium. Both β_1 and β_2-adrenoceptor subtypes were present. The relative proportions of the subtypes were: dog 69.7 ± 3.3% β_1 and 30.3 ± 3.2% β_2; pig 71.1 ± 3.2% β_1 and 28.7 ± 3.3% β_2 (means ± S.E.M.). In the gracilis artery, in both dog and pig, labelling by ICYP was associated with both smooth muscle and endothelium, the latter being more intense. The beta-adrenoceptors were mainly of the β_2-subtype (approximately 95% of total beta-adrenoceptors) and were of comparable density to β_2-adrenoceptors seen in the coronary arteries.

The present results suggest the coexistence of β_1 and β_2-adrenoceptors in the large coronary arteries of the dog and pig, and the results are in good agreement with the findings of Schwartz and Velly (1983). Beta-adrenoceptors in the gracilis arteries, however, were predominantly of the β_2-subtype. Similar densities of β_2-adrenoceptors were seen in both the coronary arteries and gracilis arteries, hence the greater density of beta-adrenoceptors seen in the coronary arteries was due to the large population of β_1-adrenoceptors. Also of interest is the finding of β_2-adrenoceptors on the endothelium of gracilis arteries but not coronary arteries.

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Schwartz, J. & Velly, J. (1983) Br.J.Pharmac. 79, 409-414.
Carstairs, J.R., et al (1985) Am.Rev.Resp.Dis. 132, 541-547.

INITIAL CALCIUM RELEASE IN RAT AORTA DOES NOT APPEAR TO CONTRIBUTE TO THE SUSTAINED CONTRACTION

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We have previously described a selective reduction by prazosin (PZ) of the initial, transient EGTA-resistant contraction of rat aorta to noradrenaline (NA) (Downing et al, 1983), an effect not seen with corynanthine (COR). Subsequently, we have shown no differential reduction by prazosin of the second, sustained phase of the EGTA-resistant response (Downing et al, 1986). We have therefore investigated the importance of the initial calcium release in this preparation as a putative component of the maintained tonic contraction to alpha-adrenoceptor agonists in normal calcium.

2-3mm circular preparations of thoracic aortae from male Wistar rats (180-200g) were denuded of endothelium, suspended under a resting tension of 2g in Krebs' solution (2.5mM Ca^{++}) containing propranolol (10^{-6}M) and gassed with 5% CO_2 in O_2. The effect of verapamil (VER) (10^{-6}M) was determined on responses to $3x10^{-6}$M (NA) alone, given as a bolus dose or by cumulative addition ($1x10^{-9}$ to $3x10^{-6}$M), and also on a bolus dose to NA following 30 min incubation with either COR ($1x10^{-6}$M) or PZ ($5x10^{-9}$M). In some experiments EGTA-resistant responses were elicited to NA following 5 min exposure to calcium free Krebs' solution containing 0.5mM EGTA.

Both PZ and COR caused a similar ($p>0.05$) small reduction of the maintained tonic contraction to NA; 15.0±2.8% (n=8) and 11.6±2.4% (n=8) respectively, but the reduction of the initial fast component was significantly greater ($p<0.001$) following PZ than following COR (100% and 12.0±5.3% (n=8) respectively). The EGTA-resistant responses to bolus addition of $3x10^{-6}$M NA were biphasic with an initial transient peak after 15 to 20 seconds and a slowly developing secondary sustained phase which reached a peak at 10-15 mins. In the presence of EGTA, cumulative addition of NA produced a slowly developing sustained phase only which was not significantly different from the sustained phase following bolus addition ($p>0.05$).

VER caused a similar reduction of the maintained tonic contractions to NA irrespective of whether NA was given as a bolus or by cumulative addition (36.3±6.4 and 33.6±1.8% reduction respectively). VER caused a greater reduction of the maintained tonic responses in preparations pretreated with PZ or COR (48.4±4.2% and 48.5±4.8% respectively) but there was no significant difference between these two values ($p>0.05$).

These observations show that when the initial calcium release to α-adrenoceptor activation in rat aorta has been prevented by prazosin or by cumulative addition of NA, there is an unchanged sensitivity of the tonic contraction to verapamil, suggesting that the initial calcium release to NA does not contribute to the tonic contraction.

Downing, O A et al (1983) Br J Pharmac. 80, 315-322
Downing, O A et al (1986) Ibid. 89, 841P.

THE EFFECTS OF SKF 104078 AT PRE- AND POST-JUNCTIONAL α_2-ADRENOCEPTORS *IN VITRO*.

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The α_2-adrenoceptor antagonist SKF 104078 has been reported to discriminate between pre-and postjunctional α_2-adrenoceptors in a variety of species (Ruffolo et al, 1987; Daly et al, 1988). In these studies, presynaptic activity was determined indirectly using nerve-stimulated tissue responses. Other workers (Connaughton et al, 1988a and b), who have additionally used stimulation-evoked ^{3}H-noradrenaline release as a direct measure of presynaptic activity, have failed to demonstrate any post-junctional selectivity for SKF 104078. In the present work, using both direct and indirect methods, we find that SKF 104078 distinguishes between pre-and postjunctional α_2-adrenoceptors in both rabbit and rat in vitro.

Postjunctional α_2-adrenoceptor antagonist potency was assessed by the ability of rauwolscine or SKF 104078 to produce competitive shifts of the noradrenaline concentration-response curves (CRC) in the rabbit saphenous vein. Prejunctional α_2-adrenoceptor antagonist potency was estimated directly by the ability of rauwolscine or SKF 104078 to reverse a clonidine-induced inhibition of stimulation-evoked ^{3}H-noradrenaline release from the superfused rabbit aorta, or by the antagonists' ability to enhance stimulation-evoked ^{3}H-noradrenaline outflow from the rabbit aorta or rat vas deferens in the absence of agonist. In addition, the effects of rauwolscine and SKF 104078 on the BHT-920-induced inhibition of the twitch response in the rat vas deferens were used as an indirect measurement of prejunctional α_2-adrenoceptor antagonism.

Rauwolscine (0.01-1 μM) and SKF 104078 (0.01-1 μM) both produced concentration-dependent shifts to the right of the noradrenaline CRC in the rabbit saphenous vein, giving Schild plots with gradients not significantly different from unity and pA_2 values of 7.9 and 6.6 respectively. Rauwolscine (0.01-0.1 μM) reversed the clonidine-induced inhibition of stimulation-evoked ^{3}H-noradrenaline release from the rabbit aorta with a pA_2 value of 8.4, while SKF 104078 (0.1-10μM) failed to produce any such reversal of clonidine-induced inhibition. Furthermore, in contrast to rauwolscine, SKF 104078 (0.01-10μM) failed to cause an increase in stimulation-evoked ^{3}H-noradrenaline release in the absence of agonist in both the rabbit aorta and rat vas deferens. BHT-920 (0.001-1.0μM) produced a dose-related inhibition of the twitch response in the rat vas deferens which was antagonised by rauwolscine (0.01-1 μM) but not by SKF 104078 (0.01-10μM).

In conclusion, the standard α_2-adrenoceptor antagonist rauwolscine failed to discriminate between pre-and postjunctional α_2-adrenoceptors in vitro whereas SKF 104078 is a selective postjunctional α_2-adrenoceptor antagonist.

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Connaughton, S. et al. (1988), Br. J. Pharmacol. Suppl. 94, 433P.
Connaughton, S. et al. (1988), Br. J. Pharmacol. Suppl. (in press).
Daly, R.N. et al. (1988), J. Pharmacol. Exp. Ther. 247, 122-128.
Ruffolo, R.R. et al. (1987), Naunyn-Schmiedebergs Arch. Pharmacol. 336, 415-418.

ISOLATION OF POST-JUNCTIONAL α_2-ADRENOCEPTORS IN RABBIT ISOLATED DISTAL SAPHENOUS ARTERY: INFLUENCE OF ANGIOTENSIN-II

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Under normal experimental conditions, responses to UK-14304 are mediated via postjunctional α_1-adrenoceptors in the isolated rabbit distal saphenous artery (DSA). However in the presence of angiotensin-II (A II) a component of the response to this agonist, which is mediated via postjunctional α_2-adrenoceptors, is revealed (Dunn *et al.*, 1989). We have now attempted to isolate a population of postjunctional α_2-adrenoceptors in this preparation using a protocol similar to that employed in the rabbit lateral saphenous vein by Daly *et al.*, (1988).

3-4mm segments of DSA were placed in Krebs solution containing 1μM propranolol and 10μM cocaine, gassed with 95% O_2: 5% CO_2 at 37°C, under a resting tension of 0.6-0.8 g.wt. After obtaining a control cumulative concentration response curve (CRC) to noradrenaline (NA) attempts were made to isolate a population of postjunctional α_2-adrenoceptors by receptor protection. Briefly, tissues were exposed to 1μM rauwolscine 5 min before addition of 0.3 μM phenoxybenzamine. Both antagonists were allowed to remain in contact with the tissue for 60 min. Subsequently the tissues were washed a minimum of 7 times over the period of 1 hour. 1μM rauwolscine was present during the 1st two washes to maximise receptor protection. Thereafter, responses to NA, UK-14304 or phenylephrine (PE) were obtained both in the absence and presence of A II (0.05μM). In experiments involving the use of A II, this compound was added 15 min prior to the α-adrenoceptor agonists. In some experiments prazosin or rauwolscine were added 45 min before the onset of a CRC to NA in the presence of A II.

In the absence of A II no responses were observed to either NA, PE or UK-14304 after attempting to isolate a population of postjunctional α_2-adrenoceptors. However in the presence of A II (0.05μM), a concentration producing a transient contraction which returned to baseline after 6-11 min, all three agonists produced concentration dependent contractions. For NA and UK-14304, under these conditions, the maximum response observed increased from 0 to 45 ± 8% of the initial control maximum response to NA, prior to the protection protocol. The order of potency for these agonists was : UK-14304 > NA > PE. In addition, prazosin (0.1μM) did not antagonise responses to NA under these conditions but rauwolscine (1μM) produced an approximately 2 log unit shift to the right in the CRC to NA after attempted isolation of postjunctional α_2-adrenoceptors in the presence of A II.

In conclusion, attempted isolation of a discrete population of postjunctional α_2-adrenoceptors in the rabbit isolated distal saphenous artery was not possible under normal experimental conditions. If however the tissues were exposed to an appropriate physiological stimulus, in this case A II, concentration-dependent contractions to α-adrenoceptor agonists were revealed. The relative potencies of the agonists UK-14304 > NA > PE and of the antagonists rauwolscine > prazosin against responses to NA indicate that in the presence of A II a quiescent population of postjunctional α_2-adrenoceptors become functional. These results are consistent with the necessary presence of A II in demonstrating responses to UK-14304 mediated via postjunctional α_2-adrenoceptors in the DSA (Dunn *et al..*, 1989) and the enhancement of α_2-adrenoceptor mediated function with A II in the rabbit saphenous vein (Schümman & Lues. 1983).

Daly, C. J., Dunn, W. R., McGrath, J. C. & Wilson, V. G. (1988) Br.J.Pharmacol. **95**, 501-511.
Dunn, W. R., McGrath, J. C. & Wilson, V. G. (1989) Br.J.Pharmacol. **96**, 259-261.
Schümman, H. & Lues, I. (1983) Naunyn.Schmiedebergs.Arch.Pharmacol. **323**, 328-334.

REVERSIBILITY OF β-ADRENOCEPTOR DESENSITIZATION IN GUINEA-PIG RIGHT ATRIA AND THE EFFECT OF PERSISTENT AGONIST

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Desensitization of β-adrenoceptors has been demonstrated in vitro in isolated respiratory (Omni et al. 1985; Goldie et al. 1986) and cardiac tissues (Kaumann & Birnbaumer 1976; Broadley & Herepath 1987). However, little attention has been paid to monitoring the removal of agonist from the tissue after incubation and before challenge to test for sensitivity. In this study we use right atrial rate to measure β-adrenoceptor-mediated responses and to follow the adequacy of agonist removal following incubation with isoprenaline.

Guinea-pig right atria were set up in Krebs-bicarbonate solution containing ascorbic acid (1mM) at 37.5°C and gassed with 5% CO_2 in O_2. Spontaneous rate of contraction was recorded. A cumulative concentration-response curve (CRC) was constructed for isoprenaline (ISO) and after reaching either 10^{-6} or 10^{-5}M, this concentration was left in contact with the tissue for 2 or 4h. The tissue was then washed twice and again at regular intervals for 0.5, 1, 1.5 or 3h, before repeating a CRC for ISO. Control experiments, performed identically except that ISO was washed out BEFORE commencing the sham incubation period, were used to correct the pre-incubation CRCs of test experiments. Increases in rate were plotted as % of the pre-incubation maximum and EC50 values and dose-ratios (DR) determined. $n \geq 4$ throughout.

After 2h incubation with 10^{-6}M ISO and 0.5h washout, the ISO curve was significantly displaced to the right (DR, 10.6±2.1) indicating desensitization. However, extending the washout to 1h almost completely reversed this desensitization (DR, 3.8±0.9). After 4h incubation with 10^{-6}M ISO and 1h washout, the CRC was still displaced (DR, 17.4±6.2) indicating poorly reversible desensitization.

Increasing the concentration to 10^{-5}M (4h incubation, 1h wash) showed an apparent increase in the rightwards shift and a depression of the maximum response to 58.3±10.2%. However, the resting rate (291.7±5.9 bpm) had failed to return to the preincubation level (272.8±9.0 bpm, corrected), resulting in the artefactually depressed curves. The 1h washout was thus inadequate for removal of ISO at 10^{-5}M. Longer washout times were therefore used after 4h incubations and the removal monitored from the spontaneous rate of contraction. The resting rates fell to 291.0±7.6, 264.2±9.7 and 254.2±11.0 bpm respectively after 1.5h (5 washes), 1.5h (10 washes) and 3h (10 washes) compared with the corresponding corrected preincubation values of 265.5±9.2, 244.2±8.7 and 231.6±13.5bpm. They were all therefore still significantly elevated and exerted artefactual effects upon postincubation CRCs. Thus even 10 washes in 3h failed to remove the isoprenaline. In contrast, when 10^{-5}M ISO was immediately removed from the bath in control experiments (no incubation), 3h wash returned the rate (280.0±5.5bpm) to the resting level (282.5±6.6 bpm). After 10^{-6}M ISO, the resting rate was restored within 0.5h, whether control or tests (4h incubation) were examined.

This study therefore serves as a warning when using concentrations of ISO in excess of 10^{-6}M for β-adrenoceptor desensitization, in that inadequate removal of agonist may interfere with the subsequent assessment of sensitivity.

Broadley, K.J. & Herepath, M.L. (1987) Br. J. Pharmac. 90, 197P
Goldie, R.G. et al. (1986) J. Pharmac. exp. Ther. 237, 275-282
Kaumann, A.J. & Birnbaumer, L. (1976) N.S. Arch. Pharmac. 293, 199-202
Omini, C. et al. (1985) Eur. J. Pharmac. 106, 601-606

ABSENCE OF FUNCTIONAL 'DOWN-REGULATION' OF CARDIAC β-ADRENOCEPTORS IN LOW-OUTPUT FAILURE IN THE RABBIT

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Bristow et al. (1982) proposed a decrease in ß-adrenoceptor density in failing human hearts leading to subsensitivity to ß-adreneroceptor stimulation, i.e. 'down-regulation' of cardiac ß-adrenoceptors. We have investigated whether functional 'down-regulation' of cardiac ß-adrenoceptors occurs in a rabbit model of chronic low-output failure (Wanless et al., 1987). Rabbits were dosed for seven weeks with adriamycin (AD) then left either two (AD (7+2)) or six (AD (7+6)) weeks before haemodynamic assessment of the conscious animal. Animals were killed 1-2 days later; left and right papillary muscles were removed, suspended in modified Krebs-Henseleit solution at 32°C under approximately 1.2g resting tension and electrically stimulated (0.5Hz, 5 ms, suprathreshold voltage). Cumulative concentration-response curves to isoprenaline and the $ß_1$-adrenoceptor 'partial' agonist, Ro363 (McPherson et al., 1984), were constructed. Similar studies were carried out on papillary muscles taken from untreated rabbits, implanted with either saline-filled or isoprenaline-filled mini-osmotic pumps (SMOP and IMOP, respectively). Pumps delivered isoprenaline at 0.1mg/kg/hr for 7 days. All data is expressed as arithmetic mean ± s.e.m. and was analysed by Student's unpaired "t"-test.

Compared with control rabbits, AD (7+2) rabbits had a significantly ($p<0.05$) reduced cardiac index (219±13 vs 336±40 ml/min/kg, respectively) which showed a further significant reduction after six weeks recovery (179±13 vs 255±10 ml/min/kg, respectively). Additionally after six weeks recovery there was a significant increase in total peripheral resistance index (0.549±0.028 vs 0.380±0.014 TPR units.kg, respectively). AD-treated rabbits were judged to be in moderate heart failure which was progressive. Receptor 'down- regulation' should lead to a parallel rightwards displacement of the concentration-response curve to a 'full' agonist and to a depression of the maximum of that to a 'partial' agonist (Kenakin & Ferris 1983). In IMOP rabbits, sensitivity to isoprenaline and the maximum response to Ro363 were significantly reduced compared with SMOP rabbits. In contrast, there was no significant difference in sensitivity to isoprenaline or maximum response to Ro363 in the other four groups of rabbits, nor were they different from the results obtained in SMOP rabbits (Table 1).

Table 1: Sensitivity to isoprenaline and Ro363 in control (C), adriamycin-, saline- and isoprenaline-pretreated rabbits.

TREATMENT GROUP	SMOP	IMOP	C (7+2)	AD (7+2)	C (7+6)	AD (7+6)
Isoprenaline pD_2	7.78 ±0.14	6.95* ±0.06	7.51 ±0.08	7.37 ±0.08	7.52 ±0.09	7.49 ±0.10
Ro363 as % Isoprenaline max.	66 ±9	9* ±1	56 ±6	48 ±4	55 ±5	57 ±7
n	4	4	8	8	6	9

* significantly different from SMOP group.

Thus although cardiac ß-adrenoceptors can be functionally 'down-regulated' in the rabbit, this does not happen in adriamycin-induced heart failure. This confirms and extends the work of Woodcock et al. (1988) which showed that the cardiac ß-adrenoceptor density and adenylate cyclase activity in the AD-treated rabbit were unaltered.

Bristow, M.R. et al. (1982), N. Engl. J. Med., 307 (4), 205-211.
Kenakin, T.P. and Ferris, R.M. (1983), J. Cardiovasc. Pharmacol., 5(1), 90-97.
McPherson, G.A. et al. (1984), Br. J. Pharmac., 82, 897-904.
Wanless, R.B. et al. (1987), Cardiovasc. Res., 21, 7-13.
Woodcock, E.A. et al. (1988), J. Mol. Cell. Cardiol., 20, 771-777.

β-ADRENOCEPTOR RESPONSIVENESS OF ISOLATED VENTRICULAR MYOCYTES FROM ISOPRENALINE- OR ADRIAMYCIN-TREATED RABBITS

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In order to study beta-adrenoceptor desensitisation in cardiac failure, we have been examining the contractile responses of single isolated myocytes from human and animal hearts. The present experiments were performed in order to determine whether altered contractile function of single cells reflected changes that had been observed in vivo or in intact muscle (Gurden et al, 1989). A model of chronic low-output failure was used in which rabbits were dosed for seven weeks with adriamycin, then left two weeks to recover (AD(7+2)) before removal of the heart for cell isolation (Wanless et al 1987). A separate group of rabbits had either saline-filled or isoprenaline-filled mini-osmotic pumps implanted (SMOP and IMOP, respectively). Pumps delivered isoprenaline at 0.1mg/kg/hr for 7 days. Myocytes were isolated from the hearts of these animals by perfusion with a low calcium solution (9uM), followed by treatment with collagenase and either protease or hyaluronidase. Isolated cells were superfused with a modified Krebs-Henseleit solution at 32^{o}C and electrically stimulated (0.5Hz, 5ms, suprathreshold voltage). Contraction amplitude was measured with a video camera/edge detection system linked to an inverted microscope (Harding et al., 1988), and is expressed as percentage change in cell length. Cumulative concentration-response curves to isoprenaline were constructed. All data is expressed as arithmetic mean ± s.e.mean. and was analysed by Student' unpaired t-test. When results were obtained from several cells from one animal, the data was pooled.

Cells from AD(7+2) (n=11) rabbits had a significantly lower maximum contraction amplitude when maximally activated with calcium than cells from controls (n=14) (11.1 ± 0.9% v 13.6 ± 0.5%, $p<0.05$). There was no evidence of specific beta-adrenoceptor desensitisation in the treated animals. Depression of the isoprenaline-induced contractile maximum was no greater than that observed in the presence of maximum calcium (11.6 ± 0.6% v 13.1 ± 0.4%, $p<0.05$). There was no difference in sensitivity to isoprenaline between AD(7+2) and controls (Table 1). In contrast, in IMOP rabbits (n=6), the sensitivity to isoprenaline (Table 1) and the maximum contraction amplitude during beta-adrenoceptor stimulation was significantly reduced compared to SMOP (n=5) (5.4 ± 1.1% v 12.0 ± 1.9%, $p<0.02$). Between-animal variation was significantly greater than between-cell variation for individual animals (eg EC50 for isoprenaline in AD(7+2) plus control (C), $p<0.002$, ANOVA).

Table 1. Sensitivity to isoprenaline in control, adriamycin-, saline- and isoprenaline-pretreated rabbits. Number of animals in brackets.

TREATMENT GROUP	SMOP	IMOP	C	AD(7+2)
Isoprenaline pD_2	8.10 ± 0.10(5)	6.65* ±0.47(5)	8.12 ±0.08(11)	8.14 ±0.10(11)

*significantly different from SMOP group, $p<0.05$.

Changes in beta-adrenoceptor sensitivity in single cells reflected those in papillary muscle; desensitisation was demonstrated in isoprenaline-treated but not in adriamycin-treated rabbits.

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Gurden, J.M., Drew, G.M., Sullivan, A.T., & Poole-Wilson, P.A. (1989) British Pharmacological Society, this meeting.
Harding, S.E., Vescovo, G., Jones, S.M., Gurden, J. & Poole-Wilson, P.A.. (1988), J. Mol. Cell. Cardiol. 20, 635-647
Wanless, R.B., Anand, I.S., Poole-Wilson, P.A. & Harris, P. (1987), Cardiovasc. Res., 21, 7-13.

EFFECTS OF CO-ADMINISTRATION OF XAMOTEROL AND ISOPRENALINE ON RAT VENTRICULAR β-ADRENOCEPTOR DOWN-REGULATION

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Xamoterol (X) is a β1 adrenoceptor (AR) selective partial agonist recently introduced for the treatment of cardiac failure. Chronic therapy with this agent in rats does not lead to down-regulation of cardiac βAR in contrast to treatment with the full agonist isoprenaline (I) (Barnett & Maguire, 1986). We have studied the effect of co-administration of X and I on β AR down-regulation and recovery in order to simulate the situation of the use of X in chronic cardiac failure which is associated with reduced β AR density (Bristow et al, 1982).

Using subcutaneous osmotic minipumps I (40ug/kg/hr) and X (600ug/kg/hr) were administered separately, simultaneously or successively for various time periods to groups of AS rats (200-220g). Rats were sacrificed after 72hrs treatment with I or X alone and after 24hrs and 72hrs treatment with I plus X to assess effects on β AR down-regulation. For recovery experiments I loaded pumps were removed after 72 hrs infusion and rats were either simply resutured (control) or given further treatment with X. Animals were finally sacrificed at 24hrs or 96hrs after 2nd treatment. All X treated animals received a single IP loading dose of X (10mg/100g). Control animals for each group were sham operated.

Ventricular membranes were prepared using previously described methods (Baker et al, 1980). Maximal binding capacities (Bmax) of ventricular β1 and β2 AR were obtained by saturation analysis of 125I-pindolol binding using CGP 20712A as previously described (Lü & Barnett, 1988). Blood samples for X levels were taken by cardiac puncture under anaesthesia prior to sacrifice in representative animals from each group of experiments.

Compared to control total Bmax was reduced by approximately 30% by 24hrs and 50% by 72hrs I treatment but was unaffected by X alone (Table). Co-administration of X did not affect the rate or extent of down-regulation by I on either βAR subtype.

Bmax (fmol/mg protein) of 125I-pindolol binding to rat ventricular membranes after 72hrs subcutaneous infusions (mean ± SEM)

	Control n = 9	Xamoterol n = 3	Isoprenaline n = 3	Isoprenaline & Xamoterol n = 3
Total	20.4 ± 1.2	19.6 ± 0.9	10.0 ± 0.5*	11.7 ± 0.8*
β1	15.0 ± 1.1	13.5 ± 0.9	8.2 ± 0.5*	9.6 ± 0.9*
β2	5.3 ± 0.2	6.2 ± 0.2	1.8 ± 0.1*	2.0 ± 0.2*

* significantly different from control, $P < 0.05$

Following removal of the minipumps recovery of β1 AR in control animals was slower than β2 AR but was complete for both subtypes by 96hrs. The rate or extent of recovery of both subtypes was not significantly affected by co-administration of X.

Plasma X levels measured at the end of all treatment periods (range 100-300 nM) were similar to those producing maximal functional response in rat heart (in vitro) suggesting full receptor occupancy by the partial agonist during the infusion experiments. These results indicate that X does not enhance βAR down-regulation induced by I or impede recovery to the control state following treatment. It seems likely therefore that chronic X therapy in cardiac failure would similarly not adversely effect AR regulation in the human myocardium.

Bristow M, Ginsberg R et al 1982 New Engl J Med, 307, 205-211
Baker SP, Boyd HM, Potter LT 1980 Brit J Pharmacol, 68, 57-63
Lü X, Barnett DB 1988 Brit J Clin Pharmacol, 95, 820P

EFFECTS OF FLOSEQUINAN, A NOVEL ARTERIOVENOUS DILATING AGENT, AND ITS METABOLITE ON BOVINE HEART PHOSPHODIESTERASE ACTIVITY

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Flosequinan, a novel hypotensive agent (Sim et al 1988), is currently under clinical trial for use in both hypertension and heart failure. The vasorelaxant activity of flosequinan has been shown to be associated with increases in cGMP (Allcock et al 1988) and cAMP (unpublished) in rat aortic strips. In isolated guinea-pig atria flosequinan has weak inotropic activity (Yates et al 1988) and in guinea-pig ventricle 6.5 x 10^{-4}M flosequinan produced a 16% and a 24% increase in cAMP and cGMP respectively (unpublished). The sulphone metabolite of flosequinan, BTS 53 554 has a similar pharmacological profile and may be responsible for a major part of the clinical response to flosequinan. In this study the possibility that flosequinan and BTS 53 554 cause an inhibition of cyclic nucleotide phosphodiesterase (PDE) activity was investigated.

A commercial preparation (Sigma) containing all three isoenzymes of PDE from bovine heart was used and activity measured by the method of Thompson et al (1979) over a 10 minute incubation period at 1 μM substrate concentration pH 8.0 and 30°C. Drugs were dissolved in DMSO giving a final assay DMSO concentration of 2.5%. This concentration of DMSO inhibited enzyme activity by 10%. Each drug was tested over the range 1 x 10^{-7}M to 5 x 10^{-3}M and its effect upon both cAMP PDE and cGMP PDE activity measured.

Table 1 Concentration causing 10% inhibition of cAMP and cGMP PDE (IC10) (n=6).

Drug	cAMP PDE		cGMP PDE	
	IC10	Potency	IC10	Potency
Flosequinan	1.0 x 10^{-4}M	1	8.5 x 10^{-5}M	1
BTS 53 554	2.0 x 10^{-4}M	0.5	2.4 x 10^{-4}M	0.3
IBMX	2.3 x 10^{-7}M	434	3.0 x 10^{-7}M	283
Milrinone	8.0 x 10^{-7}M	125	1.1 x 10^{-5}M	8
M & B 22948	5.0 x 10^{-6}M	20	7.5 x 10^{-7}M	113

At IC10 values, IBMX, a non selective PDE inhibitor, and both flosequinan and BTS 53 554 were equipotent against cAMP PDE and cGMP PDE but milrinone, a selective PDE III inhibitor and M&B 22 948 a selective PDE I inhibitor showed selectivity towards cAMP PDE and cGMP PDE respectively (Table 1).

BTS 53 554 was a slightly less potent PDE inhibitor than flosequinan but both compounds were very much less potent than IBMX or milrinone as cAMP PDE inhibitors and IBMX or M&B 22 948 as cGMP PDE inhibitors.

Flosequinan and its major metabolite thus appear to be non selective PDE inhibitors with low potency in this enzyme system. The implication of these findings in the mode of action of flosequinan are difficult to interpret from the use of a mixed PDE preparation. Moreover at concentrations similar to those seen in the clinic, flosequinan (10^{-5}M) had little or no effect on cAMP PDE or cGMP PDE whereas milrinone (10^{-6}M) caused a significant inhibition of cAMP PDE ($p<0.001$). The effects of flosequinan and BTS 53 554 will be further investigated using isolated isoenzymes from cardiac and smooth muscle.

Sim, M.F. et al (1988) Br. J. Pharmacol., 94 371-380.
Allcock, A.A. et al (1988) Br. J. Pharmacol. 94 430P.
Yates, D.B. et al (1988) Br. J. Pharmacol. 95 711P.
Thompson, W.J. et al (1979) Adv. Cyc. Nuc. Res., 10 69-92.

FLOSEQUINAN-INDUCED VASODILATATION OF HUMAN RESISTANCE VESSELS IS ASSOCIATED WITH DECREASED CALCIUM SENSITIVITY

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Flosequinan (7-Fluoro-1-Methyl-3-Methylsulphinyl-4-Quinolone) is a new vasodilator for use in heart failure and hypertension. Its exact mechanism of action is uncertain but it is associated with increased levels of cyclic GMP in rat aorta. Resistance vessels (mean internal diameter 217 $\pm$ 9 um, n=41) were isolated from biopsies of subcutaneous fat from 26 normotensive subjects undergoing routine surgery (mean blood pressure 132/83 $\pm$ 3/2 mmHg, mean age 47 $\pm$ 2 years, 20 females) and mounted as ring preparations on a myograph capable of measuring isometric tension.

Cumulative dose response characteristics were obtained for flosequinan (10^{-5}-10^{-2} mol/l) against contractions produced by noradrenaline (3 umol/l) in the presence and absence of the soluble guanylate cyclase inhibitor methylene blue (10^{-5} mol/l). Flosequinan produced dose dependent relaxation in vessels contracted by noradrenaline, the pD_2 (-log[ED50(mol/l)]) for flosequinan being 3.91 $\pm$ 0.11. The sensitivity of the vessels to flosequinan increased with vessel size ($r=0.622$, $P<0.002$, n=19). The soluble guanylate cyclase inhibitor methylene blue did not inhibit flosequinan induced relaxation (pD2 3.91 $\pm$ 0.2 P NS, n=7).

Vessels were depleted of calcium by repeated stimulation with either noradrenaline 5 umol/l or potassium 125 mmol/l in the presence of EGTA 0.1 mmol/l and zero calcium buffer. Dose response characteristics were then obtained for calcium in vessels activated with either noradrenaline or potassium alone and then in the presence of flosequinan 10^{-5}, 10^{-4} and 10^{-3} mol/l. The pD2 for calcium was 4.38 $\pm$ 0.11 during noradrenaline activation and 3.77 $\pm$ 0.08 during potassium activation. Increasing concentrations of flosequinan progressively depressed the calcium sensitivity of both noradrenaline and potassium activated vessels (pD_2 flosequinan 10^{-4} mol/l 4.1 $\pm$ 0.15, $P<0.002$, n=7, noradrenaline activation; 3.47 $\pm$ 0.12, $P<0.001$, n=8, potassium activation).

Thus flosequinan produced dose dependent relaxation of human resistance vessels and the sensitivity of the vessels to flosequinan was related to the vessel diameter. Although the action of flosequinan is associated with elevated levels of cyclic GMP in large vessels, inhibition of soluble guanylate cyclase in these vessels had no effect on the response to flosequinan. We conclude that stimulation of soluble guanylate cyclase does not explain the mechanism of action of flosequinan in these resistance vessels. Flosequinan may however cause relaxation by decreasing calcium sensitivity.

DOSE-RELATED EFFECTS OF COCAINE HYDROCHLORIDE ON CANINE NASAL VASCULAR AND AIRWAY RESISTANCES

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Cocaine users who frequently snort the drug usually suffer from symptoms of rhinitis medicamentosa such as nasal congestion, edema and rhinorrhea (Schwartz et al. 1989). It is not known whether such symptoms are due to the action of the drug on the nasal mucosa or the effects of adulterants which are often added to increase the profit to the cocaine distributor. The work presented here demonstrates the dose-related actions of cocaine in the control of nasal vascular and airway resistances.

In sodium pentobarbitone anaesthetized dogs (n=6), nasal vascular resistance (R_{nv}) was measured by constant-flow perfusion of the terminal internal maxillary artery, the main arterial supply to the nasal mucosa; nasal airway resistance (R_{na}) was assessed by monitoring the transnasal airway pressure with constant airflow through each of the nasal cavities (Lung & Wang, 1984). Cocaine hydrochloride, in doses ranging from 0.05 to 5000 $\mu g\ kg^{-1}$, was infused intra-arterially via the perfusion circuit at the rate of 0.1ml min^{-1}.

Table 1

Doses of cocaine ($\mu g\ kg^{-1}\ min^{-1}$)	R_{nv} (% change)	R_{na} (% change)
0.05	5±2.3	-5±2.5
0.5	20±3.0*	-15±2.4*
5.0	75±4.5*	-30±3.6*
50	85±5.6*	-60±4.5*
500	-20±3.9*	+5±5.5
5000	-60±4.8*	+13± 4.0*

Results are means±S.E.M. of % change from controls. *P<0.05.

Cocaine in low doses (0.05 to 50 $\mu g\ kg^{-1}\ min^{-1}$) increased R_{nv} and decreased R_{na} whereas in high doses (500 to 5000 $\mu g\ kg^{-1}\ min^{-1}$) decreased R_{nv} and increased R_{na}. The results suggest that cocaine in low doses causes constriction of both resistance and capacitance vessels; however, in high doses, the drug causes dilatation of both resistance and capacitance vessels. Since nasal blood vessels are under predominant sympathetic control (Lung and Wang, unpublished data), the vasoconstriction effect is most likely to be due to potentiation of response to circulatory catecholamines (Ritchie and Greene, 1985). The vasodilatation effect is probably the result of its well-known local anaesthetic action.

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Lung, M.A. and Wang, J.C.C. (1984). J. Pharmacol. Exp. Ther. 228, 215-219.
Ritchie, J.M. and Greene, N.M. (1985). In: The Pharmacological Basis of Therapeutics, ed. Gilman A.G. et al., pp.302-322, New York: Macmillan.
Schwartz, R.H. et al (1989). Arch. Otolaryngol. 115, 63-64.

DOSE-RELATED EFFECTS OF ADENOSINE 5'-TRIPHOSPHATE ON CANINE NASAL VASCULAR AND AIRWAY RESISTANCES

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Sympathetic nervous stimulation causes nasal mucosal vasoconstriction and a decrease in nasal airway resistance whereas parasympathetic nervous stimulation causes nasal mucosal vasodilatation and an increase in nasal airway resistance. The sympathetic response is mediated via the alpha-adrenergic receptors for the resistance vessels but via some non-adrenergic and non-cholinergic mechanism(s) for the capacitance vessels. The parasympathetic response is mediated via non-adrenergic and non-cholinergic mechanism(s) for both resistance and capacitance vessels (Lung and Wang, 1987). In 1972, Burnstock proposed that ATP was released as the principal neurotransmitter from some non-adrenergic and non-cholinergic nerves. Recently, ATP has been shown to act as a co-transmitter in sympathetic and parasympathetic neurotransmission (Burnstock, 1986). Work presented here demonstrates the action of ATP in the control of nasal vascular and airway resistances.

In sodium pentobarbitone anaesthetized dogs (n=6), nasal vascular resistance (R_{nv}) was measured by constant-flow perfusion of the terminal internal maxillary artery, the main arterial supply to the nasal mucosa; nasal airway resistance (R_{na}) was assessed by monitoring the transnasal airway pressure with constant airflow through each of the nasal cavities (Lung & Wang, 1984). ATP, in doses of 0.005, 0.05, 0.5, 5 and 50 $\mu g\ kg^{-1}\ min^{-1}$, was infused intra-arterially via the perfusion circuit at the rate of 0.1 ml min^{-1}.

Table 1

Doses of ATP ($\mu g\ kg^{-1}\ min^{-1}$)	R_{nv} (% change)	R_{na} (% change)
0.005	-2±1.4	0
0.05	-12±2.8*	2±1.6
0.5	-26±2.9*	6± 1.8*
5	-36±3.1*	10±2.1*
50	-49±3.5*	-9±2.0*

Results are means±S.E.M. of % change from controls. *P<0.05.

ATP in low doses (0.005 to 5 $\mu g\ kg^{-1}\ min^{-1}$) decreased R_{nv} but increased R_{na} whereas the highest dose (50 $\mu g\ kg^{-1}\ min^{-1}$) decreased both R_{nv} and R_{na}. The results suggest that ATP in low doses causes dilatation of both resistance and capacitance vessels (i.e. the venous sinusoidal vessels); however, the purine in high dose causes, in addition to the above actions, dilatation of the outflow venous vessels. The vasodilatatory action of ATP may be due to activation of inhibitory purinoceptors. However, the role of ATP in the autonomic control of nasal blood vessels requires further investigation.

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Lung, M.A. and Wang, J.C.C. (1984). J. Pharmacol. Exp. Ther. 228, 215-219.
Lung, M.A. and Wang, J.C.C. (1987). J. Physiol. 394, 61P.
Burnstock, G. (1972). Pharmacol. Rev. 24, 509-581.

LACK OF SELECTIVITY OF α,β–METHYLENE ATP IN REDUCING SYMPATHETIC PRESSOR RESPONSES OF THE PITHED RAT

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The stable analogue of ATP, α,β-methylene ATP (mATP), reduces selective ly responses of various isolated smooth muscle preparations to exogenously applied ATP *in vitro* by desensitising the P_{2x}-purinoceptors (Kasakov & Burnstock, 1983; Meldrum & Burnstock, 1983). Thus mATP has been used extensively to examine cotransmission by noradrenaline (NA) and ATP in a variety of sympathetically innervated tissues, including vascular smooth muscle (Machaly et al., 1988). It has been reported recently that a novel administration regime of mATP in the pithed rat was able to reduce selectively the portion of the sympathetic pressor response to spinal stimulation which was resistant to α-adrenoceptor antagonists (Bulloch & McGrath, 1988). In the present study the selectivity of this *in vivo* desensitisation procedure was tested using a variety of pressor agents.

Sprague-Dawley rats (262±6g, n=35) were pithed under ether anaesthesia and ventilated with O_2 (48 strokes/min, 1ml/100g). The pithing rod was arranged for electrical stimulation of the T6-T8 spinal outflows (Gillespie *et al.*, 1970). The left carotid artery was cannulated for monitoring of blood pressure and the external jugular veins for administration of drugs. Temperature was maintained at 37°C. Gallamine (10mg/kg+10mg.kg^{-1}.hr^{-1}) and propranolol (1mg/kg) were then administered. Pressor responses to NA (0.1μg/kg), angiotensin II (0.1μg/kg), vasopressin ($7x10^{-5}$i.u./kg), and spinal stimulation (5Hz, 0.1ms pulse duration, 50V for 5s) were obtained at 10min intervals, before and after the mATP desensitisation procedure. The mATP was administered i.v. as 3 doses of 0.05mg/kg followed by 5 doses of 0.5mg/kg, each dose being separated by 1min. In some experiments the pressor response to NA and spinal stimulation were examined in the same preparation. The doses of the pressor agents were chosen to produce a pressor response similar in magnitude to that produced by spinal stimulation.

The magnitude of the pressor response to spinal stimulation at 2min post-desensitisation with mATP was reduced to 21.2±3.2% of the control value ($P<0.01$, Students paired t-test, n=6), confirming the findings of Bulloch & McGrath (1988). The pressor response to NA (0.1μg/kg), at the same time point, was significantly reduced, to 27.2±3.4% of control ($P<0.01$, Students paired t-test, n=6). In preparations where responses to both spinal stimulation and NA were examined, the mATP desensitisation procedure produced a similar reduction of both responses. The mATP desensitisation procedure also reduced the magnitude of responses to angiotensin II and vasopressin to a similar degree. The pressor responses to all stimuli recovered in parallel towards control values and the third and consecutive responses to NA, post mATP desensitisation (22min & 32min), were not significantly different from the control response.

Thus, although mATP selectively reduces responses to ATP *in vitro*, the desensitisation procedure produced a non-selective depression of pressor responses *in vivo*.

Bulloch J.M. and McGrath J.C. (1988) Br. J. Pharmacol. 95 695-700
Gillespie, J.S., MacLaren, A. and Pollock, D. (1970) Br. J. Pharmacol. 40 257-267
Kasakov, L. and Burnstock, G. (1983) European J. Pharmacol. 86 291-294
Machaly, M., Dalziel, H.H. and Sneddon, P. (1988) European J. Pharmacol. 147 83-92
Meldrum, L.A. and Burnstock, G. (1983) European J. Pharmacol. 92 161-163
H.H.D. is an S.E.R.C. Research Student. G.A.G. was an M.R.C. Research Student. This work was supported by S.H.E.R.T. and the Wellcome Trust.

DPCPX AND PD 115,199 AS DISCRIMINATORY TOOLS FOR DIFFERENTIATING ADENOSINE RECEPTORS IN GUINEA-PIG TISSUES

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Three classes of extracellular adenosine receptor (A_1-, A_{2a}- and A_{2b}) have been delineated from studies of adenylate cyclase activity in striatal membranes and of cyclic AMP accumulation in cerebral cortical slices (Daly, 1985). The xanthine-derived compounds 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and PD 115,199 have both been shown to be high affinity adenosine receptor antagonists, with DPCPX able to distinguish between the A_1- and A_{2a}- classes of adenosine receptors (Bruns et al., 1987a,b). In this communication we report the antagonist potencies of these two compounds on the A_{2b}-receptor in guinea-pig cerebral cortex, the A_1- receptor in guinea-pig ileum (Paton, 1981) and A_2-receptor in guinea-pig trachea (Farmer et al.,1988).

The effect of DPCPX and PD 115,199 on the cyclic AMP response to 2-chloroadenosine or 5'-N-ethylcarboxamidoadenosine (NECA) was measured in guinea-pig cerebral cortical slices as described previously (Alexander et al., 1989). Antagonist equilibrium dissociation constants were also estimated from antagonism of the NECA-induced relaxation of pre-contracted (histamine, 0.1mM) tracheal rings (Farmer et al., 1988), or the adenosine-stimulated inhibition (in the presence of 1μM dipyridamole) of the transmural response (stimulus parameters: 0.1 Hz, 1 msec pulse width, 1-25 V) of guinea-pig ileum.

The apparent equilibrium dissociation constants (K_D nM) determined for DPCPX and PD 115,199 were as follows:

	DPCPX	PD 115,199
Ileum	0.30 ± 0.02 (9)	21.1 ± 1.5 (6)
Trachea	3100 ± 400 (7)	28.8 ± 3.9 (9)
Cerebral Cortex	163 ± 35 (6)	395 ± 76 (5)

DPCPX was approximately four orders of magnitude more potent at the ileal A_1-receptors than the tracheal A_2-receptor, but showed intermediate affinity for the A_{2b}-receptor of cerebral cortex. In contrast, PD 115,199 showed high affinity for both peripheral adenosine receptors, with a K_D value similar to that reported for the A_{2a}-receptor (16nM; Bruns et al. 1987b), while displaying a relatively lower affinity for the cortical A_{2b}-receptor. These data indicate that the tracheal receptor may be similar to the brain A_{2a} subclass. We conclude that DPCPX retains the ability to discriminate between adenosine receptor classes in the periphery, while PD 115,199 may prove useful as a discriminatory tool for the differentiation of A_2 receptor subclasses in both central and peripheral tissues.

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Alexander, S.P. et al. (1989) Br. J. Pharmacol. 96, 135P.
Bruns, R.F. et al. (1987a) Naun. Schm. Arch. Pharmacol. 335, 59-63
Bruns, R.F. et al. (1987a) Naun. Schm. Arch. Pharmacol. 335, 64-69
Daly, J.W. (1985) Adv. Cycl. Nucl. Prot. Phos. Res. 19, 29-46.
Farmer, S.G. et al. (1988) Br. J. Pharmacol. 95, 371-378.
Paton, D.M. (1981) J. Auton. Pharmacol. 1, 287-290.

IONIC MODULATION OF ADENOSINE HYPERPOLARISATION OF RAT SYMPATHETIC GANGLIA

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Adenosine hyperpolarises isolated rat superior cervical ganglia (Brown et al, 1979; Connolly & Stone, 1988) and inhibits calcium dependent action potentials recorded from postganglionic neurones. We have now examined the effects of altering extracellular calcium, magnesium and potassium levels on the hyperpolarising responses to adenosine (AD) and 2-chloroadenosine (2CA). DC potentials were recorded from the internal carotid nerves of desheathed ganglia in vitro by a modification of the method of Bowery and Tulett (1975), employing a grease gap. Ganglia were superfused (2-2.5 ml/min) with medium at 25°C pre-equilibrated with 5% CO_2 in oxygen. The composition of the normal medium was (mM): NaCl 125, KCl 5, KH_2PO_4 1, $CaCl_2$ 2.5, $MgSO_4$ 1, $NaHCO_3$ 25, D-glucose 10. For nominally potassium-free medium KCl was omitted and KH_2PO_4 replaced by NaH_2PO_4. A 40 minute interval was allowed between 2 minute applications of AD (10-1000 μM) or 2CA (0.3-10 μM).

Table 1 Amplitude of hyperpolarising responses to AD, 10 μM (μV)

	Calcium (mM)			
Magnesium (mM)	5	2.5	0.1	0
0		55 ± 6 (16)	61 ± 6 (8)	144 ± 36 (6)**
1	20 ± 4 (10)*	46 ± 3 (43)	86 ± 10 (12)*	93 ± 15 (12)*
10	35 ± 5 (8)	37 ± 9 (3)	60 ± 11 (4)**	60 ± 11 (6)**

* P<0.01 compared with 2.5 mM calcium
** P<0.01 compared with 1 mM magnesium
All values are mean ± s.e.m. (M)

In low calcium media (1 mM Mg) the responses to both AD and 2CA were enhanced and prolonged. Conversely in 5 mM calcium the responses were significantly reduced compared with controls. Changing the concentration of magnesium ions from 1 to 10 mM in low calcium media reduced significantly the sensitivity to adenosine. There was a tendency for AD responses to be reduced in high 12 mM) potassium (38 ± 11 μV, n = 5, for AD 100 μM) compared with controls (58 ± 9 μV, n = 8) though this was not significant. In potassium free medium the response to AD was increased to 130 ± 21 μV (n = 4, P<0.01).

The results do not reveal the same dependence on magnesium ions of postsynaptic AD responses which has been noted for presynaptic actions (Bartrup & Stone, 1988), and they also suggest that calcium ions are not important in the hyperpolarising actions of AD on this ganglion. The changes seen with alterations of potassium content however would be consistent with an increase of potassium conductance by AD.

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Bartrup, J.T. & Stone, T.W. (1988) Brain Res. 463, 374-379
Bowery, N.G. & Tulett, D. (1975) J.Physiol. 246, 20P
Brown, D.A. et al (1979) J.Physiol. 290, 441-451
Connolly, G.P. & Stone, T.W. (1988) Br.J.Pharmac. 95, 841P

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EXAMINATION OF WHETHER ADENOSINE INHIBITS THE POSITIVE CHRONOTROPIC RESPONSE TO FIELD-STIMULATED NORADRENALINE RELEASE OF GUINEA-PIG ISOLATED ATRIA

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Exogenously added adenosine has been shown to inhibit the release of noradrenaline from isolated perfused whole hearts (Wakade & Wakade, 1982; Richardt et al. 1987), presumably by a presynaptic action on sympathetic neurones. Adenosine released endogenously by ischaemia may also be inhibitory (Richardt et al. 1987). It is possible that the measurement of released noradrenaline from whole hearts is influenced by adenosine's concomitant effects on coronary flow. To overcome this possible limitation, we have used isolated atria to examine the possible effects of exogenous and endogenous adenosine on the chronotropic responses to neurotransmitter released by field stimulation.

Spontaneously beating paired guinea-pig atria were set up between platinum electrodes in 25ml organ baths. Tissues were constantly perfused (7ml min^{-1}) with Krebs bicarbonate solution containing atropine (1μM) at 38°C and gassed with 5% CO_2 in O_2. Field stimulation (2 x threshold voltage, 1ms, 1-10Hz) for 10-30s alone or alternating with bolus doses of noradrenaline (NA, 1μg) were applied at regular intervals. Rate of contraction was recorded from the isometric tension signal. After obtaining reproducible control responses, adenosine or antagonists were added to the perfusion solution.

Immediately after field stimulation (S) biphasic positive chronotropic responses were obtained. The initial peak was propranolol-sensitive (1.2μM) and therefore due to release of NA onto atrial β-adrenoceptors. The secondary plateau was removed by morphine (12.2μM) and attributed to release of an unidentified transmitter (Wilson, 1981). After 35 min perfusion with adenosine (3.75μM), the peak rate response to S (1Hz, 20s) was inhibited by only 5.6±0.1% and that to NA by 32.1±22.4%. The same result was obtained with a higher concentration (11.25μM) which was just above threshold for direct negative chronotropy. In the presence of morphine to examine only the noradrenaline-mediated response, adenosine (3.75 and 11.25μM) inhibited the responses to S by 11.1±3.2 and 9.4±9.3% and the responses to NA by 51.3±7.4 and 53.4±5.8% respectively. Since NA was also affected, the small inhibitions of S are due entirely to POST-synaptic effects of adenosine. The response to S in the presence of propranolol was unaffected by adenosine (3.75μM).

Adenosine deaminase (0.3 U ml^{-1}) did not affect responses to S (1 or 10Hz) or NA. Therefore neither exogenous nor endogenous adenosine appear to exert an inhibitory effect on the atrial responses to field-stimulated noradrenaline release. This disagrees with results from whole hearts but confirms results on noradrenaline release from mouse atria (Johnson et al. 1987).

Possible means for the lack of effect of adenosine were examined briefly. Neither neuronal uptake of NA nor autoregulatory feedback via α-adrenoceptors were responsible since adenosine was also ineffective in the presence of DMI (1.13μM) alone or with idazoxan (96nM). The technique was capable of detecting pre-synaptic inhibition since clonidine (0.13μM) abolished the response to S.

Johnson, H. et al. (1987) Br. J. Pharmac. 91, 773-781
Richardt, G. et al. (1987) Circ. Res. 61, 117-123
Wakade, A.R. & Wakade, T.D. (1982) Neuroscience 7, 2267-2276
Wilson, A.N.A. (1981) Br. J. Pharmac. 73, 294P.

EFFECT OF 8-PHENYLTHEOPHYLLINE AND ENPROFYLLINE ON THE HYPERAEMIC RESPONSE TO EXERCISE IN THE GRACILIS MUSCLE.

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The release of adenosine has been detected from the skeletal muscle of some species during exercise (Bockman et al., 1975; Ballard et al., 1987; Bockman & MacKenzie, 1983). In the present study we have investigated the contribution of adenosine to exercise hyperaemia by examining the effects of 8-phenyltheophylline (8PT), an adenosine receptor antagonist, on this vasodilator response. The effects of another xanthine (enprofylline) which has low affinity for adenosine receptors were also studied as a control.

Cats(2.3-3.0kg) were anaesthetised with sodium pentobarbital (45mg.kg^{-1} intra thoracic), maintained under anaesthesia by a continuous pentobarbital infusion (approximately 0.3mg.kg^{-1}min^{-1}) and artificially ventilated. Systemic blood pressure was measured in the right common carotid artery. The right gracilis muscle was vascularly isolated, connected to an isometric force transducer and blood flow measured with an electromagnetic flow probe. The obturator nerve was stimulated at 5 volts, 0.1 ms duration at 1.0 Hz for 20 mins and the muscle blood flow and tension measured. This was performed twice in each cat, once with vehicle (50% P.E.G./50% 0.1M NaOH, 0.1ml.min^{-1}) and repeated 40 mins into an infusion of either 8PT (n=5) or enprofylline (n=4) at 2.7x10^{-7} mol. kg^{-1}min^{-1}(i.v.).

8PT significantly reduced the hypotensive response to 2 chloroadenosine (10μg.kg^{-1}) from 37.0±0.8 to 3.4±1.4mmHg (P<0.001), whilst the response was unaffected by enprofylline (30.0±4.5 to 31.2±3.7mmHg).

The active muscle tension produced at 20 mins was unchanged at 6.5±0.6 and 7.0±0.8kg.100g muscle weight^{-1} pre and during 8PT respectively, and 8.0±1.1 and 8.1±1.3kg.100g muscle weight^{-1} pre and during enprofylline respectively.

Table 1 Gracilis muscle vascular conductance at rest and 20 mins exercise (units ml.min^{-1} 100g^{-1}.mmHg^{-1})

	VEHICLE	8PT	VEHICLE	ENPROFYLLINE
REST	0.160±0.026	0.147±0.042	0.212±0.064	0.201±0.007
EXERCISE	0.628±0.110	0.317±0.032	0.605±0.104	0.678±0.067
	P<0.05		N.S.	

All values quoted are mean ± s.e. of mean.

Conductances were not significantly different at rest (Table 1). During all exercise periods muscle conductance increased (P<0.05). However during 8PT infusion the conductance at 20 mins of exercise increased to only 46±6% of that recorded during vehicle infusion (P<0.002) whilst enprofylline had no significant effect (17±17% higher).

These results indicate that adenosine can account for approximately 50% of the vasodilatation during 1.0 Hz twitch contractions of the cat gracilis muscle.

Ballard, H.J. Cotterell, D. & Karim, F. (1987) J. Physiol. 387, 401-403.
Bockman, E.L. Berne, R.M. & Rubio, R. (1975) Pflug Arch. 355, 299-241.
Bockman, E.L. & MacKenzie, J.E. (1983) Am. J. Physiol. 244, H552-H559.
Fuchs, B.D., Gorman,M.W. & Sparks, H.V. (1986) Proc.Soc.Exp.Biol.Med. 181, 364-370.

EFFECTS OF PROPOFOL ON MEMBRANE CURRENTS AND CONTRACTION IN SINGLE MYOCYTES ISOLATED FROM GUINEA-PIG VENTRICLE

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Propofol anaesthesia has been shown to produce myocardial depression *in vivo* (Stephan *et al.*, 1986). Action potentials, membrane currents and contractions of guinea-pig ventricular myocytes were recorded in order to investigate possible mechanisms of action of propofol.

Cells were isolated by enzymic dispersion and superfused in an oxygenated balanced salt solution (36 °C; pH 7.4) containing 2.5M $CaCl_2$. Cells were impaled with glass microelectrodes containing 0.5M K_2SO_4. Resting membrane potentials were -70 to -90mV.

Propofol, in a dose-dependent fashion, depressed the plateaux and shortened the durations of action potentials induced by brief current stimuli. 50 μM propofol reduced the time taken for 20% repolarisation of the action potential by 16.3 ± 4.7% ($p<0.05$ compared with control, n=9). Cells were voltage-clamped with a single microelectrode system at a holding potential of -40mV, to inactivate the sodium current, and step depolarisations to 0mV were applied. A 200 ms depolarisation elicited currents carried by calcium. These were measured as the difference between the peak inward current and the current at the end of the step depolarisation. The associated contractions were measured using an optical method. The calcium activated current was recorded as a slow 'tail' of inward current on repolarisation, following a brief 20ms depolarisation. Student's paired t test was applied to the data. The amplitude of the membrane currents and associated contractions were depressed by propofol in a dose-dependent fashion (Table 1).

TABLE 1. Percent depression of membrane currents and contraction induced by propofol. Values are means ± s.e.m., n is the number of cells (n=6 for tail current values), *$p<0.05$ and **$p<0.005$ compared with control.

Concentration (μM)	25	50	100
n	17	19	15
Calcium current (nA)	4.5 ± 1.8*	25.0 ± 2.7**	45.6 ± 3.3**
Tail current (nA)	22.4 ± 4.6*	25.3 ± 5.4**	51.7 ± 7.4*
Contraction	23.2 ± 4.6*	37.0 ± 5.1*	57.0 ± 2.6**

The magnitude of the calcium current is thought to reflect calcium entry into the cell and the tail current may indicate the calcium transient (Mitchell *et al.*, 1987). The reduction of calcium entry by propofol and the possible influence on the cytosolic calcium transients may contribute to the observed shortening of the action potential duration and the negative inotropic effect of this anaesthetic. The actions of propofol on calcium currents appear to be similar to those of halothane (Terrar & Victory, 1988), but further investigations are required to determine whether there are any additional actions of propofol on intracellular calcium release.

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Mitchell, M. R., Powell, T., Terrar, D. A. & Twist, V. W. (1987) J. Physiol. (Lond). 391, 545-560.
Stephan, H., Sonntag, H., Schenk, H. D., Kettler, D. & Khambatta, H. J. (1986) Br. J. Anaesth. 58, 969-975.
Terrar, D. A. & Victory, J.G.G. (1988) Br. J. Pharmac. 94, 500-508.

EFFECTS OF AMIODARONE ON CALCIUM CURRENTS IN VENTRICULAR CELLS ISOLATED FROM GUINEA-PIG HEART

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Preliminary observations of acute effects of amiodarone have been reported (Nishimura et al, 1986). This has been further investigated in single muscle cells isolated from guinea-pig ventricle. Cells were isolated using collagenase and calcium currents were recorded from voltage-clamped cells in response to step depolarizations to 0 mV from a holding potential of -40 mV (Powell, Terrar & Twist, 1980; Mitchell, Powell, Terrar & Twist, 1987). Amiodarone at a final concentration of 20 μM was prepared in plasma-containing solution (following the procedure of Polster & Broekhuysen, 1976).

Figure 1A shows gradual development of the effects of amiodarone (filled circles) on the amplitude of calcium currents. Superimposed records of calcium currents before and during amiodarone are shown in Figure 1B. In five cells the decrease in amplitude of calcium currents amounted to 29 ± 8% (mean ± s.e.m.) after 2 min of exposure to amiodarone; decreases were observed in a further five cells exposed for shorter periods. Any reversibility of these effects was small and slow under the conditions of our experiments (total recording time less than 30 min). The effects of plasma alone (in equivalent concentrations to those used in the amiodarone experiments) are also shown in Figure 1A (open squares). After 2 min exposure to plasma, the amplitude of calcium currents was decreased in one cell and increased in five; the mean change was an increase of 19 ± 8%. The observed effect of amiodarone on calcium currents may contribute to its acute actions in patients.

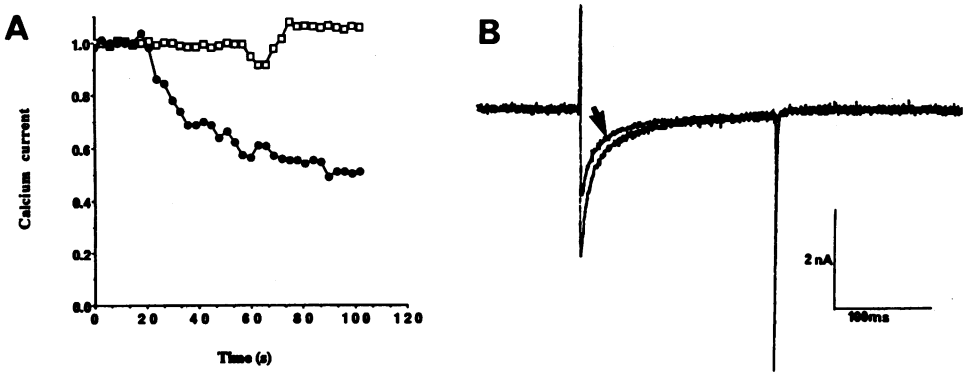


Figure 1. *A*: Development of the effect of amiodarone (20 μM; filled circles); effects of plasma alone in another cell are superimposed (open squares). The first six currents (0 to 18s) were recorded before switching solution, and current amplitudes over the whole period of recording were normalised to the mean over this period. *B*: Superimposed records of calcium currents in response to a step depolarization from -40 to 0 mV before and after two min exposure to amiodarone (arrow).

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Mitchell, M.R., Powell, T., Terrar, D.A. & Twist, V.W. (1987) J. Physiol. 391, 527-544.
Nishimura, M., Follmer, C.H., Cigan, A.L., Yeh, J.Z. & Singer, D.H. (1986) Circulation 74, Suppl. II, II-169.
Polster, P & Broekhuysen, J. (1976) Biochem. Pharmacol. 25, 131-134.
Powell, T. Terrar, D.A. & Twist, V.W. (1980) J. Physiol. 302, 131-153.

PATHWAYS FOR CALCIUM ENTRY DURING CALCIUM OVERLOAD IN CARDIAC MYOCYTES

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Calcium overload can be induced in ventricular myocytes by treatment with veratrine or veratridine (Ver Donk *et al.*,1986; Patmore, 1987), ouabain (Patmore *et al.*, 1989) or subjection to calcium paradox (Chapman and Rodrigo, 1988). These three methods depend on increasing sodium channel conductance, inhibiting sodium efflux and increasing sodium permeability (possibly through calcium channels) respectively. Thus it appears that raised levels of intracellular sodium precede calcium overload. It has been shown that certain calcium antagonists such as nicardipine and flunarizine can protect against calcium overload and that this property is not related to normal calcium channel blocking activity (Patmore *et al*, 1988). It has therefore been suggested that compounds which protect against ouabain-induced calcium overload do so by blocking sodium entry and thus prevent subsequent calcium entry : if nicardipine is applied after ouabain treatment (in the absence of extracellular calcium) and thus after sodium levels have risen on readmission of calcium, a rise in tonic tension (calcium overload) occurs (Patmore *et al.*,1989). We have examined whether this calcium entry occurs through voltage-activated calcium channels.

Embryonic chick myocytes were prepared as described by Patmore *et al.*,(1989) and calcium channel currents were recorded as described by Anderson *et al.*,(1988) We followed the experimental protocol described in fig 9 of Patmore *et al.*,(1989). Throughout this procedure calcium channel currents were evoked on depolarization from a holding potential of -80 mV to 0 mV. In untreated myocytes calcium channel currents of around 100 pA were observed. Myocytes were superfused with salt solution containing 0.5 μM TTX and 10 μM diltiazem to block normal sodium and calcium channels and to inhibit twitch contractures. They were subsequently treated with 10 μM ouabain in the absence of calcium. After 10 min, 5 μM nicardipine (a concentration which if applied before ouabain prevented calcium overload) was added to the superfusate, during this period there was no calcium channel current . After a further 10 min calcium (or barium) was readmitted, which causes a rapidly developing contracture (Patmore ,(1989). However, at this point in time the voltage-activated calcium channel current was blocked.

These data indicate that calcium entry causing calcium overload in cardiac myocytes is not via voltage-activated calcium channels but may be via non-specific cation channels or a modification of the activity of the sodium-calcium exchanger.

Anderson, A. J. *et al.*, (1988) J.Physiol., **407**, 103P
Chapman, R. A. & Rodrigo, G. C. (1988) J.Physiol., **401**, 26P
Patmore, L. (1987) J.Physiol., **381**, 90P
Patmore, L. *et al.*, (1988) Br. J. Pharmac., **93**, 71P
Patmore, L. *et al.*, (1989) Br. J. Pharmac., *(in press)*
Ver Donk, L. *et al.*, (1986) Life Sci., **38**, 765-772

EFFECTS OF ANTIOXIDANTS AND VERAPAMIL ON NORADRENALINE RELEASE AND CONTRACTURE IN THE ISCHAEMIC/REPERFUSED RAT HEART

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Myocardial ischaemia and reperfusion are associated with the development of arrhythmias and other forms of cardiac damage. The precise mechanisms contributing to this damage are poorly understood but local catecholamine release may have a role to play,possibly by enhancing the influx of calcium into cells. Recently a number of reports have shown that antioxidants and free radical scavenging drugs such as glutathione, superoxide dismutase(SOD), catalase(CAT) and mannitol(MAN) can protect the heart against ischaemic / reperfusion induced injury. This raises the possibility that these drugs may be working by reducing the local release of catecholamines from the heart. In order to examine this the effects of glutathione,and a combination of SOD/CAT/MAN ($10u.ml^{-1}$,$300u.ml^{-1}$,50mM respectively) on catecholamine release from the globally ischaemic/ reperfused rat heart have been studied. The effects of these agents on the contracture which develops following the onset of ischaemia have also been studied. Hearts from male Wistar rats were paced at 300bpm and perfused at 37°by the Langendorff technique at a flow rate of $10ml.min^{-1}$. Developed tension was measured by apex displacement at a resting tension of 2g. After a 20min. equilibration period ischaemia was induced for 60min. by reducing the flow rate to $1ml.min^{-1}$ and superfusing the heart with anoxic perfusate at$9ml.min^{-1}$. Reperfusion with oxygenated perfusate was performed for 5min. All drugs were added 10min. before the onset of ischaemia. Perfusate samples coming from the heart were assayed for noradrenaline(NA) by HPLC using electrochemical detection. Perfusion pressure,heart rate and developed tension were measured continuously. As glutathione(1mM) produced coronary dilation and a slight fall in developed tension a comparison was made with verapamil at a concentration (15nM) which produced similar changes in these parameters.

Table 1 Effects of drugs on the onset and time to maximal rise in the ischaemically induced contracture. mean+sem, *p<0.05cw control.

	n	Onset	Maximal
Control	6	7min40s±12s	15min20s±12s
GSH 0.1mM	4	9min±0s *	15min40s±20s
GSH 1.0mM	8	10min7s±21s *	18min7s±30s *
SOD/CAT/MAN	8	6min26s±17s *	13min53s±23s *
VERAPAMIL 15nM	4	11min30s±38s *	22min±1min13s *

From Table 1 it can be seen that GSH and verapamil delayed the onset and time to maximal contracture,in contrast the SOD/CAT/MAN mixture reduced these times. In control hearts basal levels of NA release (10.8+0.9pmol/min/g) were increased during ischaemia to a peak of 18+1.1pmol/min/g. During reperfusion peak release was 26.6+0.9pmol/min/g,this declined to the basal level within 5min. At the concentrations used none of the drugs had any significant effect on NA release during the ischaemic or reperfusion periods. This data indicates that the protective action of antioxidant drugs in the isolated rat heart which has been previously reported is not due to a reduction in the release of NA.

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A PHARMACOLOGICAL ACTION OF VITAMIN E UNRELATED TO ANTI-OXIDANT ACTIVITY

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The current view of vitamin E (alpha-tocopherol) as an in vivo anti-oxidant, that protects membranes from attack by free radicals generated within the cell, seems generally accepted (Lucy, 1978) and the biological potencies of tocopherols appear to be proportional to their anti-oxidant activity (Century & Horwitt, 1965).

In guinea-pig isolated hypoxic colon the vitamin was able to elicit dose-dependent contractions and restoration of mechanical activity (Kelly, 1986) and this model was used to test the hypothesis that the mechanism of action of vitamin E in hypoxic colon is related to its anti-oxidant potency. Segments of guinea-pig colon (2 cm long) were suspended vertically in tissue baths containing Krebs-Henseleit solution bubbled with a 95% O_2/5% CO_2 gas mixture. After a period of equilibration in this 'normoxic' environment the gas mixture was switched to 95% N_2/5% CO_2 and contractile activity was measured. The oxygen content of the suspending medium was also recorded. After 15 min the oxygen tension had fallen to less than 0.1% of its original level and during this 'hypoxic' period drugs were added to the bath and subsequent contractile activity was observed.

The tocopherols, alpha-, beta-, gamma- and delta-, differ in the number and position of methyl groups in the chromanol ring structure and this feature gives them different anti-oxidant capacities. Their potencies in eliciting contractions in 'hypoxic' colon were measured. Mean EC_{50} values (± s.e.mean) for (±)-alpha-tocopherol, (±)-gamma-tocopherol and (±)-delta-tocopherol were 71.7 ± 14.0, 12.7 ± 4.0 and 6.8 ± 2.7 μM respectively for 10 animals. This order of potency differs markedly from and indeed reverses the order for anti-oxidant capacities (Burton et al, 1983) and their biological potency using the normal bioassay method of foetal resorption.

In addition the anti-oxidant capacity of the chroman ring structure of the vitamin, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), is about 40% of that of vitamin E itself (Burton et al, 1983) but when tested using the colon model it was ineffective. Phytyl side chain structures, such as phytol, structurally resembling that of the vitamin but without apparent anti-oxidant capacity were potent stimulants of contractile activity. The results suggest that the stimulant action of vitamin E in the colon during hypoxia is unrelated to the vitamin's known anti-oxidant property.

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Burton, G.W., Cheeseman, K.H., Doba, T., Ingold, K.U. and Slater, T.F. (1983) Ciba Found. Symp. 101, 4-14.
Century, B. & Horwitt, M.K. (1965) Fed. Proc. 24, 906-911.
Kelly, M.J. (1986) J. Pharm. Pharmacol. 38, 66-68.
Lucy, J.A. (1978) in 'Tocopherol, Oxygen and Biomembrane': de Duve, E. & Hayaishi, O. (eds), pp 109-119. New York, Elsevier.

INCREASED RENAL RESPONSE TO FENOLDOPAM IN SPONTANEOUSLY HYPERTENSIVE RATS

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A defect in the renal dopaminergic system has been suggested to play a role in the pathogenesis of hypertension (Lee, 1986). We therefore studied the renal effects of fenoldopam (F), a selective DA-1 receptor agonist known to induce renal vasodilation and natriuresis, in both spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar-Kyoto rats (WKY). F was infused during 1 hour (1μg.kg-1.min-1) at an infusion rate of 12 ml.min-1, preceeded and followed by infusion of saline (12ml.min-1) during 30 min. Four timed 30 min urine collections were done to assess the diuretic and natriuretic effects. Renal blood flow (RBF) was measured with an electromagnetic flow meter (SKALAR MDL 1401) using perivascular flow sensors. Renal vascular resistance (RVR) was defined as blood pressure/renal blood flow. Statistical significance was assessed by two way analysis of variance. (* significantly different from baseline ($p<0.05$), + significant difference between SHR and WKY ($p<0.05$)).

Table1 Mean BP (mmHg), RVR (mmHg.min.ml-1) and urinary sodium excretion (UNa)(μmol.min-1) before and during infusion of F during 30 and 60 min.

	WKY (n=7)			SHR (n=6)		
	before	30'	60'	before	30'	60'
mean BP	129.1	118.3 *	125.4	178.6 +	160.8 *+	149.8 *+
RVR	83.74	65.90 *	60.73 *	71.19	39.83 *+	41.31 *+
UNa	0.58	0.82 *	1.08 *	0.90 +	1.81 *+	2.49 *+

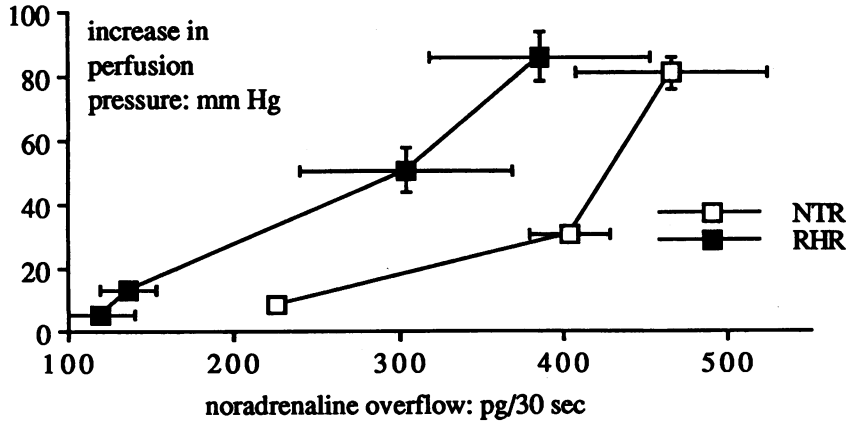
Baseline levels of mean BP, RBF and UNa were higher in SHR. F reduced BP and RVR, and enhanced UNa in both WKY and SHR. However the hypotensive effect of F, the renal vasodilatory and natriuretic response to F were more pronounced in SHR. It is concluded that the renal response to DA-1 receptor stimulation is enhanced in SHR as compared to their normotensive controls. This could be related to up-regulation or changes in affinity of renal DA-1 receptors.

Lee, M.R. (1982) Clinical Science **62**, 439-448.

VASCULAR NORADRENERGIC TRANSMISSION IN RENOVASCULAR HYPERTENSIVE RATS

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Renovascular hypertension is associated with a decrease in vasoconstrictor responses to electrical stimulation in vitro (Fouda *et al.*, 1987). This could be due to decreased release of noradrenaline and/or decreased postsynaptic sensitivity. We investigated changes in noradrenaline (determined by HPLC) content, overflow and contraction induced by electrical stimulation, in normotensive (NTR) and renovascular hypertensive (RHR, 2 kidney, 1 clip) rat tail arteries (n = 7 per group). Tail arteries were perfused and superfused at a flow rate of 2 ml/min; vasoconstriction induced by field stimulation (Fouda *et al.*, 1987) was estimated from the increase in perfusion pressure. Noradrenaline content decreased in RHR (5.8 ± 0.6 vs 8.1 ± 1.0 ng/mg; $P < 0.05$). There was no change in spontaneous noradrenaline overflow before electrical stimulation (NTR = 47 ± 5 vs RHR = 68 ± 10 pg/min). There was a decrease in vasoconstriction and noradrenaline overflow in RHR tail arteries at each frequency of electrical stimulation, e.g. 0.3 Hz, NTR = 30 ± 5 and 404 ± 58 vs RHR = 13 ± 2 mm Hg and 136 ± 17 pg/30 sec.; $P < 0.01$). A plot of vasoconstriction against noradrenaline overflow showed an increase in sensitivity to endogenous noradrenaline in RHR (see figure). Cocaïne (4×10^{-6} M) increased both contraction and noradrenaline overflow but did not alter sensitivity (i.e. contraction vs noradrenaline overflow).



These results show an increase in vascular receptor sensitivity and/or in receptor coupling efficiency, associated with a decrease in noradrenaline overflow following stimulation of sympathetic nerves, in renovascular hypertension.

Fouda A.K., Marazzi A., Boillat N., Sonnay M., Guillain H. & Atkinson J. (1987). Blood Vessels 24, 63-75.

NAFTIDROFURYL ATTENUATES VASOCONSTRICTION INDUCED BY 5-HT IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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As naftidrofuryl antagonizes the vasoconstriction induced by 5-hydroxytryptamine (5HT) (Maloteaux *et al.*, 1986) and it has been used in the chronic treatment of arterial disease (Vanhoutte & Zander, 1988), we have investigated the effects of chronic treatment of SHR with naftidrofuryl on vascular responses to 5HT in vitro. Naftidrofuryl was given as the fumarate salt (base: 30 mg/kg, ip, per day); controls received fumaric acid (7 mg/kg, ip, per day). Treatment started at 1 month of age and continued for 3 months until the SHR reached an age of 4 months. Vascular responses to 5HT were tested in tail arteries removed from the SHR following measurement of systolic arterial pressure. Proximal portions of the tail artery were perfused/superfused at a constant flow rate of 2 ml/min. A concentration-response curve was determined by injecting 0.1 ml bolus of 10^{-8} - 10^{-3} M 5HT into the perfusion circuit. A second curve was then constructed in the presence of 10^{-6} M naftidrofuryl. In preliminary experiments, 2 concentration-response curves for 5HT in the absence of naftidrofuryl gave similar results. Chronic treatment with naftidrofuryl produced the following changes: a decrease in the maximum vasoconstrictor response (240 ±18 vs. 305 ±13 mm Hg, $P < 0.05$ versus controls) and the sensitivity to 5HT (DE_{100} mm Hg: 1.8 ± 0.2 vs. 0.8 ± 0.2 x 10^{-6} M 5HT, $P < 0.05$). The in vitro sensitivity to naftidrofuryl was unchanged -log K_B 7.4 ± 0.2 vs. 7.9 ± 0.2(NS).
Naftidrofuryl did not modify systolic arterial pressure; values after 3 months' treatment were 214 ± 9 (n = 6, controls 203 ± 9, n = 6).
In conclusion chronic treatment with a dose of naftidrofuryl which does not lower blood pressure, attenuates vascular responses to 5HT.

Maloteaux J.M., Haeich J., De Campeneere D., Berta P. & Vidal N. (1986). Arzneim.-Forsch./Drug Res. 36, 1194-1198.
Vanhoutte P.M. & Zander J.F. (1988). in 'Vasodilatation: Vascular Smooth Muscle, Peptides, Autonomic Nerves, and Endothelium': Vanhoutte, P.M. (ed.), pp 281-288. New York, Raven Press.

CHARACTERISING THE 5-HT RECEPTOR MEDIATING CORONARY VASODILATATION IN THE DOG USING TRYPTAMINE ANALOGUES

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Using a series of tryptamine analogues, previous studies identified pharmacologically distinct $5HT_1$-like receptors located on the vascular smooth muscle and endothelium, which mediate vasorelaxant responses to 5HT (Martin et al, 1987). Extending this approach to the intact animals, we concluded that the systemic vasodilator response to 5HT in the anaesthetised rat was primarily mediated by the receptor located on the vascular smooth muscle (Martin et al, 1987). In the anaesthetised dog we have also observed that 5HT causes coronary vasodilatation, and therefore we have utilised these tryptamine analogues to characterise the 5HT receptor mediating this coronary vasodilatation response in vivo.

Beagle dogs (10-17kg, either sex), were anaesthetised with pentobarbitone (60mg/kg i.v.), artificially ventilated, and instrumented to measure left circumflex coronary blood flow (CBF), cardiac contractility (LVdP/dt) and rate (HR), and systemic blood pressure (BP). Coronary conductance (CC) was calculated as the reciprocal of coronary resistance x 1000 and expressed as conductance units . Each animal was pretreated with ketanserin (0.1 mg/kg i.v) and MDL-72222 (0.5 mg/kg i.v.), to block $5HT_2$ and $5HT_3$ receptors respectively, prior to generating a dose response curve to one of the following tryptamine analogues: 5-carboxamidotryptamine (5CT), 5HT, α-methyl 5HT (αT), N,N dipropyl 5-carboxamidotryptamine (DT), or GR43175 (GR, synthesised at Wellcome Research Laboratories). These analogues were administered as bolus injections via a left atrial cannula to avoid pulmonary degradation.

Table 1 Maximum haemodynamic change (mean +/- sem) and dose to produce this change for each tryptamine analogue.

Analogue (ug/kg)	CBF (ml/min)	CC (cond. units)	LVdP/dt (mmHg/sec)	HR (bpm)	BP (mmHg)	n
5CT (1)	49 ± 4	97 ± 5	400 ± 306	10 ± 3	-45 ± 5	6
5HT (100)	71 ± 14	77 ± 16	1333 ± 217	-30* ± 12	-33 ± 5	3
αT (1000)	65 ± 8	28 ± 8	11733 ± 933	70 ± 18	82 ± 10	3
DT (300)	6 ± 2	11 ± 2	-1000 ± 200	-55* ± 20	-28 ± 3	3
GR (300)	-1 ± 1	-3 ± 2	-250 ± 144	-15* ± 3	-3 ± 6	3

* transient response

As some of these tryptamine analogues produced systemic and cardiac effects, the true coronary vascular responses are indicated by the changes in CC, hence the rank order of potency for coronary vasodilatation was 5CT > 5HT> DT > αT > GR. These findings also confirm that the small increase in CC associated with the large increase in CBF (and BP), produced by αT, reflects its marked cardiac stimulant action. The poor coronary vasodilator activity of αT, relative to 5CT and 5HT, is therefore consistent with coronary vasodilatation mediated by the vascular smooth muscle $5HT_1$-like receptor (Martin et al, 1987). Furthermore, the weak activity of DT and GR suggest that this receptor is unlikely to be of the $5HT_{1A}$ subtype, for which DT has a high affinity (Markstein et al, 1986), nor does it resemble the recently identified vascular $5HT_1$ receptor subtype, for which GR is a selective agonist (Humphrey et al, 1988).

Humphrey, P.P.A. et al (1988) Br.J.Pharmacol. 94, 1123-1132
Markstein, R. et al (1986) N.S.Arch.Pharmacol. 333, 335-341,
Martin, G.R. et al (1987) N.S.Arch.Pharmacol.336, 365-373.

THE EFFECT OF ALKYLXANTHINES ON POST-ISCHAEMIC ACUTE RENAL FAILURE IN THE RAT

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Lin et al (1986) have shown that theophylline reduces the severity of post-ischaemic acute renal failure (ARF) that follows from clamping the renal artery. In this study we have compared the effect of theophylline and 8-phenyltheophylline (8-PT), a more potent adenosine antagonist, on the development of post-ischaemic ARF.

Rats were anaesthetised with thiobutabarbitone (120 mg kg^{-1} i.p.) and cannulae inserted into the jugular bein and carotid artery for infusion of saline (0.12 ml min^{-1} containing inulin 4 mg ml^{-1} and [^{3}H]-inulin 0.5 μCi ml^{-1}) and for the collection of blood. Both ureters were also cannulated. Two hours later a control 45 min urine collection was made and then the left renal artery was clamped for 45 min. One hour prior to clamping, infusions of either theophylline (20 mg kg^{-1} prime, 0.05 mg min^{-1}), 8-PT (28 mg kg^{-1}, 0.08 mg min^{-1}) or vehicle for 8-PT (2.8 ml kg^{-1}, 0.08 ml min^{-1}; 50% v/v 0.1M NaOH/polyethylene glycol) were started and continued throughout the experiment. One hour after the release of the clamp, urine was collected for three 45 min periods for the determination of [^{3}H]-inulin clearance (C_{IN}).

Table 1 The effect of theophylline and 8-PT on post-ischaemic renal function in the left kidney

	Untreated	Theophylline	Vehicle	8-PT
C_{IN} (ml min^{-1})				
Pre-ischaemia	1.17±0.13	1.09±0.05	1.31±0.05	1.21±0.08
[a]Post-ischaemia	0.06±0.02	0.15±0.02*	0.05±0.01	0.05±0.01
Urine Flow (μl min^{-1})				
Pre-ischaemia	29.5 ±5.0	34.8 ±5.0	27.9 ±1.9	17.9 ±2.5
[a]Post-ischaemia	14.5 ±4.0	37.3 ±5.5*	13.9 ±3.7	11.7 ±2.7
Left kidney weight (g)	1.70±0.03	1.36±0.04*	1.59±0.08	1.83 ±0.08

*P<0.001 relative to untreated group (t-test). [a] Post ischaemia period taken 1h 45 min after release of clamp.

Clamping the renal artery led to a severe diminution in left kidney function characterised by reductions in C_{IN} and urine flow and an increase in kidney weight (Table 1). Treatment with theophylline significantly improved these parameters but, by contrast, 8-PT had no effect. These findings suggest that the beneficial effect of theophylline on post-ischaemic renal function is not a consequence of adenosine antagonism.

Lin, J-J., et al. (1986) J. Lab. Clin. Med., 108, 150-154.

AN *IN VITRO* STUDY OF CARDIAC RESPONSES IN RATS WITH ACUTE RENAL FAILURE

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Reduced cardiac reponses to isoprenaline have been noted in experimental models of acute renal failure (ARF) (Yates et al., 1985; Mann et al., 1986). The mechanism underlying these changes is unclear. The aim of this study was to determine the basis of these reduced responses by investigating the response of isolated cardiac tissue to a range of stimulants which act at different biochemical levels within the myocardium.

ARF was induced in male Wistar albino rats (250-400g) by i.m. injection of glycerol (10ml kg^{-1}). Control rats received i.m. saline. 48 hours later, rats were killed, the heart removed and right atria and right ventricle were mounted for isometric recording in Krebs' solution at 35°C gassed with 5%CO_2 in 95%O_2. Chronotropic responses were measured with the spontaneously beating right atria and the right ventricle was stimulated (1.6Hz, 5ms, 5V) for measurement of inotropic responses. Cumulative concentration-response curves were obtained to isoprenaline (ISO), 3-isobutyl - 1-methylxanthine (IBMX), the calcium agonist BAY K 8644 and Ca^{2+}. Results are summarised in Table 1.

Table 1. The effect of ARF on chronotropic and inotropic responses of isolated cardiac preparations.

	RIGHT ATRIA				RIGHT VENTRICLE			
	Basal rate ($Beats\ min^{-1}$)		Max.↑ rate ($Beats\ min^{-1}$)		Basal tension (mg)		Max.↑ tension (%)	
	C	ARF	C	ARF	C	ARF	C	ARF
ISO	251 ± 7	281* ± 6	186 ± 11	146** ± 6	168 ± 31	194 ± 44	152 ± 30	69* ± 13
IBMX	237 ± 10	283** ± 6	211 ± 11	172** ± 7	267 ± 75	420 ± 80	80 ± 13	32* ± 8
BAY K 8644	-	-	-	-	200 ± 49	222 ± 76	142 ± 37	35* ± 8
Ca^{2+}	-	-	-	-	74 ± 18	108 ± 27	611 ± 41	187*** ± 29

Results are mean ± s.e. mean (n = 8 in each group). C = control, ARF = acute renal failure, Max.↑ rate = maximum increase in rate, Max.↑ tension = maximum increase in tension. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ relative to respective control group (Student's t-test).

Basal rate was significantly elevated in rats with ARF, but there was no significant change in basal tension. The maximum increase in rate to ISO and IBMX was reduced in ARF as was the maximum increase in tension in response to all four inotropes.

The reduced maximum increase in rate of right atria from rats with ARF may be related to the elevated basal rate in these preparations. However, reduced inotropic responses to ISO, IBMX, BAY K 8644 and Ca^{2+} suggest the existence of a defect in the myocardium which lies at a point on the stimulatory pathway common to the action of all agents tested. A possible site is that of energy production within the cell and this is at present under investigation.

Mann, J.F.E., et al. (1986). Am. J. Physiol., 250, H846-H852.
Yates, M.S., et al. (1985). J. Pharm. Pharmacol., 37, 175-179.

CHARACTERISATION AND COMPARISON OF THE GLYCEROL-INDUCED AND 5/6 NEPHRECTOMY MODELS OF RENAL FAILURE

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Renal disease has been shown to affect the metabolic clearance of many drugs (Terner et al, 1978), reduce cytochrome P450 content in rat liver (van Peer & Belpaire, 1977) and significantly alter certain pharmacokinetic parameters (Barber et al, 1989). The clinical importance of renal disease has resulted in the development of several animal models used during research into the condition. The present study was designed to fully characterise two such models and compare their features.

Male Sprague-Dawley rats (200-300 gms) were randomly assigned to glycerol or nephrectomy groups. Renal failure in the glycerol group was induced by i.m. injection of glycerol in normal saline (50% v/v) with a control group injected with saline only. All animals were used 48 hours after treatment. Animals in the nephrectomy group had one kidney removed and 2 of the 3 branches of the contralateral renal artery were ligated. Controls were sham-operated and all animals were used 1 week after surgery. All the animals were placed in metabowls overnight and food, water, urine and blood samples collected for analyses, after 48 hours of acclimatisation in the metabowls. A summary of measured and calculated parameters is shown below.

Table 1 Metabowl data for the two models of renal failure

Measurement	Glycerol (n=6)	Control (n=5)	p-value
UFR (ml/hr/kg)	5.46 ± 3.30	1.75 ± 0.56	$p < 0.05$
Cr.Cl (ml/hr/kg)	41.50 ± 45.90	287.20 ± 35.91	$p < 0.01$
NAG (nmol released/ml/hr)	789.02 ± 315.41	72.61 ± 6.36	$p < 0.01$
Urea (mmol/l)	64.7 ± 25.5	5.52 ± 1.3	$p < 0.01$
	Nephrectomy (n=6)	**Control (n=6)**	**p-value**
UFR (ml/hr/kg)	4.35 ± 0.85	1.46 ± 0.24	$p < 0.01$
Cr.Cl (ml/hr/kg)	146.61 ± 39.54	196.23 ± 45.04	N.S.
NAG (nmol released/ml/hr)	21.46 ± 7.15	28.79 ± 6.63	N.S.
Urea (mmol/l)	34.80 ± 26.30	7.90 ± 2.31	$p < 0.05$

Results are given as mean ± standard deviation
Legend: Cr.Cl = creatinine clearance, UFR = urine flow rate, NAG = N-acetyl glucosaminidase, N.S. = not significant.

The highly significant differences in creatinine clearance, urinary NAG and serum urea in glycerol rats is contrasted by much smaller changes for nephrectomised rats. These values reflect the severity of the glycerol model compared to the surgical method and suggest that the former may be superior in reliability in producing the desired effect of renal failure.

Barber H.E. et al. (1989) Br. J. Pharmac. 96 : 291P
Terner U.K. et al. (1978) Clin. Biochem. 11, 156.
van Peer A.P. & Belpaire F.M. (1977) Arch Int. Pharmacodyn, 288, 180-183

MECHANISM OF DOPAMINE-INDUCED DIURESIS IN THE ANAESTHETISED CAT

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Intravenous infusion of dopamine increases urine output in the anaesthetised cat without altering renal haemodynamics or resting blood pressure (Wassermann et al., 1980). We have re-examined the renal effects of dopamine in this model and have attempted to identify the receptor type(s) that mediates the diuresis.

Pentobarbitone anaesthetised, artificially respired cats were infused with sodium bicarbonate (0.03mmol/kg/min i.v) to prevent metabolic acidosis. Aortic blood pressure and left renal artery blood flow (RBF) were measured, and both ureters cannulated for collection of urine. Glomerular filtration rate (GFR) was calculated from creatinine clearance. Saline (30ml/kg i.v.) was infused over 2h followed by a maintenance infusion (0.1ml/kg/min i.v.). Each dose of dopamine was infused sequentially for a period of 10 minutes.

In control animals receiving saline alone (0.25ml/min i.v.) in place of dopamine little variation (<10%) occurred in measured parameters. In contrast, dopamine (10–100μg/kg/min i.v) caused a dose-related increase in mean blood pressure (MBP, 6–56%), urine output (U_V, 28–46%), and fractional sodium excretion (FE_{Na}, 5–38%) from both kidneys, and a decrease in renal vascular conductance (RVC, 20–50%). No increase in GFR was associated with the diuresis. The increase in U_V (Figure 1), MBP and FE_{Na}, and decrease in RVC caused by dopamine, were not blocked in separate cats pretreated with either the selective dopamine DA_1 or DA_2 receptor antagonists SCH23390 (30μg/kg i.v.) or domperidone (1mg/kg i.v.), respectively. However, in cats treated with the non-selective α-adrenoceptor antagonist, phentolamine (1mg/kg i.v.) dopamine (10–100μg/kg/min i.v.) caused decreases in U_V (Figure 1), GFR, MBP and FE_{Na}. Dopamine-induced decreases in RVC were only slightly attenuated in phentolamine treated cats.

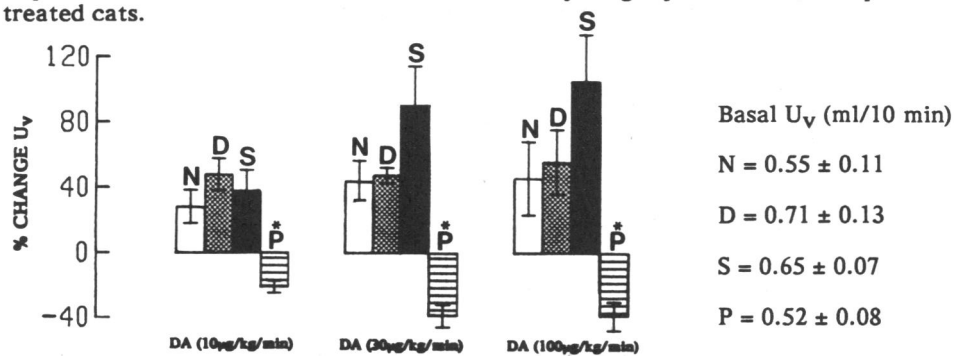


Figure 1: Basal values and effects of dopamine (DA) on Uv from the left kidney in cats with no pretreatment (N, n=6), and in cats pretreated with SCH23390 (S, n=5), domperidone (D, n=4), or phentolamine (P, n=4). Values are mean change ± s.e.m. from the post-antagonist basal value. *p<0.05, Student's t-test, antagonist treated group (D, S or P) compared to untreated group (N).

The present study has confirmed the finding of Wassermann et al. (1980) that intravenous infusion of dopamine causes diuresis in the anaesthetised cat. However, in contrast to these workers we found no convincing evidence for an involvement of dopamine receptors in this effect; rather the diuretic effect of dopamine appears to be linked to stimulation of α-adrenoceptors.

Wassermann, K. et al., (1980). Naunyn-Schmiedeberg's Arch. Pharmac., 312, 77–83

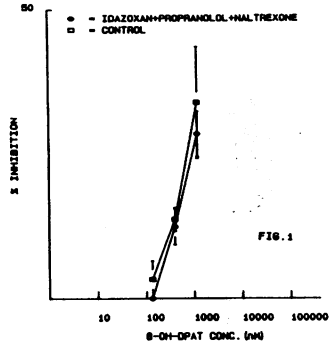
DOPAMINE AGONIST ACTIVITY OF 8-OH-DPAT

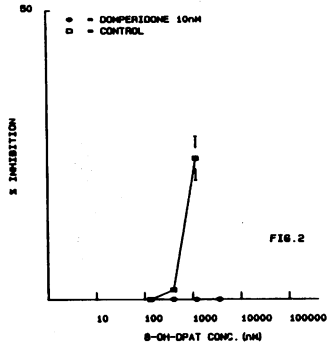
C.F.C. Smith and Susan D. Cutts (introduced by P.W. Dettmar), Reckitt & Colman plc, Dansom Lane, Hull, HU8 7DS, N. Humberside, England.

8-OH-DPAT was first identified as a 5-HT agonist by Hjorth et al. (1982) who investigated the compound in rats and reported it to produce a marked behavioural syndrome which was antagonised by methiothepin. These workers failed to see any significant dopaminergic effects and concluded that the compound was devoid of dopamine agonist activity. A short time later Middlemiss and Fozard (1983) designated the compound as a selective 5-HT_{1A} agonist on the basis of binding studies performed using rat frontal cortex. Since then the compound has been widely used as a prototype 5-HT_{1A} agonist.

While investigating this compound in a range of isolated tissue preparations in order to determine whether we could identify the existence of a population of 5-HT_{1A} receptors in a peripheral tissue, we discovered it possessed a biphasic dose response curve (initial inhibition followed by potentiation at higher doses) in the mouse isolated electrically stimulated vas deferens preparation (MVD). This communication reports the results of the investigation to determine the nature of the receptor system producing the initial inhibitory phase of the DRC.

Vasa deferentia from mice of strain MF1/OLA and weight <30 grams were set up in Krebs solution in a 50 ml organ bath and stimulated with 3 msec rectilinear pulses at 0.1 Hz. DRC's to 8-OH-DPAT were constructed every 50 mins and the effects of the antagonists determined by introducing the test antagonist into the Krebs solution immediately after the second DRC. A minimum of four DRC's were constructed on each preparation.





A combination of propranolol (300 nM), idazoxan (300 nM) and naltrexone (300 nM) had no effect on the response of the MVD to 8-OH-DPAT (Figure 1) indicating that the effect of the compound is not mediated via β, α_2 or opioid receptors. Domperidone (10 nM) (Figure 2), sulpiride (300 nM) and fluphenazine (30 nM), on the other hand, all produced a marked antagonism of the effects of 8-OH-DPAT. This is consistent with the inhibitory effect of 8-OH-DPAT being due to agonist activity at the dopamine receptors present in the MVD (Smith and Rowland, 1988).

A comparison of 8-OH-DPAT and apomorphine in the same preparations showed 8-OH-DPAT to possess 0.01 the potency of apomorphine.

Hjorth, S. et al. (1982), J. Neural Transmission, 55, 169-188
Middlemiss, D.N. and Fozard, J.R. (1983), Eur. J. Pharmacol., 90, 151-153.
Smith, C.F.C. and Rowland P.B. (1988), Br. J. Pharmac. Proc. Suppl., 96, 259P.

THE GUINEA-PIG ILEUM AS A MODEL FOR 5-HT$_{1A}$ AGONISTS: ANOMALOUS EFFECTS WITH RS-30199

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5HT$_{1A}$ receptors in the guinea-pig ileum myenteric plexus preparation have been shown to inhibit acetylcholine release following field stimulation and 8-hydroxy-n-dipropyl-aminotetralin (8-OH-DPAT) inhibits the contractile responses to field stimulation (Fozard & Kilbinger, 1985). We have modified this assay by using the more potent and selective agonist dipropyl-5-carboxyamido-tryptamine (DP-5CT) to investigate the roles of agonists and antagonists at 5HT$_{1A}$ receptors.

Guinea-pig ileum preparations were set up in 30ml isolated organ baths filled with Tyrode solution (0.9mM Ca^{2+}_o, 37°C, gassed with 100%O_2) and stimulated via two parallel platinum electrodes, one of which passed through the lumen, at 0.1Hz (1msec pulse duration, supramaximal voltage). Cumulative concentration-response curves were obtained to 8-OH-DPAT (threshold 10nM) and DP-5CT (threshold 0.1nM, maximal 30nM) only once in any tissue to avoid desensitisation; antagonists were preincubated for 40min. The concentration-response curve to 8-OH-DPAT was biphasic and only the first phase (10-300nM, 20-30% inhibition) was sensitive to spiperone (100nM). In contrast the concentration-response curve to DP-5CT (1-300nM) was monophasic with a maximal inhibition of 35-50% of the response to field stimulation; the responses were antagonised by spiperone and resistant to idazoxan (1μM).

Buspirone and ipsapirone caused inhibition of the response to field stimulation (1μM 19%±1%, n=14, & 16%±1%, n=6, respectively) and the inhibition faded over 30min after which the preparations were less sensitive to 8-OH-DPAT and DP-5CT. The inhibitory responses to buspirone and ipsapirone (1μM) were resistant to idazoxan (1μM) and abolished by pretreatment with a threshold dose of 8-OH-DPAT (10nM). These results are consistant with cross-desensitisation occuring between buspirone and 8-OH-DPAT.

Although RS-30199-193 (Brown et al, this meeting) and buspirone have equivalent affinity for the 5HT$_{1A}$ site in rat cerebral cortex, RS-30199-193 (0.1-10μM) caused a slight augmentation of the reponses to field stimulation (1μM, 15±4%, n=8) and this response was resistant to pretreatment with 8-OH-DPAT (10nM). RS-30199-193 (1μM for 20min) did not affect the sensitivity of the tissues to 8-OH-DPAT or to DP-5CT, indicating that RS-30199-193 may interact with a different subset of 5HT$_1$ receptors than buspirone. These findings indicate that binding to 5HT$_{1A}$ sites may not always correlate with functional effects.

Fozard, J.R. & Kilbinger, H. (1985). Br.J.Pharmacol., 86, 601P.

THE PUTATIVE 5-HT$_{1A}$ RECEPTOR IN GUINEA-PIG ILEUM: DIFFERENTIAL CLASSIFICATION USING TRYPTAMINES

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Fingerprints comprising tryptamine affinity and efficacy estimates provide a quantitative and economical means of identifying positively, and at the same time differentiating 5-HT receptor subtypes (Leff & Martin, 1988). Here we consider the classification of the 5-HT receptor mediating inhibition of electrically-evoked twitch in the guinea-pig isolated ileum (GPI), using tryptamine fingerprints to compare and contrast this receptor with four other 5-HT receptors currently allocated to the 5-HT$_1$-like, 5-HT$_2$ and so-called 'orphan' receptor classes.

Segments of proximal ileum were obtained from male Dunkin-Hartley guinea-pigs (250-450g). Changes in isometric force were recorded from tissues mounted for transmural stimulation (0.5msec, supramaximal voltage, 0.1Hz) in Krebs solution (37°C) containing 0.63mM Ca^{++}. Experiments were performed in the presence of ketanserin (0.1μM) and BRL24924A (30μM).

5-HT-induced inhibitions of twitch (p[A_{50}] = 6.6, max = 68%) were mimicked by 5-carboxamidotryptamine (5-CT) with higher potency (p[A_{50}] = 8.2, max = 71%). Ketanserin (0.1μM) and BRL24924A (30μM) were without effect on these responses, but methiothepin (0.03μM) caused surmountable antagonism (Δ p[A_{50}] = 0.5). Spiperone behaved as a simple competitive antagonist. Estimates of the antagonist dissociation constant (pK_B) were agonist-independent (vs 5-HT : 7.94 ± 0.07, 16df; vs 5-CT : 8.21 ± 0.11 , 24df) and were unchanged when experiments were performed in the presence of 2.5mM Ca++ (vs 5-CT: 7.91 ± 0.07, 16df).

Affinity (pK_A) and relative efficacy (τ) estimates were obtained for 5-HT, 5-CT, (±) α-methyl-5-HT (α-Me-5-HT) and N,N-di-n-propyl-5-CT (N-DP-5-CT) using direct model-fitting methods as described elsewhere (Black *et al*, 1985). The results, shown below, are compared with estimates obtained at other 5-HT receptor types previously differentiated using tryptamines (Leff and Martin, 1988).

ASSAY	Receptor		5-HT	5-CT	a-Me-5-HT	N-DP-5CT
GPI	5-HT$_1$-like	pK_A	6.39	7.90	<5.00	7.36
		τ	*1.00*	*1.10*	-	*0.38*
RbSV	5-HT$_1$-like	pK_A	7.12	7.53	5.68	6.22
		τ	*1.00*	*0.98*	*0.93*	*0.30*
DCA	5-HT$_1$-like	PK$_A$	6.20	6.66	<4.50	5.56
		τ	*1.00*	*4.70*	-	*0.55*
RbJV+E	'orphan'	pK_A	8.36	7.51	8.14	5.17
		τ	*1.00*	*0.95*	*1.04*	*0.32*
RbA	5-HT$_2$	pK_A	6.92	5.90	6.59	5.09
		τ	*1.00*	*0.35*	*0.79*	*0.26*

Key : RbA, rabbit aorta : RbSV, rabbit saphenous vein ; RbJV + E, endothelium-intact rabbit jugular vein ; DCA, beagle dog coronary artery.

These results show that the receptor in GPI is 5-HT$_1$-like, consistent with its proposed 5-HT$_{1A}$ classification (Fozard & Kilbinger, 1985). Tryptamine fingerprints identify this receptor positively and enable it to be differentiated quantitatively from other types of peripheral 5-HT receptor regardless of their current appellation.

Black, J.W. *et al* (1985) Br. J. Pharmacol., 84, 561-571.
Leff, P. & Martin, G.R. (1988) Med. Res. Rev., 8, 187-202.
Fozard, J.R. & Kilbinger, H. (1985) Br. J. Pharmacol., 86, 601P.

THE EFFECT OF VASOACTIVE DRUGS ON THE CIRRHOTIC RAT PORTAL VEIN

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Although beta blockers and serotonin antagonists have been tested in the treatment of portal hypertension in man, little is known of their direct effect on the portal vein. Beta$_2$ receptors in the normal rat hepatic vasculature mediate vasodilatation (Burns et al, 1989a). In man blockade of such receptors may be undesirable when the aim of treatment is to lower portal venous pressure (Ppv). We have examined the effects of propranolol (Pro), salbutamol (S), ketanserin (K) and prazosin (Pra) on Ppv in the portal vein of the cirrhotic rat using a constant flow isolated perfused rat liver preparation (IPRL).

The livers (mean weight (sd) 12.2 (2.2)g) of 46 cirrhotic male Wistar rats were isolated and perfused in situ as described previously (Burns et al, 1989). The animals were rendered cirrhotic by the administration of carbon tetrachloride and phenobarbitone (Burns et al, 1989b). Ppv was monitored continuously; flow 10 ml/min; perfusate volume 100ml. After a 30 min stabilisation period a noradrenaline (NA) infusion (200ng/min) was started and 30 min later the test drug was added to the reservoir of the perfusion apparatus. The initial perfusate concentrations are shown in the table with the mean (sd) changes in Ppv produced by the drugs.

Drug	n	Conc. (uM)	Pretreatment Ppv (cm water)	% rise in Ppv with NA	Change in Ppv after drug (% of Ppv after NA) 4min	10min
NA	6		11.8 (1.9)	41 (30)	1 (1)$_{*}$	1 (1)$_{**}$
Pro	7	100	11.5 (2.4)	67 (11)	-4 (4)$_{***}$	-4 (4)$_{***}$
S	7	100	11.7 (4.2)	45 (24)	-9 (2)$_{**}$	-9 (4)$_{**}$
S	6	10	10.6 (3.4)	32 (38)	-4 (2)$_{**}$	-5 (3)$_{**}$
S	8	1	9.7 (4.3)	23 (14)	-6 (4)$_{***}$	-5 (5)$_{***}$
K	6	13	11.6 (2.8)	39 (20)	-18 (7)$_{***}$	-19 (6)$_{***}$
Pra	6	0.2	10.4 (2.6)	37 (21)	-16 (7)	-17 (7)

*p<0.02, **p<0.01, ***p=0.001 for differences between NA alone and NA+Pro, NA+S, NA+K or NA+Pra.

Analysis of variance did not show any difference between the pretreatment pressures or the rise in Ppv with the NA infusion. The pressure changes with S are consistent with the findings in the normal rat liver. Pro has no effect on Ppv in the normal rat liver (Burns et al, 1989) but lowered Ppv in the cirrhotic liver. The concentrations of K and Pra were selected as they are equipotent in their systemic effects in portal hypertensive rats (Cummings et al, 1988). K and Pra produced similar changes in Ppv. It has been suggested that K acts by lowering cardiac output (Cummings et al, 1988), but in the constant flow IPRL preparation changes in Ppv reflect changes in resistance. Thus, the results of the present study suggest K may have a direct effect on the hepatic vasculature. It is possible that the effect of these agents may be useful in augmenting the portal hypotensive effect of drugs that lower portal inflow.

Burns et al (1989a) Br J Pharmac in press
Burns E (1989b) Clin Sci in press
Cummings et al (1988) Hepatology 8, 1112

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AGE-RELATED ALTERATIONS IN THE INHIBITORY ACTIONS OF 5-HT$_1$ RECEPTOR AGONISTS IN THE RAT VAS DEFERENS

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Prejunctional inhibitory 5-HT receptors in rat brain cortex (Engel et al., 1986) have been reported to resemble the 5-HT-1B binding site. Although we have previously failed to identify the inhibitory prejunctional 5-HT receptor of rat vas deferens (Docherty & Warnock, 1986), it has recently been reported that these prejunctional inhibitory 5-HT receptors of rat vas deferens resemble 5-HT-1B binding sites (Feniuk & Pickles, 1988). As a result, we have re-investigated the prejunctional effects of 5-HT receptor agonists in the rat vas deferens.

Epididymal portions of rat vas deferens were obtained from male Wistar rats of 4 age groups (1.5, 3, 6 and 22 month), and were treated with nifedipine (10 μM) to eliminate postjunctional actions of 5-HT receptor agonists. Tissues were stimulated with a single electrical pulse (supramaximal voltage, 0.5 ms pulses) every 5 min, and agonist concentration-response curves (0.5 log unit increments) were obtained in the absence or presence of antagonist

In epididymal portions of rat vas deferens, 5-carboxamidotryptamine (5-CT) and flesinoxan produced concentration-dependent inhibition of stimulation-evoked contractions. The IC_{50} (concentration producing 50% inhibition of stimulation-evoked contractions) of flesinoxan did not differ between 1.5 and 3 month rats, with values of 0.69 μM (n=4) and 1.20 μM (n=14), respectively. Effects of flesinoxan were not antagonised by cyanopindolol (1 μM) (5-HT-1B ligand)nor by yohimbine (0.3 μM) (alpha-2 adrenoceptor antagonist).

For the agonist 5-CT, analysis of data revealed that potency of 5-CT divided into two distinct ranges: IC_{50} lower than 0.1 μM and IC_{50} higher than 1 μM. In 1.5 month rats 5-CT had high potency in 7 from 9 experiments, but in 3 month rats 5-CT had high potency in 4 from 10 experiments (with 1 intermediate value). In 6 month and 22 month rats, 5-CT showed only low potency. The IC_{50} of 5-CT was 0.060μM (n=9), 0.89 μM (n=10), 3.89 μM (n=6) and 17.8 μM (n=3) in 1.5, 3, 6 and 22 month rats, respectively. Since IC_{50} values in 1.5 and 3 month animals did not follow a normal distribution, data were assessed employing the non-parametric Mann- Whitney U-test, and 5-CT was significantly more potent in 1.5 month than in 3, 6 and 22 month old animals ($P<0.05$ in each case).

Cyanopindolol (1 μM) shifted the potency of 5-CT only in experiments in which 5-CT showed high potency, so that cyanopindolol was ineffective in all 6 and 22 month animals. In 1.5 and 3 month animals, cyanopindolol shifted the potency of 5-CT with an apparent pA_2 of 7.29 (using only experiments in which 5-CT showed high potency in vehicle controls).

In conclusion, the 5-HT-1A ligand flesinoxan produces inhibition of contractions of rat vas deferens resistant to cyanopindolol, whereas the 5-HT-1 agonist 5-CT had two components to its inhibition, a high potency component antagonised by cyanopindolol and present only in 1.5-3 month rats, and a low potency component resistant to cyanopindolol. Hence, the component of the response to 5-CT which resembles a 5-HT-1B response is lost in maturation.

Docherty, J.R. & Warnock, P. (1986). Br. J. Pharmacol., 89, 843P.
Engel, G. et al. (1986). Naunyn-Schmiedeberg's Arch. Pharmacol., 332, 1-7.
Feniuk, W & Pickles, R.J. (1988). Proc. Cong. Cardiovasc. Pharmacol of 5-HT

THE METABOLISM OF LEUKOTRIENES AND OF REACTIVE OXYGEN SPECIES IN SENSITISED GUINEA-PIG LUNGS

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The cysteinyl leukotrienes (LT), C_4, D_4 and E_4, are major candidates for the mediation of bronchial asthma, and are released on allergen challenge of sensitised human and animal lungs. LTs are not stored intracellularly and their release depends on de novo synthesis from arachidonic acid. Before the role of LTs in asthma can be fully understood it is important to assess their synthesis and metabolism in this abnormal physiological state. We have, therefore, measured the metabolism of LTC_4 and LTD_4 in subcellular fractions prepared from sensitised guinea pig lungs. In addition, the efficiency with which sensitised lung tissue can detoxify reactive oxygen species has been determined.

Male Dunkin-Hartley guinea pigs (250-300g) were injected intraperitoneally with 10 μg ovalbumin in 20% (w/v) $ALOH_3$ as adjuvant and used for experiments after 14 days. Control animals received adjuvant alone. Subcellular fractions were prepared by ultracentrifugation and LT metabolism quantified by HPLC analysis. The activities of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase, the enzymes involved in detoxification of reactive oxygen species, were measured in the cytosol.

The recovery of microsomes was 3.88±0.28 mg protein/g lung from control animals and 4.21±0.34 mg protein/g lung from sensitised animals. The conversion of 16 μM LT C_4 to LTD_4 by microsomes from sensitised guinea pig lungs was significantly increased, whilst the metabolism of 20 μM LTD_4 to both LTC_4 and LTE_4 by lung homogenate was significantly decreased in the sensitised animals as shown in Table 1. The overall result of these three alterations is an increase in LTD_4 synthesis and a reduction in its catabolism. This may lead to an increase in the concentration of LTD_4 in sensitised guinea pig lungs.

Table 1. Leukotriene metabolism in guinea pig lungs

Reaction	Control	Sensitised
C_4 to D_4	68.2±5.4(6)	93.1±7.8(6)*
D_4 to C_4	21.8±2.8(8)	14.5±0.8(6)*
D_4 to E_4	61.5±3.4(8)	50.8±0.9(6)*

Values are mean ± S.E.M., with the number of animals used in parentheses. Reaction rates are pmol/min/g lung. *P<0.05, by non-paired Student's t test.

The activities of catalase and glutathione reductase were not significantly altered in sensitised guinea pig lungs. In contrast, the activity of cytosolic superoxide dismutase was decreased in sensitised guinea pig lungs (583.7±76.9 units/g lung in control animals (n=6) compared with 368.6±39.1 units/g lung in sensitised animals (n=6; P<0.05)). The activity of selenium-dependent glutathione peroxidase was unaltered by sensitisation, whereas that of the selenium-independent peroxidase was significantly increased (2.49±0.30 μmol/min/g lung in control animals (n=5) compared with 3.55±0.26 μmol/min/g lung in sensitised animals (n=5, P<0.05)). Thus, sensitised lungs may be more susceptible to the effects of superoxide, although they can detoxify organic hydroperoxides more efficiently.

EFFECTS OF EP092 ON CONTRACTIONS TO U46619, 5-HT AND OXYGEN IN THE HUMAN UMBILICAL ARTERY

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The human umbilical artery will contract to an increase in oxygen tension above 16mmHg (low) oxygen (McGrath *et al* ., 1986). This contraction is blocked by the action of the cyclo-oxygenase inhibitors indomethacin, flurbiprofen and aspirin (McLennan *et al* ., 1988). Also contractions to 5-hydroxytryptamine (5-HT) show a biphasic concentration response curve in high oxygen (pO_2 = 120mmHg) (McGrath et al 1988). The lower part of the curve is blocked by the cyclo-oxygenase inhibitors, is absent in low oxygen and is thus dependent on high oxygen and cyclo-oxygenase. In 16mmHg oxygen the 5-HT curve can be competitively inhibited by the $5\text{-}HT_2$ antagonist ketanserin. In 120mmHg the lower part of the curve which is absent in low oxygen is resistant to ketanserin and suggests a $5\text{-}HT_1$-like "receptor" MacLennan *et al* . (in press). This current study was carried out to determine the role, if any, of thromboxane in the contraction to oxygen and the biphasic response to 5-HT by using the specific thromboxane antagonist EP092.

Umbilical cords collected directly after birth were stored in ice-cold Krebs for up to 24 hrs. Isometric tension was recorded from longitudinal strips of artery in Krebs at 37°C. To mimic physiological blood gas tensions *in utero*, the Krebs was bubbled with 2.5% O_2, 8% CO_2, balance N_2 to give pO_2 16mmHg ± 3mmHg. pO_2 was measured continuously by an IL1302 O_2 electrode placed in one of the organ baths. Cumulative concentration response curves (CCRC) to U46619 were carried out in controls or in the presence of EPO92; 10nM, 0.1μM or 1μM . Alternatively a contraction to an increase in oxygen tension to 20% (~120mmHg) was obtained, then repeated in the presence of one of the three EPO92 concentrations or alone as a time control. Thirdly a CCRC to 5-HT was carried out in both low 16mmHg and high 120mmHg oxygen either alone or in the presence of one of the three concentrations of EPO92 .

At physiological pO_2 (16mmHg) U46619 contracted the artery over the range 0.3nM-0.3μM (pD_2 8.82 ± 0.04). EPO92 10nM had little effect on the contraction, with 0.1μM shifting the curve to the right (pD_2 8.1± 0.08). EPO92(1μM) almost abolished the response to U46619 with the maximum response reduced from 2,920 ± 560mg to 785 ± 200mg. EPO92 10nM & 0.1μM reduced the contraction to 20% oxygen with 1μM completely abolishing the response. Finally this concentration of antagonist also abolished the increase in sensitivity to 5-HT normally seen in high oxygen (20%) when compared with low oxygen (2.5%) (McGrath *et al* ., 1988). However EP092 did not affect the contractions to 5-HT in low pO_2, demonstrating that it was selective against U46619 and the responses dependent on high oxygen.

In conclusion these experiments would seem to suggest that the contraction to oxygen is almost entirely if not exclusively due to thromboxane release. This is also borne out by the results with 5-HT where the antagonist inhibits the initial component of the contraction which only becomes apparent in high oxygen.

McGrath, J.C., MacLennan, S.J., Mann, A.C., Stuart-Smith, K. & Whittle, M.J. (1986) J.Physiol., 380; 513-519.
McGrath, J.C., MacLennan, S.J. & Whittle, M.J.(1988) Quart.J.Exp.Physiol., 73; 547-559.
MacLennan, S.J., McGrath, J.C. & Whittle, M.J. (1988) Prostaglandins, 36 (5); 711-729.
MacLennan, S.J., McGrath, J.C. & Whittle, M.J. (1989) Br.J.Pharmacol., (in press).

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PROSTAGLANDIN E_2 RECEPTORS IN RAT UTERUS

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Prostaglandin E_2 causes contraction of the rat uterus *in vitro*. This effect may be mediated via a prostaglandin E_2-sensitive (EP) receptor. EP-receptors can be divided into EP_1, EP_2 and EP_3-receptor subtypes (Coleman et al., 1987). The purpose of this study was to characterise the EP receptors present in rat uterus.

Uterine horns were obtained from Sprague-Dawley rats which had been pretreated with diethylstilboestrol (1 mg kg^{-1}, s.c.) 18h previously. Both horns were bissected longitudinally providing 2.5 cm strips which were set up as described by Massele & Senior 1981 and superfused at a rate of 2 ml min^{-1} with oxygenated (95% O_2/5% CO_2) Krebs solution at 37° and which contained indomethacin (2.8 μM).

Uterine strips initially showed spontaneous activity but this usually disappeared within one hour of setting up the tissue. PGE_2 caused contraction of uterine strips (ED_{50} (range) 9.6 (2.29-29.12) nmoles) and the EP_1/EP_3-receptor selective agonist, sulprostone (Coleman et al., 1987) also caused contraction (ED_{50} (range) 7.23 (2.15-14.62) nmoles). Responses to sulprostone were qualitatively similar to those obtained with PGE_2.

The EP_1/IP-receptor selective agonist, iloprost (Coleman, 1987) also caused contraction in 30% of all preparations but the contraction appeared non-dose related and was variable in its action.

In order to study the effects of agonists on the EP_2-receptor which normally causes relaxation of the tissues in response to stimulation some tissue activity had to be induced in the rat uterus preparation. This preparation has no tone and to induce rythmic activity similar to the spontaneous activity seen at the beginning of the experiment, oxytocin (2.5 milliunits ml^{-1}) was added to the superfusate. The EP_2-receptor selective agonist, butaprost (Gardiner, 1986) produced no inhibition in the activity of these oxytocin-driven uterine strips.

The EP_1-receptor blocking drug, AH6809 (10 μM) (Coleman et al., 1985) did not affect responses to either PGE_2 (ED_{50} (range) 13.41 (4.76-28.28) nmoles) or sulprostone (ED_{50} (range) 7.26 (3.01-11.61) nmoles).

In conclusion it appears that there are very few, if any, EP_1-receptors and no EP_2-receptors present in this tissue. Our results support the presence of contractile EP_3-receptors in the rat uterus.

Coleman, R.A. (1987) Prostaglandins and Related Substances, a Practical Approach, ed, C. Benedetto et al., IRL Press, Oxford 267-303
Coleman, R.A. et al. (1985) Br. J. Pharmac. 85, 273P
Coleman, R.A. et al. (1987) Br. J. Pharmac. 91, 407P
Gardiner, P. (1986) Br. J. Pharmac. 87, 45-56
Massele, A.Y. & Senior, J. (1981) Br. J. Clin. Pharmac. 12, 285P.

PROSTANOID RECEPTORS IN GUINEA-PIG UTERUS

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Guinea-pig uterus (GPU) is highly sensitive to the contractile effects of various natural and synthetic prostanoids, particularly those of the E class (Whalley & White 1980a&b; Elger et al., 1981; Eglen et al., 1988). Eglen et al (1988) attempted to characterise the receptors mediating contraction, and demonstrated that GPU is sensitive to TP-, EP-, and FP-receptor agonists but not to those most active at the IP- and DP-receptors. In the present study we have examined the actions of a range of natural and synthetic prostanoids, and have attempted to characterise the receptors present.

Specimens of GPU were obtained from virgin Dunkin-Hartley guinea-pigs (600-800g) pre-treated with diethylstilboestrol ($1mkg^{-1}$ day^{-1} i.p. for two days) to synchronise the stage of the oestrous cycle. Two 1.5cm muscle strips were obtained from each uterine horn and mounted under an initial tension of 2g. Preparations were superfused at a rate of $2ml\ min^{-1}$ with oxygenated (95% 0_2/5% $c0_2$) Krebs solution at 37°C containing indomethacin (2.8μM).

The order of potency of the natural prostaglandins and the stable thromboxane mimetic U-46619 (11,9-epoxymethano PGH_2) was as follows; PGE_2 > U-46619 > PGI_2 = PGD_2 = $PGF_2\alpha$ (ED_{50} (95% C.L.) nmoles = 0.040 (0.034-0.048, n=6), 0.70 (0.51-0.97, n=5), 5.1 (2.4-10.8, n=4), 8.0 (2.5-25.6, n=5), and 10.7 (3.1-37.2, n=5), respectively). At a concentration of 1μM, the TP-receptor blocking drug GR32191 antagonised responses to U-46619 (dose ratio ~ 1000), and in all subsequent experiments, GR32191 (1μM) was included in the superfusion fluid to prevent TP-receptor involvement. Responses obtained to PGE_2, $PGF_2\alpha$, PGD_2 or PGI_2 in the presence of GR32191 were not significantly different from those obtained in its absence. To investigate the involvement of FP-, DP-, and EP-receptors, a selection of synthetic receptor-selective agonists were tested. Whilst both the FP-receptor agonist ICI81008, and the DP-receptor agonist BW245C (Coleman 1987) were inactive, the EP-receptor selective compounds sulprostone (EP_1/EP_3) and rioprostil (EP_2/EP_3) (Coleman et al 1988) and the IP/EP_1-selective compound iloprost (Coleman et al 1987) were all contractile agonists (ED_{50} (95% C.L.) nmoles = 0.43 (0.05-3.89, n=6) 0.10 (0.03-0.30, n=6), 4.7 (0.7-31.3, n=5), respectively), although substantial variability in potency was observed. The EP_1-receptor blocking drug AH6809 (10μM) had no effect against contractions to PGE_2 and sulprostone but did exhibit some antagonism of responses to iloprost, particularly at the lower end of the iloprost concentration-effect curve.

These data support the presence in GPU of both TP- and EP-receptors mediating contraction. Furthermore, the results suggest that the EP-receptors comprise a mixed popultion of EP_1 and EP_3-receptor subtypes, but that the EP_3-receptors appear to predominate.

Coleman, R.A. et al. (1988). Br. J. Pharmac. 95, 724P.
Coleman, R.A. (1987) in Prostaglandins and Related Substances, A Practical Approach. ed. C. Benedetto et al. IRL Press, Oxford, 267-303.
Eglen, R.M. et al (1988) FASEB J. 2, 1572.
Elger, W. et al (1981). Prostaglandins 21, 2, 259-266.
Whalley, E.T. & White, S.K. (1980a). Br. J. Pharmac. 68, 150p-151p.
Whalley, E.T. & White, S.K. (1980b). Br. J. Pharmac. 69, 309p.

DUAL ACTIVITY OF THE THROMBOXANE A_2 ANALOGUE, STA_2, ON HUMAN PLATELETS

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In characterising thromboxane (TP-) receptors, it would appear prudent to estimate the agonist potencies of stable analogues of both PGH_2 and TXA_2. We wish to point out a potential problem with the use of STA_2 (9,11-thia-11a-carba TXA_2) (Katsura et al, 1983), a potent full agonist on thromboxane-sensitive smooth muscle preparations (Jones et al, 1989).

On washed human, rat and rabbit platelets, STA_2 is between 3 and 6 times more potent as an aggregator than the PGH_2 analogue U-46619 (Jones et al, 1987). However we have observed reduced responses to STA_2 on human platelets at concentrations above that required for maximal aggregation (Fig.1). This effect was not seen with either U-46619 on human platelets or STA_2 on rat and rabbit platelets. In the presence of a high concentration of EP 092 (6μM), STA_2 inhibited PAF(100nM)-induced aggregation of human platelets (Fig.1). U-46619 up to 3000nM did not affect PAF action. STA_2 had little effect (inhibition <20%) on ADP(1μM)-induced aggregation of rat platelets or PAF(10nM)-induced aggregation of rabbit platelets.

pA_{10} values for block of platelet TP-receptors by EP 092 using U-46619, STA_2 and PGH_2 (synthetic, 80μM dazoxiben present) as agonists were as follows: human 6.77, 7.85, 6.61; rat 6.84, 6.86, 6.58; rabbit 5.75, 6.04, 6.02. The high pA_{10} value for EP 092 vs STA_2 on human platelets may be due to an inhibitory activity of STA_2 itself at the higher concentrations required to overcome the receptor block.

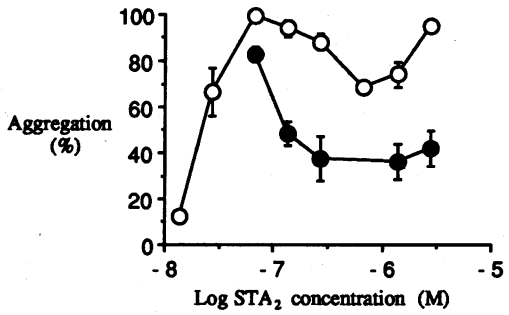


Figure 1. Aggregation of washed human platelets by STA_2 (○) and the effect of STA_2 on PAF aggregation in the presence of 6μM EP 092 (●). Means ± s.e.m (n = 4).

We have previously shown that two other analogues of TXA_2, CTA_2 and PTA_2, have inhibitory activity against non-thromboxane agents on human platelets (Armstrong et al, 1985). However, in the case of STA_2 acting alone, the inhibitory effect may be less obvious since it is opposing a full agonist action rather than a partial agonist action. This dual effect on human platelets may contribute to the low aggregating potency reported of STA_2 in some studies; for example, on human PRP the STA_2 EC_{50} = 1.1μM, 2.3 times greater than that of U-46619 (Mais et al 1985).

Armstrong, R.A. et al (1985) Br.J.Pharmacol., 84, 595-607
Jones, R.L. et al (1987) Br.J.Pharmacol., 90, 226P
Jones, R.L. et al (1989) Br.J.Pharmacol., 96, 875-887
Katsura, M. et al (1983) Adv.Prost.Thromb.Leuk.Res., 11, 351-357
Mais, D.E. et al (1985) J.Pharmacol.Exp.Ther., 233, 418-424

IS TOLFENAMIC ACID A DUAL INHIBITOR OF CYCLO-OXYGENASE AND 5-LIPOXYGENASE?

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Arachidonic acid metabolites derived from both cyclo-oxygenase (CO) and 5-lipoxygenase (5LO) pathways are mediators of inflammmation. Inhibitors of CO are clinically effective anti-inflammatory agents and dual inhibitors of 5LO and CO may have greater therapeutic efficacy in inflammatory diseases. Recently, therapeutic concentrations of tolfenamic acid, a non steroidal anti-inflammatory drug, were reported to inhibit 5LO and CO in plasma-free leukocyte suspensions in vitro (Moilanen et al, 1988). We have investigated the effects of tolfenamic acid on CO and 5LO in vitro and ex vivo and we conclude that in the presence of physiological concentrations of plasma proteins the drug is a selective CO inhibitor.

Release of arachidonic acid metabolites from human leukocytes and human or rat blood was induced by calcium ionophore A23187 (McMillan et al, 1985; McMillan et al, 1986). In some experiments studies with leukocytes were performed in the presence and absence of plasma. Release of the 5LO product, LTB_4, and the CO product, TXB_2, was measured by radio-immunoassay.

In plasma-free suspensions of human leukocytes, tolfenamic acid potently inhibited A23187-induced release of the CO product TXB_2 (IC_{50} = 0.05uM) and at higher concentrations in this system the drug also inhibited release of the 5LO product LTB_4 (IC_{50} = 10uM). In human blood, tolfenamic acid was less potent at inhibiting release of TXB_2 (IC_{50} = 6.5uM) and no significant inhibition of release of LTB_4 was observed at concentrations up to 100uM. In studies with rat blood in vitro, tolfenamic acid also inhibited release of TXB_2 (IC_{50} = 5uM) but not LTB_4. In addition, oral administration of tolfenamic acid (100mg/Kg, p.o.) inhibited ex vivo synthesis of TXB_2 in rat blood (97%; $P<0.001$) but did not significantly inhibit release of LTB_4.

In accord with previous reports, these data demonstrate that tolfenamic acid inhibits LTB_4 release from human leukocytes (Moilanen et al, 1988) but this is observed at more than 100 times the concentrations required to inhibit synthesis of CO products. Tolfenamic acid is extensively bound to plasma proteins, as demonstrated by the marked decrease in CO inhibitory potency in whole blood, and in whole blood studies we found no evidence for inhibition of 5LO. We conclude that at therapeutic blood levels (7-20 uM; Pentikainen et al, 1981) tolfenamic acid is a selective CO inhibitor and that neither its therapeutic efficacy nor its low gastric irritancy is likely to be a consequence of 5LO inhibition.

McMillan, R.M., Masters, D.J., Sterling, W.W. and Bernstein, P.R. (1985). IN "Prostaglandins, Leukotrienes and Lipoxins" (Ed. J.M.Bailey), pp655-668, Plenum Press: New York.
McMillan, R.M., Millest, A.J., Proudman, K.E. and Taylor, K.B. (1986). Br. J. Pharmacol. 87, 53P
Moilanen, E., Alanko, J., Seppala, E. and Vapaatalo, H. (1988). Agents and Actions, 24, 387-394.
Petikainen, P.J., Neuvonen, P.J. and Backman, C. (1981). Eur. J. Clin. Pharmacol., 19, 359-365.

PROBENECID INCREASES URINARY PGE_2 EXCRETION IN THE ANAESTHETISED RAT

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Probenecid, an inhibitor of organic acid transport in the proximal tubule, is well known to inhibit the tubular secretion of prostaglandins (Bito & Baroody 1978). Probenecid however is also a uricosuric agent inhibiting both the tubular reabsorption and the secretion of urate (Frankfurt & Weinman 1977). More recently, experiments using probenecid in man have identified a pathway for the tubular reabsorption of the eicosanoid,TXB2 (Zipser & Smorlesi 1984). Since considerable controversy exists over the importance of the tubular reabsorption of PGE2 in determining its urinary excretion rate, we have investigated the effect of probenecid on urinary PGE2 excretion in the anaesthetised rat.

Male Wistar rats (300-350G) were anaesthetised with thiobutabarbitone (175mg/kg) and infused with 0.154M sodium chloride at 6ml/h through a cannula placed in the jugular vein for a 6 hour period. Urine was collected from a catheter placed in the bladder and assayed for PGE2 as described previously (Haylor et al 1984). Probenecid was administered by an I.V. bolus injection(100mg/kg) followed by an I.V. infusion at 100mg/kg/hr. The dose of probenecid employed was the minimal dose required to produce the maximal inhibition of proximal PAH secretion. PAH secretion was measured by the local renal injection of isotopically labelled PAH and inulin in a separate series of experiments. Urine measurements were compared in the 1-hour periods before and after probenecid administration, probenecid being administered after 5h of saline infusion.

In a control series (n=5) receiving the drug vehicle, urinary PGE excretion remained unchanged at 9.6 ± 3.5 compared to 9.7 ± 3.8 pmol/h. In the experimental series (n=16) however, urinary PGE excretion increased ($P<0.01$) from 13.1 ± 1.3 to 105.0 ± 23.2 pmol/h during probenecid administration. Probenecid administration also produced a significant fall in urine flow from 4.7 ± 0.4 to 1.6 ± 0.2ml/h ($P<0.001$) while urine pH remained unchanged. Systemic blood pressure fell by a mean of 23 mmHg. However, in a separate series of experiments the fall in systemic blood pressure induced by probenecid remained unaffected by the administration of indomethacin 10mg/kg I.V..

The large increase in urinary PGE excretion following probenecid is consistent with a high degree of tubular reabsorption for PGE2 of some 85%. However, a number of other explanations are equally plausible including the possibility that probenecid could increase the renal delivery of PGE2 by reducing its systemic metabolism.

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Bito,L.Z. & Baroody,R.A. (1978) Am. J. Physiol. 234 F80-F88.
Frankfurt & Weinman (1977) Proc. Soc. Exp. Biol. Med. 155 554-557.
Haylor,J., et al (1984) Clin. Sci. 66 675-681.
Zipser,R.D. & Smorlesi,C. (1984) Prostaglandins 27 257-267.

PHARMACOLOGICAL REACTIVITY OF MYOFIBROBLAST CONTAINING TISSUES FROM A RANGE OF HUMAN PATHOLOGICAL CONDITIONS

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Myofibroblasts are unique mesenchymal cells capable of both contraction and collagen production. The morphological, histochemical and functional evidence first reported by Gabbiani et al. (1971) identified these cells as being responsible for the force behind wound contracture. Unfortunately, situations arise when myofibroblast accumulation and proliferation leads to excessive contraction, collagen production and scar formation often resulting in functional impairment and deformity. Conditions caused or exacerbated by myofibroblast accumulation include keloid formation and hypertrophic scarring, Dupuytren's disease and contraction of capsules surrounding mammary implants (for review see Lipper et al., 1980). This study investigated the pharmacological reactivity of myofibroblast containing tissues from these conditions together with those from actively granulating human wounds. Normal skin and 'mature' scar being used as controls.

Tissue samples (Keloid n = 6, hypertrophic scar n = 2, 'mature' scar n = 5, palmar nodules of Dupuytren's disease n = 14, breast expander capsules (<6 months since insertion) n = 28, granulation tissue n = 9, and normal dermis (post auricular n = 9, palmar n = 2) were obtained from patients undergoing surgery at the Plastic Surgery Department of St Luke's Hospital, Bradford. Each tissue was used within 60 minutes of excision, cleared of extraneous material and cut into strips (approx 25 mm x 2 mm x 2 mm), along, where recognisable, the longitudinal axis. All tissues were superfused with Krebs Henseleit solution (37°C and flow rate = 2ml min^{-1}) for isometric (1g preload) recording (Grass FT03C). The effects of a range of agonists were studied by comparing dose/concentration-effect relationships, considering (a) the magnitude of the maximum tension generated (mg) and (b) the duration of the effect (mins). Agonists administered in bolus doses (<100μl) were potassium chloride (1-8 mg), barium chloride (1-8 mg), mepyramine (100μg-8mg) and on a limited number of tissues 5-hydroxytryptamine (5-HT) (1-20μg).

Only 3 of the granulating wound samples showed any responsiveness to potassium or barium ions, all other tissues were insensitive at all doses. Mepyramine produced contractile, dose-dependent, reproducible and reversible responses in all myofibroblast containing tissues. Normal skin and mature scar samples were insensitive to this agent. 5-HT failed to produce responses in any of the samples in which it was tested (breast capsule (n = 14), Dupuytren's nodules (n = 4) and granulation tissue (n = 1)).

This study demonstrates that although the myofibroblast containing tissues were obtained from such a wide range of pathological conditions they all exhibited a similar pharmacological profile. The responsiveness to the agonists used agrees with predictions from studies employing other myofibroblast containing tissues including human placenta (Cross et al 1988a), Wharton's jelly (Cross et al. 1988b) and rat testicular capsule (Chander et al. 1985). The study also clearly demonstrates that myofibroblasts do not behave pharmacologically the same as smooth muscle cells, a proposal made on the basis of the very limited experimental evidence of Gabbiani et al. (1971).

Chander, C.L. & Naylor, I.L. (1985) Br. J. Pharmacol. 86, 517P
Cross, S.E. & Naylor, I.L. (1988a) Br. J. Pharmacol. 95, 632P
Cross, S.E. & Naylor, I.L. (1988b) Br. J. Pharmacol. 95, 831P
Gabbiani, G., Ryan, G.B. and Majno, G. (1971) Experientia 27, 549-50
Lipper, S., Kahn, L.B. & Reddick, R.L. (1980) Path. Ann. Pt(1), 409-441

INEFFECTIVENESS OF β-ADRENOCEPTOR ANTAGONISTS IN RAT ADJUVANT-INDUCED ARTHRITIS

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It has recently been reported that the mixed β1/β2 adrenergic antagonist propranolol, and the β2 antagonists ICI 118,551 and butoxamine can attenuate rat adjuvant arthritis when assessed radiologically at 28 days (Levine et al 1988). These findings are of importance since they suggest that β antagonists may find a role in the therapy of rheumatic diseases. We have therefore examined the effects of propranolol, ICI 118,551, metoprolol, the β2 agonist salbutamol and the NSAID indomethacin on adjuvant arthritis in the Alderley Park (AP) Wistar-derived rat.

Adjuvant arthritis was induced in male AP rats by subplantar injection of 500μg Mycobacterium tuberculosis in mineral oil into the right hind foot. Animals were treated from two days before adjuvant administration to twenty one days post-injection with metoprolol (50mg/kg ip), ICI 118,551 (25mg/kg ip), propranolol (20mg/kg ip), or salbutamol (5mg/kg ip) given three times daily or indomethacin (3mg/kg po) given once daily. Arthritis was monitored by measurement of hind paw thickness, cumulative weight gain, serum acute phase protein levels (alpha 1 glycoprotein), clinical score, radiological evaluation of the hind paws on days 14 and 21, and terminal histology of the hind paws.

In contrast to the finding of Levine et al, propranolol and ICI 118,551 had no effect on radiological damage in the hind paws on days 14 or 21.Treatment with metoprolol and salbutamol also had no effect on radiological damage at either time. Indomethacin, however, inhibited radiological damage at both timepoints ($p<0.01$). None of the β antagonists had any effect on right foot swelling, weight gain, serum alpha 1 glycoprotein, clinical score or histological damage. Propranolol,unlike ICI 118,551 or metoprolol, inhibited left foot swelling on days 14 and 21 ($p<0.05$). Interestingly, salbutamol, a β2 agonist, also inhibited left foot swelling and histological damage but had no effect on the other disease parameters. Indomethacin, as the positive control, inhibited left and right foot swelling ($p<0.05$), clinical score ($p<0.01$), lowered serum alpha 1 glycoprotein levels ($p<0.01$), and increased weight gain relative to controls ($p<0.05$), but had no effect on histological damage.

In conclusion, we were unable to demonstrate, using a multi-parameter assessment, that β adrenoreceptor antagonists have a biologically significant inhibitory effect on adjuvant arthritis in the AP rat. Further studies are required to identify the reasons for the differences between these data and those of Levine et al.

Levine et al PNAS (1988) 85, 4553-4556.

THE EFFECT OF α_1-ACID GLYCOPROTEIN ON QUININE DISPOSITION IN THE ISOLATED PERFUSED RAT LIVER PREPARATION

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α_1-Acid glycoprotein (AAG) is known to increase non-specifically in response to certain pathophysiological changes (Kremer *et al* 1988). During malaria, plasma AAG is elevated with a compensatory fall in albumin (Silamut *et al* 1985). Quinine binds to AAG rather than albumin *in vitro* (Mihaly *et al* 1987) so changes in AAG concentrations may influence the disposition of quinine. Malaria infection alters quinine disposition in the isolated perfused rat liver (IPRL) preparation (Mansor *et al* 1989).

We have studied the effect of 2 different concentrations of AAG on quinine disposition in livers isolated from 3 groups of male Wistar rats (200-250g); (A) Control, without the addition of bovine AAG (N=6), (B) Addition of AAG of 50 mg/100ml; N=4 (normal concentration) and (C) Addition of AAG of 200 mg/100ml; N=4 (the concentration observed during malaria infection (Voulgari *et al* 1982)). Following bolus administration of quinine (1mg), perfusate was sampled at intervals to 4 h in each group and perfusate plasma assayed by HPLC. Additional samples were taken hourly to measure protein binding by ultrafiltration.

Pharmacokinetic parameters are tabulated below

Parameter	Control	50 mg/100ml	200 mg/100ml
AUC (nghr/ml)	1856 ± 460	2006 ± 659	6035 ± 2374**
CL (ml/min)	9.57 ± 2.87	8.89 ± 2.38	3.10 ± 1.17**
Vd (ml)	1198 ± 416	1690 ± 330	466 ± 95**
T½ (hr)	1.47 ± 0.39	2.27 ± 0.44	1.88 ± 0.64

Table 1: Pharmacokinetic parameters (mean ± s.d.) for quinine in the IPRL. (**P < 0.01 compared with control, ANOVA, accepting P < 0.05 as significant).

Significantly higher perfusate concentration of quinine, as evidenced by an increased AUC were observed in perfusate from Group C (200 mg/100ml) compared to controls. No significant difference in AUC was observed between controls and Group B (50 mg/100ml). The significant difference in AUC (P < 0.05) of Group B and C is related to change in Vd (P < 0.005) and Cl (P < 0.01) as there is no difference in T½ between 50 and 200 mg/100ml; AAG. Higher concentrations of AAG (200 mg/100ml) resulted in a significant decrease in Cl (68%) and Vd (61%) but not T½ compared to control. At lower concentrations (50 mg/100ml) compared to control, there was no significant difference in Cl, Vd and T½. Percentage unbound quinine in control and experimental perfused livers 1h after drug administration was 64 ± 3% (control), and 51 ± 4% (50 mg/100ml; AAG), and 20 ± 5% (200 mg/100ml; AAG). Over 4h, the percentage of unbound drug increased in all groups.

These data indicate that changes in AAG can affect the disposition of quinine in the IPRL and emphasises the importance of alterations in unbound drug concentrations in disease and their relation to the pharmacological response.

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Kremer J.M.H. *et al.* (1988) Pharmac. Rev. 40, 1-47.
Silamut K. *et al.* (1985) Am. J. Trop. Med. Hyg. 3, 681-686.
Mihaly G.W. *et al.* (1987) Br. J. clin. Pharmac. 24, 769-774.
Mansor S.M. *et al.* (1989) Br. J. Pharmac. 96, 296P.
Voulgari F. *et al.* (1982) Br. Heart J. 48, 352-356.

TIME-COURSE CHANGES IN LIVER CYTOCHROMES P-450 FOLLOWING INJECTION OF EMULSIFIED PERFLUOROCHEMICALS IN RATS

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Emulsified perfluorochemicals (PFCs) are attracting interest as potential physiological gas transport fluids and have been tested for oxygenation of ischaemic tissues and isolated organs (Lowe, 1987). Components of such emulsions are known to induce cytochromes P-450 (P-450) activity in the liver (Obraztsov et al., 1985) although the time course of such changes, possible differences between male and female animals, and effects of emulsion composition have not been examined in detail. We have therefore studied the effects of low doses of different PFC emulsions on this enzyme system in rats.

Male or female Wistar rats (body weight (b.w.): 180-310g; n = 143) were used. They were injected intravenously (i.v.) via a tail vein with 10 ml/kg b.w. of one of the following: (i) saline (0.9% w/v NaCl); (ii) Fluosol-DA 20% (F-DA; Green Cross, Japan); (iii) Oxypherol (FC-43; Green Cross); and (iv) a novel 20% (w/v) perfluorodecalin (FDC) emulsion containing 1% (w/v) of a C-16 oil additive and 4% (w/v) Pluronic F-68 (Sharma et al., 1987). At 24hr, 72hr and 7 days after injection, groups of animals were killed and their livers removed and weighed. Tissues were homogenized in 1.15% (w/v) Tris/HCl buffered KCl solution and hepatic microsomal protein and P-450 concentrations measured using standard assays (Omura & Sato, 1964).

Mean liver weights in male rats were increased by 27% and 32% ($P < 0.01$) at 72hr and 7 days respectively following injection of the novel emulsion. Liver weight was similarly increased by 17% at 7 days after injection of F-DA ($P<0.05$); no comparable changes occurred following injection of FC-43. In female rats, liver weight was also increased by 10% at 72 hr following injection of novel emulsion ($P<0.05$) but was unaffected by injection of either F-DA or FC-43. Mean liver P-450 concentrations in male rats were increased up to 3 fold at 72 hr following injection of either F-DA ($P<0.05$) or the novel emulsion ($P<0.02$); P-450 was up to 65% greater ($P<0.01$) than the corresponding control mean value (0.96 ± 0.12 nmol/mg protein; n = 4) at 7 days after injection of novel emulsion in male rats. P-450 concentrations in female rats did not change in response to injection of F-DA, FC-43 or the novel emulsion; overall mean liver P-450 in the 3 groups of control female rats was 0.64 + 0.09 nmol/mg protein (n = 12).

These results show that emulsion composition is an important determinant of liver microsomal P-450 induction by emulsified PFCs in rats. The increase in P-450 following injection of F-DA or novel emulsion was consistent with previous findings that FDC is the active principle involved (Obratzsov et al., 1985). We speculate that the marked variation in response between male and female rats is due, at least in part, to differences in circulating androgens which are known to affect liver P-450 activity in this species (Kato & Onoda, 1970).

Kato, R. & Onoda, K. (1970) Clin. Pharmac. 19, 1649-1660.
Lowe, K.C. (1987) Comp. Biochem. Physiol. 87A, 825-838.
Obratzsov, V.V., Shekhtman, D.G., Sologub, G.R. & Beloyartsev, F.F. (1985) Biochem. U.S.S.R. 50, 1041-1048.
Omura, T. & Sato, R. (1964) J. Biol. Chem. 239, 2370-2385.
Sharma, S.K., Bollands, A.D., Davis, S.S. & Lowe, K.C. (1987) In Oxygen Transport to Tissue, Vol. IX (eds. Silver, I.A. & Silver, A.), pp. 97-108. London, Plenum.

EMULSIFIED PERFLUOROCHEMICALS AS ADJUNCTS TO PHOTODYNAMIC THERAPY IN MICE

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Photodynamic therapy (PDT) is being evaluated for the treatment of tumours in man. It depends on the use of tumour-localising porphyrins that are activated by visible light to produce tumour destruction (Fingar et al., 1987). PDT is oxygen dependent and recent attention has focussed on the value of emulsified perfluorochemicals (PFCs) for improving tumour oxygenation and thereby increasing the effectiveness of PDT. We have therefore studied the effects of PDT on skin and tumours in mice previously injected with a novel perfluorodecalin (FDC) emulsion.

Inbred BALB/c female mice with subcutaneous implants of a plasma cell tumour (Berenbaum et al., 1986) were given various doses of a new, potent photosensitizer, meta-tetra(hydroxyphenyl)porphyrin (m-THPP) (Berenbaum et al., 1986). 24h later, tumours and skin were exposed to 10 J cm ^{2} of light at the activating wavelength (648 nm) from a dye laser. Some mice were given, at 2.5 hr before PDT, 0.5 ml/10g b.w. of a 20% (w/v) emulsion of FDC stablized with 1% (w/v) of a C-16 oil additive, perfluoroperhydrofluoranthrene (Sharma et al., 1987). The next day, mice were injected i.v. with 0.2 ml of 1% (w/v) Evans Blue in saline and killed 1 hr later. The depth of necrosis was measured in tumour slices as reported previously (Berenbaum et al., 1986). Additional groups of animals were injected with m-THPP either 24 hr or 48 hr before light and emulsion either 2.5 hr or 24 hr before light. 4 hr after illumination, they were killed and a 1 cm disc of skin was removed and weighed; skin Evans Blue concentration was measured by absorbance at 625 nm following sodium sulphate-acetone extraction (Berenbaum et al., 1986).

The mean depth of tumour necrosis 24 hr after PDT was 2.5 ± 0.1 mm (n = 7) in mice given FDC emulsion and 2.14 ± 0.6 mm (n = 7) in controls; this difference was not significant ($P > 0.05$). However, PDT-induced skin damage was significantly less in mice injected with FDC emulsion, as shown by shifts in the dose-response curves for skin disc weight (oedema) and uptake of Evans blue dye (vascular permeability). For example, in mice injected with 12.5 μm/kg m-THPP 24 hr before light and emulsion 2.5 hr before light, mean skin disc weight was up to 60% lower than control. Moreover, in animals injected with 12.5-50 μm/kg m-THPP 48 hr before light and emulsion 24 hr before light, skin Evans Blue concentration was up to 44% lower than control.

These results show that, while pretreatment with emulsified fluorocarbons does not alter PDT-induced tumour necrosis in mice, it nevertheless provides protection against photodynamic skin damage. The protective effects of the emulsion may be due to inhibition of leucocyte-mediated inflammatory responses in accord with previous observations (Lowe, 1987). Alternatively, the apparent skin protection may simply reflect haemodilution effects of the emulsion. Further studies are in progress to distinguish between these possibilities.

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Berenbaum, M.C., Akande, S.L., Bonnett, R., Kaur, H., Ioannou, S., White, R.D. & Winfield, U.-J. (1986) Br. J. Cancer 54, 717-725.

Fingar, V.H., Mang, T.S. & Henderson, B.W. (1988) Cancer Res. 48, 3350-3354.

Lowe, K.C. (1987) Comp. Biochem. Physiol. 87A, 825-838.

Sharma, S.K., Bollands, A.D., Davis, S.S. & Lowe, K.C. (1987) In Oxygen Transport to Tissue, Vol. IX (eds Silver, I.A. & Silver, A.), pp. 97-108. London, Plenum.

589C80, A POTENT LONG-ACTING LEFT-SHIFTER OF THE OXYGEN SATURATION CURVE OF HAEMOGLOBIN *IN VIVO* IN THE RAT

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A compound which left-shifts the oxygen saturation curve of haemoglobin is of potential utility in the prophylaxis of sickle cell disease (Dean & Schechter, 1978) and may also be useful in cancer therapy (Adams et al, 1986). 12C79 (5-(2-formyl-3-hydroxyphenoxy)pentanoic acid) was designed to produce such a left-shift by a preferential binding between the terminal amino groups of the α-chains in the oxy conformation of haemoglobin (Beddell et al, 1984). 589C80 (4-(2-formyl-3-hydroxyphenoxymethyl)benzoic acid) is a close analogue in which the tetramethylene chain of 12C79 is replaced by a benzyl group. This change results in an increased potency of interaction with haemoglobin and a much longer duration of action in vivo.

Oxygen saturation curves were measured at 37°C for dilute human haemoglobin solutions (23μM) using a Hemox Analyzer (TCS Medical Products) and for whole human blood using a Hem-O-Scan (Aminco). In both solution and whole blood a fully left-shifted curve was obtained with 589C80 to haemoglobin ratios of 4 to 1 (100μM in solution and 10 mM in whole blood). The solution curve was devoid of cooperativity with a Hill Coefficient of 1 and an oxygen affinity equivalent to that of the oxy conformation of the haemoglobin tetramer (P50 ca 1 mm Hg). This suggests that 589C80 binds more tightly to oxy haemoglobin than does 12C79 since the fully shifted curve elicited by 12C79 still displays cooperativity. At lower concentrations of 589C80 the oxygen saturation curves were markedly biphasic which enabled estimation of the amount of haemoglobin reacted (%MOD) as described previously for 12C79 (FitzHarris et al, 1985).

Groups of 5 male rats were treated i.v. with either 12C79 (87.5 mg/Kg) or 589C80 (100 mg/Kg) (i.e. mole equivalent) and blood samples taken by tail bleed at various times. The %MOD values were estimated from the measured oxygen saturation curves and plotted against time. Peak %MOD levels were achieved rapidly (< 15 min) for both compounds but there was a very marked difference in duration of effect. The $t_{\frac{1}{2}}$ was estimated as 2.8 h for 12C79 and 16 h for 589C80. An even longer duration of action with 589C80 was observed after oral dosing but in this case the peak %MOD level was attained only after several hours. Thus, 589C80 is a potentially useful compound for assessing the effects on disease states of a long term left-shift of the oxygen saturation curve.

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Adams, G.D. et al. (1986) Int. J. Radiation Oncology Biol. Phys. 12, 1299 - 1302.
Beddell, C.R. et. al. (1984) Br. J. Pharmac. 82, 397 - 407.
Dean, J. & Schechter, A.N. (1978) New Eng. J. Med. 299, 752 - 763, 804 - 811 & 863 - 870.
FitzHarris, P. et. al. (1985) Br.J. Clin. Pharm. 19, 471 - 481.

5-HT_3 RECEPTORS MEDIATE INHIBITION OF [^{3}H]ACETYLCHOLINE RELEASE FROM RAT ENTORHINAL CORTEX *IN VITRO*

CONSEQUENCES OF A STEADY-STATE TERNARY COMPLEX MODEL

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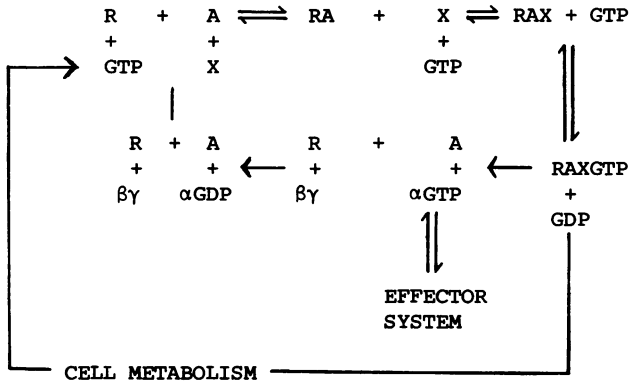
In the past, comparison of the properties of agonists and their use in receptor classification has been based mainly on ideas and equations developed using the occupancy model of drug action. It has become increasingly clear that interpretations of concentration-response curves in terms of this model cannot be regarded as satisfactory. Attempts have been made recently (Mackay,1987,1988) to develop null equations based on a version of the ternary complex model but this model too has serious limitations. Steady-state analysis has therefore been performed on the more realistic ternary complex model shown below, to derive null equations for the comparison of agonists and for estimating apparent affinity constants of agonists using irreversible antagonists.

Symbols

R = receptor

A = agonist

X = G-protein, which is a complex of α, β and γ sub units



The null equations developed in this way have been shown to have the same mathematical form as those developed previously using the occupancy model of drug action. However these new null equations predict that in general, for any one receptor-effector system, the apparent affinity constants of agonists (estimated from curve displacements produced by irreversible antagonists) may depend on the concentration of G-protein in the cell membranes and on the intracellular concentration of GTP. The same conclusion applies to estimates of relative potencies of agonists, though these are not likely to be quite so dependent on these tissue variables.
It is concluded that relative potencies, efficacies and apparent affinity constants of agonists, estimated from concentration-response curves, do not provide a totally dependable method for classifying receptor-effector systems.

Mackay,D. (1987) In Perspectives on Receptor Classification, (Black,J.W., Jenkinson,D.H., & Gerskowitch, V.P.eds.), pp.103-114, A.R.Liss.
Mackay,D. (1988) Trends Pharmacol. Sci.9,156-157

EVIDENCE IN FAVOUR OF THE STEADY-STATE TERNARY COMPLEX MODEL

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Competitive binding experiments have been carried out on rat atrial membranes. Suspensions of the washed membranes, ^{3}H-N-methyl-scopolamine (NMS) (3nM), and various concentrations of carbachol, oxotremorine or pentyl-trimethyl-ammonium (pentyl-TMA) were incubated in Krebs' solution for 30 min. at 27°C. The tube contents were then rapidly diluted and filtered. Bound NMS was estimated by liquid scintillation counting. Non-specific binding was taken to be that remaining when atropine (1 μM) was also present. The data were analysed in terms of the equilibrium ternary complex model (see e.g. Ehlert, 1985). The results are shown below. R_T and X_T are the total concentrations of receptor and of G-protein. K_{1A} is the affinity of agonist A for its receptor and K_{2A} is the affinity of the RA complex for X.

Agonist	K_{1A} x 10^{-4}M	$K_{2A} \cdot X_T$	X_T/R_T	n
Oxotremorine	509 ± 62	45.1 ± 5.7	0.48 ± 0.03	6
Carbachol	33.7 ± 4.2	56.2 ± 10.1	0.55 ± 0.03	6
Pentyl-TMA	5.7 ± 0.3	32.3 ± 13.3	0.47 ± 0.03	3

Each value is given as mean ± s.e.m. based on n results.

Apparent affinity constants of these agonists for the muscarinic receptors were estimated by comparing concentration-response curves determined at 27°C in the presence of 20 nM isoprenaline, on rat atria paced at 180 beats/min., before and after treatment of the tissue with propylbenzilyl-choline mustard. The bathing medium in these experiments was the same as that used in the ligand binding experiments. The values of these apparent affinity constants, K_{Aapp}, are shown below, together with values calculated from the null equation derived on the basis of the equilibrium ternary complex model (Mackay, 1988).

Agonist	K_{Aapp} x 10^{-4}M (Observed)	K_{Aapp} x 10^{-6}M(Calculated)
Oxotremorine	45.9 ± 3.6	235
Carbachol	4.7 ± 0.4	19.2
Pentyl-TMA	5.3 ± 1.3	1.9

Each observed value is given as mean ± s.e.m. based on 4 results.

The apparent affinity constants measured on the intact tissues are much less than those predicted by the equilibrium ternary complex model. The various discrepancies can be explained in terms of the steady-state ternary complex model (Mackay, 1989) since the concentration of guanosine-5'-triphosphate in rat atria has been found, by HPLC, to be approximately 150 μmole/kg surface-dried tissue.

Ehlert, F.J. (1985) Mol. Pharmacol., 28, 410-421.
Mackay, D. (1988) Trends in Pharmacol. Sci., 9, 156-157.
Mackay, D. (1989) Communication C49 at this Meeting.

POTENT INHIBITION OF RECEPTOR-LINKED PGI_2 SYNTHESIS BY NSAIDS IN THE RAT AORTA: EVIDENCE FOR EFFECTS ON CALCIUM MOBILISATION

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Although the role of prostanoids (PGs) in mediating vascular reactivity has been largely investigated using cyclooxygenase inhibitors (COIs) such as indomethacin, COIs exert effects on vascular reactivity that appear not to be PG-mediated (Altura & Altura, 1976; Hadhazy *et al.*, 1988). Studies have also indicated that COIs exert actions on intracellular systems such as G proteins, protein kinase C and calcium binding at the plasmalemma (Hadhazy *et al.*, 1988; Peplow, 1988). It has recently been established that receptor-linked prostacyclin (PGI_2) synthesis in blood vessels is mediated by G proteins, protein kinase C and cell surface calcium channels (Jeremy *et al.*, 1988). We therefore investigated the effect of three COIs (indomethacin, tiaprofenic acid and ibuprofen) on PGI_2 synthesis by the rat aorta when stimulated by noradrenaline (NA), phorbol ester dibutyrate (PDBU; a protein kinase Ca^{2+} activator), sodium fluoride (NaF; a G protein activator), calcium ionophore A23187 (A23187; a creator of artificial Ca^{2+} channels), arachidonate (AA; a PG precursor) and trauma (sonication: stimulates PGI_2 synthesis via extracellular Ca^{2+} activation of phospholipase A_2; Jeremy *et al.*, 1986).

Aortic tissue was processed for the assessment of PGI_2 stimulation/inhibition as previously described (Jeremy & Dandona, 1989). Tissue was preincubated with COIs for 1 h prior to the addition of PGI_2 stimulators ([final concentrations] NA[10 μM], NaF [10 mM], PDBU [1 μM], A23187 [20 μM], AA [20 μM]) or sonication, and further incubated at 37 °C for 1 h. PGI_2 (as 6-oxo-$PGF_{1\alpha}$) released into the supernatant were measured by specific radioimmunoassay.

Table 1 IC_{50}s (μM) of COIs on different stimulators of PGI synthesis.

	NA	NaF	PDBU	A23187	Trauma	AA
Indomethacin	0.009	0.01	0.01	0.01	0.33	0.30
Ibuprofen	0.05	0.05	0.05	0.05	1.21	1.25
Tiaprofenic acid	0.03	0.03	0.03	0.03	0.45	0.48

The present study demonstrates that although there was no difference in the IC_{50}s of the COIs when stimulated with NA, NaF, PDBU and A23187, there was a marked right shift in the IC_{50}s of the COIs when PGI_2 was stimulated by sonication (trauma) and AA. Since both trauma and AA-stimulated PGI_2 synthesis bypass Ca^{2+} channel-mediated stimulation of PG synthesis (Jeremy *et al.*, 1986), the present data suggest that the COIs are exerting inhibitory actions not only on cyclooxygenase but also (and more potently) on the mobilisation of Ca^{2+} linked to PGI_2 synthesis. Furthermore, since calcium is a universal second messenger, these data may not only be of importance to rationalising anomalous effects of COIs on vascular reactivity but also to the antiinflammatory efficacy and deleterious side effects of COIs.

Altura, B.M. & Altura, B.T. (1976) Fed. Proc. 35, 2360-2370.
Hadhazy, P., Malomvolgyi, B. & Magyar, K. (1988) Prost. Leuk. Ess. Fatty Acid Revs. 32, 175-186
Jeremy, J.Y., Mikhailidis, D.P. & Dandona, P. (1986) Naunyn Schmiedeberg's Arch. Pharmacol. 332, 70-73.
Jeremy, J.Y., Mikhailidis, D.P. & Dandona, P. (1988) Prost. Leuk. Ess. Fatty Acid Revs. 34, 215-218
Jeremy, J.Y. & Dandona, P. (1989) Br. J. Pharmac. 96, 243-250.
Peplow, P. (1988) Prost. Leuk. Ess. Fatty Acid Revs. 33, 239-252

PROSTANOID RECEPTORS MEDIATING CONTRACTION AND RELAXATION OF HUMAN ISOLATED UTERINE ARTERY

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We have shown previously that prostanoids can both relax and contract guinea pig uterine arterial smooth muscle and that relaxation is mediated via IP-receptors, and contraction by both TP- and EP_3-receptors (Baxter et al. 1988). Prostanoids can also constrict human uterine artery (Wilhelmsson et al., 1981; Vincent et al., 1983). However, the receptor population in this tissue has not yet been defined. In the present study we have examined the action of prostanoids on human isolated uterine artery and attempted to characterise the prostanoid receptors present.

Specimens of ascending uterine artery were obtained from non-pregnant, pre-menopausal patients at hysterectomy. Uterine arteries were cut into rings approximately 3mm in length and mounted under 1g tension in organ baths containing oxygenated (95% O_2/5% CO_2) Krebs solution at 37°C and indomethacin (2.8μM).

The stable thromboxane-A_2 mimetic U-46619 (11,9-epoxymethano PGH_2) was a potent contractile agonist (EC_{50} (95% C.L.) = 3.5 (1.8-6.7) nM, n = 6), whereas the natural prostanoids $PGF_{2\alpha}$, PGE_2 and PGD_2 were considerably weaker (equipotent concentration ratios, (U-46619 = 1); 743 (207-2667) n = 5, ≥3800 n = 5 and ≥900 n = 6, respectively). In a few preparations, PGI_2 caused small contractions at the highest concentration used (30μM). The TP-receptor blocking drug GR32191 caused concentration dependent rightward shifts of contractile concentration-effect curves to U-46619 (pA_2[range] = 8.96 [8.35-10.22] slope[range] = 0.84 [0.6-1.0], n = 3). At a concentration of 1μM GR32191 abolished contractile responses to all the natural prostanoids tested. GR32191 did not influence concentration effect curves to phenylephrine and was included in the Krebs solution throughout all subsequent experiments. Under these conditions the selective FP- and DP-receptor agonists ICI81008 and BW245C (Coleman, 1987) and the selective EP-receptor agonists sulprostone, rioprostil (Coleman et al., 1988) and butaprost (Gardiner, 1986) were inactive up to the highest concentration used (30μM). In preparations contracted with phenylephrine PGI_2 caused concentration-dependent relaxations of uterine arteries (EC_{50} = 12.7 (4.8-34.0)nM, n = 6). The potent IP-receptor agonists iloprost and cicaprost were more potent than PGI_2 (EPC = 0.09 (0.05-0.18), n = 4, and 0.08 (0.01-0.53), n = 4 respectively) whereas BW245C was considerably less potent (EPC>1000). Rioprostil and butaprost were inactive (EPC>30,000).

These data are consistent with the presence of only TP- and IP-receptors in human uterine artery mediating contractile and relaxant responses respectively. Although the naturally-occurring prostanoids, $PGF_{2\alpha}$, PGE_2 and PGD_2 produce constriction of the artery, it appears that these responses are mediated via TP-receptors.

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Baxter, G.S. et al. (1988) Br. J. Pharmac. 96, 71P.
Coleman, R.A. et al. (1988) Br. J. Pharmac. 95, 742P.
Coleman, R.A. (1987) in Prostaglandins and Related Substances, a Practical Approach, ed. C. Benedetto et al., IRL Press, Oxford: 267-303.
Gardiner, P.J. (1986) Br. J. Pharmac. 87: 45-56.
Vincent, J.E. et al. (1983) Advances in Prostaglandin and Leukotriene Research Vol. 12, edited by Samuelsson, Paoletti and Ramwell, Raven Press, N.Y.: 417-422
Wilhelmsson, L. et al. (1981) Prostaglandins, 22: 223-233.

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RENAL VASCULAR RESPONSES TO ARACHIDONIC ACID IN EXPERIMENTAL DIABETES

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Diabetes mellitus (DM) is accompanied by alterations in arachidonic acid (AA) metabolism and vascular responsiveness. We used isolated perfused kidneys of male Wistar rats, 4-6 weeks and 20-24 weeks after the induction of DM with streptozotocin (STZ, 70 mg/kg i.v.), to address renal vascular responses to AA and the endoperoxide analogue, U46619. Responses were compared to those obtained in kidneys from age-matched vehicle-treated rats. A minimum of 5 rats/group were used unless otherwise stated. Blood glucose levels in rats treated with STZ were greater than 350 mg/dl. In both control and diabetic rat kidneys AA (0.3-10 μg) and U46619 (10-1000 ng) produced dose-dependent increases in perfusion pressure. At 4-6 weeks and 20-24 weeks after STZ the vasoconstrictor responses to AA were enhanced compared to the corresponding control. Thus, 4-6 weeks after STZ the approximate ED_{50} for AA-induced vasoconstriction was 0.66 μg compared to 2.1 μg for the control. Similarly, 20-24 weeks after STZ or vehicle treatment, 3 μg AA elevated perfusion pressure by 143$\pm$27 mmHg and 40$\pm$10 mmHg, respectively ($p<0.01$). The enhanced vasoconstrictor response to AA in 4-6 week diabetic rats was associated with increased conversion of AA to immunoreactive prostanoids. Thus, in diabetic rat kidneys (n=3) 3 μg AA increased the efflux of PGE_2 and 6-keto-$PGF_{1\alpha}$ by 7.1$\pm$1.2 ng/min and 6.0$\pm$0.6 ng/min, respectively, compared to control values (n=4) of 3.9$\pm$0.5 ng/min ($p<0.05$) and 2.7$\pm$0.4 ng/min ($p<0.01$), respectively. In addition, vasoconstrictor responses to U46619 were increased in kidneys from diabetic rats. Thus, 4-6 weeks and 20-24 weeks after STZ, 100 ng U46619 increased perfusion pressure by 146$\pm$7 mmHg and 140$\pm$13 mmHg, respectively, compared to values in age-matched controls of 75$\pm$17 mmHg and 54$\pm$19 mmHg, respectively ($p<0.01$). Vasoconstrictor responses to AA and U46619 in diabetic and control rat kidneys were unaffected by inhibition of thromboxane synthase with CGS-13080 (5 μM) but were significantly reduced by the PGH_2/TxA_2 receptor antagonist, SQ 29548 (1 μM). This study demonstrates that the renal vasoconstrictor response to AA is increased in D.M. and may result from increased sensitivity of the renal vasculature to endoperoxides and, possibly, enhanced conversion of AA to endoperoxides (Quilley et al., 1989).

Quilley, J., McGiff, J.C. and Nasjletti, A. (1989) Brit. J. Pharmacol. 96:111-116.

A SINGLE α_1-ADRENOCEPTOR, DISTINCT FROM THE α_{1A} AND α_{1B} SUBTYPES, MAY MEDIATE CONTRACTION OF THE RAT AORTA TO PHENYLEPHRINE

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Two subtypes of α_1-adrenoceptor have recently been proposed (Morrow and Creese, 1986; Han et al., 1987). One, showing relatively high affinity for WB 4101 and linked to nifedipine-sensive Ca^{++} influx, is designated α_{1A}; the other, showing relatively low affinity for WB 4101 and linked to inositol phospholipid turnover, is designated α_{1B}. The α_{1B} site can be selectively inactivated by the alkylating agent, chloroethylclonidine. The rat aorta contracts to α_1-adrenoceptor agonists by both nifedipine sensitive and insensitive mechanisms (Chiu et al., 1987) suggesting mediation by both α_{1A} and α_{1B} sites. The present experiments were carried out to verify this hypothesis.

Spiral strips were cut from the thoracic aortae of male, Sprague-Dawley rats killed by cervical dislocation. The endothelium was removed and the tissues suspended under 1 g tension in a modified Tyrode solution (Fozard and Mobarok Ali, 1978). Concentration-response curves to phenylephrine were generated cumulatively in the absence and presence (20 min preincubation) of vehicle (no significant sensitivity change) or antagonists.

Consistent with the literature, incubation of rat aortae in nifedipine, 10^{-6} M, reduced both the EC50 (by 6.6-fold) and the maximum response (by 26%) to phenylephrine (n = 7). A summary of the effects of a number of putative antagonists obtained in the absence and presence of nifedipine 10^{-6} M is presented in Table 1.

Table 1 Effects of antagonists on the response to phenylephrine in the absence and presence of nifedipine

	No nifedipine			nifedipine 10^{-6} M		
	pA_2	Schild slope	n	pA_2	Schild slope	n
prazosin	9.69 ± 0.07	- 1.06 ± 0.09	4	10.03 ± 0.10	- 1.00 ± 0.13	4
yohimbine	6.54 ± 0.14	- 1.10 ± 0.18	4	6.54 ± 0.14	- 1.41 ± 0.14	4
phentolamine	8.24 ± 0.09	- 0.84 ± 0.11	4	8.50 ± 0.08	- 1.03 ± 0.10	4
WB 4101	9.14 ± 0.08	- 1.06 ± 0.10	7	9.57 ± 0.04	- 0.99 ± 0.05	6
5-methylurapidil	7.54 ± 0.15	- 1.31 ± 0.16	4	7.71 ± 0.11	- 1.13 ± 0.14	4
chloroethylclonidine	5.22 ± 0.01*		4	5.19 ± 0.13*		4

* pD'_2 values estimated after 30' incubation in chloroethylclonidine followed by repeated washing

The results confirm that the contractile response to phenylephrine is mediated through an α_1-adrenoceptor linked to both nifedipine sensitive and insensitive mechanisms. However, the fact that both surmountable (WB 4101 and 5-methylurapidil) and insurmountable (chloroethylclonidine) discriminatory antagonists yield similar data in the presence or absence of nifedipine is not consistent with the response being mediated by the putative α_{1A} and α_{1B} subtypes. A further α_1-adrenoceptor subtype with a unique pharmacology and linked to both nifedipine sensitive and insensitive mechanisms is implicated in the contractile response of the rat aorta to phenylephrine.

Chiu, A.T., Bozarth, J.M. and Timmermans, P.B.M.W.M. (1987) J. Pharmacol. exp. Ther. 240, 123 - 127.
Han, C., Abel, P.W. and Minneman, K.P. (1987) Nature 329. 333 - 335.
Morrow, A.L. and Creese, I. (1986) Mol. Pharmacol. 29, 321 - 330.
Fozard, J.R. and Mobarok Ali, A.T.M. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 301, 223 - 235.

HETEROGENEITY OF α_1-ADRENOCEPTOR BINDING SITES IN THE RAT HEART - SUBTYPE-SELECTIVE AGONISTS AND ANTAGONISTS

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Distinct α_1-adrenoceptor binding sites showing high (α_{1A}) and low affinity (α_{1B}) for WB-4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4 benzodioxane) and 5-methyl-urapidil can be distinguished in various tissues of the rat (Morrow & Creese, 1986, Minneman et al., 1988, Gross et al., 1988). In the present study we determined the distribution of these binding sites on membranes of the rat heart by using [^{3}H]prazosin as radioligand and several unlabelled adrenergic agents.

Table 1. Inhibition of [^{3}H]prazosin binding to rat heart membranes

	$pK_{I\,high}$	$pK_{I\,low}$	$\%_{high}$
(+)Niguldipine	10.7 ± 0.1	7.6 ± 0.1	19 ± 1
5-Methyl-urapidil	9.2 ± 0.3	7.4 ± 0.1	22 ± 5
YM-12617*	10.3 ± 0.2	8.9 ± 0.1	25 ± 1
WB-4101	10.3 ± 0.3	8.9 ± 0.1	22 ± 4
Oxymetazoline	8.9 ± 0.1	6.6 ± 0.1	16 ± 1
Amidephrine	6.5 ± 0.2	4.4 ± 0.1	13 ± 1

*5-(2-((2-(2-ethoxyphenoxy)ethyl)amino)propyl)-2-methoxybenzenesulfonamide HCl. Means ± s.e.mean of at least 3 separate experiments

Prazosin, rauwolscine and butanserine displaced [^{3}H]prazosin binding monophasically, whereas several other compounds gave biphasic inhibition curves which allowed calculation of the proportion of sites with high and low affinity (Fig. 1). (+)Niguldipine which was recently introduced by Boer et al. (1989) proved to be the most subtype-selective compound presently known. Incubation of membranes or right ventricle strips with chloroethylclonidine (CEC) according to Minneman et al. (1988) resulted in a 71 % loss of [^{3}H]prazosin binding sites. The majority of the remaining binding sites showed high affinity for niguldipine, 5-methyl-urapidil, YM-12617, oxymetazoline and amidephrine and apparently belonged to the α_{1A} subtype. In order to evaluate the functional role of α_{1A}- and α_{1B}-binding sites, the inotropic response to adrenaline (in the presence of propranolol) was recorded in right ventricle strips preincubated with or without CEC. This treatment increased rather than decreased the maximum response to α_1-adrenoceptor stimulation and resulted in a significant increase in the potency of 5-MU but not of prazosin.

These results demonstrate the existence of two distinct α_1-adrenoceptor binding sites in the rat heart and suggest that both sites may be functionally relevant and seem to interact in a complex manner which is not yet clearly understood.

Boer, R. et al. (1989) Naunyn-Schmiedeberg's Arch. Pharmacol.339,R91
Gross, G. et al. (1988) Eur. J. Pharmacol. 151, 333
Minneman, K.P. et al. (1988) Mol. Pharmacol. 33, 509
Morrow, A.L. & Creese, I. (1986) Mol. Pharmacol. 29, 321.

EFFECT OF DURATION OF DIABETES ON CARDIAC ADRENOCEPTOR-MEDIATED INOTROPIC RESPONSES

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There are many conflicting reports concerning the effect of experimental diabetes on cardiac adrenoceptor-mediated responses. We have examined rat left atrial and papillary muscle responses to isoprenaline and phenylephrine to determine whether the effects of diabetes are dependent on the cardiac tissue examined and/or the duration of diabetes.

Diabetes was induced in female wistar rats (200g) by a single intraperitoneal injection of streptozotocin (50 $mgKg^{-1}$) (STZ). Age matched controls were injected with citrate buffer vehicle. 2, 4 or 12 weeks later animals were killed and left atria (LA) and papillary muscles (PM) set up in aerated Krebs solution (11.7mmol l^{-1}) at 37°C. Tissues were paced at 1Hz and isometric developed tension recorded. Cumulative concentration-response curves to isoprenaline and phenylephrine (in the presence of 1 μM propranolol) were constructed in the presence of desipramine (1 μM) and metanephrine (10 μM). The remaining ventricular tissue was assayed for [^{3}H] dihydroalprenolol or [^{3}H] prazosin binding.

TABLE 1: EC_{50} values (nM) with 95% confidence limits for isoprenaline

Tissue	Duration of diabetes 2 weeks	4 weeks	12 weeks
Left Atria			
control	38.4 (21.9 - 67.3)	49.7 (8.5 - 291.5)	13.7 (7.2 - 26.0)
diabetic	5.1 (2.7 - 9.7)*	10.6 (8.8 - 12.7)*	76.8 (33.8 - 174.5)*
Papillary muscles			
control	57.6 (27.6 - 119)	77.5 (40.7 - 147.8)	85.0 (34.6 - 208.5)
diabetic	10.6 (3.5 - 32.6)*	8.0 (3.3 - 18.9)**	35.8 (10.6 - 120.8)

*$P < 0.05$ **$P < 0.01$

2 and 4 weeks after STZ-pretreatment, the responses of both LA and PM's to isoprenaline were enhanced and EC_{50} values were significantly reduced compared with controls (Table 1). The increase in responsiveness to isoprenaline at 2 weeks was accompanied by an increase in the density of ventricular [^{3}H] dihydroalprenolol binding sites from 40.6 ± 3.9 to 80.2 ± 12.8 fmol mg^{-1} protein ($P < 0.05$) while the dissociation constant for binding was unaffected. After 3 months of diabetes, the sensitivity of PM's to isoprenaline was similar to controls while responses of LA were significantly depressed with isoprenaline EC_{50} values being greater than in LA from age-matched controls (Table 1).

Responses of LA to phenylephrine were similar to controls at all time points examined, although responses of PM's to phenylephrine were enhanced 12 weeks after STZ-treatment. Phenyelphrine EC_{50} values at this time point were reduced although not significantly, the values being 15.2 (4.0 - 58.0) μM and 5.4 (0.2 - 151.2) μM in tissues from control and diabetic rats respectively, while the maximum response to phenylephrine relative to the isoprenaline maximum was increased from 45.1 ± 6.0% to 81.3 ± 6.7% in diabetic tissues ($P < 0.01$). The increase in α-adrenoceptor responsiveness at 12 weeks was not associated with a change in [^{3}H]prazosin binding. Neither the density of binding sites nor the dissociation constants were different between ventricular tissues from control (118.3 ± 11.1 fmol mg^{-1}, 0.10 ± 0.04 nM) and diabetic animals (126.8 ± 13.2 fmol mg^{-1} protein; 0.11 ± 0.03 nM).

These results indicate that the effect of diabetes on cardiac adrenergic responsiveness is dependent on the duration of diabetes and the tissue examined.

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SMOOTH MUSCLE PROLIFERATION FROM RABBIT AORTA IS INHIBITED BY 8-Br-cAMP BUT NOT 8-Br-cGMP

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Intimal proliferation of arterial smooth muscle cells leads to the development of atherosclerotic fibrous plaques and there are few agents known to inhibit it. To replicate, smooth muscle cells must transform from the 'contractile' phenotype to a 'synthetic' phenotype, and most previous studies of growth regulation have used synthetic phenotype cells. We have quantified proliferation of contractile smooth muscle cells from explants of rabbit aortic media and report that proliferation is inhibited by 8-Br-cAMP. The endothelium of segments of rabbit thoracic aorta was removed by abrasion and strips of underlying smooth muscle were pulled away from the adventitia. Smooth muscle strips were sliced into precise 1mm^2 explants using a tissue chopper and plated individually into 96-well microtitre plates containing 0.1 ml of DMEM tissue culture medium supplemented with 20% foetal calf serum. Explants were cultured at 37°C for up to 10 days ([^{3}H] thymidine (1μCi/ml) was added for the last 18 h) and were then extracted with 0.1 ml of ice cold 10% trichloroacetic acid (w/v). Extracts from 8 explants were pooled and centrifuged at 1500xg for 5 min at 4°C. The ATP concentration in the supernatants was measured as an assay of biomass and cell viability. The DNA concentration and radioactivity of the pellet were measured as assays of proliferation. Freshly isolated rabbit aorta had an ATP concentration (nmol/μg DNA) of 0.29±0.03 (SE, n=21) whereas explants before culturing had an ATP concn of 0.11±0.006 (n=42). In a second experiment (Table), the ATP concentration in explants increased 3 fold after 3 days in culture and 11 fold after 10 days. This was associated with a pronounced increase in thymidine incorporation, although DNA concentration rose nonsignificantly. The increases in ATP concentration and thymidine incorporation were attenuated dose-dependently by 8-Br-cAMP (10^{-5} to 10^{-3}M, some data not shown) and DNA concentration was decreased. No effect was observed with 8-Br-cGMP (10^{-5} to 10^{-3}M).

Days	Control	8-Br-cAMP (10^{-4}M)	8-Br-cGMP (10^{-4}M)
	ATP concentration (nmoles / 8 explants)		
0	0.17±0.02	N. D.	N. D.
3	0.53±0.09	0.37±0.09	0.58±0.09
10	2.1±0.4	0.75±0.07 *	2.4±0.2
	Thymidine incorporation (1000 dpm / 8 explants)		
1	1.2±0.2	N. D.	N. D.
3	55±4	4.2±0.2 *	67±17
10	139±9	20±1 *	164±21
	DNA concentration (μg / 8 explants)		
0	5±1	N. D.	N. D.
3	5±1	5±1	6±1
10	7±1	2.4±0.4 *	7±1

N. D., not determined; * $p<0.02$ vs corresponding control value (n=3 aortas)

Preparation of explants decreased cell viability by 60 to 90%. Despite this, culturing led to a reproducible increase in ATP and thymidine incorporation, thereby allowing cell proliferation to be quantified. The rise in DNA concentration appeared, however, to be obscured by a fall in DNA concentration in necrotic cells. Cell proliferation was inhibited by 8-Br-cAMP (and by forskolin, unpublished observations), suggesting a similar role for endogenous cAMP-elevating agents including adenosine, adrenaline and prostacyclin.

ANTAGONIST PROFILE OF SK&F104078 AT PRE- AND POST-JUNCTIONAL α_2-ADRENOCEPTORS

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Initial reports suggested that SK&F104078 (6-chloro-9-[(3-methyl-2-butenyl)oxy] -3-methyl-1H-2,3,4,5,-tetra-hydro-3-benzazepine) was an antagonist at post-junctional, but not pre-junctional, α_2-adrenoceptors (Hieble et al., 1986). Subsequent studies cast doubt on this proposal (Connaughton et al., 1988; Bertie et al., 1989). We have re-evaluated its antagonist profile against two well-established α_2-adrenoceptor agonists, B-HT933 and xylazine, at pre- and post-junctional α_2-adrenoceptors and compared it with that of yohimbine.

Antagonist potency at pre-junctional α_2-adrenoceptors in rat vas deferens (prostatic portion) and guinea-pig ileum was determined as described by Drew (1977, 1978 respectively). Its potency at post-junctional α_2-adrenoceptors in rabbit ear vein was established as described by Daly et al. (1988). All tissues were suspended in modified Krebs-Henseleit solution at 37°C, bubbled with 95% O_2/5% CO_2. Cumulative concentration-response curves to agonists were constructed in the absence of, and after 45 min exposure to, antagonist.

Table 1 Potency of SK&F104078 at α_2-adrenoceptors in vitro

Tissue	Antagonist Potency against			
	B-HT933		Xylazine	
	pA_2	slope	pA_2	slope
Rat Vas Deferens	6.48 (6.22-6.84)	0.96 (0.69-1.24)	nc	-
Guinea-Pig Ileum	<5.50	-	<5.50	-
Rabbit Ear Vein	6.67 (6.56-6.77)	1.04 (0.94-1.15)	6.70 (6.51-6.89)	1.09 (0.99-1.19)

Results are mean values (with 95% confidence intervals; n > 4).
nc - not calculated

SK&F104078 ($1x10^{-6}$-$1x10^{-5}$M) competitively antagonised B-HT933 at pre-junctional α_2-adrenoceptors in rat vas deferens but not guinea-pig ileum (up to 3 x 10^{-6}M). It was almost ineffective against xylazine in either tissue, but antagonised both agonists at post-junctional α_2-adrenoceptors in rabbit ear vein (Table 1). Yohimbine ($3x10^{-8}$-$1x10^{-6}$M) antagonised xylazine and B-HT933 with approximately the same potency in all preparations (pA_2 range 7.46-8.00). In pithed rats, SK&F104078 (10mg/kg i.v.) antagonised xylazine, but not B-HT933 at pre-junctional α_2-adrenoceptors in the heart. Yohimbine (1mg/kg i.v.) was equipotent against both agonists.

In summary, the antagonist activity of SK&F104078 was not confined to post-junctional α_2-adrenoceptors, but its effects at pre-junctional α_2-adrenoceptors were agonist and tissue dependent.

Bertie, K. et al., (1988). Br. J. Pharmac., 94, 433P.
Connaughton, S. et al., (1988). Br. J. Pharmac., 95, 702P.
Daly, C.J., et al., (1988). Br. J. Pharmac., 94, 1085-1090.
Drew, G.M., (1977). Eur. J. Pharmac., 42, 123-130.
Drew, G.M., (1978). Br. J. Pharmac., 64, 293-300.
Hieble, J.P. et al., (1986). J. Hypertens., 4, (suppl 6), S189-S192.

We thank Dr D. Butina, Chemistry Dept, GGR Ware, for synthesis of SK&F 104078.

DIFFERENTIAL EFFECT OF COCAINE ON ELECTRICALLY EVOKED TRITIUM OVERFLOW FROM MOUSE VAS DEFERENS AND RAT BRAIN CORTEX SLICES

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Blockade of neuronal uptake increases evoked noradrenaline (NA) overflow in rat brain cortex slices (Gothert et al., 1983) but not in mouse vas deferens (Hagan and Hughes, 1981). To investigate this discrepancy isolated mouse vasa deferentia (Tuck No. 1, 30-35g) and rat brain cortex slices (Wistar, 150-200g) were incubated with ^{3}H-NA and washed as detailed elsewhere (Hagan and Hughes, 1981). Electrical stimulation (vas deferens, 2.5 Hz, 2 ms width, 400 mA for 90 s every 14 min; brain slices, 3 Hz, 2 ms width, 20 mA for 120 s every 20 min) enhanced tritium overflow from both tissues. Drugs were added after a control period of stimulation and drug effects are expressed as the ratio of the fractional evoked tritium overflow in the presence of a drug to that in its absence. Results are given as mean ± s.e. mean.

Idazoxan (0.01-1μM) produced a concentration dependent increase in evoked tritium overflow from vas deferens (control 0.94±0.03; idazoxan 1μM 2.08±0.15; n=6; $P<0.001$; Student's t-test) and brain slices (control 0.94±0.04; idazoxan 1μM 2.03±0.16; n=6; $P<0.001$). Cocaine (0.1-10μM) failed to increase evoked tritium overflow from vas deferens (cocaine 10μM 0.95±0.08; n=6; $P>0.05$ compared to control) while in brain slices a concentration dependent increase was obtained (cocaine 10μM 1.81±0.14; n=6; $P<0.001$ compared to control). Idazoxan (1μM) + cocaine (10μM) greatly increased evoked tritium overflow (vas deferens 3.03±0.24; brain slices 3.21±0.36; n=5; $P<0.05$ compared to idazoxan alone values).

Increasing the frequency of stimulation (0.1, 0.5, 1, 3 and 6 Hz) resulted in a progressive increase in the evoked tritium overflow. In the presence of cocaine (10μM) fractional evoked tritium overflow from brain slices was significantly ($P<0.05$) increased at every frequency except 6Hz while in vas deferens no significant changes were produced by cocaine at any frequency tested ($P>0.05$). Bathing the vas deferens with the physiological saline used for brain slices resulted in about a 50% reduction in evoked tritium overflow but cocaine (10μM) still had no effect. Cocaine 10μM had no effect on evoked tritium overflow from vas deferens superfused in the apparatus normally used for brain slices (control 0.90±0.008; treated 0.93±0.03; n=4; $P>0.05$).

Unmetabolized ^{3}H-NA represented 54±8% and 63±1% (n=4) of the tritium in the evoked overflow from vas deferens and brain slices respectively as determined by column chromatography. Cocaine (10μM) increased markedly the amount of ^{3}H-NA in the evoked overflow from brain slices (dpm; control 2505±228; treated 4518±579; n=5; $P<0.01$) but not in vas deferens (control 3656±177; treated 3714±175; n=5; $P>0.05$). The overflow of endogenous NA, as determined by HPLC, was also increased significantly ($P<0.05$) by cocaine in brain slices (control 0.15±0.02ng; treated 0.28±0.03ng; n=6; $P<0.05$) but not in vas deferens (control 0.34±0.03ng; treated 0.31±0.06; n=4; $P>0.05$).

In conclusion, the different effect of cocaine in the two tissues is not due to any impairment in the function of the presynaptic α_2-adrenoceptors or neuronal uptake processes and is independent of the frequency of electrical stimulation and the experimental apparatus. The effect of cocaine is not due to a difference in the amount of NA contributing to the total tritum overflow and is still apparent when the overflow of endogenous NA is measured.

Gothert, M., Schlicker, E. and Kostermann, F. (1983). Arch Pharmacol., 332, 121-128.
Hagan, R.M. and Hughes, I.E. (1981). J. Auton. Pharmacol., 1, 385-390.

RESPONSES TO CATECHOLAMINES IN RAT DISTAL COLON MAY BE MEDIATED, IN PART, BY 'ATYPICAL' β–ADRENOCEPTORS.

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Responses to catecholamines which are resistant to blockade of classical α- and β-adrenoceptors have been reported in a number of isolated animal tissues. Several reports in the literature suggest that these responses to catecholamines are mediated via β-adrenoceptors which are 'atypical' in nature, like those mediating lipolysis in rat adipocytes (Stanton, 1972; Harms *et al*, 1977). Tissues in which 'atypical' β-adrenoceptors have been reported to exist include the guinea-pig ileum (Bond *et al*, 1986; Bond and Clarke, 1988) and the guinea-pig gastric fundus (Coleman *et al*, 1987). Evidence presented here supports the hypothesis that relaxatory responses to noradrenaline and isoprenaline in the rat distal colon (RDC) may be mediated, in part, via 'atypical' β-adrenoceptors.

Portions of the distal 6cm of the rat colon were suspended in Krebs' PSS at 37°C containing cocaine (3 μM), ascorbic acid (30 μM) and EDTA (30 μM), and were contracted by the hypertonic addition of KCl (30-40 mM) to the organ baths. Responses to noradrenaline and isoprenaline were measured in the presence of propranolol (1 μM) and phentolamine (1 μM), respectively.

Noradrenaline relaxed KCl-induced tone in the RDC in a concentration-dependent manner, with a pIC_{50} (mean ± se) of 6.3 ± 0.18 (n=4). These relaxations to noradrenaline were not antagonised by phentolamine (0.01-1 μM). This action of noradrenaline could not, therefore, be attributed to an action at α-adrenoceptors. Neither cirazoline (0.01-30 μM) nor UK 14,304 (0.01-30 μM) relaxed KCl-induced tone in the RDC. Isoprenaline relaxed KCl-induced tone in the RDC in a concentration-dependent manner, with a pIC_{50} (mean ± se) of 8.6 ± 0.19 (n=5). These relaxations to isoprenaline were antagonised in a non-competitive manner by propranolol (0.01-1 μM), the slope of the Schild plot being significantly less than unity (0.56 ± 0.38, 95% C.L., n = 17). In the presence of propranolol (1 μM) and phentolamine (1 μM), the selective 'atypical' β-adrenoceptor agonist BRL 37344 (Arch *et al*, 1984) relaxed KCl-induced tone in the RDC in a concentration-dependent manner, with a pIC_{50} (mean ± se) of 6.97 ± 0.16 (n=6).

In the presence of phentolamine (1 μM) and propranolol (1 μM), BRL 37344 (1 μM) desensitised responses to noradrenaline and isoprenaline, causing mean rightward 18- and 19-fold shifts of the concentration-response curves (CRC's) to noradrenaline and isoprenaline respectively ($p < 0.01$, n = 4). However, BRL 37344 had no effect on the relaxatory response to papaverine in the RDC, suggesting that its effect was not simply a non-specific effect on all relaxatory responses in the RDC. In the presence of phentolamine (1 μM), tachyphylaxis of responses to BRL 37344 itself was also evident, with a second CRC shifted to the right approximately 14-fold.

The weak, non-competitive antagonism displayed by propranolol and phentolamine against relaxatory responses to catecholamines in the RDC, together with the clear tachyphylactic action of BRL 37344 on both its own response and on the responses to noradrenaline and isoprenaline under conditions of α- and β-adrenoceptor blockade, support the hypothesis that responses to noradrenaline and isoprenaline in the RDC may be mediated, in part, by 'atypical' β-adrenoceptors similar to those present in rat adipocytes and other tissues.

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Arch, J.R.S., Ainsworth, A.T., Cawthorne, M.A., Piercy, V., Sennitt, M.V., Thody, V.E., Wilson, C. & Wilson, S. (1984) Nature 309, 163-165.
Bond, R.A., Charlton, K.G. & Clarke, D.E. (1986) J. Pharmacol. Exp. Ther. 236, 408-415.
Bond, R.A. & Clarke, D.E. (1988) Br. J. Pharmac. 95, 723-734.
Coleman, R.A., Denyer, L.H. & Sheldrick, K.E. (1987) Br. J. Pharmac. 90, Proc. Suppl., 40P.
Harms, H.H., Zaagsma, J. & de Vente, J. (1977) Life Sci. 21, 123-128.
Stanton, H.C. (1972) Arch. Int. Pharmacodyn. 196, 246-258.

COMPARATIVE INHIBITORY EFFECTS OF FPL 63547 AND ENALAPRIL ON RAT TISSUE CONVERTING ENZYME

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FPL 63547 is a novel inhibitor of angiotensin converting enzyme (ACE) which has antihypertensive effects of long duration in spontaneously hypertensive rat (Carr et al., 1988). It is currently thought that local renin angiotensin systems exist in many tissues (Dzau, 1987) and there is evidence that inhibition of tissue ACE and hence local production of angiotensin II may contribute to the cardiovascular actions of ACE inhibitors (Unger et al., 1984). We have compared the inhibitory effect of FPL 63547 and enalapril on tissue ACE in various sites in the rat after oral dosing.

In preliminary studies FPL 63547 (15 and 50 µg/kg p.o.) and enalapril (0.5 and 1.5 mg/kg p.o.) were administered to conscious normotensive rats (male Sprague-Dawley, 250-350g). At each of the fixed time points after dosing (1, 3, 5, 15, 24 and 48h), rats (n = 3) were killed, dissected rapidly, and the relevant tissues examined for the extent of inhibition of ACE. In the heart, kidney, lung, skeletal muscle and plasma FPL 63547 was more potent and of longer duration of effect compared with enalapril. Both compounds lacked inhibitory activity in the brain.

Further studies were performed to compare a range of doses of FPL 63547 (3-50 µg/kg p.o.) and enalapril (5-2000 µg/kg p.o.) at the time of maximum effect (3h). Drug effects were compared with vehicle treated controls in groups of 5 rats. Dose versus inhibition curves were constructed for FPL 63547 and enalapril for the heart, kidney and lung. Regression analysis showed no significant deviation from parallelism for the kidney and lung. The potency ratios (FPL 63547: enalapril) were 3.1:1 (95% confidence intervals, (c.i.)=2.1-4.5) and 6.6:1 (95% c.i.=5.3-8.4) respectively. However, for the heart in three independent experiments, the dose response curves were non-parallel; enalapril having the shallower slope. The difference between FPL 63547 and enalapril was greater for the heart than either of the other organs as exemplified by the ratio of ID_{50} values of 20.3:1 (95% c.i.= 10.7-38.3).

Relative to enalapril, FPL 63547 may show selectivity for the heart compartment of ACE. The possible pathophysiological role of the cardiac renin angiotensin system and implications of local inhibition of ACE have been reported (Dzau, 1987). Further studies will be necessary to determine whether these differences in activity between FPL 63547 and enalapril have functional significance.

Carr, R.D. et al., (1988) Br. J. Pharmacol. 95 487P.
Dzau, V.J. (1987) Amer. J. Cardiol. 59 59A-65A.
Unger, T. et al. (1984) J. Cardiovasc. Pharmacol. 6 872-880

THE EFFECTS OF PINACIDIL AND FELODIPINE ON RAT PULMONARY ARTERY ARE ALTERED IN MONOCROTALINE-INDUCED PULMONARY HYPERTENSION

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The responsiveness of isolated pulmonary blood vessels to spasmogens is known to be altered in rats with monocrotaline (MCT)-induced pulmonary hypertension (Altiere *et al.*, 1986; Wanstall & O'Donnell, 1989). In this study the calcium entry blocking drug, felodipine (FEL), and the potassium channel opening drug, pinacidil (PIN), have been compared as vasorelaxants on isolated preparations of pulmonary artery from rats with MCT-induced pulmonary hypertension.

MCT-treated rats (105 mg/kg s.c. 4 weeks before the experiment) had pulmonary artery pressures of 30±2.7 mm Hg, n=20, compared with saline-treated rats (17±0.8 mm Hg, n=27), and had right ventricular hypertrophy. Single ring preparations (3mm long) of main pulmonary artery (endothelium not removed) were set up in physiological salt solution (37°C, 95%O_2/5%CO_2) at resting forces (MCT, 20 mN; saline, 10 mN) corresponding to the respective pulmonary artery pressures. Cumulative concentration-response curves were obtained to noradrenaline (NA), 5-hydroxytryptamine (5-HT) or endothelin (ET) in the absence and presence of 10 nM FEL or 3 μM PIN (30 min contact).

MCT-induced pulmonary hypertension caused significant ($P<0.05$, Student's *t* test) increases in the potency (neg log EC50) of NA (saline 7.98±0.07, n=9; MCT 8.48±0.08, n=8) and 5-HT (saline 5.19±0.05, n=8; MCT 6.53±0.11, n=7), and significant decreases in the maximum responses (mN/mm²) to NA (saline 15.1±1.5, n=9; MCT 10.7±1.04, n=8) and ET (saline 29.1±1.8, n=5; MCT 10.6±1.7, n=4). In both groups of rats FEL and PIN each caused non parallel shifts in the concentration-response curves to NA and 5-HT with depressions in maximum response. PIN was more effective than FEL. The depression in NA maximum by FEL was more pronounced in MCT-treated rats (68% reduction) than in saline-treated rats (28% reduction), and both FEL and PIN caused a decrease in NA potency in MCT-treated rats (FEL 0.60±0.08 log units, n=3; PIN 0.68±0.06 log units n=4) which was not seen (FEL 0.04±0.03 log units, n=3) or was significantly ($P<0.05$) less (PIN 0.37±0.06 log units, n=4) in saline-treated rats. Similarly, when 5-HT was the spasmogen, the effects of FEL and PIN were enhanced in pulmonary hypertension. FEL and PIN had no effect on the concentration-response curves to ET except for a small (37%) depression in ET maximum by PIN in MCT-treated rats.

In conclusion, in contrast to the vasodilators acetylcholine and isoprenaline, which are reported to be less effective in MCT-induced pulmonary hypertension (Altiere *et al.*, 1986), FEL and PIN showed increased vasorelaxant activity, at least against contractions induced by NA or 5-HT. The potency of NA and 5-HT as spasmogens was also increased in MCT-induced pulmonary hypertension. If these two effects of MCT-treatment are related, it could indicate an increase in the involvement of voltage-dependent calcium channels in the spasmogenic effects of NA and 5-HT in MCT-induced pulmonary hypertension.

Altiere, R.J., Olson, J.W. & Gillespie, M.N. (1986) *J. Pharmac exp.Ther.*, 236, 390-395.
Wanstall, J.C. & O'Donnell, S.R. (1989) *Clin. exp. Pharmac. Physiol.* (in press)

EFFECTS OF K^+-CHANNEL OPENERS ON BOVINE TRACHEAL SMOOTH MUSCLE

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The novel class of compounds which initiate relaxation of smooth muscle by opening K^+-channels in the cell membrane have therapeutic potential as bronchodilators (Arch et al 1988). One means of studying the properties of these compounds is by measuring their effect on ^{86}Rb efflux (as a marker for changes in K^+-permeability). While such an approach has been successful for vascular smooth muscle, where large changes in the rate of Rb efflux occur, the effect of cromakalim (CRK) on Rb efflux from guinea-pig tracheal muscle is relatively small (Allen et al 1986, Foster 1989). We have examined the actions of CRK, pinacidil (PIN) and diazoxide (DZ) on Rb efflux and mechanical activity in bovine trachea - a richer source of muscle than guinea-pig trachea.

Small strips of tracheal muscle (approx 15mg wet weight) were prepared. For tissue bath studies these were suspended under an initial tension of 1g in Krebs at 37°C. The tissues were contracted with KCl (25 or 80mM) and, once tension had stabilised, compounds were added cumulatively at 10-fold concentration increments. For efflux studies, the muscle strips were attached to a gassing manifold and incubated for 60min in Krebs at 37°C before transfer to Krebs containing ^{86}Rb (1μC_i/ml) for a further 120min. Tissues were then transferred at 4 min intervals through a series of tubes containing Krebs, with compound under test added to three tubes in the middle of the run. The rate of Rb efflux was calculated as a fractional rate constant (KRb) for each sample.

CRK, PIN and DZ caused relaxation of bovine trachea contracted by 25mM KCl with IC_{50} values of 0.4μM, 1.5μM and 45μM respectively (n=5). In contrast, none of the compounds at concentrations up to 100μM (300μM for DZ) relaxed tissues which had been contracted by 80mM KCl. Following 45min incubation with the K^+-channel blocker glibenclamide (GBC, 1μM) the IC_{50}'s for CRK, PIN and DZ were 12μM, 18μM and 270μM respectively (n=5). CRK (1-30μM) and PIN (3-30μM) markedly increased the rate of Rb efflux. Thus, for example, CRK (10μM, n=8) and PIN (30μM, n=5) produced 2.8 and 2.5 fold increases in KRb respectively. Stimulation of Rb efflux by CRK and PIN was blocked by GBC (0.3-1μM). DZ (1mM), isoprenaline (1μM) and aminophylline (1mM) each had no effect on Rb efflux.

K^+-channel openers stimulate Rb efflux from bovine trachea to a much greater extent than from guinea-pig trachea, despite similar potency as muscle relaxants in the two tissues. Bovine tracheal smooth muscle therefore offers considerable advantages over guinea-pig tissue for studies of Rb efflux. DZ is a considerably weaker relaxant of this tissue than either CRK or PIN although the effects of all three compounds were inhibited by GBC. The failure of DZ to stimulate Rb efflux suggests that a part of its relaxant activity may be due to actions unconnected with the opening of Rb-permeable K^+ channels. We conclude that CRK and PIN relax bovine trachea by opening a Rb-permeable, glibenclamide-sensitive K^+-channel.

Allen SL, Boyle JP, Cortijo J, Foster GP, Small RC. (1986) Brit.J.Pharmac. 89 395-405
Arch JRS, Buckle DR, Bumstead J, Clarke GD, Taylor JF, Taylor SG. (1988) Brit.J.Pharmac. 95 763-770
Foster KA. (1989) Brit.J.Pharmac. 96 233P

COMPARISON OF THE EFFECTS OF GASTRIN/CHOLECYSTOKININ-RELATED PEPTIDES IN RAT GASTRIC MUCOSA AND GUINEA-PIG ILEUM

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Gastrin (G-17) and cholecystokinin (CCK-8) have diverse actions in the gastrointestinal tract including stimulation of gastric acid secretion and contraction of smooth muscle. Binding studies using gastrin/CCK related peptides have suggested that these peptides mediate their effects via three receptor types: CCK_A, CCK_B (Innis and Snyder, 1980) and gastrin receptors (Praissman et al., 1983). Comparable studies of gastrin/CCK receptors have not been made in functional studies: In the present study the rank order of agonist potencies of gastrin/CCK related peptides have been investigated in rat isolated gastric mucosa (RGM) and guinea-pig isolated ileum longitudinal muscle myenteric plexus (GPI-LMMP) to provide such a functional comparison.

RGM was prepared for measurement of acid secretion as described previously (Reeves and Stables, 1985). Strips of GPI-LMMP were suspended in organ baths (resting tension, 0.8g) and contractions recorded isometrically. All tissues were bathed in Krebs-Henseleit solution, containing phosphoramidon (1μM) and bestatin (100μM), gassed with 95% O_2:5% CO_2 and maintained at 37°C. Concentration-response curves were constructed for agonists (cumulatively for RGM and sequentially for GPI-LMMP) and could be repeated after washing.

Table 1 shows rank order of agonist potencies for the peptides in both preparations. In RGM, the gastrins (G-17, non-sulphated G-17) and pentagastrin (PTG) were potent full agonists. CCK-8 was a partial agonist, producing only 29% of the PTG maximum response at a concentration of 10nM. In addition, CCK-8 (100nM) inhibited subsequent responses to PTG (100nM) by 71±13% (n=4). The activities of the peptides in GPI-LMMP differed from those observed in RGM. In GPI-LMMP, CCK-8 was greater than 1000 times more potent than the other peptides (Table 1). Non-sulphated G-17 was virtually inactive and at a concentration of 1μM produced a small inhibition of CCK-8 responses (concentration ratio = 2.6, n=3).

Table 1 Rank orders of agonist potency of gastrin/CCK related peptides in RGM and GPI-LMMP

RGM	G-17	(=CCK-8)	nsG-17	PTG	nsCCK-8
	0.21	p.a.	0.61	1	11
GPI-LMMP	CCK-8	G-17	nsCCK-8	PTG	nsG-17
	1	1220	2000	78000	10000

Values are equipotent molar ratios (geometric mean, n=4 to 6) relative to pentagastrin in RGM (EC_{50}=26[15-45]nM) and CCK-8 in GPI-LMMP (EC_{50}=0.7 [0.3-1.4]nM). pa = partial agonist, ns = non-sulphated.

Comparison of agonist rank orders of potencies in these isolated preparations is consistent with the view that the effects of gastrin/CCK peptides in RGM to stimulate acid secretion are mediated by gastrin or CCK_B receptors whilst their contractile effects in GPI-LMMP are mediated by CCK_A receptors. These functional preparations may be useful systems with which to determine the activities of gastrin/CCK agonists and antagonists.

Innis, R.B. and Snyder, S.H. (1980), Proc. Natl. Acad. Sci., 77, 6917-6921.
Praissman, M. et al. (1984), J. Receptor Res., 3, 647-665.
Reeves, J.J. and Stables, R. (1985), Br. J. Pharmac., 86, 677-684.

INTERACTION OF PARAFLUOROHEXAHYDROSILADIFENIDOL (PFHHSID) AT MUSCARINIC RECEPTORS *IN VITRO*

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Muscarinic receptors exist in multiple subtypes, 5 of which have been identified from genomic cloning studies (m1, m2, m3, m4 and m5; see Bonner et al. 1988 for references) and 3 of which have been postulated from radioligand binding and functional studies (M_1, M_2 and M_3; Doods et al. 1987). Pirenzepine and methoctramine are M_1 and M_2 selective antagonists respectively (see Eglen et al. 1988 for references), whilst pFHHSiD has been postulated (Lambrecht et al. 1988) to be the first selective M_3 antagonist.

We have characterised pFHHSiD in guinea-pig ileum and left, paced atria (Eglen et al. 1988). Endothelial dependent relaxations were elicited in rings of rat aorta, rabbit jugular vein and dog femoral artery when precontracted with 0.1 μM phenylephrine (Eglen et al., 1988). Rings of dog femoral vein, in which contractile responses only are observed to muscarinic agonists, were also prepared (O'Rourke and Vanhoutte, 1987). Carbachol was used as the agonist and pA_2 values (Arunlakshana and Schild, 1959) were calculated using 60 min equilibration at each of the 4 antagonist concentrations employed.

pFHHSiD dextrally shifted the concentration-response curves in a parallel fashion in all preparations with no significant depression in the maxima. The mean (± s.e. mean) pA_2 values for pFHHSiD are shown in table 1.

Table 1 pA_2 VALUES AND SCHILD SLOPES IN VARIOUS IN VITRO PREPARATIONS

Preparation	Subtype	pA_2	Schild Slope	n
Femoral Vein	M_1	7.10 ± 0.09	1.1	10
Left Atria	M_2	6.14 ± 0.08	1.2	6
Ileum	M_3	7.86 ± 0.04	1.0	6
Aorta	M_3	7.68 ± 0.07	1.0	8
Jugular Vein	M_3	7.74 ± 0.11	1.0	8
Femoral Artery	M_3	7.90 ± 0.10	1.1	7

The pA_2 values were similar in ileal and vascular preparations indicating the presence of M_3 receptors. The pA_2 value in the left atria was in accordance with the presence of an M_2 receptor, whilst the intermediate value in the dog femoral vein was indicative of an M_1 receptor. pA_2 values in this latter tissue for pirenzepine, 7.85 ± 0.10 (1.0) and methoctramine, 6.1 ± 0.05 (1.2), were also indicative of M_1 stimulation. pFHHSiD may represent a useful probe in the functional and molecular biological classifications of muscarinic receptor subtypes.

Arunlakshana, O and Schild, H.O. (1959) Br. J. Pharmac. 14, 48-58.
Bonner, T.I. et al (1988) Neuron 1, 403-410.
Doods, H.N. et al (1987) J. Pharm. exp. Ther. 242, 257-262.
Eglen, R.M. et al (1988) Br. J. Pharmac. 95 1031-1038.
Lambrecht, G. et al (1988) Eur. J. Pharmac. 152, 193-194.
O'Rourke, S.T. and Vanhoutte, P.M. (1987) J. Pharm. exp. Ther. 241, 64-67.

ANTAGONISM BY METOCLOPRAMIDE AND BRL 43694 OF 5-HT-INDUCED RESPONSES IN RABBIT ISOLATED VAGUS NERVE

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There is uncertainty as to the mechanism of action of 5-HT_3 receptor antagonists on vagus nerve (see Ireland & Tyers, 1987; Richardson et al.,1985). One possible reason is that two different 5-HT-induced responses have been measured, i.e. 5-HT-induced depolarization and inhibition of C-spike amplitude, respectively. In these experiments we have compared the action of metoclopramide, reported to be a competitive antagonist at 5-HT_3 receptors (Ireland & Tyers, 1987), and BRL 43694, a potent 5-HT_3 receptor antagonist (Fake et al., 1987) on both of these 5-HT-induced responses recorded simultaneously. Extracellular recordings were made from rabbit vagus nerve using the method described by Marsh et al. (1987). The action of the antagonists (metoclopramide 0.3-10 μM, pre-incubation one hour; BRL 43694 0.3-10 nM, pre-incubation three hours) was assessed from cumulative concentration-response curves (CCRC) to 5-HT (0.1-300 μM), using the method of Schild (Arunlakshana & Schild, 1959). The results are shown in the table.

5-HT action.	Depolarization	Vagal C-spike inhibition
METOCLOPRAMIDE.		
Slope.	0.70 (0.9-0.69)	0.74 (0.93-0.54)
pA_2.	7.41 (7.57-7.25)	7.63 (7.97-7.28)
pA_2*.	7.04 ± 0.04	7.13 ± 0.06
	(20 dose-ratio determinations from 5 preparations).	
BRL 43694.		
Slope.	0.91 (1.24-0.58)	0.74 (1.08-0.39)
pA_2.	10.16 (10.58-9.74)	10.86 (11.49-10.24)
pA_2*.	10.03 ± 0.09	10.31 ± 0.1
	(20 dose-ratio determinations from 16 preparations).	

Slopes and apparent pA_2 values from Schild plots, mean values (95% confidence levels). pA_2* from Schild equation, means ± s.e.mean.

Although the antagonists appeared to be more potent on 5-HT-induced C-spike inhibition than on depolarization, differences were not significant. Metoclopramide did not seem to act competitively in that the slope of the Schild plot was significantly less than unity and there was a marked increase in the maximal response of the 5-HT CCRC. These features remain unexplained, but the shallow Schild plot was not due to an uptake mechanism, as citalopram (0.1-10 μM, n=4) had no effect on the 5-HT CCRC. BRL 43694, on both 5-HT-induced responses, caused parallel rightward shifts with no change in maximal response, except at 10 nM which reduced the maximal 5-HT-induced depolarization by about 40%. The slope of the Schild plots were not significantly different from unity. Thus, BRL 43694 at concentrations below 10 nM appears to behave competitively.

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Arunlakshana,O. & Schild,H.O. (1959) Br.J.Pharmac.14:48-58.
Fake,C.S. et al. (1987) Br.J.Pharmac.91:335P.
Ireland,S.J. & Tyers,M.B. (1987) Br.J.Pharmac.90:229-238.
Marsh,S.J. et al. (1987) Neuroscience 23:275-289.
Richardson,B.P. et al. (1985) Nature 316:126-131.

A RECEPTOR FOR 5-HT IN HUMAN ATRIUM

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Little is known about the nature of the myocardial receptors that mediate the positive inotropic effects of 5-hydroxytryptamine (5-HT). 5-HT and (+)lysergic acid diethylamide (LSD) increase force of mollusc hearts (Greenberg,1960), presumably through an increase in the concentration of cyclic AMP (Sawada et al., 1984). In cat heart, methysergide blocks the positive inotropic effects of 5-HT on atria (K_B=20 nM) but not on papillary muscles, indicating that atrial and ventricular 5-HT receptors are distinct (Kaumann,1985). Does 5-HT augment contractile force of human heart and if so, through what receptors ? To answer these questions, we carried out experiments on right atrial appendages obtained from patients with coronary artery disease undergoing bypass surgery.

Atrial strips were paced at 0.5 Hz in Krebs' solution (containing 0.2 mM ascorbate) as described (Gille et al.,1985). Usually, 4 strips were prepared from each atrial appendage. Two strips were used for force measurements, one as control and the other incubated with a blocking agent for a 5-HT receptor type. A cumulative concentration-effect curve for 5-HT was determined for each and the experiment terminated with 200 µM (-)isoprenaline (ISO). The other 2 strips were incubated with 0.4 µM (±)propranolol. One strip served as control. The other was exposed to a single concentration of 5-HT for 5 min. which caused an equilibrium inotropic response. All four strips were then freeze-clamped and extracts assayed for cyclic AMP (radioimmunoassay) and for cyclic AMP-dependent protein kinase (cA-PrK) activity ratio (Murray et al.1988).

5-HT increased contractile force in a concentration-dependent manner. The effects of 5-HT were not blocked by ketanserin (0.4 µM), methysergide (1 µM), LSD (0.4 µM), methiothepin (0.4 µM), yohimbine (4 µM), (±)propranolol (0.4 µM), (-)pindolol (0.4 µM) or MDL 72222 (2 µM), excluding an involvement of 5-HT receptors of type 1,2 or 3 (Bradley et al.,1986). However, ICS 205930 (2 µM) surmountably antagonised the positive inotropic effect of 5-HT, increasing the log (EC_{50}) for 5-HT from -6.94±0.09 to -5.82±0.16 (mean ± s.e.m., n=5 ß-blocker-treated patients) without altering the maximal response to 5-HT (57±5% and 51±6% of ISO response in the absence and presence of ICS 205930 respectively). 5-HT (1-30 µM) increased cyclic AMP from 11.0±1.5 to 19.7±2.7 pmol.(mg protein)$^{-1}$ ($p<0.05$) and the cA-PrK activity ratio from 0.27±0.02 to 0.35±0.03 ($p<0.01$). These are 21% and 40% of the corresponding effects of (-)isoprenaline.

Conclusions: The observed blocking potency of ICS 205930 (K_B = 0.2 µM) is lower than would be expected from its affinity for $5\text{-}HT_3$ receptors (Bradley et al.,1986) but similar to that observed by Dumuis et al.(1988) against 5-HT-induced stimulation of adenylate cyclase in brain (K_B = 0.5 µM). The 5-HT receptors of human atrium may therefore resemble the cerebral 5-HT receptors, designated $5\text{-}HT_4$ by Dumuis et al. Our results are consistent with the pathway:
5-HT → $5\text{-}HT_4$ → ↑ cyclic AMP → ↑cA-PrK activity → ↑ force.

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Bradley, P.B. et al. (1986). Neuropharmacology 25, 563-576.
Dumuis, A. et al. (1988). Mol. Pharmacol. 34, 880-887.
Gille, E. et al. (1985). Naunyn-Schmiedeberg's Arch. Pharmacol 331, 60-70.
Greenberg, M.J. (1960). Br. J. Pharmac. 15, 375-388.
Kaumann, A.J. (1985). J.Cardiovasc. Pharmacol. 7(Suppl. 7), S76-S78.
Murray, K.J. et al. (1988). Biochem. Soc. Trans. 16, 355.
Sawada, P.R. et al. (1984). J. Neurophysiol. 51, 361-374.

CONTRIBUTION OF AMINE RE-UPTAKE TO OBSERVED DIFFERENCES IN HISTAMINE AND 5-HT SECRETION IN RAT MAST CELLS

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We have shown (Cohen *et al*, 1987; Purcell *et al*, 1988a) that compound 48/80 induces a greater percentage release of histamine than 5-HT, from rat mast cells isolated from different anatomical locations, despite differences in the ratio of histamine:5-HT within these cells. This suggests differential secretory mechanisms for the two amines, although this does not accord with convincing evidence that they share similar granular storage sites (Uvnas, 1972). Mast cells can take up exogenous histamine and 5-HT (Purcell *et al*, 1988b), and we have addressed the question of whether post-secretory uptake of amines may explain observed differences in their evoked secretion.

Mixed peritoneal cells were obtained by lavage from female Wistar rats, and the mast cells purified (>90%) over bovine serum albumin (38%). Purified cells were incubated (37ºC, 15 min) with varying concentrations of clomipramine (10^{-8}-10^{-5} M, 5 min preincubation), or fluoxetine (10^{-6}-10^{-4} M, 5 min preincubation) in HEPES buffered Locke's solution (pH 7), and either compound 48/80 (1.0 μg/ml) in secretion experiments, a mixture of histamine (1.0 μg/ml) and 5-HT (0.2 μg/ml) in uptake experiments, or buffer alone. The supernatant was assayed fluorimetrically for the presence of histamine and 5-HT (Cohen *et al*, 1987). Results are corrected for the respective spontaneous amine release, with or without the drugs.

Similar gravimetric amounts of amine were removed from the supernatant, despite the fact that histamine was in a five-fold excess of 5-HT: thus 66 ± 14 ng of histamine (6 nmol) and 58 ± 7 ng of 5-HT (3 nmol) was subject to uptake by the cells (n=6). Clomipramine (10^{-7}-10^{-5} M) and fluoxetine (10^{-6}-10^{-4} M), significantly inhibited 5-HT uptake and were without effect upon histamine uptake. Compound 48/80 (1.0 μg/ml) induced a greater percentage release of histamine (63%, n=6) than 5-HT (33%, n=6). Both clomipramine and fluoxetine increased the observed percentage release of 5-HT, at concentrations which inhibited uptake of this amine: histamine secretion was unaffected by incubation with either drug. To determine whether this phenomenon was dependent upon the extent of amine release, purified mast cells were preincubated with clomipramine (10^{-5} M) or fluoxetine (10^{-4} M), concentrations which produced marked reduction in 5-HT uptake, prior to the addition of compound 48/80 (0.1-30.0 μg/ml). In the presence of these drugs the concentration effect curves of histamine and 5-HT were collinear, such that there was no difference between the percentage output of 5-HT and that of histamine.

These results suggest measured differences in amine release reflect selective 5-HT reuptake rather than differential amine output, and imply a single secretory mechanism for histamine and 5-HT.

Cohen, DL *et al* (1987) Br. J. Pharmacol. **90**, 101P
Purcell, WM *et al* (1988a) Br. J. Pharmacol. **95**, 673P
Purcell, WM *et al* (1988b) Br. J. Pharmacol. **94**, 370P
Uvnas, B (1972) Proc. Int. Congr. Pharm. **4**, 306-326

THE STIMULATORY EFFECT OF 5-HT ON HUMAN OSTEOBLASTS IS ANTAGONISED BY KETANSERIN TARTRATE AND IMIPRAMINE

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Osteoblasts are the principal cells involved in the formation of new bone, and play a crucial role in the reparative process following a bone fracture. Serotonin (5-HT) is contained in platelet granules and is released in large amounts by blood platelets at sites of injury. It has also been shown to increase cell proliferation in fibroblast and endothelial cells and may act as a growth factor (D'Amore & Shepro, 1977). We therefore carried out a study to assess the effect of 5-HT and anti-5-HT drugs on two markers of osteoblast function: alkaline phosphatase (ALP) production and [^{3}H]thymidine incorporation.

Isolation and culture of bone cells from trabecular fragments of human bone was carried out as described by Beresford *et al.* (1984), and standard cell culture techniques were used as previously described by us (Khokher & Dandona, 1988). The effect of drugs was assessed by incubating cells, $5x10^4$ per well, with and without test substances, for 24 h. ALP activity was measured in supernatants using an Encore™ Centrifugichem® and a kit from Baker Instruments Ltd (Egham, Surrey, UK). [^{3}H]thymidine incorporation was assessed by detaching the cells with trypsin centrifuging and liquid scintillation counting at the end of the incubation.

5-HT stimulated ALP production and [^{3}H]thymidine incorporation in human osteoblasts in a dose-dependent manner (ALP $EC_{50} \simeq 0.75$ μM). This effect could not be demonstrated with tryptophan, 5-OH-tryptophan or 5-hydroxyindole acetic acid at similar concentrations. The 5-HT-induced stimulatory effect ([5-HT] = 1 μM) on ALP production and [^{3}H]thymidine incorporation were blocked in a dose-dependent manner by ketanserin tartrate and imipramine with similar IC_{50}s (ALP $IC_{50} \simeq 1$ μM, [^{3}H]thymidine incorporation $IC_{50} \simeq 0.1$ μM). Both drugs were without effect on their own. Pargyline and the 5-HT antagonist BRL-43694 were also unable to block the 5-HT-induced stimulatory effects consistently and were without effect on their own.

These results suggest that 5-HT stimulates secretion and mitosis by osteoblasts and that these effects may be mediated by 5-HT receptors; and/or that the effect of 5-HT on osteoblast function involve captation of this amine. Further studies with more selective 5-HT antagonists and other 5-HT uptake inhibitors are in progress.

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D'Amore, F. & Shepro, D. (1977) J. Cell. Physiol. 92, 177-184
Beresford, J.N., Gallagher, J.A., Poser, J.W. & Russell, R.G.G. (1984) Metab. Bone Dis. Rel. Res. 5, 229-234.
Khokher, M.A. & Dandona, P. (1988) Br. J. Rheum. 27, 291-294.

HETEROGENEITY OF THE REGULATION OF PHOSPHOLIPASE C IN T LYMPHOCYTES

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Protein kinase C (PK-C) activation by phorbol esters potentiates, whilst PK-C inhibition by staurosporine inhibits UCHT1 (anti-CD3 antibody; O'Flynn et al, 1985) - induced elevation of cytosolic free calcium levels ($[Ca^{2+}]_i$) in human peripheral blood derived T lymphoblasts (Ward et al, 1988). However, in the human leukaemic T cell line Jurkat, activation of PK-C has been shown to inhibit CD3-induced elevation of $[Ca^{2+}]_i$ (Cantrell et al 1989). In the present study, we have examined the consequences of PK-C activation on CD3-mediated phosphatidylinositol (PI) metabolism in both human T lymphoblasts and the Jurkat cell line to see whether the differences between the cells in PK-C regulation of receptor-operated $[Ca^{2+}]_i$ levels are reflected in the regulation of phospholipase C-mediated PI metabolism.

Inositol labelling of the cells (10^8 cells/ml) and inositol (poly)phosphate(s) extraction and determination was essentially as described previously (Cockcroft et al, 1987). The cells were washed and resuspended in Hanks balanced salt solution (whole cells) or permeabilisation buffer (for composition see Stutchfield and Cockcroft, 1988) prior to use. Four minute pre-treatment with the PK-C activator phorbol dibutyrate (PDBU 100 nM) caused a marked inhibition ($p<0.05$) of UCHT1 (0.25-10µg/ml)-induced elevation of total [^{3}H]-inositol phosphates in Jurkat. The inhibition by 100nM PDBU of 4 and 10µg/ml UCHT1 responses was 40±11% and 64±8% inhibition (n-8) respectively. The effect of PDBU was dose-dependent (1-100nM) but could not be mimicked by the inactive phorbol ester 4 α phorbol (100nM,n=4). In marked contrast, PK-C stimulation had no inhibitory effect on UCHT1 (0.25-10µg/ml) induced elevation of total [^{3}H] inositol phosphates in human T lymphoblasts. In Streptolysin-O permeabilised Jurkat, but not T lymphoblasts, significant ($p<0.05$) total [^{3}H] inositol phosphate generation (5-7 fold enhancement above basal levels) was induced by physiological levels of Ca^{2+} (50-150nM). Calcium induced (50, 100, 150nM) phospholipase C activity in Jurkat was inhibited by 4 minute pre-treatment with 100nM PDBU (38±9, 39±2, 49±10% inhibition (n-4) respectively), whereas 4α phorbol (100nM) had no effect.

The different calcium sensitivity of PI metabolism and the different effect of PK-C stimulation on calcium and receptor-operated PI metabolism in Jurkat and T lymphoblasts indicates heterogeneity of phospholipase C regulation in T lymphocytes. Multiple isozymes of phospholipase C have been decribed (Pann-Ghill et al, 1988) and it remains to be determined whether the differential regulation of phospholipase C reflects different phospholipase C isozyme coupling to CD3 in Jurkat and T lymphoblasts.

Cantrell DA et al (1989) J. Immunol. (In Press)
Cockcroft S et al (1987) Immunology 60:209-212
O'Flynn K et al (1984) Biochem.J. 219:661-666
Pann-Ghill S et al (1988) Cell 54: 161-169
Stutchfield J & Cockcroft S (1988) Biochem.J. 250:375-382
Ward SG et al (1988) FEBS Letts. 239:363-366

DISSOCIATION OF 5-LIPOXYGENASE INHIBITION AND METHAEMOGLOBINAEMIA INDUCTION BY INDAZOLINONES

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We have previously reported selective 5-lipoxygenase inhibition by ICI207968 (2-(3-pyridylmethyl)-indazolinone) and related compounds (McMillan et al, 1989). In common with other lipoxygenase inhibitors, 2-substituted indazolinones have the potential to participate in redox reactions and this property may contribute to their mechanism of action. Other redox inhibitors have been reported to induce methaemoglobinaemia (MHb)(Fort et al,1984). We report here on induction of MHb with ICI207968 and on structural modifications which dissociate MHb from lipoxygenase inhibition.

Inhibition of 5-lipoxygenase in vitro and ex vivo were investigated in anti-coagulated blood stimulated with calcium ionophore A23187 (McMillan et al, 1986). MHb in whole blood was monitored spectrophotometrically and was expressed as a percentage of total haemoglobin.

Oral administration of ICI207968 to rats produced inhibition of ex vivo LTB_4 release by whole blood with ED_{50} values of 2.5, 10 and 25 mg/Kg p.o.respectively at 1, 3 and 5 hours after dosing. MHb was not detected following oral dosing in the rat even in a sub acute study with twice daily dosing at 100mg/Kg p.o.for 10 days. Oral administration of ICI207968 (5mg/Kg) to dogs produced significant inhibition of ex vivo LTB_4 release: mean inhibitory values of 95, 90 and 75% were obtained at 1, 3 and 6 hours respectively ($P<0.01$ in each case). At this dose, ICI207968 induced mean MHb of 25 and 20% at 1 and 3 hours respectively but MHb was not detected at 6 hours. At 1mg/Kg p.o., ICI207968 produced 80% inhibition of ex vivo LTB_4 release ($P<0.01$) 1 hour after dosing and this was not accompanied by induction of MHb. At this dose ex vivo LTB_4 release had returned to control values within 3 hours. In vitro studies demonstrated that ICI207968 had comparable inhibitory potency against LTB_4 release by dog, human and rat blood (IC_{50} values 0.5-1.5uM) but there were marked species differences in the sensitivity to MHb induction with EC_{25} values in dog, human and rat blood of 7.5, 80 and 250uM respectively. The 4-(N-n-pentyl carboxamido) analogue of ICI207968 had similar potency against LTB_4 release in dog blood (IC_{50}= 1.5uM) but did not induce significant MHb at concentrations up to 250uM. Disssociation of LTB_4 inhibition and MHb induction was also observed with the 4-(N-n-pentyl carboxamido) analogues of the 2-methyl and 2-unsubstituted indazolinones.

These data demonstrate that ICI207968 induces species-dependent MHb induction and, in view of the sensitivity of dog blood to MHb induction with ICI207968, in vivo studies in this species are not recommended. The demonstration of amidic analogues that retain LTB_4 inhibitory potency but do not induce MHb indicates that different structural features are critical for LTB_4 inhibition and MHb induction by 2-substituted indazolinones.

Fort, F.L., Pratt, M.C., Carter, G.W., Lewkowski, J.P., Heyman, I.A., Cusick, P.K. and Kestertson, J.W. (1984). Fund. & Appl. Toxicol. 4,216.
McMillan, R.M., Millest, A.J., Proudman, K.E. and Taylor, K.B. (1986). Br.J.Pharmacol. 87, 53P.
McMillan, R.M., Masters, D.J., Vickers, V.C., Proudman, K.E., Foster, S.J., Pilgrim, W.P. and Bruneau, P. (1989). Br. J. Pharmacol. 96, 36P.

EFFECT OF NABUMETONE AND ITS PRINCIPAL METABOLITE ON RAT AND HUMAN GASTRIC MUCOSAL PROSTANOID SYNTHESIS AND PLATELET FUNCTION

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Non-steroidal antiinflammatory drugs (NSAIDs) cause gastric erosion and ulcerogenesis through inhibition of the gastroprotective prostaglandins (PGs) I_2 and E_2. In order to obviate these deleterious side effects, "prodrugs" such as nabumetone have been developed. Although weak inhibitors of cyclooxygenase, prodrugs are converted by the liver to metabolites which are more potent inhibitors of cyclooxygenase. Nabumetone may therefore obviate the occurrence of gastric erosion while maintaining efficacy as an antiinflammatory drug systemically. To further investigate prodrugs and their metabolites, we compared the effects of nabumetone and 6-methoxy-2-naphthylacetic acid (6MNA; the principle metabolite of nabumetone) with naproxen and indomethacin on *in vitro* synthesis of PGI_2 and PGE_2 by rat and human gastric mucosa and rat gastric mucosa *ex vivo*. To study the effect of 6MNA on peripheral target tissues, the effect of this metabolite on human platelet aggregation and thromboxane A_2 (TXA_2) synthesis was also investigated.

Gastric mucosa (GM) was obtained from male Sprague Dawley rats and from patients undergoing partial gastrectomy. GM discs were then prepared using a 5 mm diameter punch, and incubated with NSAIDs in Kreb's Ringer bicarbonate buffer (KRB) for 1 h at 37 °C. Supernatants were collected for estimation of PGs by specific radioimmunoassay (Jeremy *et al.*, 1985). In the *ex vivo* rat studies, NSAIDs (indomethacin, naproxen and nabumetone) were administered by gavage and the animals killed by a blow to the hea d at 30 min or 2 h. GM discs were prepared, incubated, and PG output assessed as described above. Platelet aggregation was carried out using standard aggregometry methods (Mikhailidis *et al.*, 1983). Platelet TXA_2 synthesis by platelet pellets was assessed as previously described (Jeremy *et al.*, 1988).

In vitro PGI_2 and PGE_2 synthesis by rat and human GM was inhibited by indomethacin, naproxen and 6MNA (in this order of potency), whereas nabumetone was completely without effect. In the *ex vivo* studies, indomethacin and naproxen significantly inhibited GM PGI_2 and PGE_2 synthesis at 30 min post gavage at a dose of 1 $mg.kg^{-1}$, whereas nabumetone was without significant effect even at the highest dose of 80 $mg.kg^{-1}$ at 2 h post gavage. Platelet aggregation and TXA_2 synthesis were similarly inhibited by the NSAIDs (indomethacin > naproxen > 6MNA >>> nabumetone).

These results support the view that nabumetone does not inhibit gastroprotective prostanoids, whereas its active metabolite 6MNA is an effective inhibitor of prostanoid synthesis in peripheral target tissues.

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Jeremy, J.Y., Mikhailidis, D.P. & Dandona, P. (1985) Agents & Actions 17, 205-208.
Jeremy, J.Y., Mikhailidis, D.P., Thompson, C.S. & Dandona, P. (1988) Diab. Res. 8, 177-181
Mikhailidis, D.P., Jeremy, J.Y., Barradas. M.A., Green, N & Dandona, P. (1983) Br. Med. J. 287, 1495-1498

THE EFFECT OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON IL-1 SECRETION FROM MURINE MACROPHAGES

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Non-steroidal anti-inflammatory drugs (NSAIDs) eg. aspirin, indomethacin etc., are widely used to treat the symptoms of rheumatoid arthritis (RA) but may increase pathogenic mechanisms (Kunkel *et al.*, 1986; Dale & Penfield, 1987). The former found that NSAIDs increased interleukin-1 (IL-1) secretion from macrophages (MØ). They and others have suggested that prostaglandin E_2 (PGE_2) is a down-regulator of IL-1 secretion. However, Otterness *et al.* (1988) were unable to show any significant potentiation of IL-1 secretion by NSAIDs. The above authors used the thymocyte co-stimulator bioassay to quantitate IL-1. The assay is strongly inhibited by PGE_2 and also responds to other lymphokines such as IL-6, IL-2, IL-4 and TNF_α. Thus increases in proliferation of the thymocytes may not necessarily represent increased IL-1 secretion since cyclooxygenase inhibition will increase thymocyte proliferation to IL-1.

We have re-evaluated the effect of NSAIDs on IL-1 secretion using a sensitive and selective bioassay designed by Gearing *et al.* (1987). CTLL proliferation was measured as outlined by Tada *et al.* (1986). MØ monolayers were produced by adherence of 1×10^6 peritoneal exudate cells, from 8-12 week BKW mice, to a 24 well flat-bottomed tissue culture plate and rinsing. The monolayer was overlaid with 1ml of serum-free RPMI-1640 containing L-glutamine 2mM; gentamicin 50$\mu g ml^{-1}$ and lipopolysacchride 1$\mu g ml^{-1}$. Varying concentrations of NSAIDs were added and the cell-free supernatants were collected after 20 h incubation at 37°, 5% CO_2 95% air and 100% humidity. The PGE_2 concentration in the supernatants was quantitated using a radioimmunoassay. All values for IL-1 secretion are expressed as percentage of the secretion observed with LPS 1$\mu g ml^{-1}$ in the table below.

ASP (μM)	IL-1	PGE_2 $ng ml^{-1}$	IND (μM)	IL-1	PGE_2 $ng ml^{-1}$	PIR (μM)	IL-1	PGE_2 $ng ml^{-1}$	NaMec (μM)	IL-1	PGE_2 $ng ml^{-1}$
100	113±12	0.17	100	305±42*	0.018	100	170±10*	0.03	—	—	—
10	110±9	2.7	10	176±6*	0.012	10	130±22	0.04	10	280±33*	0.053
1	99±8	2.3	1	150±9*	0.012	1	116±30	0.033	1	217±13*	0.089

*Significant difference from LPS control $P<0.05$ (Students t-test).
IL-1 mean ± sem (n=3); PGE_2 mean (n=2).

The results show that indomethacin (IND) and sodium meclofenamate (NaMec) produce dose-dependent increases in IL-1 secretion which are only partially related to PGE_2 inhibition. Piroxicam (PIR) 10μM although producing similar levels of PGE_2 inhibition to NaMec 10μM is far less potent at increasing IL-1 secretion. Aspirin (ASP) had no significant effect on IL-1 secretion. Addition of PGE_2 10ng to the NSAID-treated monolayers inhibits potentiated IL-1 secretion induced by PIR 100 μM and diminishes but does not ablate increased IL-1 secretion by IND 100 μM and NaMec 10 μM.

Dale, M.M., Penfield, A. (1987) *Br. J. Pharmac.*, 92:63-68.
Gearing, A.J.H. *et al.* (1987) *J. Immunol. Methods* 99:7-11.
Kunkel, S.L. *et al.* (1986) *J. Immunol.* 136:186-192.
Otterness, I.G. *et al.* (1988) *Cell Immunol.* 114:385-397.
Tada, H. *et al.* (1986) *J. Immunol. Methods* 93:157-165.

THE POTENTIATION OF HUMAN NEUTROPHIL SUPEROXIDE GENERATION BY SODIUM MECLOFENAMATE

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The non-steroidal anti-inflammatory drugs (NSAIDs) are thought to mediate their anti-inflammatory effects by inhibition of cyclooxygenase, but a number of anomalous results suggest that there may be additional effects which could increase inflammatory mechanisms. We have shown that the NSAID, indomethacin, increases superoxide production from neutrophils activated with post-receptor stimuli (Dale & Penfield, 1985). Here we present another example, sodium meclofenamate, which increases both post-receptor and receptor-mediated superoxide generation.

Superoxide generation by human neutrophils was measured spectrophotometrically using the superoxide-dismutase inhibitable reduction of ferricytochrome *c* to ferro-cytochrome *c*. Neutrophils were prepared from human blood by Ficoll-Isopaque separation with subsequent hypotonic lysis to remove the red blood cells. Neutrophil viability was greater than 99% and purity greater than 97%. Cells were pre incubated with sodium meclofenamate for 20 minutes at 37°C before stimulation.

Sodium meclofenamate (10-100μM) produced a marked left shift of the dose-response curve to the post-receptor stimuli: diC_8, a diacylglycerol analogue, and fluoride, a G-protein activator. A similar pattern of results was seen with the receptor stimuli: platelet activating factor, opsonized zymosan, IgG and heat-aggregated IgG. Although sodium meclofenamate produces a significant potentiation of O_2^- generation with these stimuli, it has no effect on unstimulated cells.

It has been reported that another NSAID, benoxaprofen, which produces enhanced superoxide generation from neutrophils, mediates its effects by an action on protein kinase C (Lukey *et al.*, 1988). However, when tested with a sensitive Triton mixed micellar assay (Hannun et al., 1985), we could not show protein kinase C activation with either sodium meclofenamate or benoxaprofen. We propose that sodium meclofenamate and possibly benoxaprofen work by inhibiting the diacylglycerol (DAG) metabolising enzyme, DAG kinase, thereby increasing DAG levels and promoting protein kinase C activation. Similar results to sodium meclofenamate were found with the specific DAG kinase inhibitor, R59022, in that both potentiated all these stimuli (Muid *et al.*, 1987).

Toxic oxygen metabolites generated by active phagocytic cells have been implicated in tissue damage. Superoxide produced by neutrophils can modify IgG molecules causing them to form aggregates which, in turn, can act as stimuli for superoxide generation (Lunec *et al.*, 1985). In addition, these complexes could function as auto-antigens, thus triggering a self-perpetuating cycle of auto-immune reactions thought to underlie rheumatoid arthritis. Non-steroidal anti-inflammatory drugs which increase receptor-mediated O_2^- release could possibly exacerbate tissue damage.

Dale, M.M. & Penfield, A. (1985) FEBS Letts. 185, 213-217.
Hannun,Y.A., Loomis,C.R. & Bell,R.E. (1985) J. Biol. Chem. 260, 10039-10043.
Lukey, P.T., Anderson, R. & Dippenaar, U.H. (1988) Br. J. Pharmac. 93, 289-294.
Lunec, J., Blake, D.R., McCleary, S.J., Brailsford, S. & Bacon, P.A. (1985) J. Clin. Invest. 76, 2084-2090.
Muid, R.E., Penfield, A. & Dale, M.M. (1987) Biochem. Biophys. Res. Commun. 143, 630-637.

OPERATION OF A NA^+/H^+-EXCHANGE MECHANISM IS NOT A PREREQUISITE FOR HUMAN PLATELET ACTIVATION

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It has been known for some years that activation of human platelets results in an uptake of Na^+ and an increase in cytosolic pH. Recently, Siffert and Akkerman (1987) have suggested that these effects result from the operation of a Na^+/H^+ antiporter in the platelet, the activation of which is necessary to trigger an elevation of cytosolic Ca^{2+} ($[Ca^{2+}]_i$) and thereby induce cell activation. However, this hypothesis appears largely to rely upon studies performed with thrombin as the platelet stimulant. We have therefore extended these studies by examining the ability of a range of agents to elevate $[Ca^{2+}]_i$ and cause platelet aggregation in human Fura-2-loaded gel-filtered platelets (GFP) under conditions where a Na^+/H^+ exchange mechanism would be expected to be operative (external Na^+ concentration ($[Na^+]_o$) = 150mM) or inoperative ($[Na^+]_o$ <1mM).

Human GFP were loaded with Fura-2 as described elsewhere (Watts et al., 1987) and suspended in a physiological salt solution containing either 150mM Na^+ or one in which all Na^+ salts were replaced with either choline or N-methyl-D-glucamine (NMDG) salts. The elevation of platelet $[Ca^{2+}]_i$ induced by thrombin, adenosine diphosphate (ADP), platelet activating factor (PAF) and U-46619 was monitored in each of the three media (Watts et al., 1987). Platelet aggregation to the same agonists as well as to adrenaline was also monitored in parallel utilising an electronic platelet counting technique. In GFP in which Na^+ was replaced by choline or NMDG, thrombin-induced elevation of $[Ca^{2+}]_i$ was inhibited with an 8-fold rightward displacement of its concentration-effect curve. In contrast elevation of $[Ca^{2+}]_i$ by ADP, PAF and U-41669 was unaffected. Platelet aggregation to thrombin and adrenaline was also inhibited in both low Na^+ media (e.g. mean concentration-ratio values of 9 and 38 for the two agonists respectively in the choline-containing GFP) whereas that to ADP, PAF and U-46619 was unaffected. Further evidence for a possible involvement of a Na^+/H^+ exchange process being involved in thrombin-induced $[Ca^{2+}]_i$ mobilisation and platelet aggregation was sought using the claimed selective inhibitor of this process, ethylisopropylamiloride (EIPA)(Vigne et al., 1983). The effect of the compound upon elevations in $[Ca^{2+}]_i$ could not be assessed since, over the concentration range 20-100μM, it markedly quenched fluorescence in Fura-2-loaded GFP. However, EIPA (100μM) was without effect upon thrombin-induced platelet aggregation, a finding inconsistent with results utilising the low $[Na^+]_o$ media. In contrast, over the range 10-100μM, EIPA inhibited adrenaline-induced platelet aggregation. This latter effect may, however, be explained by the reported α_2-adrenoceptor blocking activity of EIPA (Howard et al., 1987).

Thus, under conditions of low $[Na^+]_o$, when a Na^+/H^+ exchange system should have been inoperative, elevation of $[Ca^{2+}]_i$ and platelet aggregation induced by thrombin was inhibited whereas these same effects induced by ADP, PAF and U-46619 were unaffected. The use of EIPA failed, however, to confirm the involvement of a Na^+/H^+ exchange mechanism in platelet activation induced by thrombin. Whilst not ruling out the operation of a Na^+/H^+ exchange system being involved in platelet activation to some agonists such as thrombin, the present results indicate that other agonists can induce activation independently of such a process.

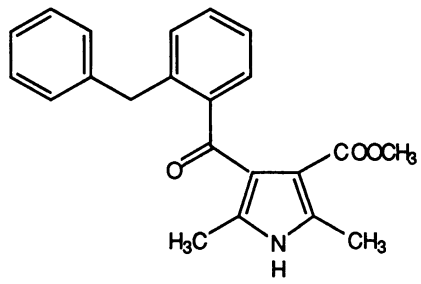
Howard, M.J. et al (1987), Am. J. Physiol., 253, F21-F25
Siffert, W. and Akkerman, J.W.N. (1987), Nature (Lond.), 325, 456-458
Vigne, P. et al. (1983), Biochem, Biophys. Res. Comm., 116, 86-90
Watts, I.S. et al (1987), Thromb. Haemostasis, 58, 466

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CALCIUM CHANNEL ACTIVATOR PROPERTIES IN A NOVEL NON-DIHYDROPYRIDINE - FPL 64176

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Bay k8644 is the prototype of a small group of structurally-related dihydropyridine calcium activators. These agents increase calcium influx through voltage-operated channels (VOC) by interacting with specific dihydropyridine binding sites on the channels (Schramm et al., 1983, Bellemann, 1984). Here we report some of the pharmacological properties of FPL 64176, a benzoyl pyrrole, which are consistent with it being the first member of a new class of calcium channel activator. In this study FPL 64176 has been compared directly with Bay k8644.



FPL 64176

In rat pituitary GH3 cells depolarised with 50mM K^+ both FPL 64176 and Bay k8644 were potent enhancers of ^{45}Ca uptake (pD_2, 7.79 ± 0.05 and 7.83 ± 0.14 respectively, n = 6), whereas uptake at physiological K^+ (5mM) was unaffected by FPL 64176 and Bay k8644 at concentrations up to 10^{-6}M. Neither FPL 64176 nor Bay k8644 contracted quiescent (5.4 mM K^+) guinea pig aortic rings up to 10^{-6}M, but both produced concentration related increases in tension in tissues pre-contracted with 30 mM K^+ (pD_2: FPL 64176 6.90 ± 0.02, n = 8; Bay k8644 - 7.8 ± 0.02M, n = 8).

In paced guinea pig atria (1 Hz, 1.5 x threshold v, 5 ms) FPL 64176 was a potent inotropic agent producing changes in developed tension equivalent to 50% of the isoprenaline maximum at 7.31 ± 0.06 (n = 7), compared with isoprenaline (7.74 ± 0.21, n = 7), ouabain (7.06 ± 0.15, n = 4), Bay k8644 (5.71 ± 0.25, n = 8) and milrinone (<3, n = 4). [^{3}H]-Isradipine (4.6 x 10^{-11}M) binding in cardiac sarcolemmal membranes was displaced in a concentration dependent manner by isradipine (IC_{50} 2.6 x 10^{-10}M, n = 3), and Bay k8644 (IC_{50} 1.1 x 10^{-8}M, n = 3). FPL 64176 produced only weak displacement at concentrations above 10^{-6}M (IC_{50} 3.9 x 10^{-6}M, n = 3).

We have demonstrated that FPL 64176 enhances calcium uptake and contraction in depolarised, but not quiescent tissues, and is a positive inotropic agent, properties which are compatible with activation of VOC. In these respects it was qualitatively similar to Bay k8644. However, the relative potencies of FPL 64176 and Bay k8644 varied between the different tissues studied and, unlike Bay k8644, FPL 64176 did not displace [^{3}H]-dihydropyridine binding at pharmacologically relevant concentrations. These observations suggest a novel mode of action on the channel for FPL 64176.

Schramm, M., Thomas, G., Towart, R. and Franckowiak, G. (1983) Nature, 303, 535-537.
Bellemann, P. (1984) FEBS Lett., 167, 88-92.

SK&F 96365, A NOVEL INHIBITOR OF RECEPTOR-MEDIATED CALCIUM ENTRY AND AGGREGATION IN QUIN2-LOADED HUMAN PLATELETS

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Ca^{2+} can enter the cytosol by discharge of intracellular stores and by various forms of receptor-mediated Ca^{2+} entry (e.g. Hallam & Rink, 1989). Compounds that inhibit Ca^{2+} mobilisation by either means might be expected to reduce Ca^{2+}-dependent cellular responses such as platelet aggregation. Such compounds might therefore have potential as antithrombotics that would be effective against all agonists that work through an elevation in $[Ca^{2+}]_i$. SK&F 96365 (1-{β-[3-(p-Methoxyphenyl)-propyloxy]-p-methoxyphenethyl} -1H-imidazole hydrochloride) selectively inhibits Ca^{2+} entry into platelets (as well as other cell-types) in response to various agonists. Aggregation of quin2-loaded platelets stimulated by the same agonists is also inhibited by similar concentrations of SK&F 96365.

Freshly isolated human platelets were loaded with quin2 to a cytosolic concentration of 1mM in order to buffer heavily changes in $[Ca^{2+}]_i$. Under these conditions ADP (20uM) or thrombin (1U/ml) stimulate a small rise in $[Ca^{2+}]_i$ (from c.100nM to c.200nM) in the absence of extracellular Ca^{2+} and a larger rise (from c.100nM to c.1000nM) in the presence of extracellular Ca^{2+} (Rink et al, 1982; Hallam & Rink, 1985a). The additional response in the presence of extracellular Ca^{2+} is attributed to Ca^{2+} entry. SK&F 96365 inhibited ADP-stimulated Ca^{2+} entry with an IC_{50} of 8.5uM and the response to thrombin was inhibited with an IC_{50} of 11.7uM. Release of Ca^{2+} from intracellular stores (i.e. the rise in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}) in response to both ADP and thrombin was only inhibited by very high concentrations of SK&F 96365 (IC_{50} = 120uM). A more direct measure of agonist-stimulated divalent cation influx across the plasma membrane involves the use of extracellular Mn^{2+}, which quenches cytosolic fura-2 upon entry into the cell. ADP, thrombin and U46619 stimulate Mn^{2+} entry into fura-2 (or quin2)-loaded platelets (Hallam & Rink, 1985b), and Mn^{2+} entry in response to each of these agonists was inhibited by SK&F 96365 with IC_{50} values of 27uM, 23uM and 18uM respectively.

The above results show that SK&F 96365 inhibits Ca^{2+} entry into platelets in response to various agonists. Platelet aggregation is known to be associated with elevations in $[Ca^{2+}]_i$ (Rink et al, 1982). When platelets are loaded with quin2, aggregation is largely dependent on Ca^{2+} entry (because rises due to internal release are heavily buffered). Under these conditions, SK&F 96365 inhibited aggregation stimulated by U46619 with an IC_{50} of 19uM.

Hallam, T.J. & Rink, T.J. (1985a) J. Physiol. 368, 131-146.
Hallam, T.J. & Rink, T.J. (1985b) FEBS Lett. 186, 175-179.
Hallam T.J. & Rink, T.J. (1989) Trends in Pharm. Sci. 10, 8-10.
Rink, T.J., Smith, S.W. & Tsien, R.Y. (1982) FEBS Lett. 148, 21-26.

[^{3}H] IMIPRAMINE AND [^{3}H] PAROXETINE BINDING TO FRESH AND FROZEN HUMAN PLATELET MEMBRANES

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[^{3}H]Imipramine ([^{3}H]IMIP) labels sites associated with 5-HT uptake in brain and platelets. Although numerous studies have investigated platelet [^{3}H]IMIP binding as a putative peripheral marker of depression, the findings are inconsistent (Mellerup & Plenge, 1988). These inconsistencies and the wide variations in binding parameters reported for control subjects may in part be related to methodological differences such as platelet preparation and storage. In this study we have compared [^{3}H]IMIP binding to human platelet membranes prepared from freshly isolated platelets and platelets from the same subject stored frozen prior to assay. In parallel we studied the binding of the selective 5-HT uptake inhibitor [^{3}H]paroxetine ([^{3}H]PAR), which is thought to label a different subunit to [^{3}H]IMIP within the 5-HT transport complex (Mellerup et al, 1985).

Blood samples (60 ml) were obtained from 20 (10 for each ligand) healthy drug-free volunteers. Samples were divided into two equal portions and platelets isolated. One portion was stored at -80^{o}C for 30 days, the other was immediately washed, platelet membranes prepared by hypotonic lysis/homogenisation and assayed. The frozen samples were subsequently processed in a manner identical to that used for fresh platelets. Aliquots of platelets were incubated with [^{3}H]IMIP (0.2-8.7 nM) or [^{3}H] PAR (0.01-1 nM) in 50 mM Tris-HCl pH 7.5 containing 120 mM NaCl and 5 mM KCl for 75 and 90 min respectively. Specific binding was defined with 100 μM desipramine ([^{3}H]IMIP) or 1 μM citalopram ([^{3}H]PAR). Bmax and Kd values were determined by computerised non-linear analysis of saturation plots.

Table 1 The binding of [^{3}H]IMIP and [^{3}H]PAR to fresh and frozen platelets

	[^{3}H]IMIP			[^{3}H]PAR		
	Bmax	Kd	Protein	Bmax	Kd	Protein
Fresh	920 ± 52	0.54 ± 0.02	104 ± 7	870 ± 59	0.035 ± 0.005	93 ± 13
Frozen	832 ± 63	0.52 ± 0.05	80 ± 5*	815 ± 29	0.021 ± 0.005	47 ± 8*

Values are means ± SEM, n = 10 for each group. Kd = nM, Bmax = fmol/mg protein, protein = μg protein/assay. * $p < 0.05$ by paired t-test.

Both ligands labelled similar numbers of sites. The mean number of [^{3}H]IMIP and [^{3}H]PAR binding sites did not differ significantly between fresh and frozen platelets (Table 1). The Kd of [^{3}H]PAR was an order of magnitude lower than that of [^{3}H]IMIP and was lower ($p = 0.055$) in frozen than fresh platelets. In contrast the Kd of [^{3}H]IMIP was not influenced by freezing. Freezing caused a significant reduction in total protein content.

The number of 5-HT uptake sites in blood platelets are not significantly altered by freezing/storage for 30 days and thus this is unlikely to be an important factor in explaining discrepancies in values reported for controls or depressed subjects.

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Mellerup, E.T. et al (1985) Eur. J. Pharmacol. 106, 411-413.
Mellerup, E.T. & Plenge, P. (1988) Acta. Psychiat. Scand. Suppl 345, 61-68.

APPARENT SEPARATION OF BENZODIAZEPINE TOLERANCE AND DEPENDENCE: FLUMAZENIL DOES NOT ABOLISH THE WITHDRAWAL EFFECT

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We recently demonstrated that the intravenous pentylenetetrazole (PTZ) test, with which benzodiazepine tolerance can be demonstrated, could also be used to study benzodiazepine dependence in mice (Feely et al., 1989). Tolerance was represented by a reduced anticonvulsant effect after repeated dosing: dependence was represented by a proconvulsant effect after withdrawal. In this report, we describe the results of a comparison between lorazepam, nitrazepam and clonazepam and the effects of the benzodiazepine antagonist flumazenil (Ro 15-1788) on the withdrawal response.

Convulsive threshold was measured using a protocol described previously (Gent et al., 1986). Adult male mice (Tuck No. 1, 25-35 g) were used throughout the study. To assess tolerance, 5 animals per group were used, but to measure withdrawal effects, we used groups of 10 in order to assess more accurately the significance of the inevitably small differences from control values. Preliminary experiments were carried out with all drugs to establish suitable doses and to study the time courses of action. All three benzodiazepines were given s.c. 30 min before testing with PTZ. When flumazenil was used, it was given i.p. 5 min before the test. Lorazepam and clonazepam were given in saline, nitrazepam and flumazenil in a propylene-glycol based vehicle. Pretreatment with the three benzodiazepines consisted of twice daily dosing i.p. for 3 days.

The results are expressed as the minimum convulsant dose (MCD) of PTZ in mg kg^{-1} (mean $\pm$ s.e. mean) which elicits a clonic seizure; significance was assessed with analysis of variance.

Drug (dose)	1st dose	7th dose	Controls	12h Withdrawn
Lorazepam ($0.2mg\ kg^{-1}$)	108.0 $\pm$ 2.5	93.3 $\pm$ 2.0	41.65 $\pm$ 1.1	35.1 $\pm$ 0.8
Nitrazepam ($0.6mg\ kg^{-1}$)	89.6 $\pm$ 2.1	80.3 $\pm$ 0.8	41.65 $\pm$ 1.1	37.1 $\pm$ 1.0
Clonazepam ($0.2mg\ kg^{-1}$)	88.5 $\pm$ 1.3	92.2 $\pm$ 1.7	40.59 $\pm$ 1.0	34.1 $\pm$ 1.1

Significant ($P<0.05$) tolerance occurred to the effects of lorazepam and nitrazepam but not to clonazepam. However, a significant proconvulsant effect after withdrawal ($P<0.01$) occurred in all cases. Since a marked fall in convulsive threshold occurred with lorazepam, we measured the MCD of PTZ in lorazepam withdrawn animals after injection of flumazenil (4 mg kg^{-1} i.p.; a dose which completely blocked the acute effects of lorazepam but which had no effect on its own). The MCD of PTZ in the withdrawn goup given flumazenil (37.2 $\pm$ 1.1) was not significantly different from that in the withdrawn group not given flumazenil; both were significantly lower than that in controls.

Thus lorazepam and nitrazepam both induced tolerance, and withdrawal of all 3 drugs led to a proconvulsant effect which may be taken as an indication of dependence. The lack of significant tolerance to clonazepam was unexpected, but not surprising since we have previously shown that tolerance develops only slowly to this drug (Gent et al., 1985). That a withdrawal reponse should occur after clonazepam was an interesting finding, since it suggests not only that tolerance and dependence can be separated, but that dependence may occur without obvious tolerance. Since flumazenil did not significantly alter the withdrawal response we measured after lorazepam, it seems unlikely that an endogenous ligand is involved in this case.

Feely, M., Peaker, S., Gent, J.P. & Boyland, P. (1989) Br. J. Pharmacol. (Proceedings, in press)
Gent, J.P., Bentley, M. Feely, M. & Haigh, J.R.M. (1986) Eur. J. Pharmacol. 128, 9-15
Gent, J.P., Feely, M. & Haigh, J.R.M. (1985) Life Sciences 37, 849-856

CHRONIC BENZODIAZEPINE AGONIST TREATMENT INCREASES THE ACTIONS OF THE INVERSE AGONIST FG7142 ON THE HIPPOCAMPAL SLICE

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The mechanisms of the adaptations that occur on chronic treatment with benzodiazepine agonists are not well understood. Receptor down-regulation occurs only after high doses of agonists. Chronic benzodiazepine agonist treatment increased effects of benzodiazepine inverse agonists, such as FG7142 (Little et al, 1987, 1988). Evidence suggested that this was an increase in the intrinsic properties of the inverse agonist rather than precipitated withdrawal. We have now studied the effects of the partial inverse agonist, FG7142, on field potentials in the hippocampal slice, *in vitro*, following chronic treatment of mice *in vivo* with the benzodiazepine agonist flurazepam. The treatment schedule was the same as that used in our earlier behavioural studies.

Male mice, CD1 strain (30-35g) were given seven daily i.p. injections of flurazepam, 40 mg/kg i.p. Controls received saline injections. Hippocampal slices were prepared 48h after the last chronic treatment injection, a time when the actions of FG7142 *in vivo* were greatly increased and our evidence showed that there was no benzodiazepine agonist in the CNS (Little et al, 1987). Extracellular recordings were made from stratum pyramidale, CA1 area, with orthodromic paired pulse stimulation (50 msec interval) of Schaffer collateral/commissural fibres, by an observer blind to the chronic treatment. Input/output curves were constructed in normal medium, then in successively increasing concentrations of FG7142 (10 nM to 25μM) perfused for 20 mins. In order that comparisons could be made between preparations from control and flurazepam-treated mice, the areas of the population (pop.) spikes for each preparation, and any secondary discharges, were calculated as percentages of the maximum population spike in normal medium (first reponse of the pair). The effects of FG7142 were then expressed as the differences between the responses in normal and in FG7142-containing medium. The effects of FG7142 were compared by nonparametric analysis of variance for slices prepared from control and flurazepam-treated mice, n = 6 per group (Meddis, 1984)

Effects of FG7142, 50 nM Results as mean ± s.e.m. for changes in % responses, **P<0.001

Stimulation as multiple of threshold:-		*1.0*	*1.5*	*2.0*	*3.0*
Control slices:	First pop.spike:	4 ± 9	6 ± 11	8 ± 19	-5 ± 20
	secondary discharges:	-8 ± 8	-1 ± 11	28 ± 15	61 ± 37
	Second pop.spike:	11 ± 20	-20 ± 13	-4 ± 13	24 ± 14
	secondary discharges:	-17 ± 11	34 ± 29	6 ± 28	-7 ± 19
Flurazepam:	First pop.spike:	14 ± 13	40 ± 27	13 ± 20	16 ± 26
treatment	secondary discharges:	0 ± 0	7± 5	37 ± 16	48 ± 15
	Second pop.spike:	6 ± 7	4 ± 20	7 ± 11	11 ± 17
	secondary discharges**:	11 ± 1	104 ± 56	73 ± 53	115 ± 72

Chronic flurazepam treatment increased the production of secondary discharges by FG7142, particularly following the second response of the slices to the paired pulse stimulation. This reflects the ability of cells to repolarise after stimulation. and may also indicate decreased inhibition. GABA-mediated inhibition would normally affect the second response to the paired stimulation. In our earlier behavioural studies this chronic flurazepam treatment schedule changed the actions of FG7142 *in vivo* from proconvulsant to convulsant. It is therefore possible that alterations in repolarisation and/or in inhibition may be involved in the increased action of FG7142 *in vivo* seen after chronic benzodiazepine agonist administration.

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Little,H.J.,Nutt,D.J.,Gale,R.,Sellars,N. & Taylor,S.C.1988 Neuropharmacology,27,383-389
Little, H. J., Nutt, D. J. & Taylor, S. C. 1987, Brain Res. Bull., 19, 371 - 378
Meddis, R. 1984, "Statistics Using Ranks" Basil Blackwell Ltd

THE CENTRAL ADMINISTRATION OF THE GABA-B RECEPTOR AGONIST, BACLOFEN, INDUCES FEEDING IN THE SATIATED PIG.

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GABA exerts its effects by acting at two pharmacologically distinct receptor subtypes, viz. GABA-A and GABA-B receptors (see Meldrum, 1987). In recent years, considerable evidence has accumulated to support the view that a central GABAergic mechanism is involved in the control of food intake and pharmacological studies have revealed that this action is mediated via central GABA-A receptors (Morley, 1987). However, very little attention has been paid to the GABA-B receptor subtype, and as far as we are aware, no attempt has been made to examine the effects of GABA-B agonists and antagonists on feeding. In the present study, we investigated the effects of the GABA-B agonist baclofen on food and water intake in satiated pigs.

Prepubertal large white pigs (10 male, 8 female; body weights: 30-60kg) were housed individually in metabolic cages. The animals were trained to press operant switch panels located in the cage with their snouts on a fixed ratio of 5 for food and water reinforcements. Each pig was implanted, under halothane anaesthesia, with a stainless steel guide tube aimed at the lateral cerebral ventricle for subsequent intracerebroventricular (ICV) injections of drugs (see Baldwin and Thornton, 1986 for details). In addition, 2 pigs were also prepared with intravenous catheters in the external jugular vein for the i.v. administration of drugs. Food and water were available ad lib., and consummatory behaviour was continously monitored on a data logger. ICV injection of baclofen (25, 50 and 100 nmol) or vehicle (physiological saline) were carried out between 1000h and 1130h each weekday. The criterion for drug administration was that the pig had not eaten for at least 30 min. prior to injection. Drug sessions were conducted twice a week, and control sessions bracketed each drug session. The effects of drug treatment on food and water intake for each pig was compared with the saline control values recorded on the preceding day using the paired t-test.

Baclofen (25-100 nmol) caused a dose-related increase in food intake in the satiated pigs. The 25 nmol dose had no significant effect on feeding compared with control data. In contrast, 50 nmol baclofen increased feeding ($P<0.01$) during the first 15 min. after icv injection, while the 100nmol dose increased feeding ($P<0.01$) during the first 30 min. Eating usually began 2-5 min after injection but there was no significant effect on water intake. Baclofen (50 and 100 nmol) caused the pigs to display signs of ataxia about 10-20 min after injection and they eventually went into a deep sleep for between 2-3h. When they awoke they displayed no signs of illness and usually began eating and drinking again. The effects of baclofen (50 nmol) on feeding could be completely abolished by pretreating the animals with the novel GABA-B receptor antagonist phaclofen (500 nmol icv). In addition, the observation that i.v. administration of baclofen (100 nmol) to 2 pigs had no effect on food intake, eliminated the possibility that baclofen may have a peripheral mode of action. The results of this study have indicated that baclofen can induce feeding in sated pigs by acting on central GABA-B receptors. It remains to be determined whether this action has a physiological role in the regulation of food intake.

Baldwin, B.A. and Thornton, S.N. (1986) Physiol. Behav. 36, 325-328
Morley, J.E. (1987) Endoc. Rev. 8, 256-287
Meldrum, B. (1987) J. Psychopharm. 1, 1-5

ACTIONS OF 5-HT$_{1A}$ LIGANDS AND STANDARD ANXIOLYTICS ON MOUSE EXPLORATORY BEHAVIOUR IN A TWO-COMPARTMENT LIGHT:DARK ARENA

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Although buspirone is a clinically effective anxiolytic (eg: Rickels et al., 1982) it, and other 5-HT$_{1A}$ ligands are not consistently active in a range of animal anxiety models (Gardner, 1988). Using a model of anxiety based on the exploratory behaviour of mice in a two-compartment light:dark arena (Costall et al., 1988) we have examined the activity of several 5-HT$_{1A}$ ligands, ranging from the agonists/partial agonists 8-OH-DPAT, buspirone, gepirone and ipsapirone, to the putative antagonists BMY 7378 (Yocca et al., 1987) and MDL 73005 (Hibert et al., 1988).

The test apparatus (based on the design of Costall et al., 1988) consisted of an open-topped box (81 x 36 x 27 cm high), one third of which was painted black, illuminated under dim red light and partitioned from the remainder of the box, which was painted white and brightly lit with a 60W spotlight located 40cm above the floor. An opening (7.5 x 7.5 cm) was located in the centre of the partition at floor level. The floor of the box was lined into a grid pattern of 9 cm squares. Female albino Tuck (T/O strain) mice (22-30g; n=8 per treatment), which were housed in the darkened experimental room for 18h prior to experimentation, were dosed s.c. with either saline or the test compounds (0.01-5.0 mg/kg) 30 min prior to being placed in the centre of the light compartment. The number of line crossings, rears, the time spent in each section of the box and the number of transitions between the two compartments were recorded in a 5 min test period.

Vehicle control mice were markedly less active in the light compartment relative to the dark, spending between 34-44% of the total time in the light. The ranges of control values (mean ± s.e.m. per 5 min) in these experiments were:- light (rears 8.8 ± 2.1 - 14.0 ± 1.8; line crossings 47.4 ± 7.7 - 70.6 ± 11.6), dark (rears 31.9 ± 2.4 - 44.6 ± 3.2; line crossings 103.8 ± 15.0 - 125.8 ± 4.5). The most consistent and reproducible effects of standard anxiolytics were to markedly and significantly ($p < 0.05$, ANOVA/Dunnett's test) increase rearing and motor activity in the light, at doses which did not modify dark compartment activity. Effective dose ranges (mg/kg s.c.) were:- chlordiazepoxide (0.5-5.0), diazepam (0.2-3.0 p.o.), meprobamate (50). In all experiments involving 5-HT$_{1A}$ ligands a chlordiazepoxide or diazepam treatment group was included as a positive control. With the exception of gepirone, 5-HT$_{1A}$ ligands also significantly ($p < 0.05$) increased light compartment rearing and motor activity. The doses which induced maximal effects (with percentage increases, relative to vehicle controls, in light compartment rearing/line crossings) were:- buspirone 1.0 (86/54); ipsapirone 1.5 (123/83); BMY 7378 1.0 (121/55); MDL 73005 2.0 (58/36); 8-OH-DPAT 0.1 (134/97). Higher doses of these compounds (0.3 mg/kg or greater for 8-OH-DPAT, 3.0-5.0 mg/kg for all other compounds) were sedative, in that frequencies of rearings and line crossings were decreased in both compartments (though more markedly in the dark).

Our data on buspirone in this model are in reasonable agreement with those obtained by Costall et al., 1988. The mechanism by which buspirone and other 5-HT$_{1A}$ ligands induce these effects may involve either a presynaptic agonist action (to attenuate 5-HT release) and/or a postsynaptic antagonist action. Both 5-HT$_{1A}$ agonist and antagonist properties of these ligands have been demonstrated in various functional models of the 5-HT$_{1A}$ receptor.

Costall, B. et al., (1988). J. Pharm. Pharmacol., 40, 494-500.
Gardner, C.R. (1988). Gen. Pharmacol., 19, 347-356.
Hibert, M. et al., (1988). Br. J. Pharmacol., 93, 2P.
Rickels, K. et al., (1982). J. Clin. Psychiat., 43, 81-86.
Yocca, F.D. et al., (1987). Eur. J. Pharmacol., 137, 293-294.

THE DIFFERENTIAL EFFECTS OF RU24969 UNDER HALOTHANE COMPARED TO CHLORAL HYDRATE ANAESTHESIA

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Previously it has been shown that RU24969 (5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl) IH indole succinate, 5-HT_1 agonist) at a dose of 10 mg/kg i.p. decreased both release and metabolism of 5-hydroxytryptamine (5HT) (Brazell et al., 1985) in chloral hydrate (600 mg/kg) anaesthetized rats. In a study of the effects of RU24969 on dorsal raphe neuronal firing we observed that doses of RU24969 administered without problems under chloral hydrate were lethal to animals anaesthetized with halothane (1.5-2%). This observation led us to compare the effects of RU24969 given intravenously (i.v.) and intraperitoneally (i.p.) under each anaesthetic.

Male Wistar rats in the weight range 350-400 g were anaesthetized with either halothane (1.5-2%) or chloral hydrate (600 mg/kg), venous cannulae were implanted in all sixteen rats together with a glass recording electrode (4-6 MΩ) implanted in the dorsal raphe nucleus for extraneuronal electrophysiological recordings. 5HT dorsal raphe neurones were characterized by their slow (0.5 - 2 spikes/sec) regular firing pattern. Rats under halothane and given RU24969 (40 ug/kg i.v.) died within the following 10 minutes. When RU24969 (20 ug/kg i.v.) was administered unit activity in the dorsal raphe nucleus was successfully recorded. Rats anaesthetized with chloral hydrate and given RU24969 at a dose of 0.5 mg/kg i.v. survived for several hours and were used for unit activity recordings with RU24969 again producing no change in 5HT neuronal firing, though a dose of 1 mg/kg i.v. proved fatal within 15 min. A further group of rats were anaesthetized with halothane (1.5-2%) and were given RU24969 (10 mg/kg i.p. or 5 mg/kg i.p.). Those with the larger dose died within 15 minutes of the injection whereas those given 5 mg/kg lasted several hours. In contrast 10 mg/kg i.p. could be safely administered to rats anaesthetized with chloral hydrate as shown in previous studies (Brazell et al., 1985; Martin & Marsden, 1986). RU24969 following i.p. administration failed to alter 5HT neuronal firing either under halothane or chloral hydrate anaesthesia.

Thus, it would seem that a twenty-five times greater dose of RU24969 can be intravenously administered to rats anaesthetized with chloral hydrate compared to those under halothane while double the dose of RU24969 can be administered intraperitoneally to rats anaesthetized with chloral hydrate compared to halothane. As both chloral hydrate and halothane suppress respiration, it would seem unlikely that the addition of RU24969 exacerbates this phenomenon in one but not the other. As yet the reason for the different effects of RU24969 under these two anaesthetics is unclear and warrants further investigation.

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Brazell, M.P., Marsden, C.A., Nisbet, A.P. and Routledge, C. (1985). Br. J. Pharmac. 86, 209-216.
Martin, K.F. and Marsden C.A. (1986). Eur. J. Pharmac. 121, 135-139.

RS-30199, AN AZEPINE WITH AFFINITY FOR THE 5-HT$_{1A}$ SITE

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Adrenoceptors show structural similarities to 5HT$_{1A}$ receptors and we have shown that agents with high affinity for 5HT$_{1A}$ receptors can distinguish between α_2-adrenoceptor subtypes (Brown et al., 1989). We now show that slight structural modifications of an α_2-adrenoceptor antagonist can confer affinity for 5HT$_{1A}$ sites. Radioligand binding assays were performed essentially as described by Hoyer et al. (1985) using the appropriate ligands and tissues as shown in Table 1. RS-30199-193 (5-chloro-2methyl-1,2,3,4,8,9, 10,10a-octahydronaphth-[1,8-cd]-azepine) is an analogue of SKF-86466 (DeMarinis et al., 1983); (pKi: α_2 7.0; 5HT$_{1A}$ 7.9) with an additional 6-membered ring. The analogue with a 5-membered ring (RS-10869-193; 5-chloro-2 methyl-1,3,4,8,9,9a-hexahydro 2H-indeno [1,7-cd] azepine), although potent had less selectivity (pKi α_2 8.2; 5HT$_{1A}$ 7.6). RS-30199-193 has high affinity for the 5HT$_{1A}$ site.

Table 1. Affinity values of RS-30199-193 (pKi mean±s.e.mean)

5HT$_{1A}$	[^{3}H]-8-OH-DPAT		rat cortex	7.91±0.08
5HT$_{1B}$	[^{125}I]-cyanopindolol		rat striatum	<5
5HT$_{1C}$	[^{3}H]-mesulergine	pig	choroid plexus	<5
5HT$_{1D}$	[^{3}H]-5HT		baboon cortex	<5
5HT$_2$	[^{3}H]-ketanserin	rat	frontal cortex	5.9±0.1
α_1	[^{3}H]-prazosin		rat cortex	6.2±0.1
α_2	[^{3}H]-yohimbine		rat cortex	7.0±0.1
β_1	[^{3}H]-dihydroalprenolol		guinea-pig heart	6.2±0.2
β_2	[^{3}H]-dihydroalprenolol		guinea-pig lung	5.7±0.2
D_2	[^{3}H]-spiperone		rat striatum	6.0±0.3
CEB	[^{3}H]-nitrendipine		rat striatum	<5

In guinea pig hippocampal slices RS-30199-193 (3-100nM) behaved as a partial agonist in that it increased submaximal forskolin-induced cyclic AMP changes, but with a lower maximal effect than that induced by 8-hydroxy-n-dipropylaminotetralin or 5HT. In an in vivo binding assay, RS-30199-193 (3 & 10mg/kg, s.c.) was as potent as buspirone in causing inhibition of [^{3}H]-8-OH-DPAT (administered i.v. by the tail vein 30 min post dose) binding in the frontal cortex of the rat. However, RS-30199-193 was 3-fold less potent than buspirone in causing antagonism of flat body posture, fore-paw treading, head-weaving and increased locomotion induced by 8-OH-DPAT (300μg/kg, s.c.) in the rat; the compound did not induce these behaviours per se at doses up to 30mg/kg s.c.. The weaker potency against the 8-OH-DPAT-induced behaviours may be a consequence of effects at a different subtype of receptor (see Small et al., Redfern et al., this meeting).

Brown, C.M. et al.,(1989) Br.J.Pharmacol. (in press)
DeMarinis, R.M. et al., (1983). J.Med.Chem., 26, 1213-1219.
Hoyer, D. et al., (1985). Eur.J.Pharmacol., 118, 13-23.

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ANXIOGENIC-LIKE PROPERTIES OF RS-30199 IN THE RAT

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RS-30199 (Brown *et al*, 1989) is an azepine derivative with a similar affinity to buspirone for the 5-HT_{1A} binding site in the rat brain, but which does not have the same agonistic effects in the guinea pig ileum preparation as buspirone, ipsapirone or 8-hydroxy-di-n- propyl-aminotetralin (8-OH-DPAT; Small & Spedding, 1989). The compound displaces [^{3}H]-8-OH-DPAT from cerebral cortex in *in vivo* binding assays following intravenous administration to the rat, implying good penetration of the central nervous system (Brown *et al.*, 1989). We now describe its effects on the activity of rats in tests designed to distinguish between anxiety-related and other central effects.

In male Sprague-Dawley rats, RS-30199 (10mg/kg s.c.; n=10) reduced spontaneous locomotor activity, measured over a 9 min period 40 min after injection, by 60% compared to the saline-treated control group (n=10; $p < 0.001$). This was not due to motor impairment as there was no significant effect on the performance of these rats in an accelerating rotarod test (saline group: 97 $\pm$ 16 s; RS-30199 group: 75 $\pm$ 13 s) . In order to study the effect on exploratory behaviour in more detail, rats which had been housed singly for 4-8 days were placed sequentially in an elevated X-maze and then in a holeboard containing a shaded corner (Redfern & Williams, 1989). There were 11-14 rats in each group. RS-30199 (0.3-30 mg/kg i.p.) produced a dose-related suppression of open arm (X-maze) and open field (holeboard) activity. In the X-maze (5 min), a dose of 30mg/kg i.p. produced a >50% reduction in the total number of arm entries ($p < 0.002$), in the proportion of open:enclosed arm entries ($p < 0.002$) and in the time spent on the open arms ($p < 0.02$). In the holeboard (12 min), the initial emergence latency from the shaded corner was elevated after a 3 mg/kg dose of RS-30199 ($p = 0.05$). At a dose of 30 mg/kg i.p there was a >50 % reduction in line crossings ($p < 0.05$). Forays, head dips, rears and time in the open area were similarly reduced, but not significantly. There were no signs of drowsiness or hypotonia in the RS-30199 treatment groups during handling.

Taken together, these data indicate that, in the rat, RS-30199 is anxiogenic rather than sedative, and has effects opposite to those of buspirone in the holeboard (Redfern & Williams, 1989). Further investigation of the actions of this drug in other behavioural tests is required in order to clarify this.

Brown, C. *et al* (1989) Proc. Brit. Pharmac. Soc. (this meeting).
Redfern, W.S. & Williams, A. (1989) Proc. Brit. Pharmac. Soc. (this meeting).
Small, C. & Spedding, M. (1989) Proc. Brit. Pharmac. Soc. (this meeting).

ACUTE EFFECTS OF CENTRALLY-ACTING DRUGS ON THE BEHAVIOUR OF RATS IN AN ELEVATED X-MAZE AND A PARTIALLY-SHADED HOLEBOARD

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Exploratory behaviour in a novel environment is disinhibited by non-sedative doses of anxiolytic drugs (File, 1985). One test of such behaviour is the elevated X-maze (Handley & Mithani, 1984). As it is unwise to rely on one test alone, we first tested rats in an X-maze and then placed them in a modified holeboard which combines an emergence latency test with a holeboard activity test.

Male Sprague-Dawley rats (140-230g) were housed singly for 4-8 days before testing. The rats were transferred to the testing room on the day before the experiments and were handled by the experimenter on the day before and 0.5h before the test session (09.00-12.30h). Rats (6-18 per group) were injected (i.p.) with either drug or vehicle, and 0.5h later each rat was placed on the elevated X-maze comprising 2 open arms (length 50 cm, width 10 cm) facing each other, and 2 enclosed arms (length 50 cm, width 10 cm, height 20 cm) with an open roof, and was elevated 50 cm from the floor. Observations were made over 5 min., then the rat was transferred to the holeboard (length 75 cm, width 46 cm, height 30 cm) containing a small roof (12 x 15 cm) fixed 10 cm above the floor in one corner. The floor was delineated into 8 sectors and contained 8 holes (diameter 3.5 cm). The rat was placed in the shaded corner and observations were made of the initial emergence latency, forays from the shaded corner, lines crossed, rears, head dips and time in the open area, over 12 min.

X-maze: Buspirone (1mg/kg) reduced the time on the open arms ($p < 0.02$; U-test) and a dose of 10 mg/kg reduced the total number of arm entries ($p < 0.002$). Lower doses (0.1 & 0.3 mg/kg) had no effect. Diphenhydramine (30 mg/kg) decreased the total number of arm entries ($p < 0.002$) without affecting the proportion of open:enclosed arm activity. Picrotoxin (5 mg/kg) reduced the total number of arm entries ($p < 0.002$), the ratio of open:total arm entries ($p < 0.02$) and the time on the open arms ($p < 0.002$). Chlordiazepoxide (2.5 mg/kg), picrotoxin (2 mg/kg) and amphetamine (1 mg/kg) had no significant effects in the X-maze.
Emergence latency: Buspirone (10 mg/kg) and picrotoxin (5 mg/kg) increased ($p < 0.02$) the emergence latency from the shaded corner; other drugs had no effect.
Holeboard: Buspirone (1 mg/kg) increased the number of forays ($p < 0.05$) and line crossings ($p < 0.05$). Amphetamine (1 mg/kg) increased all activities in the holeboard ($p < 0.05$). Picrotoxin (2 & 5 mg/kg) reduced head dips ($p < 0.05$) and line crossings ($p < 0.05$). Chlordiazepoxide (2.5 mg/kg; $p = 0.05$) and diphenhydramine (30 mg/kg; $p < 0.05$) reduced head dipping.

In conclusion, use of the modified holeboard in conjunction with the X-maze allows analysis of the effects of drugs on exploratory activity, and may help to distinguish between effects on anxiety and arousal, and other central effects which influence activity in an unfamiliar environment.

File, S.E. (1985) Neurosci. Biobehav. Rev. 9, 45-54.
Handley, S.L. & Mithani, S. (1984) N-S Arch. Pharmacol. 327, 1-5.

WHICH 5-HT RECEPTORS ARE INVOLVED IN THE INHIBITION OF OBJECT BURYING BEHAVIOUR BY 5-HT UPTAKE INHIBITORS?

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5-HT reuptake inhibitors selectively inhibit object burying behaviour at doses which do not affect immersion-induced grooming in mice, according to Broekkamp and Jenck (1987). We have therefore started to investigate which 5-HT receptor(s) might be involved.

Female MF1 mice (25-35 g) were placed individually in polypropylene cages (42 x 24 x 12 cm) containing 20 glass marbles evenly spaced on 5 cm deep sawdust and the number of marbles at least 2/3 buried counted 30 min later. Motor activity was assessed separately by 5 min exposure to a circular runway placed on an Animex activity recorder. All drugs were injected ip 30 min before test except 8-OHDPAT (10 min) and 5-MeODMT (15 min). Antagonists were injected 10 min before the test drug. ED50s, calculated as the dose in mg/kg, to reduce marble burying to 50% of that of the saline controls are given below with 95% confidence limits where log-dose/response curves were shown to be linear.

Zimeldine (7.14 [1.5-33.5]), citalopram (12.5), fluvoxamine (3.96), indalpine (4.89), fenfluramine (3.15 [.22-42]), DOI (.18 [.004-7.9]), mCPP (.58 [.13-2.6]) and TFMPP (.46 [.35-5.9]) all reduced marble burying at doses which did not affect locomotion. 5-HTP (7.2), 5-MeODMT (2.47), 8-OHDPAT (2.13) and gepirone (10.06) reduced both marble burying and locomotion at all effective doses. RU24969 (.60 [.21-1.5]) reduced marble burying while increasing motor activity. Buspirone was ineffective below 20 mg/kg, a dose which also reduced locomotion. Ipsapirone (1-20 mg/kg) was without effect on marble burying.

Ritanserin (10 mg/kg), and ICI 169369 (10 mg/kg) prevented DOI-induced inhibition of burying, but together with ICS 205-930 (10 mg/kg) or pindolol (5 mg/kg) these agents not only failed to abolish the response to zimeldine, but actually increased zimeldine inhibition of burying (eg marbles buried: saline 5.3 (s.e.m. 1.2), zimeldine 3 mg/kg 4.0 (1.4), ritanserin 5 mg/kg 8.7 (2.3), zimeldine 3 mg/kg + ritanserin 5 mg/kg 1.3 (0.4) ($p<0.01$). The drug combinations did not affect locomotion.

The potent activity of DOI, mCPP and TFMPP in selectively inhibiting burying suggests the possible involvement of 5-HT_{1C} receptors (Hoyer, 1988) in this effect, without eliminating the possibility that 5-HT_2 and 5-HT_{1B} receptors are also involved. Multiple receptor involvement may explain the potentiating effects of the selective antagonists on zimeldine inhibition of burying.

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Broekkamp C L & Jenck F (1987). Behavioural Pharmacology of Serotonin, International Congress, Amsterdam, S12.
Hoyer D (1988). Trends Pharmac. Sci., 9, 89-94.

THE EFFECT OF 5-HT ON RAT PIAL ARTERIOLES *IN VIVO*.

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In vitro studies have shown 5-hydroxytryptamine (5-HT) to be a potent constrictor of large cerebral arteries in a number of animal species including man (Edvinsson et al., 1983). However, there is evidence from studies using direct and indirect methods of assessment which indicate that 5-HT in vivo may cause only dilatation of smaller cerebral arteries and arterioles (Harper and MacKenzie, 1977, Saxena & Verdouw, 1985). We have therefore compared the effect in vivo of topical application of 5-HT with a number of 5-HT agonists on rat pial arterioles using a cortical cup technique.

Male Wistar rats (300-400g) were anaesthetised with thiopentone (120mg/kg i.p.). A tracheostomy was performed and the left femoral artery and vein cannulated for blood pressure measurement and drug injection respectively. The heart rate was measured from the E.C.G. A craniotomy was performed over the left parietal cortex and dura incised to expose the underlying cortex. Pial vessels on the surface of the cortex were irrigated with artificial CSF (pH = 7.3) and visualised using a microscope and closed-circuit television system. Individual pictures were sequentially digitised and computer enhanced on-line and the diameter of the selected vessel automatically measured at predetermined intervals by the appropriate software. 5-HT or one of the following agonists 5-carboxamidotryptamine (5-CT), (+)α-methyl-5-HT (α-Me-5-HT), GR43175 (prepared at W.R.L.) was then applied directly on to the vessel in incremental doses (1.25-20nmol) from five-barrelled micropipettes (overall tip size of 20 µm). One barrel always contained artificial CSF. This method of application produced no significant effect on either systemic blood pressure or heart rate.

		% change of initial diameter (mean + s.e.m.)				
	n	Control	2.5 nmol	5nmol	10nmol	20nmol
5-HT	5	-0.3±0.7	4.7±0.4**	7.5±0.7**	12.7±0.9**	17.6±0.7**
α-Me-5-HT	3	-0.6±1.4	4.0±0.8	6.2±0.6**	12.7±0.7**	15.3±2.1**
5-CT	4	-0.6±0.5	-0.7±1.3	-2.4±0.7	0.3±1.2	6.6±1.3 **
GR43175	3	-1.5±0.9	-8.1±2.6*	-11.2±2.3**	-14.4±3.5*	

*p<0.05, **p<0.01 Average initial arteriole diameter = 49.5± 1.2 µm.

Preliminary studies showed that on small extraparenchymal arterioles (diam.<20µm) 5-HT had little or no effect. Similarly, on the large vessels (diam.>90µm) 5-HT produced variable and inconsistent responses in that small dilatations and constrictions were noted in the same preparation. However, on vessels with a diameter between 20 - 90µm, 5-HT always produced dose-related dilatation. This effect was produced in a dose range higher than that usually associated with in vitro bioassay experiments and always followed a slight delay (12-20 sec). Similarly, α-Me-5-HT produced only dilatation of arterioles 20 - 90 µm in diam., being approximately equipotent with 5-HT. 5-CT also induced dilatation but only at doses 5-7 times higher than those of 5-HT. In contrast, the 'atypical' 5-HT$_1$-like agonist GR43175 (Humphrey et al, 1988) caused the constriction of those vessels which respond to 5-HT with a dilatation. The relative order of potency of the compounds studied suggests that the dilatatory effect of 5-HT is mediated through a receptor similar to that described by Ieff et al, (1987) and Martin et al, (1987) which induces endothelium dependent relaxation of rabbit jugular vein.

Edvinsson, L. et al. (1983) In Vascular Neuroeffector Mechanisms Eds. J.A.Bevan p251-258.
Harper, A.M. & MacKenzie, E.T. (1977) J. Physiol. (Lond) 271, 735-746.
Saxena, P.R. & Verdouw P.D. (1985) Br. J. Pharmac. 84, 533-544.
Humphrey, P.P.A. et al. (1988) Br. J. Pharmac. 94, 1123-1132.
Ieff P. et al (1987) Br. J. Pharmac. 91, 321-331.
Martin G.R. et al (1987) N.S. Arch. Pharmac. 336, 365-373.

ACTIVATION OF THE 5-HT$_2$ RECEPTOR SUBTYPE INCREASES RAT PLASMA ACTH CONCENTRATION

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1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI) is a potent hallucinogen which is reported to function as a selective agonist at the 5-HT$_2$ receptor (Glennon, 1986). Because of the implication of this receptor subtype in the mediation of the effects of psychotomimetic drugs, as well as its association with a number of psychiatric illnesses, the search for physiological markers of 5-HT$_2$ receptor subtype activation is of interest. McCall and Harris(1988) have examined the effects of DOI on the frequency of sympathetic nerve discharge (SND) in anaesthetized cats, and concluded that 5-HT$_2$ activation was responsible for measured increases in SND, since DOI shows high affinity at 5-HT$_2$ sites and the effects were reversed by the 5-HT$_2$ antagonists ketanserin and LY 53857. However, Hoyer and Karpf (1988) have recently shown that DOI has a high affinity for 5-HT$_{1C}$ receptors as it is a potent displacer of both [^{125}I]-SCH 23982 and [^{3}H]-mesulergine; compounds with high 5-HT$_{1C}$ affinity. Taken together, these data suggest the possibility of an important role for the 5-HT$_{1C}$ receptor subtype in mediating some effects of DOI which heretofore have been ascribed to the 5-HT$_2$ receptor subtype. We have thus investigated the plasma ACTH response to DOI in the rat, with the aim of distinguishing the role of 5-HT$_2$ from 5-HT$_{1C}$ receptors in the mediation of measured changes.

Male Sprague-Dawley rats (200$\pm$50g.) were sacrificed by cardiac puncture under halothane anesthesia 30 minutes after s.c. injection with DOI or vehicle (n=5-7 per dose). DOI significantly increased plasma ACTH in a dose-dependent manner (ED$_{80}$=0.8 mg/kg). The ED$_{80}$ dose was used in subsequent experiments which consisted of the s.c. administration of antagonist or vehicle at time zero, followed by s.c. administration of DOI at 30 minutes, and cardiac puncture at 60 minutes. Rat plasma ACTH was measured by two-site immunoradiometric assay (Euro-Diagnostics BV, Apeldoorn, Holland) (See Gilbert, et al., 1988).

The ACTH response to DOI was blocked by the 5-HT$_2$ receptor antagonists ICI 170,809, mesulergine, spiperone, and ketanserin, whilst the 5-HT$_{1A}$/5-HT$_{1B}$ receptor antagonist, (-)-pindolol, had no effect (Table 1).

Table 1: Effects of 5-HT Antagonists on the Plasma ACTH Response to DOI in the Rat.

Drug	*ED$_{50}$*	*Dose Range (mg/kg)*	*N (per dose)*
Spiperone	.009	0, .01, .05, .25, 1.25	7
Mesulergine	.014	0, 0.01, .05, 0.1, 1.00	4-9
Ketanserin	.064	0, .01, .05, .25, 1.25	7
ICI 170,809	.29	0, .01, .05, .25, 1.25	9
(-)-Pindolol	>5	0, 0.50, 1.00, 2.00, 5.00	9

The lack of demonstrable inhibition by (-)-pindolol, a drug with high affinity at 5-HT$_{1A}$/5-HT$_{1B}$ sites but not 5-HT$_{1C}$/5-HT$_2$ sites effectively minimizes the former receptor subtypes' importance, as well as beta-receptor involvement in the mediation of the DOI effect on ACTH. Additionally, spiperone has approximately 600-fold greater affinity for the 5-HT$_2$ than for the 5-HT$_{1C}$ site (Hoyer, 1988), and ketanserin, which shares nanomolar affinity at the 5-HT$_2$ site but not at dopaminergic sites, also inhibited the DOI-mediated ACTH increase. Thus it appears that 5-HT$_2$ receptor stimulation is more important than that of the 5-HT$_{1C}$ receptor in the production of the DOI-induced ACTH response.

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Gilbert, F., Dourish, C.T., Brazell, C., McClue, S., & Stahl, S.M.(1988) *Psychoneuroendocrinology*, 13, 471-478.
Glennon, R.A.(1986) *Life Sciences*, 39, 825-830.
Hoyer, D.(1988) *Journal of Receptor Research*, 8(1-4), 59-81.
Hoyer, D., & Karpf, A.(1988) *European Journal of Pharmacology*, 150, 181-184.
McCall, R.B., & Harris, L.T.(1988) *European Journal of Pharmacology*, 151, 113-116.

COMPARISON OF DOI-INDUCED SCRATCHING AND HEAD-TWITCH BEHAVIOURS: DIFFERENTIAL ACTION OF 8-OH-DPAT

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We have previously shown that DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane), a selective 5-HT_2/5-HT_{1C} agonist (Hoyer, 1988) produces scratching as well as head-twitching behaviour in mice (Heaton et al., 1988). Head-twitching is thought to be mediated by 5-HT_2 receptors and is powerfully modulated by a variety of other transmitters (Handley & Singh, 1986). We have therefore begun to investigate whether DOI-induced scratching resembles (head-) twitching in its pharmacology.

Male MF1 mice (20-30g) received DOI ip and were videotaped in groups of 3 over the next 10 minutes (data is for at least 6 animals/dose group). Twitch frequency was counted as previously (Handley & Singh, 1986), scratching was recorded as number of bouts in 10 min. Each bout lasted for 1-3 sec and consisted of continous rapid scratching of the flank or abdomen by the ipsilateral hindlimb. Other drugs were injected ip 30 min before DOI unless otherwise stated.

Dose-related scratching occurred between 1-10 mg/kg DOI compared with 0.1-1.0 mg/kg for head-twitching. 2.5 mg/kg DOI was therefore used to induce scratching and 0.5mg/kg for twitching. Ritanserin antagonised both twitching (ID_{50} 0.24 [.05-.86] mg/kg) and scratching (ID_{50} 0.03 [.0001-2.93] mg/kg).

Twitching and scratching were similarly affected by FLA63 40 mg/kg (-4h) (62% and 95% inhibition respectively) and clonidine 0.05 mg/kg (97% and 61% inhibition), although idazoxan (0.5 mg/kg) potentiated twitching (to 134%) but not scratching (90%). The chief difference was found in the response to the 5-HT_{1A} agonist 8-OHDPAT (0.1-1.0 mg/kg) which potentiated scratching but inhibited twitching (eg 8-OHDPAT 1.0 mg/kg increased scratching to 188% control and reduced twitching to 25% control).

In view of the low dose of ritanserin required to antagonise it, the scratching response to DOI is likely to be mediated by 5-HT_2 receptors. Scratching appears to resemble head-twitching in its modulation by noradrenergic mechanisms but the two behaviours contrasted sharply in their response to the 5-HT_{1A} agonist 8-OHDPAT. Inhibition of DOI head-twitching by 8-OHDPAT has previously been reported by Arnt & Hyttel (1989). Since the same dose of 8-OHDPAT had opposite effects on twitching and scratching, it is likely that simple behavioural interference by the '5-HT syndrome' can be eliminated as a cause of these interactions. This effect of 8-OHDPAT also suggests that 5-HT_2 receptor effects are not all modulated in the same way by 5-HT_{1A} receptor activation.

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Handley, S. L. & Singh, L. (1986) Trends Pharmac. Sci. 7, 324-8
Heaton, J. C. P. Njung'e, K. & Handley, S. L. (1988) Br. J. Pharmac. 94, 388P
Hoyer, D. (1988) Trends Pharmac Sci. 9, 89-94
Arnt, J. & Hyttel, J. (1989) Eur. J. Pharmac. 161, 45-51

CHRONIC ADMINISTRATION OF THE 5-HT$_2$ ANTAGONIST RITANSERIN PRODUCES ELEVATION OF 5-HT-MEDIATED PI HYDROLYSIS

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Chronic administration of 5HT$_2$ antagonists eg, ritanserin, causes "down-regulation" of 5HT$_2$ receptors in rat cerebral cortex (Leysen et al, 1986; Twist et al, 1988). This antagonist induced "down-regulation" is unusual and the mechanisms involved are unclear. We hypothesised that chronic administration of an antagonist would cause "up-regulation" of the receptor-linked 2nd messenger system and that the reported receptor "down-regulation" is a direct effect on the receptor rather than one mediated by the 2nd messenger system. As 5HT$_2$ receptors are linked to phosphoinositide hydrolysis (Conn and Sanders-Bush, 1984), this 2nd messenger system was examined by measuring inositol monophosphate (IP$_1$) accumulation in brain slices.

Wistar rats (350 g) received ritanserin (10 mg/kg/day) or vehicle (tartaric acid, 50 mM, pH 5) for 28 days (Twist et al, 1989). After a 3 day drug free period, animals were sacrificed and frontal cortices were cross-chopped into 300 x 300 µM prisms using a McIllwain chopper. Slices were preincubated with [^{3}H]myo-inositol (50 µCi) in oxygenated Tyrodes buffer for 1 hour before incubation in Tyrodes buffer containing LiCl (10 mM) +/- 5HT (1 µM, 100 µM or 1 mM) for 1 hour at 37°C. Reactions were stopped with trichloroacetic acid (15%). Samples were washed with diethylether (x 3) and [^{3}H]IP$_1$ was extracted from the aqueous phase by ion exchange chromatography (Berridge, 1983). Free inositol was eluted using 12 x 1 ml H_2O and [^{3}H]IP$_1$ was eluted using 12 x 1 ml ammonium formate (200 mM) in formic acid (100 mM). Samples were counted in a scintillation counter using Optiphase Hisafe III (12 ml). Data on IP$_1$ accumulation is shown below.

	5HT (1 µM)	5 HT (100 µM)	5HT (1 mM)
Control	118.44 ± 5.77(9)	124.21 ± 3.59(14)	114.80 ± 10.47(5)
Ritanserin	124.33 ± 8.22(9)	139.79 ± 5.30(14)*	175.80 ± 41.97(5)

Values are expressed as % change from corresponding basal levels (100%) ± SEM (paired t-test - *$p<0.05$)

There was no difference in the basal levels of [^{3}H]IP$_1$ accumulation in the controls compared to the ritanserin treated animals. In the presence of 5HT however, the accumulation of [^{3}H]IP$_1$ was greater in the ritanserin group compared to controls and at 100 µM 5HT, this difference was statistically significant ($p<0.05$).

The results support our hypothesis that the ritanserin mediated "down-regulation" of receptors is a direct effect but that the overall effect of chronic administration of a receptor antagonist is a supersensitivity in the system.

Leysen, J.E., Van Gompel, P., Gommeren, W., Wostenborghs, R. and Janssen, P.A.J. (1986) Psychopharmacol. 88: 434-444.
Twist, E.C., Hirst, A.D., Brazell, C., Stahl, S.M. and Campbell, I.C. (1989) Brit. J. Pharmacol. 96: 301p.
Berridge, M.J. (1983) Biochem. J. 212: 849-858.
Conn, P.J. and Sanders-Bush, E. (1984) Neuropharmacol. 23: 993-996.

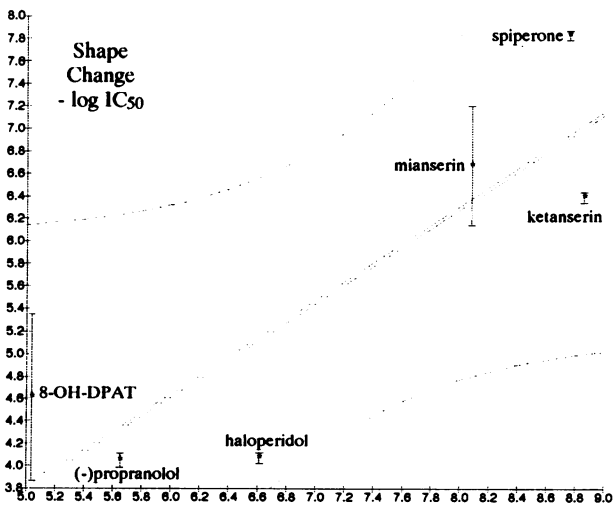
5-HT-INDUCED PLATELET SHAPE CHANGE IN THE RAT: POTENCY OF INHIBITION CORRELATES WITH 5-HT$_2$ BINDING AFFINITY

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The platelet has been proposed as a model for the serotonergic neuron because, like the neuron, there exists a mechanism for active transport of 5-HT, the subcellular vesicular storage of 5-HT, and the presence of a 5-HT transporter which is imipramine sensitive (Pletscher and Laubscher, 1980). Importantly, a receptor of the 5-HT$_2$ subtype is also demonstrable in neurons and platelets both in man and in animals including the cat and rabbit (see De Clerck and Herman, 1983 (review)). Stimulation of these receptors in vitro leads to specific, measureable physiological events like platelet shape change which may be followed by platelet aggregation (Graf and Pletscher, 1979; Peters and Grahame-Smith, 1980). Despite the abundance of existant data regarding the binding affinity of 5-HT antagonists to rat membrane preparations, the functional utility of 5-HT-induced rat platelet shape change has heretofore not been characterized.

Male Sprague-Dawley rats (250-300g.) were sacrificed by cardiac puncture under halothane anaesthesia yielding a volume of 12 ml whole blood. Blood was collected via a 16 g needle into a plastic syringe preloaded with 1 ml acid citrate dextrose anticoagulant, and then split into equal volumes in capped plastic tubes for centrifugation at 40 x g. for 15 minutes. The platelet-rich plasma supernatant (PRP) was collected into a plastic tube, covered and incubated at 37°C pending the completion of a second 15 min. centrifugation of the red cell fraction at 400 x g. which yielded platelet-poor plasma supernatant (PPP). A sample of PRP was counted using a Scquoia-Turner Cell-Dyn 900 semi-automated counter, and then diluted with autologous PPP to 200ul aliquots with platelet concentration of 350,000/ml in the micro-volume siliconized glass cuvettes of a PAP-4 platelet aggregometer (Bio-Data Corp.). The aggregometer was connected via the analogue output to an IBM PC, and voltages generated by the aggregometer light transducer sampled as previously described (Brazell, et al., 1988).

FIGURE 1: 5-HT-Induced Shape Change in Rat Platelets v. Binding Affinity at the 5-HT$_2$ Receptor



5-HT$_2$ antagonists were added in volumes of 2.0 ul, 90 seconds prior to addition of 10^{-5} M 5-HT, a previously determined EC$_{100}$ (2.0 ul). Measured platelet shape change was expressed as a percentage of control values (5-HT alone), and dose-response curves from at least 3 animals were pooled for the determination of drug IC$_{50}$ values.

The 5-HT antagonists tested appear in Figure 1. A significant correlation exists between the potency of inhibition of 5-HT-induced platelet shape change and these antagonists' binding affinity at the cortical 5-HT$_2$ ($r = .75$, $p < .03$), but not at 5-HT$_{1A}$, 5-HT$_{1B}$, or 5-HT$_{1C}$ binding sites (Hoyer, 1988).

Thus, it appears that 5-HT-induced platelet shape change is a useful model for the assessment of 5-HT$_2$ mediated events in the rat.

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Brazell, C., McClue, S., & Stahl, S.M.(1988) *Br. J. Pharmac.* 93, 189P.
De Clerck F.F., & Herman, A.G.(1983) *Fed. Proc.* 42, 228-232.
Graf, M., & Pletscher, A.(1979) *Br. J. Pharmac.* 65, 601-608.
Hoyer, D.(1988) *J. Receptor Res.*, 8(1-4), 59-81.
Peters, J.R. & Grahame-Smith, D.G.(1980) *Eur. J. Pharmac.* 68, 243-256.
Pletscher, A., & Laubscher, A.(1980) *J. Neural Transm., Suppl.* 16, 7-16.

α_2-ADRENOCEPTORS AND K-OPIATE RECEPTORS DECREASE ^{45}Ca ENTRY INTO RAT CORTICAL SYNAPTOSOMES BY CLOSURE OF N-TYPE CA^{2+} CHANNELS

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In adult rat brain cortical synaptosomes prepared by the method of Dunkley et al (1988), clonidine (0.1-100μM) and the selective K-opiate agonist U50488H(1-100μM) signigficantly reduced ^{45}Ca uptake in a time- and dose-dependent manner. In physiological medium, the maximum inhibition (50% and 55% at 100μM clonidine and U50488H) occurred after 2 min: IC_{50} values were 80nM and 90nM, respectively.

The L-type Ca^{2+} channel antagonist nifedipine(1μM) had no significant effect on ^{45}Ca influx in physiological medium (containing 5mM K^+), though there was an approximately 20% decrease in the presence of high concentrations of the drug(100μM). Nifedipine, however, caused a significant blockade of ^{45}Ca entry in medium containing 10-15mM K^+, demonstrating that L-type Ca^{2+} channels on synaptosomes are operational when the membrane is depolarised: under these conditions, there were additive inhibitory effects on ^{45}Ca entry into synaptosomes when clonidine(1μM) and nifedipine (1μM) or U50488H(1μM) and nifedipine(1μM) were co-incubated.

Treatment of synaptosomes in physiological medium with the N- and L-type Ca^{2+} channel blocker w-conotoxin fraction GVIA(w-CgTx,0.5μM) (Olivera 1984), resulted in a 35% reduction in ^{45}Ca uptake. w-CgTx(0.5μM) or w-CgTx(0.5μM) plus the α_2-adrenergic antagonist idazoxan(1μM) or plus the low affinity K-opiate antagonist naloxone(20μM) abolished the inhibitory effects of clonidine or U50488H on ^{45}Ca uptake, although idazoxan or naloxone alone did not alter the blockade of L-type Ca^{2+} channels caused by nifedipine.

The data supports our earlier studies (Adamson et al 1987; Xiang et al 1988) and demonstrates that under depolarising but not resting conditions, there are functional L-type Ca^{2+} channels on CNS nerve endings. Secondly, the results show that clonidine and U50488H decrease $[Ca^{2+}]_i$ by preventing Ca^{2+} entry into nerve terminals and suggest that these inhibitory effects occur by closure of N- but not L-type Ca^{2+} channels. The data provide a biochemical rationale for the effects of α_2-adrenergic agonists and K-opiate agonists on neurotransmitter release and possibly for their mode of action as sedative as well as hypotensive and antinociceptive drugs, respectively.

Adamson, P. et al. (1987) Eur.J.Pharmac. 142:261-266.
Dunkley, P. R. et al. (1988) Brain Res. 441:59-80.
Olivera, B. M. et al. (1984) Biochem. 23:5087-5092.
Xiang, J-Z. et al. (1988) Br.J.Pharmac. 95:597p.

PRESYNAPTIC MUSCARINIC CHOLINOCEPTORS INCREASE $[CA^{2+}]_i$ BY PROTEIN KINASE C-DEPENDENT OPENING OF L-TYPE CA^{2+} CHANNELS

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Stimulation of muscarinic acetylcholine receptors (mAChR) may increase intracellular free calcium ($[Ca^{2+}]_i$) in neuroblastoma cell lines (Lambert et al., 1989) and inhibit calcium currents in certain neurones (Wanke et al., 1987). Some subtypes of mAChRs are coupled to inositolphospholipid hydrolysis, while other inhibit adenylate cyclase (Bonner, 1989, for review).

As studies have demonstrated that phorbol esters (by mimicking the action of diacylglycerol on protein kinase C) increase $[Ca^{2+}]_i$ in synaptosomes via opening of an L-type calcium channel (Adamson et al., 1989) and that presynaptic muscarinic receptors hydrolyse phosphoinositides (Brammer et al., 1988), we examined the effect of carbachol mediated stimulation of presynaptic mAChR on $[Ca^{2+}]_i$.

Using fura-2 loaded synaptosomes from rat cerebral cortex (Adamson et al., 1989), we observed that carbachol (5 min incubation) produced a dose-dependent (1-100µM) increase in synaptosomal $[Ca^{2+}]_i$ with a maximum response of 22% and an EC_{50} of 58µM. The effect of carbachol (100µM) on $[Ca^{2+}]_i$ was antagonised by atropine with an IC_{50} of 200nM.

The rise in $[Ca^{2+}]_i$ produced by carbachol (100µM) was effectively blocked by the L-type calcium channel blocker nifedipine: the IC_{50} for nifedipine was 13nM.

Carbachol (100µM) induced increases in $[Ca^{2+}]_i$ was reduced if the synaptosomes were preincubated for 30 minutes with the protein kinase inhibitor H7 (Hidaka et al., 1984), (50µM): 4.0±1.4% increase (n=4) as opposed to 17.3+2.8% (n=4;solvent chloroform) in the control or with the more selective protein kinase C inhibitor staurosporine (Tamaoki et al., 1986), (1µM): 3.7+3.7% increase (n=3), control 20.0+1.5% (n=12; solvent DMSO). Values are ± SEM

These results support the conclusion that (in a manner analogous to the effect of phorbol esters) stimulation of muscarinic acetylcholine receptors in synaptosomes from rat cerebral cortex increases $[Ca^{2+}]_i$ by protein kinase C dependent activation of L-type calcium channels.

Adamson, P., Hajimohamadreza, I., Brammer, M. J., & Campbell, I. C. (1989) Eur.J.Pharmacol., 162, 59-66.
Bonner, T. I. (1989) Trends Neurosci. 12, 148-151.
Brammer, M. J., Hajimohammadreza, I., Sardiwal, S., Weaver, K. (1988) J.Neurochem. 51, 514-521.
Hidaka, H., Inagaki, M., Kawamoto, S., Sasaki, Y. (1984) Biochemistry, 23, 5036-4041.
Lambert, D. G. et al. (1989) Br.J.Pharmacol. 96, 132P.
Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. & Tomita, F. (1986) Biochem.Biophys.Res.Commun. 135, 397-402.
Wanke, E., Ferroni, A., Malagaroli, A., Ambrosini, A., Pozzan, T. & Meldolesi, J. (1987) Proc.Natl.Acad.Sci.USA 84, 4313-4317.

BILATERAL ABDOMINAL VAGOTOMY ABOLISHES BINDING OF [^{3}H]-BRL 43694 IN FERRET DORSOVAGAL COMPLEX

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5-HT_3 receptor antagonists are effective in treating vomiting due to radiation and cytotoxic drugs. The early descriptions of 5-HT_3 receptors were in peripheral tissues but recent attention has focussed on the existence of displaceable binding sites in the dorsal vagal complex and on the role of such "central receptors" in the vomiting response. The precise distribution of 5-HT_3 binding sites within the dorsal vagal complex is unclear. Pratt and Bowery have provided evidence that such binding sites may be located on terminals of vagal afferents in the medulla of the rat (a non-vomiting species). We have investigated the effects of bilateral abdominal vagotomy on the binding of ^{3}(H)-BRL 43694 in the dorsal vagal complex of the ferret, an animal with well characterized emetic behaviour.

Adult male ferrets with bilateral abdominal vagotomy, bilateral abdominal splanchnectomy and sham operated controls were anaesthetized 18 days after the initial surgery and perfused with saline. Brains were removed and frozen immediately. Cryostat sections were incubated in 10nM (^{3}H)-BRL 43694 (s.a. 21.2 Ci/mmol) in HEPES buffer at room temperature for 45 minutes. Adjacent sections were similarly incubated with the addition of 100μM GR 38032F. Sections were air dried and exposed to Amersham Hyperfilm for 6 weeks along with tritium standards to allow quantitative densitometric analysis.

High levels of specific binding were seen in the NTS of the 3 control animals (118.2,130.5 and 135.0 fmol/mg tissue) and in the 2 splanchnectomy-treated animals (128.4 and 121.6 fmol/mg tissue). Specific binding in the AP of controls (82.0,73.0 and 58.4 fmol/mg tissue) was similar to the splanchnectomy group (49.8 and 63.8 fmol/mg tissue). No specific binding was detectable in the NTS or AP in any of the 4 ferrets which had undergone bilateral subdiaphragmatic vagotomy.

Grant and Arvidsson have shown that lesions of peripheral branches of primary sensory neurones result in transganglionic degeneration of the central afferent projections. Since degeneration of medullary vagal afferent terminals most probably followed the vagal lesions described in this study, we hypothesise that the loss of 5-HT_3 binding sites observed was due to their location on these terminals.

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Pratt G.D. and Bowery N.G. (1989) Br. J. Pharmac.,97,414P.
Grant G. and Arvidsson J. (1975) Brain Res.,95,265-279.

GR38032F IMPROVES PERFORMANCE IN A MOUSE HABITUATION TEST, AND INHIBITS CHOLINERGIC-LINKED DEFICITS

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Studies investigating the role of 5-hydroxytryptamine (5-HT) in cognition using compounds with a non-selective action on 5-HT receptors have produced contradictory results. 5-HT receptor agonists and antagonists have either failed to influence or cause an enhancement or impairment of performance in animal tests (see Ogren et al 1981). In the present work we used the selective 5-HT_3 receptor antagonist GR38032F (Butler et al 1988) to investigate the role of 5-HT_3 receptors in moderating behaviour in a mouse habituation test.

Young adult (6 to 8 weeks old) and aged (8 to 10 months old) male albino BKW mice were used. Behavioural testing was carried out daily when mice were taken from a dark environment and placed into the centre of the white brightly illuminated area of a two compartment white and black test box. The latency of the initial movement from the aversive white area to the black section was determined from video recording. Mice received treatment with scopolamine (0.25 mg/kg i.p. b.d.), or electrolytic or ibotenic acid (IB) lesions of the nucleus basalis (NB) (the latter reduced cortical ChAT activity by 34-57%)

Table 1. The effect of GR38032F (10ng/kg i.p. b.d.) on the habituation response of young adult and aged mice and antagonism of the impairment induced by scopolamine and NB lesions

Treatment Groups	Latency to enter the dark(s)					
	day 1		day 3		day 6	
	Vehicle	GR38032F	Vehicle	GR38032F	Vehicle	GR38032F
Young adult mice	12.0±1.3	10.1±1.1	11.6±1.7	<1.0**	2.2±0.3	<1.0**
+ Scopolamine	13.1±1.6	10.2±0.8	16.6±2.1	2.4±0.4**	18.0±1.9	<1.0**
+ Electrolesion NB	12.5±1.4	9.0±0.8	10.1±1.3	3.8±0.4**	12.9±1.3	<1.0**
+ IB lesion NB	11.2±1.3	7.8±0.9	19.3±2.0	4.0±0.4**	17.1±1.8	<1.0**
Aged mice	9.9±1.2	4.5±0.6*	9.6±1.3	1.7±0.2**	9.4±1.0	1.1±0.2**

n = 10. <1.0s = latency less than time taken to operate remote video system. *P<0.05, **P<0.001 (decreased latency compared to vehicle, Dunnett's t test).

Mice normally habituate to the test situation over a 4 to 6 day period, and this is measured as decreased latency to enter the dark. Mice treated with GR38032F (10ng/kg i.p. b.d.) habituate more rapidly (see data for day 3). Scopolamine treatment or NB lesions impair habituation (see data for day 6). This impairment is inhibited by GR38032F, and the improved habituation can be seen on days 3 and 6 (Table 1). Aged mice similarly fail to habituate except when treated with GR38032F and improved habituation can be seen on days 1, 3 and 6 (Table 1). It is concluded that GR38032F can improve basal performance of young and aged mice in the habituation test and inhibit the impairments caused by a cholinergic deficit. This may correlate with the ability of the 5-HT_3 receptor antagonists to prevent the inhibitory action of 5-HT on acetylcholine release (Barnes et al 1989).

Barnes, J.M. et al (1989) Nature in press
Butler, A. et al (1988) Br. J. Pharmac. 94: 397-412
Ogren, S. et al (1981) Adv. Expl. Med. Biol. 133: 681-705
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SURFACE N-WAVE INHIBITION BY OXOTREMORINE-M IS MEDIATED BY PRE-SYNAPTIC M1 RECEPTOR STIMULATION IN GUINEA-PIG OLFACTORY CORTEX

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Radioligand binding studies have demonstrated the existence of more than one type of muscarinic binding site. According to these results, high affinity sites for pirenzepine were shown in the higher structures of the brain, superior cervical ganglia and in the enteric nervous system and were called M_1 receptors whereas low affinity sites were identified in atrial and ileal tissues, and named M_2 receptors (Hammer et al, 1980). These data were confirmed by autoradiographic studies (Wamsley et al, 1984; Spencer et al, 1986). Recently, using an extracellular recording technique, Williams & Constanti (1988) described the inhibitory effects of muscarinic agonists on the surface-negative field potential (N-wave) evoked by lateral olfactory tract (LOT) stimulation in guinea-pig olfactory cortex slices maintained in vitro. These inhibitory effects appeared to be mediated via presynaptyc M_1 muscarinic receptor stimulation since pirenzepine, but not gallamine, antagonized them in a potent and competitive fashion. Moreover, oxotremorine, was found to be a weak competitive antagonist on this system. Oxotremorine-M, a quaternary analogue of oxotremorine, has been reported to be a potent muscarinic agonist (Bebbington et al, 1966) and stimulant of brain PI hydrolysis, an effect also induced by carbachol (CCh) (Fisher et al, 1984). The aim of the present study was to test the effects of OXO-M on the evoked N-wave amplitude, recorded in guinea-pig olfactory cortex slices.

Bath superfusion (2 min) with OXO-M (5-80 uM; n=8 slices) produced a dose-dependent reduction of the N-wave amplitude, without affecting the LOT compound action potential. The peak effect was observed approx. 7 min after the drug application and complete recovery was usually obtained 45 min after. In comparison with CCh (5-300 uM; n=12 slices), OXO-M was 4.6 times more potent in reducing the N-wave amplitude (IC_{50}=87.9±2.4 and 19.2±1.5 uM, respectively) (means±s.e.m.). Telenzepine (5-100 nM), a new selective M_1 antagonist, competitively antagonized the effects of OXO-M (pA_2=7.91; n=9 slices) and of CCh (pA_2=8.19; n=12 slices), whereas methoctramine (300 nM) and AF-DX 116 (up to 500 nM), two cardio-selective M_2 antagonists, were ineffective in this respect.

In conclusion, our experiments suggest that OXO-M inhibits the surface-negative field potential by stimulating presynaptic M_1 muscarinic receptors in the olfactory cortex slice.

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Bebbington, A. et al (1966) Br. J. Pharmacol. 26, 56-67.
Fisher, S.K. et al (1984) J. Neurochem. 43, 1171-1179.
Hammer, R. et al (1980) Nature 283, 90-92.
Spencer, D.G. et al (1986) Brain Res. 380, 59-68.
Wamsley, J.K. et al (1984) Life Sci. 34, 1395-1402.
Williams, S.H. & Constanti, A. (1988) Br.J.Pharmacol. 93, 855-862.

ANTI-CHOLINERGIC AND ANTI-HISTAMINIC PROPERTIES OF CHLORPROMAZINE AND FIVE METABOLITES DETERMINED ON GUINEA-PIG ILEUM

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Chlorpromazine (CPZ) has numerous pharmacological properties, including antagonism at muscarinic and H-1 receptors (Courvoisier et al 1953). The compound is extensively metabolised and some of the metabolites are pharmacologically active. To see to what extent metabolites might contribute to the anti-cholinergic and anti-histaminic properties, and to investigate any structure activity relationships that might exist, pA_2 values were determined using guinea-pig ileum.

7-Hydroxychlorpromazine (7OHCPZ), monodesmethylCPZ (MDCPZ), didesmethylCPZ (DDCPZ), CPZ amine oxide (CPZNO) and CPZ sulphoxide (CPZSO) were provided by Dr A.A.Mainian, NIH, Bethesda, USA. Octanol partition coefficients (P) were obtained using standard methods (Leo et al 1963). Ionisation constants of CPZ, MDCPZ and DDCPZ were determined from their limiting solubilities and the pKa for CPZSO determined spectrophotometrically (Albert & Serjeant, 1962). pH-Partitioning into heptane was used to derive the pKa for 7OHCPZ (Whelpton, 1989).

Table 1.: Physicochemical properties and pA_2 values for CPZ and 5 metabolites.

Compound	Log P	pKa	pA_2 (s.d.)*	
			Acetylcholine	Histamine
CPZ	5.30	9.3	6.29 (0.08)	7.75 (0.05)
7OHCPZ	4.63	9.2	5.91 (0.08)	7.71 (0.14)
MDCPZ	4.94	10.2	5.79 (0.11)	6.61 (0.18)
DDCPZ	4.47	9.9	5.45 (0.08)	5.61 (0.22)
CPZNO	2.73	4.7	5.19 (0.04)	6.59 (0.09)
CPZSO	2.32	9.0	4.91 (0.10)	6.61 (0.07)

* n=5

None of the metabolites was more potent than the parent compound. The anti-cholinergic properties and octanol partition coefficients correlated ($r = 0.92$). Analysis of variance (Goodford, 1973) showed this to be highly significant, $F(1,4) = 22$, $p<0.01$. No such relationship existed for the anti-histaminic properties. However, demethylation had a marked effect, the potency fell by a factor of approximately 10 for each N-methyl group removed. This does not appear to be due to differences in pKa. The more polar CPZNO and CPZSO were also less potent than the parent compound.

Albert, A. & Serjeant, E.P. (1962) Ionisation Constants of Acids and Bases, Methuen & Co. Ltd., London.
Courvoisier, S., et al (1953) Arch. Int. Pharmacodyn. 92, 305-361.
Goodford, P.J. (1973) Adv. Pharmacol. Chemother. 11, 51-97.
Leo, A., Hansch, C. & Elkins, D. (1971) Chem. Rev. 71, 525-553.
Whelpton, R. (1989) Trends Pharmacol. Sci. 10, 182-183

REGIONALLY SELECTIVE EFFECTS OF CHRONIC NICOTINE ON RAT BRAIN TYROSINE HYDROXYLASE ACTIVITY: A TIME-COURSE STUDY

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The cholinergic agonist nicotine (NIC) stimulates the release of catecholamines from in vitro preparations of various areas of rat brain (e.g. Westfall et al. 1974). Given in vivo acutely, it stimulates dopamine release and synthesis in the nucleus accumbens (n.acc), and catecholamine synthesis in the hypothalamus and hippocampus (Mitchell et al. 1987, Brazell et al. 1989). Chronic pretreatment potentiates this effect selectively in the hippocampus (Mitchell et al. submitted). 28 days treatment also increases the activity of tyrosine hydroxylase (TH) selectively in this area, although acute nicotine is without additional effect on TH (Joseph et al. submitted). A time course study of the effects of chronic NIC on TH activity in this area, and various related catecholaminergic areas was therefore undertaken.

Separate groups (n=7) of male Sprague-Dawley rats (Haarlan-Olac), initial weight 250-300g, received daily injections of nicotine bitartrate (0.8 mg/kg s.c. as free base) neutralised in saline, or the vehicle alone for 3, 7, 14, 21 or 28 days. Sacrifice was 24h later; brain regions were dissected from 1.5-2.0 mm coronal slices on ice and immediately frozen pending analysis. TH activity was assayed with saturating concentrations of co-factor (6MPH4) and ascorbate as reductant (Lerner et al. 1978), and alumina separation of DOPA produced (Hendry and Iversen, 1971).

The activity in dopaminergically innervated areas (bed nucleus, caudate, n.acc) was unaffected by NIC at any time point studied. In noradrenergically innervated areas, TH was significantly increased ($p<0.02$ or better). The increase was maximal at 7 days (207% of control) in the cerebellum, at 21 days (147%) in the hypothalamus and reached 205% and 131% of control at 28 days in the hippocampus and frontal cortex respectively. It was also significantly increased (223% of control), but only at 7 days, in the locus coeruleus. This pattern is very similar to that reported after single dose reserpine (Zigmond, 1979) or repeated stress, and is consistent with increased enzyme synthesis in the cell bodies, followed by transport to the terminals. Although the site of action of this effect of NIC remains to be determined, it may be important in the behavioural adaptation that occurs to repeated NIC administration in rats (Stolerman et al, 1973), and indeed in man.

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Brazell, M.P. et al (1989) Br.J.Pharmac. 96, 339P
Hendry, I.A. and Iversen, L.L. (1971) Brain Res. 29, 159-162
Lerner, P. et al (1978) Neurochem.Res. 3, 641-651
Mitchell, S.N. et al (1987) Br.J.Pharmac. 91, 430P
Stolerman, I.P. et al (1973) Psychopharmac. 30,329-342
Westfall, T.C. (1974) Neuropharmac. 13, 693-700
Zigmond, R.E. (1979) J.Neurochem. 32, 23-29

IDENTIFICATION OF THE HYPOTHALAMIC RECEPTORS INVOLVED IN THE INCREASE IN BLOOD PRESSURE DURING STIMULATION OF THE C_1 AREA.

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Immunohistochemical techniques have demonstrated a population of neurones containing the adrenaline synthesizing enzyme in a restricted zone (C1 cell group) of the medulla oblongata (Hokfelt et al., 1974). Several studies have demonstrated that the adrenergic neurones in C1 are an important central vasomotor pathway (Ross et al., 1983; Goodchild et al., 1984). It is established that neurones in the C1 region project both to the brain (including the posterior hypothalamus (PH)) and to the intermediolateral region of the thoracolumbar spinal cord. It has been demonstrated that stimulation of C1 results in an increase in blood pressure (BP) which is attenuated by the central injection of a B_2 antagonist but enhanced by the central injection of an α_2 antagonist, suggesting that the increase in BP might involve B2 and α2 adrenoceptors (Ward-Routledge et al., 1988). However, these observations do not rule out the possibility that other neurotransmitters present in the C1 neurones or present in the PH could be affected by C1 stimulation and participate in the pressor effect. The present study further examines the hypothalamic neurotransmitters involved in the C1 induced increase in BP.

Anaesthetized, male Wistar rats were placed in a stereotaxic frame and an injection cannula was unilaterally implanted in the PH (rostral-caudal -3.8, lateral + 0.5, vertical -8.2 relative to bregma). A concentric needle electrode (SNE 100) was implanted into the C1 region (rostral-caudal -11.8, lateral +1.6, vertical -10.5 relative to bregma) ipsilateral to the cannula. The C1 region was electrically stimulated for 5 min (2 V, square wave pulses 1 ms, 40 Hz for 10 s every 60 s) following a 30 min stabilisation period. BP was monitored via a catheter previously implanted in the abdominal aorta through the caudal artery. 15 min after the first C1 stimulation, saline or drugs were injected i.v. or into the PH (1 μl over a period of 1 min) then 15 min later the C1 region again was stimulated. Differences between the increase in BP produced by C1 stimulation before and after saline or drug injection were examined using Student's paired t-test.

The increase in BP produced by C1 stimulation was significantly attenuated by pentolinium (1 mg/kg i.v.), propranolol (20 μg into the PH) and clonidine (8 μg into the PH). The C1 stimulation induced rise in BP was not affected by d-propranolol (20 μg), clonidine (3 μg) or idazoxan (66 μg); however the inhibition produced by clonidine (8 or 27 μg) was significantly reduced by prior administration of idazoxan (66 μg). These results confirm that stimulation of the C1 area provides excitatory drive to the preganglionic sympathetic neurones of the spinal cord and to the posterior hypothalamus with increased BP which is in part centrally modulated by α_2 and B adrenoceptors. The effect of C1 stimulation on BP was also significantly attenuated by the administration into the PH of atropine (8 μg) and methysergide (10 μg) suggesting a cholinergic and serotoninergic involvement in the pressor response. On the other hand the C1 response was not altered by cimetidine (11 μg), chlorphenydramine (12 μg) or naloxone (10 μg) excluding the participation of endogenous histamine and opioids in the pressor effect.

Goodchild, A.K. et al. (1984) Neurosci. Lett. 45, 267-272.
Hokfelt, T. et al. (1974) Brain Res. 66, 235-251.
Ross, C.A. et al. (1983) Brain Res. 273, 356-361.
Ward-Routledge, C. et al. (1988) Br. J. Pharmacol. 94, 609-619.

SUBTYPES OF α_1-ADRENOCEPTOR BINDING SITES IN HUMAN BRAIN: RADIOLIGAND BINDING AND RECEPTOR AUTORADIOGRAPHY

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Radioligand binding studies suggest that α_1-adrenoceptor recognition sites labelled by [^{3}H]prazosin are heterogeneous. Several adrenergic agents, e.g. WB-4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane), phentolamine and 5-methyl-urapidil discriminate between two α_1-adrenoceptor binding sites in various tissues of the rat (Morrow & Creese, 1986; Minneman et al., 1988; Gross et al., 1988). The sites with high and low affinity for these compounds were designated α_{1A} and α_{1B}, respectively (Morrow & Creese, 1986). Chloroethylclonidine was shown to irreversibly inactivate α_{1B}-binding sites (Minneman et al., 1988). Thus far, α_1-adrenoceptor subtypes have only been reported in experiments using rat tissues and it remained unknown whether different α_1-adrenoceptor recognition sites can also be distinguished in tissues of other species including man.

Therefore, we investigated radioligand binding to α_1-adrenoceptor recognition sites of rat and guinea pig tissues and studied α_1-adrenoceptors of human brain tissue obtained during surgery or post mortem by conventional radioligand binding techniques and receptor autoradiography. α_1-Adrenoceptors were labelled by 0.2 nM [^{3}H]prazosin, non-specific binding was defined by 10 µM phentolamine. 5-Methyl-urapidil which has a 80- to 90-fold higher affinity for α_{1A}- as compared to α_{1B}-adrenoceptor binding sites displaced [^{3}H]prazosin biphasically in rat and guinea pig cerebral cortex, hippocampus and vas deferens indicating two distinct binding sites (α_{1A} and α_{1B}) but monophasically in rat and guinea pig spleen (α_{1B}). In membranes of human cerebral neocortex, 5-methyl-urapidil also discriminated between two distinct α_1-recognition sites. In order to visualize α_{1A}- and α_{1B}-binding sites autoradiographically, [^{3}H]prazosin binding to human brain slices was determined after preincubation with chloroethylclonidine or in the presence of unlabelled 5-methyl-urapidil in a concentration which occupies about 90 % of α_{1A}-binding sites but hardly affects α_{1B}-sites. It is shown that in human hippocampus the distribution of chloroethylclonidine-resistant [^{3}H]prazosin binding sites (α_{1A}) differs from that of binding sites with low affinity for 5-methyl-urapidil (α_{1B}).

Our results demonstrate the existence of two distinct populations of [^{3}H]prazosin binding sites in brain tissue of different species including man which may represent separate α_1-adrenoceptor subtypes.

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Gross, G. et al.(1988) Eur. J. Pharmacol. 151: 333
Minneman, K.P. et al. (1988) Mol. Pharmacol. 33: 509
Morrow, A.L. & Creese, I. (1986) Mol. Pharmacol. 29: 321

CHRONIC GUANABENZ TREATMENT DOWN-REGULATES α_2-ADRENOCEPTORS IN RABBIT BRAIN

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Clonidine and guanabenz have been reported to act by stimulation of central alpha$_2$-adrenoceptors leading to a reduction in catecholamine turnover and reduced sympathetic outflow (Kobinger 1978). Prolonged activation of alpha$_2$ receptors may lead to desensitisation (Brodde et al 1982) and it has been proposed that the sympathetic hyperactivity following withdrawal of chronic clonidine treatment may be mediated by down-regulation of postsynaptic alpha$_2$-adrenoceptors in the CNS (Finberg & Kopin 1987). However decreases in alpha$_2$-adrenoceptor binding in the brain after chronic agonist treatment have been hard to demonstrate (Armstrong et al 1983), although changes in response have been observed by several groups (Finberg & Kopin 1987).

We have examined some effects of chronic guanabenz treatment on alpha$_2$-adrenoceptor number and function in the rabbit brain and periphery. Guanabenz (20 umoles/kg/day) or vehicle was adminstered for 6 days to groups of rabbits (n=6-8). Pressor responses to intravenous alphamethylnoradrenaline (alpha MeNA)(5-100 ug) and depressor responses to intracisternal clonidine (1 ug/kg) (Yakubu et al 1988) were used to study peripheral vascular and central alpha$_2$-adrenoceptor-mediated functions respectively. [^{3}H] yohimbine (1.25-37 nM) binding to tissue homogenates from rabbit forebrain, hindbrain and kidney were performed (Yakubu et al 1988) to study effects of treatment on alpha$_2$-adrenoceptor number.

Both pressor responses to alpha-MeNA and the depressor response to intracisternal clonidine were significantly attenuated by chronic guanabenz treatment. The dose of alpha-MeNA required to increase mean arterial pressure (MAP) by 30 mmHg was increased from 20±5 for controls to 65±10 ug for the treated group. The maximum fall in MAP after clonidine injection was observed at 5 mins for the treated group (-14±10 mmHg) and at 15 mins for controls (-26±15 mmHg). The falls in MAP were significantly attenuated at 15, 30, and 45 mins after injection (ANOVA). Chronic guanabenz infusion significantly reduced ($p<0.05$) [^{3}H] yohimbine binding to forebrain and hindbrain (Bmax forebrain 118±22 control and 53±18 guanabenz treated; hindbrain: 109±14 control and 60±29 fmoles/mg protein treated)(unpaired t-test). There was no change in the number of kidney binding sites nor was there any significant treatment effects on K_D in any tissues.

Thus, chronic guanabenz infusion significantly attenuated both peripheral and central alpha$_2$-adrenoceptor mediated functions. The different effects of guanabenz treatment on [^{3}H]yohimbine binding in brain and kidney could be related to the different subtypes of alpha$_2$-adrenoceptor predominating in brain and kidney or to differences in drug distribution. In addition the changes in receptor density observed in the brain could contribute to withdrawal reactions observed after abrupt cessation of treatment with centrally acting drugs.

Armstrong J.M., Atkinson J., Langer S.Z., Pimoule C. (1983). Br. J. Pharmacol. 78: 26p.
Brodde O.E, Anlauf M, Graben N, Bock K.D. (1982). Eur. J. Clin. Pharmacol. 23: 403-409.
Finberg J.P.M. & Kopin I.J. (1987). Eur. J. Pharmacol. 138: 95-100.
Kobinger W (1978) Rev. Physiol. Biochem. Pharmacol.81: 31-39.
Yakubu M.A., Hamilton C.A., Reid J.L. (1988). Brain Res. 463: 289-295.

α- AND β–ADRENOCEPTOR REGULATION OF CYCLIC AMP ACCUMULATION IN A MIXED PRIMARY ASTROGLIAL CELL CULTURE

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Two morphologically distinct cell types, which appear to represent different stages of astroglial differentiation, can be detected in primary astrocyte cultures from neonatal rat brain (Pollenz & McCarthy, 1986; Trimmer & McCarthy, 1986). The predominant cell type consists of flat polygonally shaped protoplasmic astroglial cells while the second cell type (fibrous) has short processes and grows on top of a layer of protoplasmic cells. Previous studies have indicated that rat astroglia express both α- and β-adrenoceptors which respectively inhibit and stimulate cyclic AMP accumulation (McCarthy & De Vellis, 1979; Evans et al., 1984). Here we report the effects of noradrenaline (NA) and isoprenaline (ISO) on cyclic AMP accumulation in primary cultures containing (a) only protoplasmic astroglia or (b) both protoplasmic and fibrous astroglia.

Primary cultures of neonatal brain astroglia were prepared essentially as described by (Rumsby et al., 1984) from either 1 day (for mixed protoplasmic/fibrous cultures) or 5 day (for protoplasmic cultures) old rat pups and maintained in culture for 14 days in DMEM medium supplemented with 2mM glutamine and 10 % foetal calf serum at 37°C. Cyclic AMP accumulation was measured in cells prelabelled with [^{3}H]adenine as described by Donaldson et al. (1988).

NA and ISO stimulated cyclic AMP accumulation in both protoplasmic (EC_{50}[NA] = 0.7 ± 0.2 μM, n=3; EC_{50}[ISO] = 0.5 ± 0.2 μM, n=3) and mixed fibrous/protoplasmic (EC_{50}[NA] = 0.10 ± 0.02 μM, n=4; EC_{50}[ISO] = 0.29 ± 0.07 μM, n=5) cell cultures. In the mixed fibrous/protoplasmic cultures a combination of ISO (1μM) and NA (1μM) produced a cyclic AMP response which was only 29.7 ± 4.0 % of that produced by ISO alone (n=4). This attenuation of the response to ISO was antagonised by the α_2-adrenoceptor antagonist yohimbine (1μM). Similarly, the response to 1μM NA alone in these cells was augmented by 1 μM yohimbine (from 22 ± 5 % to 70 ± 3 % of the response to 1μM ISO, $p < 0.05$, n=3) but not by prazosin (1μM). In contrast, no consistent attenuation of the response to ISO (1μM) was produced by either 1μM NA (104.2 ± 22.7 % of ISO response, n=6) or 10 μM NA (103.2 ± 17.5% of ISO response, n=4) in astroglial cultures containing only protoplasmic cells.

These data indicate that there are differences in the extent of α_2-receptor-mediated inhibition of cyclic AMP accumulation between the two primary astroglial preparations. It remains to be established, however, whether this is due to a preferential expression of α_2-receptors in the fibrous cells or to an interaction between the two different cell classes.

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Donaldson, J. et al., (1988) Biochem. Pharmacol. 37, 715-723.
McCarthy, K.D. & De Vellis, J. (1979) Life Sci. 24, 639-650.
Pollenz, R.S. & McCarthy, K.D. (1986) J. Neurochem. 47, 9-17.
Trimmer, P.A. & McCarthy, K.D. (1986) Dev. Brain Res. 27, 151-165.
Rumsby, M. et al. (1984) J. Neuroimmunol. 7, 1-20.

BRAIN β-ADRENOCEPTOR LOSS AFTER A PHENELZINE INJECTION DOES NOT INVOLVE RECEPTOR INTERNALISATION

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Studies of cells in culture have shown that exposure to β-adrenergic agonists can promote rapid receptor internalisation (Toews & Perkins, 1984). The existence of this phenomenon in brain, has however, not been established. We have investigated the rapid β-adrenoceptor down-regulation which results from acute administration of a single acute high dose of phenelzine, with the object of determining whether a rapid desensitisation/internalisation period precedes the down regulation.

Male Wistar rats (200-230g) were sacrificed 1,3,6 and 20 hours after a single injection of phenelzine (50mg/kg/ip). Specific binding to β-adrenoceptors was measured using [^{3}H]dihydroalprenolol (0.1-3nM) and non specific binding was assessed in the presence of propranolol (10μM). B_{max} and Kd values were assessed from six point saturation curves. Internalised receptors have been reported to appear in light density fractions following sucrose density centrifugation and this experimental procedure was used (Koshikawa et al., 1987). Cortical homogenates were applied to sucrose gradients (0.5-1.5M) and centrifuged to equilibrium (110,000g/16h/4°C). Two ml fractions were pumped out and analysed for specific [^{3}H]DHA binding (Burgisser & Lefkowitz, 1984).

A significant reduction (17%) (P 0.01) in β-adrenoceptor number was found in homogenates (P_2) after the acute phenelzine injection: the Kd was unchanged. In vitro studies showed that phenelzine (0.2nM-2μM) had no direct effect on [^{3}H]DHA binding.

The analysis of the density gradient data showed that there was no significant migration of specific binding sites for [^{3}H]DHA at any of the times examined ie 1,3, 6 and 20 hours after the phenelzine administration, although at 3,6 and 20 hours there was a significant decrease in specific binding in the plasma membrane fraction (ie around 0.9M sucrose).

From these studies, we conclude that down regulation is promoted by a process other than receptor internalisation.

Burgisser, E & Lefkowitz, R. J. in Brain Receptor Methodologies, Marangos, P. J. et al, Eds. Acad. Press, Florida, 1984, pp 230-252.
Koshikawa, N., Durcan, M. J., Weaver, K., Lawrence, K., & Campbell, I. C. (1987) Neuropharmacol., 26, 1346-1349.
Toews, M. & Perkins, J. P. (1984) J.Biol.Chem., 259, 11844-11850.

EFFECT OF DOPAMINE ON NEUROTENSIN-STIMULATED INOSITOL PHOSPHATE ACCUMULATION IN RAT STRIATAL SLICES

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Neurotensin (NT) receptor stimulation is coupled via phospholipase C to inositol 1,4,5-trisphosphate formation in a variety of rat brain regions, including the striatum (Goedert et al., 1984). We have previously reported (Ford et al., 1989) that NT fails to influence dopamine (DA)-stimulated adenylate cyclase activity in the same brain region; in this study, we have examined the converse situation, whether DA itself has any effect on the second messenger response to NT-receptor stimulation. Other workers have demonstrated that DA-receptor stimulation may inhibit inositol phosphate production in rat striatal slices (Pizzi et al., 1987), although this was not confirmed by Kelly and co-workers (1988). Elucidation of any such DA-NT interaction may facilitate our understanding of the mechanism by which NT inhibits cerebral DA function.

Inositol phosphate (IP) production was measured using the myo-[2-^{3}H] inositol prelabelling method of Brown et al. (1984), in cross-chopped slices (0.35mm) of striata from male Sprague Dawley rats (250-350g), in the presence of 5mM lithium chloride. Agonists (NT or carbachol) were present in the incubation for a 45 min stimulation period. Antagonists were present from the commencement of the incorporation period (40 min). Dopamine was added 10 min prior to NT or carbachol. All experiments with NT included bacitracin (28 mg/l) in the Krebs-Henseleit incubation buffer.

NT (1μM) increased basal IP accumulation to 142.7±8.4% (mean ± s.e.m., n=6; $p<0.01$) which was significantly lower ($p<0.05$) than that produced by NT in the presence of DA (100μM; 177±13.9%). The α_1-adrenoceptor antagonist prazosin (1μM), which was reported to decrease the IP response to DA in the presence of elevated KCl (Kelly et al., 1988) did not affect the response to DA plus NT. Inclusion of the D_2 receptor antagonist sulpiride (50μM) in the incubation medium was also without effect on the DA-NT synergy. To assess the specificity of this response, the effect of DA (100μM) on carbachol-induced IP accumulation was examined. It was observed that carbachol (100μM) produced an IP level of 490.5±20% of basal ($p<0.01$, n=4), and that DA (100μM) significantly reduced this to 420±24% ($p<0.05$). In the presence of sulpiride (50μM) this was further lowered to 391±14% ($p<0.01$).

We conclude that DA can potentiate NT-induced phosphoinositide hydrolysis in the rat striatum in vitro, although the mechanisms involved, apparently unrelated to α_1 or D_2 receptors, remain unclear. It would appear that this response is specific for NT-receptor activation, as DA inhibits the IP production in response to muscarinic receptor activation.

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Goedert, M. et al. (1984) Brain Research, 323: 193-197.
Ford, A.P.D.W. et al. (1989) Br.J.Pharmacol. Proceedings of Bristol Meeting.
Pizzi, M. et al. (1987) Eur.J.Pharmacol., 136: 263-264.
Kelly, E. et al. (1988) J.Neurochem., 51: 918-924.
Brown, E. et al. (1984) J.Neurochem., 42: 1379-1387.

EFFECT OF N-ETHYLMALEIMIDE ON DOPAMINE D_2 RECEPTOR REGULATION OF STRIATAL ADENYLATE CYCLASE ACTIVITY AND [^{3}H]-ACETYLCHOLINE RELEASE

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Many functions of dopamine D-2 receptors are thought to be mediated by their coupling, via G_i or other pertussis toxin-sensitive G proteins, to inhibition of adenylate cyclase or to other effector systems such as K^+ channels or intracellular Ca^{2+} (Vallar & Meldolesi, 1989). The striatum contains several functionally-differing populations of D-2 receptors, including presynaptic autoreceptors regulating dopamine release and heteroreceptors regulating acetylcholine release, as well as receptors regulating adenylate cyclase activity. The alkylating agent N-ethylmaleimide (NEM) is thought to inactivate the same G proteins as pertussis toxin and, unlike the latter, is effective for short term in vitro treatment. However, recent studies have shown that NEM treatment of striatal slices inactivates D-2 receptor regulation of adenylate cyclase but not autoreceptor regulation of dopamine release (Herdon, 1988 a,b), suggesting that the latter could be occurring via a different type of G protein. The present studies have continued these investigations by comparing the effects of NEM treatment on D-2 receptor regulation of adenylate cyclase activity and ^{3}H-acetylcholine release.

D-2 receptor regulation of D-1 receptor-stimulated adenylate cyclase activity in cross-chopped rat striatal slices was measured using a ^{3}H-adenine prelabelling method as described previously (Herdon, 1988b). D-2 receptor regulation of electrically-stimulated (3Hz, 2msec, 30mA) ^{3}H-acetylcholine release from superfused slices prelabelled with ^{3}H-choline (20μCi) was assessed using a minor modification of published methods (Herdon & Nahorski, 1987). Two 4 min periods of stimulation were applied 40 min apart (S_1 & S_2); spontaneous ^{3}H release was measured before each stimulation and stimulated ^{3}H release above this level was taken as representing ^{3}H-acetylcholine release. The choline uptake blocker hemicholinium-3 (10μM) was present throughout the superfusion period. Results are presented as means ± SEM of 4-8 experiments.

Preincubation of slices for 30 min with 30μM NEM greatly reduces the enhancement of dopamine (30μM)-stimulated adenylate cyclase activity by the D-2 antagonist sulpiride (30μM) (Herdon, 1988b); in the present studies, preincubation with 20μM NEM was equally effective (% increase with sulpiride: control 76 ± 6%; NEM 11 ± 3%, $p < 0.05$), whereas 10μM NEM was much less effective. Preincubation of ^{3}H-choline-prelabelled slices with 30μM NEM produced very large increases in spontaneous ^{3}H release and greatly decreased S_2/S_1 ratios for electrically-evoked release, making it difficult to assess D-2 receptor activity (data not shown). Preincubation with 20μM NEM also produced a 4-6 fold increase in spontaneous release as well as decreasing S_2/S_1 ratios to 61 ± 4% of control values. The D-2 agonist pergolide (1μM), added 20 min before S_2, reduced electrically-evoked ^{3}H-acetylcholine release from control slices by 62 ± 4%; the reduction by pergolide of release from NEM-treated slices was slightly though significantly less (43 ± 7%, $p < 0.05$).

These results suggest that treatment with even a fairly low concentration of NEM can largely inactivate the G_i proteins coupling D-2 receptors to adenylate cyclase. The same NEM treatment produced substantial non-specific effects on ^{3}H-acetylcholine release, but did not seem to prevent the regulation of release by D-2 receptors. One possibility, therefore, is that neither presynaptic D-2 autoreceptor regulation of dopamine release (Herdon, 1988 a,b) nor heteroreceptor regulation of acetylcholine release (present studies) involve an NEM-sensitive G protein. A more likely alternative, however, is that these receptor systems may possess a substantial "G protein reserve" so that most of their available G protein can be inactivated by NEM without blocking receptor function.

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Herdon, H. & Nahorski, S. (1987). N. S. Arch. Pharmacol. 335, 238-242.
Herdon, H. (1988a). Br. J. Pharmacol. 94, 351P.
Herdon, H. (1988b). Eur. J. Pharmacol. 154, 115-116.
Vallar, L. & Meldolesi, J. (1989). Trends Pharmacol. Sci. 10, 74-77.

AGONIST-INDUCED DESENSITIZATION OF THE D_1 DOPAMINE RECEPTOR EXPRESSED BY A HUMAN ASTROCYTOMA CELL LINE

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Little is known about the regulation of the D1-dopamine receptor. In contrast, considerable advances have been made using cell culture, in our understanding of the regulation of the β-adrenoceptor, which is similarly linked to adenylate cyclase. We recently reported that the human astrocytoma cell line D384 expressed D1-dopamine receptors (Balmforth et al., 1988). In the present study we have examined whether persistent occupation of the D-1 dopamine receptor desensitizes dopamine-stimulated cyclic AMP formation in intact D384 cells. In addition we have compared the effect of potentiating agonist stimulation of cyclic AMP, by simultaneous addition of a low concentration of forskolin, on the level of desensitization attained.

Intracellular cyclic AMP formation in D384 cells was assayed using a ^{3}H-adenine prelabelling method as described previously (Balmforth et al., 1988). The cells were preincubated (1h) with agents prior to the assay of intracellular cyclic AMP formation stimulated by a maximally-effective concentration of dopamine (1mM).

Preincubation of D384 cells with dopamine resulted in a time dependent attenuation of cyclic AMP responsiveness to subsequent dopamine stimulation. This effect was agonist specific since the PGE1 stimulation of cyclic AMP of similarly treated cells remained unchanged (table 1).

Table 1. D-1 dopamine receptor desensitization time course

Time of preincubation with 1mM DA (min)	0	2	5	15	30	60
DA % response	100	87 ± 3	82 ± 2	75 ± 3	52 ± 3	37 ± 2
PGE1 % response	100	95 ± 1	102 ± 2	102 ± 4	99 ± 2	102 ± 3

Each value is the mean ± SEM (n = 3)

The attenuation by dopamine was concentration dependent (EC50=1uM), with a maximal observed at 100uM. A comparison of dopamine concentration-response curves of control and dopamine preincubated cells revealed no change in the EC50, but a marked attenuation of the maximal response to 36 ± 4% of the control. Preincubation of cells with dopamine in the presence of D-1, but not D-2 selective antagonists, partially prevented the observed loss of responsiveness to subsequent dopamine stimulation. Attenuations in dopamine responsiveness were also obtained when D384 cells were preincubated with D-1, but not D-2 receptor agonists. The level of attenuation attained appeared to be related to agonist efficiency in stimulating cyclic AMP: SKF38393 < 3,4-dihydroxynomifensine < fenoldopam < 6,7-ADTN = dopamine. However, increasing the efficiency of 3,4-dihydroxynomifensine stimulation of cyclic AMP (from 13% to 160% of dopamine maximum), via the synergistic effect of adding a low concentration of forskolin, produced no further change in the attenuation of the subsequent response to dopamine.

These results suggest that the D1-dopamine receptors expressed by D384 cells undergo homologous desensitization. Uncoupling of the D-1 dopamine receptor appears to be independent of cyclic AMP formation, analogous to the mechanism proposed for the β-adrenoceptor (Lefkowitz et al., 1986).

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Balmforth, A.J. et al. (1988) J. Neurochem. 51, 1510-1515
Lefkowitz, R.J. et al. (1986) Trends Pharmacol. Sci. 7, 444-448

REGIONAL CHANGES IN [^{3}H]-SCH 23390 BINDING TO STRIATUM FOLLOWING L-DOPA TREATMENT OF 6-OHDA LESIONED RATS

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Alteration in striatal D-1 receptors may occur in Parkinson's disease or in response to L-DOPA therapy. In rats with a unilateral 6-OHDA lesion of the nigrostriatal pathway autoradiographic studies showed no change in ^{3}H-SCH 23390 binding to rat striatal slices (Savasta et al. 1987, 1988). However, the effect of L-DOPA treatment has not been studied using this technique. We now report the effect of a unilateral 6-OHDA lesion and chronic L-DOPA treatment on ^{3}H-SCH 23390 binding to rat striatal slices.

Male Wistar rats received a unilateral 6-OHDA (8 µg) lesion of the medial forebrain bundle. Other animals underwent a sham surgical procedure using injection of 0.9% saline. Those animals showing marked contralateral rotation to apomorphine (0.5 mg/kg s.c.) 3 weeks post surgery were selected for further study. Animals were dosed for 24 weeks with either L-DOPA (173-205 mg/kg/day) plus carbidopa (21.6-25.6 mg/kg) or carbidopa alone via the drinking water. After a drug washout period of 4 days animals were decapitated and brains frozen. Serial sections (20 µM) of rat striata (level +7.5 Paxinos & Watson, 1982) were incubated with ^{3}H-SCH 23390 (0.2 nM). Non-specific binding was determined by the inclusion of 10^{-7}M cis-flupenthixol.

There was no difference observed in specific ^{3}H-SCH 23390 binding between sham-operated and 6-OHDA lesioned animals. Similarly the 6-OHDA lesion did not alter ^{3}H-SCH 23390 binding in the ipsilateral striatum compared to the intact contralateral striatum. However, in animals treated with L-DOPA for 6 months there was a selective increase in ^{3}H-SCH 23390 binding in the lateral area of the ipsilateral striatum compared to same region in the contralateral striatum (Table 1). There was no difference in ^{3}H-SCH 23390 binding in the medial area between the ipsilateral and contralateral striatum.

Table 1 Effect of 6-OHDA lesion and L-DOPA treatment on ^{3}H-SCH 23390 binding to lateral striatum

	Ipsilateral striatum (fmol/mg wet weight tissue)	Contralateral striatum (fmol/mg wet weight tissue)
6-OHDA + carbidopa (n = 5)	116.4 ± 10.0	102.1 ± 12.6
6-OHDA + L-DOPA/carbidopa (n = 5)	147.7 ± 12.8*	103.6 ± 9.4

Results were analysed using a Student's t test. * $p < 0.05$ vs contralateral striatum.

Denervation of the striatum alone does not alter the regional binding of ^{3}H-SCH 23390. However, chronic L-DOPA treatment can cause a selective regional increase in ^{3}H-SCH 23390 binding. This change may contribute to the long term complications of L-DOPA therapy in Parkinson's disease.

Savasta, M., Dubois, A., Feuerstein, C., Benavides, J., Scatton, G. (1987) Biogenic Amines 4, 419-429.
Savasta, M., Dubois, A., Benavides, J., Scatton, B. (1988) Neurosci. Lett. 85, 119-124.
Paxinos, G., Watson, C. (1982) The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

NB-355: A PRODRUG FOR L-DOPA WITH REDUCED RISK FOR DYSKINESIAS IN MPTP-TREATED PRIMATES

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Poor bioavailability, short half-life and unpredictable absorption contribute to neurological complications experienced after chronic therapy with L-DOPA ("wearing-off", "on-off" effects and dyskinesias). A prodrug with improved absorption and slow conversion to L-DOPA might overcome these problems.

NB-355 (L-3(3-hydroxy-4-pivaloxyloxyphenyl)alanine) is a catechol mono ester of L-DOPA (Ihara et al, 1989). We have compared the ability of NB-355 and L-DOPA to stimulate locomotor activity and induce dyskinesias in parkinsonian squirrel monkeys.

Locomotor activity was recorded for 4.5h in 10 min time bins in individual cages with infra-red photocell units. Dyskinesias (chorea and dystonia) in the limbs or the rest of the body were each rated on a scale of 0-4 every 15 min for 4h (max possible score = 128). Carbidopa was given as a 1h pretreatment in a fixed dopa equivalent dose ratio of 1:4 mg/kg p.o. as is used clinically. NB-355 and L-DOPA were examined in a dopa equivalent dose range of 10-80 mg/kg p.o.

Both NB-355 (20-80 mg/kg) and L-DOPA (10-40 mg/kg) increased locomotor activity. Approximately double the dopa equivalent dose of NB-355 was required to increase activity to the same level seen after L-DOPA treatment. For doses of NB-355 and L-DOPA which stimulated activity to similar levels over 4.5h, the time course indicated a more gradual increase and lower peak and longer duration of action for NB-355 than for L-DOPA. Dyskinesias were markedly less severe for 40 mg/kg of NB-355 than for 20 mg/kg of L-DOPA despite a similar efficacy in stimulating locomotion. Dyskinesias were less severe at the highest dose of L-DOPA due to the induction of stereotypic climbing which was generally not observed with NB-355.

Treatment	Dopa Equivalent Dose (mg/kg p.o.)	Activity Counts/4.5h	Dyskinesia score/4h
Control	-	1500 ± 594	ND
L-DOPA	10	3243 ± 894	11.5
	20	5057 ± 1652	57
	40	7350 ± 1472	36
NB-355	20	1980 ± 959	11.4
	40	4735 ± 1503	13.5*
	80	6394 ± 1435	38

* $p < 0.05$ compared to L-DOPA 20 mg/kg, Mann Whitney U-test.

Our findings indicate that NB-355 is capable of equivalent stimulation of locomotor activity to L-DOPA but with a longer duration of action and substantially reduced risk for induction of dyskinesias in primates.

Ihara M et al (1989). J. Pharm. Sci. in press.

EVIDENCE TO SUGGEST A DIFFERENTIAL INVOLVEMENT OF CORTICOSTEROIDS IN THE EXPRESSION OF OPIATE AND OPIOID ANALGESIA

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Several lines of evidence now suggest that the ACTH/corticosteroid axis may be involved in the expression of opiate/opioid analgesia. Of particular relevance in this context are data indicating that adrenalectomy and corticosteroids enhance opioid/opiate analgesia (Miczek et al 1986). Whilst these findings are strongly suggestive of a prime role for corticosteroids in the expression of opiate analgesia it is necessary to examine the influence of the absence of corticosteroids per se before firm conclusions can be drawn.

For these studies 25-30g DBA/2 mice were injected with the 11 B-hydroxylase inhibitor Metyrapone, which blocks the synthesis of corticosterone from 11-Deoxycorticosterone. In the absence of data concerning the effects of Metyrapone on baseline pain responding a series of titration studies were conducted to examine this. 30 mins following drug administration (0 - 160 mg/kg Metyrapone) animals were assayed for tail-flick latency (TFL) at 0,15,30,45 and 60 mins post-injection. Data were analysed by ANOVA which revealed a bi-phasic effect, with 160 mg/kg inducing significant analgesia and 1 - 20 mg/kg producing only weak and inconsistently reproduceable analgesia. In the second series of studies the influence of Metyrapone on analgesia induced by 5 mg/kg morphine was studied. ANOVA revealed that 20 - 80 mg/kg potently and 0.1 - 1 mg/kg partially blocked this form of antinociception. Finally, although the analgesia induced by exposure to 35 bite attacks in a standard Resident-Intruder Paradigm is known to be opioid-mediated, Metyrapone was without effect. Data from these studies are summarised below.

mg/kg Metyrapone

	0.05	0.1	0.5	1	5	10	20	40	80	160
Basal	-	-	-	*	*	*	*	-	-	***
Opioid							-	-	-	
Morphine	-	Partial Block(?)	Partial Block	Partial Block	-	-	Block	Block	Block	

- = no effect * = weak analgesia *** = potent analgesia

Current data demonstrate that Metyrapone has (i) biphasic effects on baseline pain sensitivity (ii) no influence on opioid analgesia at the doses thus far examined, yet (iii) fully blocks morphine analgesia at high and partially blocks this response at low doses. Thus, these data possibly suggest a difference in the mechanisms involved in the expression of opioid and opiate analgesia.

Miczek KA et al Annals of NY Acad Sci 467: 14-29 (1986).

INTERLEUKIN 1β AND LIPOPOLYSACCHARIDE EFFECTS ON PROSTAGLANDIN E_2 RELEASE FROM RAT BRAIN SLICES

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Bacterial lipopolysaccharide (LPS) produces fever by causing the synthesis and release of endogenous pyrogenic cytokines such as interleukin 1 (IL-1). The febrile responses to exogenous and endogenous pyrogens are both mediated in the brain by prostanoids, notably PGE_2, and are both blocked by inhibitors of cyclo-oxygenase (Dascombe, 1985). The effects of LPS and recombinant IL-1β on PGE_2 release from rat brain tissue have been studied to determine the mechanism by which pyrogens stimulate PGE_2 production centrally.

Male Sprague Dawley rats were killed by concussion and cervical dislocation. Cerebral hemispheres were removed, cross chopped (0.5 mm x 0.5 mm) and suspended (10 mg wet weight/ml) in sterile, pyrogen-free Krebs solution, gassed with 95% O_2/5% CO_2, containing the drugs. Following incubation at 37°C for the times indicated below, the supernatant was removed and stored at -20°C until assayed in duplicate for PGE_2 content by radioimmunoassay.

LPS from E.coli (10 μg/ml, contact time 30 min) stimulated PGE_2 release from brain slices ($P<0.05$, n=7 brains), but other concentrations (1 and 100 μg/ml) had no significant effect. Human IL-1β (DuPont, 1-100 ng/ml contact time 20 min, n=5) had no significant effect on PGE_2 release. Murine IL-1β (DuPont, contact time 20 min, n=8) caused a dose related increase in PGE_2 release (Table 1), which was inhibited by flurbiprofen (1 μg/ml, n=5).

Table 1 Effect of murine IL-1β on PGE_2 release from rat brain slices

	IL-1β (ng/ml)			
	control	1	10	100
Normal Krebs	224 ± 52	244 ± 59	314 ± 76*	413 ± 104*
Flurbiprofen	147 ± 44	95 ± 47⁺	202 ± 65	48 ± 26⁺

Values are mean ± s.e.mean PGE_2 concentrations (pg/ml), * $P<0.05$ compared to paired control, ⁺ $P<0.05$ compared to response to IL-1β in normal Krebs.

Quinacrine (1mM) had no effect on basal release of PGE_2, but reduced the responses to murine IL-1β (10 ng/ml:181 ± 28 pg PGE_2/ml, 100 ng/ml:167 ± 15 pg PGE_2/ml, n=5). In calcium-free Krebs containing EGTA (2mM), basal release of PGE_2 was increased (446 ± 41 pg/ml, n=6, $P<0.05$) and the responses to murine IL-1β (10 and 100 ng/ml, n=6) were abolished.

These results show that murine IL-1β produces a dose dependent increase in PGE_2 release from rat brain slices. The absence of an effect by human IL-1β in this study may be due to its lack of homology with rat IL-1β. Recombinant IL-1β induced release of PGE_2 from brain tissue appears to involve the activation of a calcium dependent phospholipase, possibly phospholipase A_2, as, for example, in synovial cells (Godfrey et al, 1987) and fibroblasts (Solito & Parente, 1989).

EJR is an ONO Pharmaceuticals Research Student.

Dascombe, M.J. (1985) Prog. Neurobiol. 25, 327-373
Godfrey, R.W. et al (1987) Biochem. Biophys. Res. Comm. 142, 235-241
Solito, E. & Parente, L. (1989) Br. J. Pharmac. 96, 656-660

HAEMODYNAMIC RESPONSES TO ENDOTHELIN AFTER PERIPHERAL AND CENTRAL ADMINISTRATION IN THE CONSCIOUS RAT

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Endothelin (ET-1), a recently discovered endothelial-derived peptide, is a potent vasoconstrictor in vitro and elicits a biphasic cardiovascular response in anaesthetised, ganglion-blocked rats following intravenous administration (Yanagisawa et al 1988; Wright et al 1989). The present study investigated the haemodynamic actions of ET-1 in conscious freely moving male WKY-rats (270-300 g), after intravenous (i.v.) and intracisternal (i.c.) administration.

Intracisternal guide cannulae were implanted under brietal sodium anaesthesia (60 mg kg^{-1}; i.p.) and the rats given 7 days to recover from surgery. The femoral artery and vein were cannulated on the study day for blood pressure measurement and drug administration respectively. Three doses of ET-1 (0.1,0.3, & 1.0 nmol) were given i.v. in a randomised order to each rat (n=5) in a volume of 0.1 ml over a period of 1 minute. In separate animals injections of ET-1 (0.01-0.03 nmol) were made i.c.in a volume of 2.5 ul over the same time period,one dose per rat (n=4-6).

ET-1 (0.1-1 nmol) i.v. evoked a biphasic cardiovascular response.The initial effect was a transient (⩽ 30 sec) dose-related hypotension with a corresponding tachycardia (Table 1). The initial depressor response was followed by a marked dose-related increase in MAP which was prolonged (⩽ 60 mins). No effect was observed on HR (Table 1).

Table 1: Maximum hypo (a) and hypertensive (b) responses and HR after ET-1 (i.v.).

ET-1	MAP (mmHg) + SEM			HR (b/min + SEM)		
nmol	Basal	(a)	(b)	Basal	(a)	(b)
0.1	125.8±4.5	123±6	135*±4.7	322±21.5	304*±19.7	298.5±20.2
0.3	126±5.3	107*±7.8	146*±4.3	320±23.02	377*±31.3	292±29.6
1.0	128±2.5	86*±3.3	156*±1.9	337±27.18	438*±17.4	310±7.8

ET-1 (0.01-0.03 nmol) i.c. increased MAP but had variable effects on HR (Table 2). In some cases administration of ET-1 elicited a marked increase in MAP, a fall in HR, convulsions and death.

Table 2: Maximum hypertensive response (a) & corresponding HR (b) after ET-1 (i.c.)

ET-1	MAP (mmHg + SEM)		HR (b/min + SEM)	
nmol	Basal	(a)	Basal	(b)
0.01	122.1±1.90	162.8±14.5	302.7±11.2	292±16.9
0.02	119.4±3.4	180±23.2	315±32.1	390±32.4
0.03	128.8±3.1	184.5*±15.2	322.5±9.2	305±15

Thus, increases in MAP are observed after both peripheral and central administration of ET-1. However it is not clear at present whether the central response is a direct effect on parenchymal or neuronal Endothelin receptors or secondary to cerebral vasoconstriction.

* $p < 0.05$ different from basal values, Repeated Measures ANOVA.

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Yanagisawa, M. et al (1988) Nature, 332, 411-415.
Wright, C.E. and Fozard, J.R. (1989) Proceedings of the British Pharmacological Society, April 1989.

PHARMACOLOGICAL ACTIONS OF ENDOTHELIN (ET-1) ON CARDIAC TISSUE *IN VITRO*

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Porcine endothelin (ET-1) has potent vasoconstrictor effects in vivo and in vitro (Yanagisawa *et al.*, 1988). ET-1 also has positive inotropic and chronotropic effects in guinea pig, rat and human isolated tissues (Ishikawa *et al.*, 1988; Moravec *et al.*, 1989). We have studied the action of ET-1 on cardiac tissue using functional, autoradiographical and electrophysiological techniques.

Left (paced) and right (unpaced) atria (rat, male Sprague-Dawley, 250g) were prepared for measurement of inotropic and chronotropic responses respectively (Ishikawa *et al.*, 1988). ET-1 was added in a cumulative fashion (0.1 nM-1 μM). Transverse slices (20 μm) of rat heart were prepared for autoradiographical studies (Sharif *et al.*, 1988) using 20 pM [^{125}I] ET-1 in 20 mM HEPES buffer containing 5 mM $MgCl_2$ and 0.1% BSA. Non-specific binding was defined using 0.5 μM ET-1.All values quoted are the means ± s.e.mean, n=4-10. Recordings of calcium channel currents in embryonic chick myocytes were made as described by Anderson *et al.*, (1988).

ET-1 elicited concentration-dependent positive inotropic and chronotropic responses (-log EC_{50} values of 8.0 ± 0.05 and 8.1 ± 0.10 respectively). The maximum positive chronotropic and inotropic responses were 28 ± 4 and 35 ± 5 % respectively of that attained by 0.1 μM isoprenaline. The time taken to attain an equilibrium response to ET-1 were significantly ($p<0.05$) longer than that for isoprenaline (0.1 μM ET-1 : 25 ± 2 min; 0.1 μM isoprenaline 8 ± 3 min). High density specific binding of labelled ET-1 was located on the atrial appendages and the aortic arch (6.3 ± 0.16 and 2.2 ± 0.6 amol.mm^{-2} respectively. ET-1 (25-100 nM) did not enhance the calcium channel currents evoked by depolarisation to 0 mV from a holding potential of -80 mV. Instead ET-1 (100 nM) reduced the amplitude of the calcium channel current by around 40%.

The positive chronotropic and inotropic responses are in good agreement with the data of Ishikawa *et al.* (1988) but in contrast to Moravec *et al.*(1989) who observed no positive chronotropic response in the rat. ET-1 binds to specific sites in the myocardium, through which it may exert its physiological effects. ET-1 is not a direct activator of calcium channel currents in embryonic chick myocytes suggesting the mechanism of action may be due to release of calcium from intracellular stores. ET-1 elicits a stimulation of inositol phospholipid hydrolysis in rat atria (Kloog *et al.*, 1988) which may account for the above responses.

Anderson, A. J. *et al.*, (1988) J. Physiol., **407**, 130P
Ishikawa, T. *et al.*, (1988) Am. J. Physiol., **255**, H970-973
Kloog, Y. *et al.*, (1988) Science, **242**, 268-270.
Moravec, C. M. *et al.*, (1989) Biochem. Biophys. Res. Comm., **159**, 14-18
Sharif, N. A. *et al.*, (1988) Neurosci. Lett., **86**, 272-278
Yanagisawa, M. *et al.*, (1988) Nature, **332**, 411-415

INFLUENCE OF ENDOTHELIUM ON THE CONTRACTILE RESPONSE OF ISOLATED RAT AORTA TO SYNTHETIC TETRADECAPEPTIDE RENIN SUBSTRATE

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Recently, evidence for the existence of a vascular renin-angiotensin system (RAS) has been shown (see review, Dzau, 1988). The purpose of the present work was to determine the pathway(s) of the formation of angiotensin II (ANGII) from synthetic tetradecapeptide renin substrate (TDRS) in the isolated rat aorta and to study the possible role of the endothelium in the formation of ANG II. Therefore, the contraction of aortic rings with and without endothelium to TDRS, angiotensin I (ANG I) or ANGII was studied in the presence or absence of inhibitors of the RAS.

Aorta rings were taken from male Sprague Dawley rat (300-350 g). The rings were left intact or mechanically rubbed to remove endothelial cells and mounted in 20 ml organ baths filled with a physiological salt solution at 37°C gassed with 95% O_2 : 5% CO_2. Tissues were contracted with a depolarizing solution containing 100 mM KCl, which induced a maximal contraction (1079 ± 30 mg, n=23), and challenged with ACh (1 µM) which induced a relaxation (36.8 ± 1.1%, n=23) in intact rings and no relaxation in rubbed rings. Responses to single concentrations of ANGII, ANGI and TDRS were obtained by adding the appropriate concentration into the bath, in the presence or absence of a pharmacological inhibitor of the RAS.

In the presence of a functional endothelium, TDRS induced an increase in tension which was concentration-dependent. The maximal response induced by TDRS (429 ± 49 mg, n=4) was similar to that produced by ANGI and ANGII (496 ± 46 mg and 530 ± 73 mg, n=5). The sensitivity of the aorta to TDRS (EC_{50} = 0.25 ± 0.09 µM, n=4) was about 2 times less than that of ANGI and ANGII (0.14 ± 0.05 µM and 0.13 ± 0.03 µM, n=5). However, the differences didn't reach statistical significance. ANGII receptor blockade (^{1}Sar-^{8}Ile 18 nM) completely inhibited the responses to ANGII, ANGI and TDRS (1 µM). Converting enzyme inhibition (enalaprilat 6.1 µM) had no effect on the response to ANGII (1 µM) but completely blocked the responses to ANGI and TDRS (1 µM). Renin inhibition (CGP 44099A 4 µM) had no effect on the responses to ANGII and ANGI (1 µM) but inhibited those to TDRS (1 µM). In the absence of the endothelium, the maximal response to TDRS, ANGI and ANGII were increased by about 2 fold. Also, the sensitivity of the aorta to TDRS, ANGI and ANGII was increased (EC_{50} values were decreased by 4 to 15 fold). This effect may be due to release of Endothelium-Derived Relaxing Factor since in intact preparations, methylene blue (3 µM) (Al Osachie & Godfraind, 1986) increased the contractile response to TDRS and ANGII (1 µM) to a similar level as endothelium removal. In the absence of endothelium, ANGII receptor blockade, converting enzyme inhibition and renin inhibition still inhibited the contractile response induced by TDRS (0.1 µM).

In conclusion, the formation of ANGII from TDRS in the rat aortic preparations show that converting enzyme and a renin-like aspartic proteinase are present in the aortic wall. Furthermore we show that endothelium is not essential for the conversion of TDRS to ANGII but modulates the response to the endogenously produced ANGII.

Al Osachie I. & Godfraind, T. (1986). Br. J. Pharmacol., 89, 525-532.
Dzau, V. (1988). Circ. Res., 77 (suppl.I), I-4-I-13.

LDL INHIBITS RELAXATIONS EVOKED BY THIMEROSAL IN RABBIT AORTA

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Endothelium-dependent relaxations in the rabbit aorta are accounted for by the release of endothelium-derived nitric oxide (ENDO) (Palmer et al. 1987). This response is inhibited by low-density lipoproteins (LDL) in aorta pre-contracted with noradrenaline (NA) or serotonin (5HT) (Andrews et al. 1987). Here, we investigate the inhibition of ENDO-mediated relaxations evoked by thimerosal, an agent which acts by a post-receptor mechanism (Forstermann et al. 1986).

LDL (density, 1.019-1.063 g/ml) were prepared from fresh human plasma by discontinuous density gradient ultracentrifugation in the presence of 0.3 mM EDTA but in the absence of thimerosal (Chung et al. 1980). ENDO relaxations were assayed as previously described using isolated intact rings of thoracic aorta from 6 month-old New Zealand White rabbits (Andrews et al. 1987). Tissues were pre-contracted with noradrenaline (NA) or serotonin (5HT) and relaxed by acetylcholine (ACh). After washout and equilibration for 30 min, LDL (2 mg protein/ml) or Tyrode's buffer (control) was added to the organ bath and the pre-contracted tissues were relaxed by graded doses of thimerosal. After maximal relaxation was achieved, the tissues were incubated for 60 min before a further challenge with 0.5μM ACh, washout and equilibration. A further relaxation to ACh was then performed on the pre-contracted tissues to determine the reversibility of the effects.

ACh (0.1μM) and thimerosal (0.7μM) caused a relaxation in control tissues of 90-100%. After maximal relaxation by thimerosal was achieved, the tissues slowly regained their initial tension. On challenge with ACh, both LDL-treated and control tissues were unable to relax. The dose-response curves obtained for the relaxations to thimerosal in the presence of LDL showed a 10-fold rightward shift compared with control tissues.

Thimerosal as described here, like mellitin and polyarginine exhibits a biphasic response in aortic rings (Rapoport et al. 1989). Namely, a rapid relaxations followed by slow contraction which results in desensitization to ACh. LDL inhibits the relaxation induced by thimerosal but not the desensitization. The inhibition by LDL of relaxation evoked by A23187 (Andrews et al. 1987) and thimerosal, serves to confirm a post-receptor mechanism for the effect. Furthermore, we have discovered that thimerosal, which was used previously as a anti-bacterial agent in the preparation of LDL (Andrews et al. 1987), is not readily dialysed from the LDL. Consequently, the true nature of the inhibition of EDNO responses by LDL was previously masked by desensitization (Plane et al. 1989).

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Andrews, H.E., Bruckdorfer, K.R., Dunn, R.C. and Jacobs, M. (1987). Nature 327, 237-239.
Chung, B.Y., Wilkinson, T., Geer, J.C. and Segrest, J.P. (1980). J. Lipid Res. 21, 284-291.
Forstermann, U., Goppelt-Strube, M., Frolich, J.C. and Busse, R. (1986). J. Exp. Ther. 238, 352-359.
Palmer, R.J.M., Ferrige, A.G. & Moncada, S. (1987). Nature 327, 524-526.
Plane, F. et al. (1989). This Meeting.
Rapoport, R.M., Ashraf, M. and Murad, F. (1989). Circulation Research 64, 463-473.

COMPARATIVE EFFECTS OF LIGNOCAINE AND ELECTROMAGNETIC INDUCTION ON IMPULSE CONDUCTION IN FROG ISOLATED NERVE-MUSCLE PREPARATION

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The local anaesthetic drug,lignocaine,is known to inhibit impulse conduction by a well-known mechanism,i.e.,by blocking the Na^+ channel,in nerve or muscle fibres. Recently,Brain & Wali (1988) have shown that electromagnetic induction(EMI) also can block conduction (by unknown mechanism) in the frog isolated sciatic nerve-gastrocnemius muscle preparation. The aim of the present investigation was to evaluate and compare the nerve blockade produced by these two methods in the same preparation of the frog nerve-muscle system.

The frog sciatic nerve-gastrocnemius muscle was dissected out and set up in an organ bath containing 100 ml of Ringer solution.The nerve trunk was passed through an induction coil(copper coil,20-100 turn,0.5 mm diameter and 0.6 ohm).The induction coil was wound on a P.V.C. tube,internal diameter 2 mm. The nerve trunk was moistened with Ringer solution, and the gastrocnemius muscle was laid in the organ bath,with one end pinned while the other was attached,via a thread,to a force-displacement transducer and a pen recorder. The temperature of the bath was 22-25° C. The nerve trunk was electrically stimulated at 0.5 Hz with 0.25-0.6 V(supramaximal) and 1 ms pulse duration.The pattern of electrical stimulation was interrupted by a period of EMI (from a d.c. source 1.5-4 V at a frequency of 100 min^{-1}) for 2-4 min, operated via a make-and-break switch. The contractile responses were recorded isometrically.

Electrical stimulation(ES) produced repeatable,reliable and fairly constant pattern of twitch contractions in the frog gastrocnemius muscle (control 2.4±0.1 g, tension,mean±s.e.,n=8 experiments-frogs). EMI, after a latency of 15 s, reduced and then blocked these responses in about 2 min.The inhibitory effect was reversed after the cessation of the EMI,and washing out the preparation in Ringer solution. Recovery of the blocked twitches occurred within 5-6 min. The time taken to produce 50% maximum inhibition of twitch tension by EMI was 80±5 s.

Lignocaine(0.1-100 μM) produced concentration-dependent inhibition of the twitch contractions in the frog muscle(control 3.6±0.2 g).The mean EC_{50} values(i.e., concentration to produce 50% maximum inhibition) of lignocaine-induced blockade was 0.8±0.1 μM, and 95% blockade was obtained with 8 μM lignocaine in 3±0.2 min. When compared to EMI inhibition,95% blockade by EMI was obtained in 1±0.1 min, thus blockade with EMI was faster than that with lignocaine in the same muscle.

The mechanism of lignocaine-induced inhibition of twitch tension is different from that produced by EMI,in that the latter may interfere with the ionic fluxes across the nerve membrane,probably by causing damage to the membrane,e.g.,the myeline sheath and Schwann cells.This has been found to be the case in the present study. Other workers (Kolin,1968;Oberg,1973;Polson,Barker & Freeston,1982) have considered EMI advantageous over ES. Moreover,EMI can be made to produce nerve blockade similar to that produced by an effective dose(or concentration) of a local anaesthetic drug(Scurlock,Meyamaris & Gregus,1978).

Brain,A.I.J.& Wali,F.A.(1988). Br.J.Pharmacol. 95, 843 P
Kolin,A.(1966). Physics Today, 21, 39-50
Oberg,P.A.(1973). Med.& Biol. Eng., 11, 55-64
Polson,M.J.R.,Barker,A.T.& Freeston, I.L.(1982). Med.Biol.Eng.& Comput., 20, 242-244
Scurlock,J.E.,Meyamaris,E.& Gregus,J.(1978). Acta Anaesthesiol.Scand., 22, 601-608

NORADRENALINE-INDUCED DEPOLARIZATION AND CONTRACTION IN THE RAT ISOLATED SMOOTH MUSCLE OF SEMINAL VESICLE

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The rat seminal vesicle is mainly innervated by adrenergic neurones (Bouquet & Noach, 1971), although cholinergic and peptidergic innervations have also been reported by other workers (Fedan,Besse,Carpenter & Teague,1977; Meldrum & Burnstock,1985). The aim of the present investigation, was to further analyse the mechanism of action of noradrenaline (NA), in the rat seminal vesicle, by measuring its effects on membrane potential changes (depolarization) and the subsequent muscle contraction, recorded simultaneously.

Adult, male, Sprague-Dawley rats, weighing 200-300 g, were killed by a blow to the head and bled. The seminal vesicle was dissected out and was cut spirally into strips, 2-3 cm long and 2 mm wide, for the recordings of the mechanical responses, and 8-10 cm long and 2 mm wide, for the recordings of the electrical responses. The mechanical responses were recorded isometrically, using a force-displacement transducer and a pen recorder, whereas the electrical responses were recorded by the sucrose-gap method (Keatinge,1978; Wali,1982).

Noradrenaline (1-1000 μM) produced concentration-dependent depolarization and contraction in the smooth muscle of the rat isolated seminal vesicle. A mean maximum depolarization and contraction of 4.6 ± 0.2 mV and 2.5 ± 0.1 g tension, were produced by NA (500 μM) (means$\pm$s.e.,n=8 experiments-rats). The ratio of the contraction (C) to the depolarization (D) was 6.7:1.0, in the control situation, i.e., in the absence of any antagonists, e.g.,phentolamine (1 μM). Phentolamine (1 μM) reduced both types of NA -induced responses by 3.3 -5.0 fold, and the ratio of the C/D was increased to 10:1.0 (control 6.7:1.0). The mean concentration of NA which produced 50% maximum responses (depolarization or contraction), in the rat seminal vesicle, in the absence and presence of phentolamine (1 μM) were 15 ± 1.0 μM (depolarization, control), 50 ± 6.0 μM (depolarization in phentolamine), and 100 ± 8 μM (contraction, control), 500 ± 21 μM (contraction in phentolamine), respectively. The differences between the control responses and the responses in the presence of phentolamine were significant ($P < 0.001$).

It was concluded that noradrenaline produced both depolarization and contraction in the rat isolated seminal vesicle. The differential effect of the action of noradrenaline (on depolarization and contraction responses) is not clear in the present study, but it may in part explain the mode of action of noradrenaline (and its ionic basis) in the smooth muscle.

Bouquet, J.& Noach,E.L.(1971). Arch.Int.Pharmacodyn.Ther. 189, 397-398
Fedan, J.S., Besse, J.C., Carpenter, F.G. & Teague, R.S.(1977). J.Pharmacol. Exp.Ther. 201, 285-297
Keatinge, W.R.(1978). J.Physiol.Lond. 279, 275-289
Meldrum, L.A.& Burnstock, G.(1985). Eur.J.Pharmacol. 110, 363-366
Wali, F.A.(1982). Br.J.Pharmacol. 77, 565 P

INTERACTIONS OF GALANIN WITH VASOACTIVE INTESTINAL PEPTIDE ON HUMAN COLONIC MOTILITY *IN VITRO.*

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Galanin (GAL) is a 29a.a. peptide found in the enteric nerves of a number of species including man (Rokaeus, 1987). In some instances GAL appears to co-exist with vasoactive intestinal peptide (VIP, Melander et al, 1985; Furness et al, 1987).

In the present study the presence of galanin-like immunoreactivity (GAL-IR) was confirmed in nerve fibres supplying the myenteric plexus and both the longitudinal (LM) and circular (CM) muscle layers of the human colon. Co-existence of GAL-IR with VIP-IR could be seen in nerve fibres supplying both muscle layers; in LM over 70% of GAL-IR fibres contained VIP-IR.

GAL (0.01-6.21μM) produced dose dependent, TTX (1.57μM) resistant, contractions of both LM and CM. For LM the highest dose tested (1.24μM) caused 86±6% (n=6) of a maximum contraction to acetylcholine while for CM the highest dose tested (6.21μM) caused 20±4% (n=3) of the maximum. To investigate the possibility that GAL may interact with VIP, responses of muscle strips to VIP only and VIP plus GAL were compared. GAL (0.01μM) reduced submaximal relaxations to VIP (0.1μM, LM; 0.02μM, CM) by 68±24% on LM (n=6, $P<0.05$) and by 18±21% on CM (n=4, $P>0.05$). Submaximal relaxations to noradrenaline (0.45μM) were not reduced by GAL (0.01μM).

In summary GAL-IR is co-localised with VIP-IR in nerve fibres innervating human colonic muscle. GAL has a direct excitatory motor effect on both muscle layers, in contrast to VIP which causes relaxation, GAL reduces the responsiveness of LM to VIP.

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Furness, J.B., Costa, M., Rokaeus, A., McDonald, T.J. & Brooks, B. (1987). Cell Tiss. Res. 250, 607-615.

Melander, T., Hokfelt, T., Rokaeus, A., Fahrenkrug, J., Tatmedo, K. & Mott, V. (1985). Cell. Tiss. Res. 239, 253-270.

Rokaeus, A. (1987). TINS 10, 158-164.

EFFECT OF GANGLIOSIDE TREATMENT ON SUBSTANCE P-LIKE IMMUNOREACTIVITY AXONAL TRANSPORT IN RATS WITH EXPERIMENTAL DIABETES

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Ganglioside treatment prevents some neurological defects in diabetic rats which may be progenetive for diabetic neuropathy (Calcutt, Tomlinson & Willars, 1988). This study examined the effect of ganglioside treatment on deficient axonal transport of substance P-like immunoreactivity (SPLI) in sciatic nerves of diabetic rats. Control and diabetic (50 mg/kg streptozotocin i.p.) male Wistar rats were maintained for 4 weeks, with or without mixed bovine brain ganglioside treatment (10 mg/kg/day i.p.). At the end of the protocol, under halothane anaesthesia, two tight ligatures were placed 1.5 cm apart on the mid-femoral region of the left sciatic nerve to arrest anterograde and retrograde axonal transport. After 12 h the accumulation and redistribution of SPLI (mean$\pm$SEM; measured by radioimmunoassay) at and between the ligatures was measured, along with the SPLI content of the unconstricted contralateral nerve and L_4 and L_5 dorsal root ganglia. Background SPLI in unconstricted nerves of untreated controls was 90 ± 10 pg/mm and in untreated diabetics it was 72 ± 11. SPLI in the L_4 dorsal root ganglion was 671 ± 78 pg in controls and 436 ± 68 pg in diabetics ($p<0.05$). The accumulation of SPLI proximal to the upper ligature (anterograde axonal transport) and distal to the lower ligature (retrograde transport) were respectively 3331 ± 573 pg and 225 ± 49 pg in controls and 1956 ± 161 pg and 77 ± 33 pg in diabetics (both $p<0.05$). However, the mobile fraction of SPLI and its accumulation rates, as calculated using the SPLI redistribution profile between the ligatures, were similar in controls and diabetics. A similar pattern of changes was observed when the ganglioside-treated diabetics were compared with the ganglioside treated controls. Proximal accumulations were 3265 ± 648 pg (ganglioside-treated controls) and 1950 ± 182 pg (ganglioside-treated diabetics). Distal accumulations (respectively) were 260 ± 72 pg (treated controls) and 145 ± 45 pg (treated diabetics). Thus, ganglioside treatment was without effect on the observed deficits in SPLI in constricted nerves and dorsal root ganglia of diabetic rats.

Calcutt,N.A., Tomlinson,D.R. & Willars,G.B. (1988) J. Neurochem. 50, 1478-1483.

NOVEL, POTENT AND SELECTIVE AGONISTS AT NK-1 AND NK-2 RECEPTORS

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The lack of selectivity of the endogenous neurokinins substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) has created a need for potent and selective neurokinin receptor agonists to aid characterisation of neurokinin receptor-mediated actions, both *in vitro* and *in vivo*. Furthermore, in view of the susceptibility of neurokinins to inactivation by tissue peptidases (e.g. in guinea-pig isolated trachea; Ireland et al., 1988), it would be advantageous if selective agonists were also peptidase-resistant. Here, we describe the actions of two neurokinin agonists, δ-aminovaleryl[L-Pro^9,N-MeLeu^{10}]SP(7-11) (GR73632) and [Lys^3,Gly^8-R-ϒ-lactam-Leu^9]NKA(3-10) (GR64349) at NK-1 receptors in guinea-pig ileum longitudinal muscle (GPI), NK-2 receptors in rat colon muscularis mucosae (RC) (see Brown et al., 1985) and NK-3 receptors in rat everted portal vein (RPV) (see Mastrangelo et al., 1986) (Table 1).

Table 1: Mean EC_{50} values (nM) ± s.e. mean, ($n \geq 3$) for contractions induced by neurokinin agonists.

Agonist	GPI (NK-1)	RC (NK-2)	RPV (NK-3)
SP	5.6±0.7	167±20	7500±1200
GR73632	4.0±0.5	961±175	>15,000
NKA	13.7±2.1	1.7±0.3	563±99
GR64349	4237±1295	3.7±0.6	1177±446
NKB	68.0±28.0	27.3±11.9	6.3±1.3

In order to test the stability of these selective agonists to peptidases, we have utilised spiral strips of guinea-pig isolated trachea (GPT). In this preparation, the contractile response to naturally-occurring neurokinins is markedly potentiated by peptidase inhibitors (Stephens-Smith et al., 1988). Therefore, we examined the potency of the agonists to contract GPT in the absence and presence of the endopeptidase inhibitor phosphoramidon (1μM) and the aminopeptidase inhibitor bestatin (100μM). Whereas the concentration-response curves for SP and NKA were displaced markedly to the left (>50-fold shift in EC_{50}), the EC_{50} values for GR73632 (4.7±0.3nM) and GR64349 (9.9±2.2nM) were little affected (<5-fold shift).

These results suggest that GR73632 and GR64349 are potent, selective and metabolically stable agonists at NK-1 and NK-2 receptors respectively. Moreover, the potencies of these selective agonists in the GPT are consistent with the existence of both NK-1 and NK-2 receptors mediating the contractile response to neurokinins in this preparation (cf Mcknight et al., 1988, and Ireland et al., 1988).

Brown, J.R. et al. (1985) in 'Tachykinin Antagonists': Hakanson, R. & Sundler, F. (ed.), pp. 305-312. Amsterdam, Elsevier.
Ireland, S.J. et al. (1988) Regulatory Peptides 22, 93.
McKnight, A.T. et al. (1988) Regulatory Peptides 22, 126.
Mastrangelo, D. et al. (1986) Eur. J. Pharmac. 134, 321-326.
Stephens-Smith, M. et al. (1988) Regulatory Peptides 22, 177.

THE MUSCARINIC RECEPTOR SUB-TYPE MEDIATING AIRWAY RESPONSES TO ACETYLCHOLINE IN THE GUINEA-PIG

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Binding studies show that approximately 50% of muscarinic receptors in guinea-pig lung are of the M1 sub-type, having high affinity for pirenzepine (Mak & Barnes, 1988). We have investigated whether M1 receptors have a functional role in mediating changes in lung resistance (R_L) and dynamic compliance (C_{dyn}) in response to exogenous acetylcholine (ACh) in the anaesthetised guinea-pig.

The method used is an adaptation of that previously described (Gater et al, 1988). A sub-maximal dose of ACh (normally 10-20ug/Kg i.v.) causing an approximate 2-fold increase in R_L and a 50% fall in C_{dyn} and HR was established and then repeated at 10 minute intervals until responses were consistent. Cumulatively increasing i.v. doses of muscarinic antagonists were given and the ACh responses re-determined after each dose. The doses of antagonists (ug/Kg ± s.e.mean) which inhibited the ACh response by 50% (IC_{50}) are shown in the table.

IC_{50} values for muscarinic antagonists against acetylcholine induced changes in R_L, C_{dyn}, and HR

Antagonist	R_L	C_{dyn}	HR	n
Atropine	0.78 ± 0.2	0.96 ± 0.2	2.0 ± 0.08	7
Ipratropium Bromide	1.7 ± 0.4	1.5 ± 0.3	2.0 ± 0.1	3
4-DAMP	2.26 ± 0.7	2.79 ± 0.9	119.6 ± 22	7
Pirenzepine	90.4 ± 26.5	83.8 ± 23.4	244.0 ± 80	5
Methoctramine	⩾300	⩾300	21.2 ± 3.5	6

In agreement with others (Maclagan et al, 1989) we have found that pirenzepine is approximately 100 fold weaker than atropine against the ACh-induced changes in R_L, C_{dyn} and HR. Thus M1 (high pirenzepine affinity) receptors are not involved in these responses to ACh. The muscarinic receptors which have low affinity for pirenzepine include M2 (cardiac) and M3 (smooth muscle) sub-types. These can be differentiated in vitro by 4-DAMP (M3-selective) and methoctramine (M2-selective), (Mihm & Wetzel, 1987). We have shown here that 4-DAMP is markedly lung selective, whilst methoctramine has the converse selectivity. We therefore conclude that M3 receptors, but not M1 receptors, mediate the functional responses to acetylcholine in the airways of the anaesthetised guinea-pig.

Gater P.R., Moore B.A. & Alabaster V.A. (1988) Brit.J.Pharm. 93 242P
Maclagan J., Fryer A.D. & Faulkner D. (1989) Brit.J.Pharm. In press.
Mak J.C.W. & Barnes P.J. (1988) Brit.J.Pharm. 95 777P
Mihm G. & Wetzel B. (1987) Drugs of the Future 12 1127

COMPARISON OF THE *IN VIVO* POTENCY OF METHOCTRAMINE FOR PRE- AND POST-JUNCTIONAL MUSCARINIC RECEPTORS IN GUINEA-PIG LUNG.

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Stimulation of pulmonary parasympathetic nerves causes bronchoconstriction via release of acetylcholine onto M_3 muscarinic receptors on airway smooth muscle. Transmitter release is inhibited by activation of pre-junctional M_2 receptors and blockade of these autoreceptors by the muscarinic antagonist gallamine causes facilitation of vagally-induced bronchoconstriction (Fryer and Maclagan, 1987). This drug however, is also a weak antagonist for post junctional M_3 receptors, causing an under-estimate of its potency for the neuronal M_2 receptors. Recently a new muscarinic antagonist, methoctramine, has been described which has more than 100 times greater affinity for M_2 receptors in the heart than for M_3 receptors on tracheal smooth muscle *in vitro* (Giraldo et al., 1988).

In the present experiments, the effects of methoctramine have been studied in the lung to compare its potency on pre- and post-junctional muscarinic receptors. Guinea-pigs (350-450g), anaesthetised with urethane, were vagotomised and respired by positive pressure, constant volume ventilation from a Harvard rodent respirator. Bronchoconstriction was induced alternately by stimulation of the peripheral end of the right vagus nerve (30 Hz, 0.2ms, 10-40 V, 5s) or by i.v. acetylcholine (ACh 5-10ug.kg^{-1}) and recorded as an increase in tracheal pressure above the normal excursions generated by the ventilator (Fryer and Maclagan, 1987) before and during cumulative administration of methoctramine.

Methoctramine caused inhibition of the bradycardia induced by vagal nerve stimulation (ED_{50} 38 = 5 nmol.kg^{-1}, n=6). In contrast, in the lung facilitation of vagally-induced bronchoconstriction occurred at all doses (ED_{50} 58 = 7 nmol.kg^{-1}, n=6). ACh-induced bronchoconstriction, however , was inhibited at doses exceeding 60 nmol.kg^{-1} (ED_{50} 81 = 11 nmol.kg^{-1}, n=6). Methoctramine did not affect ACh-induced hypotensive responses at doses upto 240nmol.kg^{-1}.

These results suggest that in the guinea-pig *in vivo* methoctramine shows approximately a two fold greater potency for the M_2 receptors in the heart than for M_3 receptors on airway smooth muscle. Therefore the M_2/M_3 selectivity previously reported for methoctramine *in vitro* is not demonstrable *in vivo* in this model. In the lung, methoctramine also has greater potency for the pre-junctional M_2 receptors on pulmonary parasympathetic nerves compared to the post-junctional M_3 receptors of airway smooth muscle. Thus, *in vivo*, this antagonist can be used to demonstrate pre-junctional, pulmonary, neuronal muscarinic receptors, showing a potency ratio for M_2/M_3 receptors in the guinea-pig lung comparable to that previously described for gallamine (Fryer and Maclagan 1987), but methoctramine has the experimental advantage of a shorter duration of action.

Fryer, A. & Maclagan, J. (1987) Naunyn-Schmied Arch. Pharmac. *335*, 367-371.
Giraldo, E., Micheletti, R., Montagna, E. et al (1988) J. Pharmac. Exp. Ther. *244* (3) 1016-1020.

ACTIONS OF Ro 31-6930, A NOVEL POTASSIUM CHANNEL OPENER, ON GUINEA-PIG TRACHEAL SMOOTH MUSCLE PREPARATIONS

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Ro 31-6930 2-(6-cyano-2,2-dimethyl-2H-1-benzopyran-4-yl)pyridine-1-oxide is a novel potassium channel opener possessing smooth muscle relaxant and antihypertensive properties (Paciorek et al., 1989). The aim of this study was to evaluate the actions of Ro 31-6930 on guinea pig tracheal smooth muscle preparations both in vitro and in vivo.

Tracheal rings were opened out and suspended in Krebs (37°C, 95% O_2/ 5% CO_2) under 1 g tension for the isometric recording of tone. Tracheae were incubated with indomethacin (3 µM) prior to being exposed to ≈EC_{75} concentrations of agonists. Test agents were administered cumulatively and relaxation was expressed as a percentage of the maximal relaxation to 1 mM aminophylline. Ro 31-6930 (0.001-1.0 µM), cromakalim (0.03-3.0 µM), salbutamol (0.001-1.0 µM) and theophylline (0.3-100 µM) evoked dose related reductions in spontaneous tracheal tone with IC_{50} values of 0.044 ± 0.019, 0.2 ± 0.006, 0.021 ± 0.001 and 21 ± 2.0 µM respectively (mean ± s.e.mean n = 4). Over a similar dose range all four bronchodilators evoked dose related reductions in leukotriene D_4 (0.02 µM) induced tone with IC_{50} values of 0.043 ± 0.009, 1.19 ± 0.34, 0.012 ± 0.004 and 7.2 ± 3.0 µM respectively. Tone induced by 0.1 µM U46619, a thromboxane A_2 mimetic (Coleman et al., 1981) was reduced in a dose related fashion by all four agents with IC_{50} values of 0.091 ± 0.023, 0.61 ± 0.13, 0.016 ± 0.001 and 53.5 ± 7.8 µM respectively. Betahistine (10 µM) induced tracheal tone (Brown et al., 1986) was also reduced in a dose related fashion with IC_{50} values of 0.053 ± 0.013, 0.35 ± 0.12, 0.0059 ± 0.0014 and 7.6 ± 2.4 µM respectively.

Bronchoconstriction was assessed in urethane (1.75 gkg^{-1} i.p.) anaesthetised, pithed, vagotomised guinea pigs by the air overflow method (Konzett & Rössler, 1940). ED_{65} doses of 5-HT (1.5-8.0 µg kg^{-1} i.v.) were administered every 5 min until reproducible bronchospasm was established. Ro 31-6930 (0.01-0.1 $mgkg^{-1}$ i.v.), cromakalim (0.03-1.0 $mgkg^{-1}$ i.v.), salbutamol (0.001-0.01 $mgkg^{-1}$ i.v.) and theophylline (1.0-30.0 $mgkg^{-1}$ i.v.) were then administered cumulatively 10 min prior to each 5-HT challenge. Agents evoked dose related reductions in bronchoconstriction with ID_{50} values of 0.016 ± 0.003, 0.123 ± 0.028, 0.004 ± 0.001 and 15.6 ± 2.3 $mgkg^{-1}$ respectively.

These results demonstrate that Ro 31-6930 compares favourably with salbutamol as a bronchodilator, and provides further evidence to suggest that potassium channel openers may be useful in the treatment of asthma (Buckle et al., 1987).

Buckle, D.R. et al (1987) Br.J.Pharmacol. 92, Proc.Suppl. 744P.
Brown, E.A. et al (1986) Br.J.Pharmacol. 87, 569-578.
Coleman, R.A. et al (1981) Br.J.Pharmacol. 73, 773-778.
Konzett, H. & Rössler, R. (1940) Arch.Exp.Path.Pharmak. 195, 71-74.
Paciorek, P.M. et al (1989) This meeting.

COMPARISON OF THE EFFECTS OF CROMAKALIM (BRL 34915) ON RABBIT ISOLATED BASILAR ARTERY AND THORACIC AORTA.

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Cromakalim (CROM) is a novel vasodilator which appears to act by opening K^+ channels in smooth muscle cells (Hamilton et al, 1986). CROM has been reported to relax potently a variety of precontracted, isolated peripheral blood vessels and to inhibit spontaneous mechanical activity in the portal vein from a variety of species. However, relatively little is known about the effects of CROM in isolated cerebral blood vessels. The aim of the present study was to characterise the relaxant properties of CROM in a cerebral artery, the basilar artery, and to compare this with effects in thoracic aorta, a peripheral artery.

Rings of rabbit isolated basilar artery or thoracic aorta were mounted for isometric tension recording in Krebs' bicarbonate buffer bubbled with 95%O_2–5%CO_2 at 37°C. The tissues were precontracted with EC_{50} concentrations of noradrenaline (NA), 5–hydroxytryptamine (5–HT), neuropeptide Y (NPY) or KCl and then challenged with CROM (0.01–10μM) applied cumulatively.

CROM (0.01–10μM) produced dose–related relaxations of basilar arteries precontracted with 5–HT (0.06μM), KCl (20mM) and NPY (0.05μM). The IC_{50} values and maximum % relaxations induced by CROM are shown in table 1. NA produced small, transient contractions of basilar arteries and so was not studied further in this tissue.

In rabbit thoracic aortae precontracted with NA (0.3μM), KCl (30mM) or 5–HT (1μM), CROM (0.01–10μM) produced small, dose–dependent relaxations which did not reach 50% relaxation even at CROM (10μM) (see table 1). NPY (up to 3μM) had no contractile effect on aorta.

Table 1 IC_{50}(M) for CROM against the following agonists:

Tissue	5HT	KCl	NA	NPY
Basilar	$1.8(\pm0.5)\times10^{-7}$	$2.5(\pm0.8)\times10^{-7}$	–	$(2.1\pm0.1)\times10^{-7}$
	(131±13.2%)	(100±0%)	–	(123±3.3%)
Aorta	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$	–
	(27±9.1%)	(44±4.8%)	(49±12.0%)	–

(Values in parentheses below IC_{50} values represent max. % relaxations obtained).

The results show that CROM produces potent, dose–related relaxations of the precontracted rabbit basilar artery irrespective of the spasmogen used to induce tone. In contrast, CROM produced relatively small relaxations of precontracted rabbit aorta. The effects of CROM in the cerebral vessel are interesting and require further investigation.

Hamilton, T.C. et al (1986). Br. J. Pharmacol., 88, 103–111.

Ro 31-6930, A NOVEL POTASSIUM CHANNEL OPENER WITH SMOOTH MUSCLE RELAXANT AND ANTIHYPERTENSIVE PROPERTIES

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Ro 31-6930, 2-(6-cyano-2,2-dimethyl-2H-1-benzopyran-4-yl)pyridine-1-oxide is a novel pyridine N-oxide derivative with potassium channel opening properties. The aim of this study was to evaluate some of the in vivo and in vitro pharmacology of this compound.

Portal veins were removed from male Sprague Dawley rats (≈250 g) and suspended in normal Krebs (37°C, 95% O_2/5% CO_2) under 0.5 g tension for the isometric recording of tone. Tissues were contracted with 20 mM KCl and inhibitory dose response curves constructed to cumulative administration of Ro 31-6930 or cromakalim (Hamilton, Weir & Weston, 1986). When activity was abolished the organ bath concentration of KCl was increased to 80 mM and the increase in tone recorded. Ro 31-6930 0.01-0.3 µM and cromakalim 0.1-3.0 µM evoked dose related inhibition of 20 mM KCl responses (IC_{50}: 0.038 ± 0.002 µM and 0.24 ± 0.03 µM) respectively, and had no effect on responses evoked by 80 mM KCl (mean ± s.e.mean n = 4).

When portal veins were loaded with ^{86}Rb, 1.5 µCi ml^{-1} for 90 min and the ^{86}Rb allowed to efflux into ^{86}Rb free MOPS PSS, the efflux rate coefficient (3.81 ± 0.51% 3 min^{-1}) became constant after approximately 20 min. Tissues were then challenged with Ro 31-6930 (5 µM) and a marked increase in the ^{86}Rb efflux rate coefficient (6.52 ± 0.92% 3 min^{-1}, peak effect) was detected (mean ± s.e.mean n = 5), $p<0.05$, unpaired t-test.

Male spontaneously hypertensive rats (300-350 g) were anaesthetised with methohexitone (50 $mgkg^{-1}$ i.p.). The right carotid artery was cannulated with a polyethylene catheter which was exteriorised at the back of the neck. Rats were allowed to recover and used the following day for measurements of mean arterial pressure (MAP) and heart rate. Ro 31-6930 0.01, 0.03 and 0.1 $mgkg^{-1}$ p.o. evoked significant reductions in MAP of 61.3 ± 5.0, 82.3 ± 3.3 and 98.2 ± 4.5 mmHg, 15 min after dosing. MAP remained below control values for 8-24 h following dosing. The reductions in MAP were accompanied by reflex tachycardia of 81.7 ± 7.9, 61.7 ± 15 and 103 ± 21 beats min respectively. Cromakalim 0.1, 0.3 and 1.0 $mgkg^{-1}$ p.o. evoked significant reductions in MAP of 44.3 ± 4.7, 97.5 ± 8.2 and 97.3 ± 8.2 mmHg, 15 min after dosing. MAP returned to control values between 4-8 h later. The reductions in MAP were accompanied by reflex tachycardia of 43.3 ± 6.1, 100 ± 5.8 and 90.0 ± 15 beats min^{-1} respectively (mean ± s.e.mean n = 6), $p<0.05$, split plot ANOVA.

These results show that Ro 31-6930, a novel potassium channel opener, possesses a similar profile of pharmacological activity to cromakalim but has a greater potency, and possesses a longer duration of action as an antihypertensive agent in the spontaneously hypertensive rat.

Hamilton, T.C., Weir, S.W. & Weston, A.H. (1986) Br.J.Pharmacol. 88, 103-111.

ANTAGONISM OF VASORELAXATION TO CROMAKALIM AND BRL 38227 BY NON-SULPHONYLUREA HYPOGLYCAEMIC AGENTS

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Relaxation of noradrenaline(NA)-contracted rabbit isolated mesenteric artery (RIMA) by the K^+ channel activator, cromakalim, can be antagonised by sulphonylurea hypoglycaemic agents such as glibenclamide (Wilson 1989). Two non-sulphonylurea hypoglycaemic drugs, meglitidine (Garrino et al, 1985) and pirogliride (Tutweiler et al, 1978), and glibenclamide, have now been examined as antagonists of vasorelaxation induced by cromakalim or its active (-)-enantiomer, BRL 38227 (Buckingham et al., 1986).

Isometric tension was recorded from rings of RIMA (n=5-6 per treatment) suspended in Krebs-Henseleit solution at 37°C (Clapham & Wilson, 1987). Tissues were contracted with NA, (0.1 mM) then relaxed by the cumulative addition of either cromakalim or BRL 38227. After a 30 min washout period, the tissues were again contracted with NA then incubated for a further 15 min with either pirogliride, meglitidine, glibenclamide or vehicle. The concentration- response curve to cromakalim or BRL 38227 was then repeated. IC_{50} values for cromakalim and BRL 38227 were obtained pre- and post-treatment and, after correcting for the increase in sensitivity observed in the controls, concentration ratios were calculated. Where parallel shifts of the curves to the right were obtained, pA2 values were calculated. Otherwise the reduction in maximum response was noted.

Meglitidine produced concentration-related parallel rightward shifts of the cromakalim concentration-response curve to yield a pA2 of 5.66 (Table 1); glibenclamide produced a similar effect against BRL 38227 with a pA2 of 6.86. Pirogliride produced non-parallel concentration-related rightward shifts of the BRL 38227 curve, the maximum responses being reduced by 2.4, 11.4 and 37.5% by pirogliride 1, 3 and 10 µM respectively when compared with the vehicle controls.

Table 1. Antagonism of cromakalim- or BRL 38227-induced vasorelaxation.

Antagonist	Concentration	Concentration ratio	Mean pA2
Meglitidine	1 µM	1.59	
(Vs cromakalim)	10 µM	5.08	5.66 ± 0.06
	100 µM	39.7	
Glibenclamide	0.3 µM	3.14	
(Vs BRL 38227)	1 µM	6.62	6.86 ± 0.07
	3 µM	29.9	
Pirogliride	1 µM	1.35	-
(Vs BRL 38227)	3 µM	5.83	
	10 µM	27.7	

Glibenclamide exhibits apparently competitive antagonism of BRL 38227-induced vasorelaxation with similar potency to that observed previously against cromakalim (Wilson, 1989). This result suggests a common site of action for cromakalim and its (-)-enantiomer, BRL 38227. Meglitidine, a structural analogue of glibenclamide but lacking the sulphonylurea moiety, also demonstrated apparently competitive antagonism. Pirogliride, a structurally unrelated hypoglycaemic agent, was a potent antagonist against BRL 38227, but inhibition appeared non-competitive. It remains to be determined whether this qualitative difference is a function of the antagonist drug or is related to a different site of inhibition.

Buckingham, R.E. et al (1986) Br. J. Pharmac. 87, 78P.
Clapham, J.C. and Wilson, C. (1987) J. Autonom. Pharmac. 7, 233-242.
Garrino, M.G. et al (1985) Diabetologia 28, 697
Tutweiler, G.R. et al (1978) Diabetes 27, 856.
Wilson, C. (1989) J. Autonom. Pharmac. 9, 71-78

POTASSIUM CHANNELS OF THE T-LYMPHOCYTE HYBRIDOMA CELL LINE DO-11.10.

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T-cell hybridomas such as the DO-11.10 cell line can be activated by mitogens to produce interleukin-2 (IL-2) (Shimonkevitz et. al. 1983) and thus can be used as a model for studying the mechanisms of action of drugs that promote or block T-cell mitogenesis. Mature T-cells require functional K channels to respond to mitogenic stimuli (Lewis & Cahalan 1988a). In this communication we present data to show the presence of such channels in DO-11.10 cells and show some of the basic properties of these channels.

Cells were held at a potential of -70 or -80mV using the whole-cell patch-clamp recording technique and depolarising steps were applied for 150-500ms every 15s to various test potentials. This protocol induced an outward current which inactivated slowly and which became detectable when the test potential was at values more positive than -50mV. The maximum whole-cell conductance obtained was 4.91±0.71nS (mean±s.e.m., n=8 cells). This outward current showed use-dependent inactivation. If test potentials were applied for 500ms every 1s to a potential of +20mV the peak current progressively declined until it was completely inactivated. This observation suggests that the current is carried by the "n" or normal-type K channels present in developing and activated T cells of the mouse but virtually absent in adult mouse quiescent T cells (Lewis & Cahalan 1988b). The current could be blocked by replacing internal K ions with Cs ions and was virtually abolished by external application of 100μM quinine.

The single channels that make up the whole-cell K current were observed using outside-out and cell-attached patches. These channels had a main conductance value of 14-16pS, however, rare but distinct subconductance states were also observed. Quinine (50μM) produced a flickery block of these channels without any reduction in the observed single channel conductance. This can be contrasted with TEA which has been shown to reduce the observed single channel conductance of the K channels of mouse T cells (Lewis & Cahalan 1988a).

While many drugs that promote (PHA) or block (TEA, 4-AP, quinine) mitogenesis act on these K channels (Chandy et. al. 1984), D-penicillamine (1-3mM) and auranofin (10nM) which promote IL-2 production and auranofin (10μM-1mM) which blocks it (Oben et. al. 1988) had no detectable effect on the whole-cell K current or its gating.

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Chandy, K.G., DeCoursey, T.E., Cahalan, M.D., McLaughlin, C. & Gupta, S. (1984) J. Exp. Med. 160 369-385.
Lewis, R.S. & Cahalan, M.D. (1988a) Science 239 771-775.
Lewis, R.S. & Cahalan, M.D. (1988b) Trends in Neurosci. 11 214-218.
Oben, J.A., Wallace, G.R., Chain, B.M. & Foreman, J.C. (1988) Br. J. Pharmac. 95 534P.
Shimonkevitz, R., Kappler, J., Marrack, P. & Grey, H. (1983) J. Exp. Med. 158 306-316.

LOCALIZATION OF β-ADRENOCEPTOR SUBTYPES IN THE CORONARY ARTERIES AND GRACILIS ARTERIES OF THE DOG AND PIG

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Whether large coronary arteries contain β_1 or β_2-adrenoceptors has been the subject of considerable controversy. In vitro studies have generally concluded that β_1-adrenergic receptors exert primary control over large coronary arteries. However Schwartz and Velly (1983), using radioligand binding studies, have reported a ratio of β_1:β_2-adrenoceptors of approximately 2:1 in large coronary arteries of the pig. In the present study we report the autoradiographic localization of beta-adrenoceptor subtypes in the large coronary arteries of both dog and pig. Measurements were also made in dog gracilis artery, where β_2-adrenoceptors are thought to predominate.

Hearts and gracilis muscles were excised rapidly from three dogs and three pigs anaesthetized with α-chloralose (0.1g/kg). The tissues were dissected, transferred to 0.1M phosphate buffer (pH 7.4) containing sucrose (0.32M) and rapidly frozen in Arcton 12 previously cooled to -196°C. Tissue was stored at -80°C prior to use. Frozen tissue sections (20μm thick) were cut on a cryostat and thaw-mounted on to poly-1-lysine-coated slides. Tissue sections were incubated with 25pM [^{125}I]iodocyanopindolol (ICYP) for 2 h at 37°C. Alternate sections were incubated with radioligand in the presence of either 10^{-7}M ICI 118,551 (a β_2-selective antagonist) or 10^{-7}M betaxolol (a β_1-selective antagonist) to discriminate between the beta-adrenoceptor subtypes. Non-specific binding was determined in the presence of 10^{-7}M-ICI 118,551 plus 10^{-7}M betaxolol. Autoradiograms were generated as described by Carstairs et al (1985).

In both the dog and pig there was intense labelling of large coronary arteries by ICYP. The labelling was predominantly associated with vascular smooth muscle, but not, apparently, with the endothelium. Both β_1 and β_2-adrenoceptor subtypes were present. The relative proportions of the subtypes were: dog 69.7 ± 3.3% β_1 and 30.3 ± 3.2% β_2; pig 71.1 ± 3.2% β_1 and 28.7 ± 3.3% β_2 (means ± S.E.M.). In the gracilis artery, in both dog and pig, labelling by ICYP was associated with both smooth muscle and endothelium, the latter being more intense. The beta-adrenoceptors were mainly of the β_2-subtype (approximately 95% of total beta-adrenoceptors) and were of comparable density to β_2-adrenoceptors seen in the coronary arteries.

The present results suggest the coexistence of β_1 and β_2-adrenoceptors in the large coronary arteries of the dog and pig, and the results are in good agreement with the findings of Schwartz and Velly (1983). Beta-adrenoceptors in the gracilis arteries, however, were predominantly of the β_2-subtype. Similar densities of β_2-adrenoceptors were seen in both the coronary arteries and gracilis arteries, hence the greater density of beta-adrenoceptors seen in the coronary arteries was due to the large population of β_1-adrenoceptors. Also of interest is the finding of β_2-adrenoceptors on the endothelium of gracilis arteries but not coronary arteries.

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Schwartz, J. & Velly, J. (1983) Br.J.Pharmac. 79, 409-414.
Carstairs, J.R., et al (1985) Am.Rev.Resp.Dis. 132, 541-547.

INITIAL CALCIUM RELEASE IN RAT AORTA DOES NOT APPEAR TO CONTRIBUTE TO THE SUSTAINED CONTRACTION

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We have previously described a selective reduction by prazosin (PZ) of the initial, transient EGTA-resistant contraction of rat aorta to noradrenaline (NA) (Downing et al, 1983), an effect not seen with corynanthine (COR). Subsequently, we have shown no differential reduction by prazosin of the second, sustained phase of the EGTA-resistant response (Downing et al, 1986). We have therefore investigated the importance of the initial calcium release in this preparation as a putative component of the maintained tonic contraction to alpha-adrenoceptor agonists in normal calcium.

2-3mm circular preparations of thoracic aortae from male Wistar rats (180-200g) were denuded of endothelium, suspended under a resting tension of 2g in Krebs' solution (2.5mM Ca^{++}) containing propranolol (10^{-6}M) and gassed with 5% CO_2 in O_2. The effect of verapamil (VER) (10^{-6}M) was determined on responses to $3x10^{-6}$M (NA) alone, given as a bolus dose or by cumulative addition ($1x10^{-9}$ to $3x10^{-6}$M), and also on a bolus dose to NA following 30 min incubation with either COR ($1x10^{-6}$M) or PZ ($5x10^{-9}$M). In some experiments EGTA-resistant responses were elicited to NA following 5 min exposure to calcium free Krebs' solution containing 0.5mM EGTA.

Both PZ and COR caused a similar ($p>0.05$) small reduction of the maintained tonic contraction to NA; 15.0±2.8% (n=8) and 11.6±2.4% (n=8) respectively, but the reduction of the initial fast component was significantly greater ($p<0.001$) following PZ than following COR (100% and 12.0±5.3% (n=8) respectively). The EGTA-resistant responses to bolus addition of $3x10^{-6}$M NA were biphasic with an initial transient peak after 15 to 20 seconds and a slowly developing secondary sustained phase which reached a peak at 10-15 mins. In the presence of EGTA, cumulative addition of NA produced a slowly developing sustained phase only which was not significantly different from the sustained phase following bolus addition ($p>0.05$).

VER caused a similar reduction of the maintained tonic contractions to NA irrespective of whether NA was given as a bolus or by cumulative addition (36.3±6.4 and 33.6±1.8% reduction respectively). VER caused a greater reduction of the maintained tonic responses in preparations pretreated with PZ or COR (48.4±4.2% and 48.5±4.8% respectively) but there was no significant difference between these two values ($p>0.05$).

These observations show that when the initial calcium release to α-adrenoceptor activation in rat aorta has been prevented by prazosin or by cumulative addition of NA, there is an unchanged sensitivity of the tonic contraction to verapamil, suggesting that the initial calcium release to NA does not contribute to the tonic contraction.

Downing, O A et al (1983) Br J Pharmac. 80, 315-322
Downing, O A et al (1986) Ibid. 89, 841P.

THE EFFECTS OF SKF 104078 AT PRE- AND POST-JUNCTIONAL α_2-ADRENOCEPTORS *IN VITRO*.

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The α_2-adrenoceptor antagonist SKF 104078 has been reported to discriminate between pre-and postjunctional α_2-adrenoceptors in a variety of species (Ruffolo et al, 1987; Daly et al, 1988). In these studies, presynaptic activity was determined indirectly using nerve-stimulated tissue responses. Other workers (Connaughton et al, 1988a and b), who have additionally used stimulation-evoked ^{3}H-noradrenaline release as a direct measure of presynaptic activity, have failed to demonstrate any post-junctional selectivity for SKF 104078. In the present work, using both direct and indirect methods, we find that SKF 104078 distinguishes between pre-and postjunctional α_2-adrenoceptors in both rabbit and rat in vitro.

Postjunctional α_2-adrenoceptor antagonist potency was assessed by the ability of rauwolscine or SKF 104078 to produce competitive shifts of the noradrenaline concentration-response curves (CRC) in the rabbit saphenous vein. Prejunctional α_2-adrenoceptor antagonist potency was estimated directly by the ability of rauwolscine or SKF 104078 to reverse a clonidine-induced inhibition of stimulation-evoked ^{3}H-noradrenaline release from the superfused rabbit aorta, or by the antagonists' ability to enhance stimulation-evoked ^{3}H-noradrenaline outflow from the rabbit aorta or rat vas deferens in the absence of agonist. In addition, the effects of rauwolscine and SKF 104078 on the BHT-920-induced inhibition of the twitch response in the rat vas deferens were used as an indirect measurement of prejunctional α_2-adrenoceptor antagonism.

Rauwolscine (0.01-1 μM) and SKF 104078 (0.01-1 μM) both produced concentration-dependent shifts to the right of the noradrenaline CRC in the rabbit saphenous vein, giving Schild plots with gradients not significantly different from unity and pA_2 values of 7.9 and 6.6 respectively. Rauwolscine (0.01-0.1 μM) reversed the clonidine-induced inhibition of stimulation-evoked ^{3}H-noradrenaline release from the rabbit aorta with a pA_2 value of 8.4, while SKF 104078 (0.1-10μM) failed to produce any such reversal of clonidine-induced inhibition. Furthermore, in contrast to rauwolscine, SKF 104078 (0.01-10μM) failed to cause an increase in stimulation-evoked ^{3}H-noradrenaline release in the absence of agonist in both the rabbit aorta and rat vas deferens. BHT-920 (0.001-1.0μM) produced a dose-related inhibition of the twitch response in the rat vas deferens which was antagonised by rauwolscine (0.01-1 μM) but not by SKF 104078 (0.01-10μM).

In conclusion, the standard α_2-adrenoceptor antagonist rauwolscine failed to discriminate between pre-and postjunctional α_2-adrenoceptors in vitro whereas SKF 104078 is a selective postjunctional α_2-adrenoceptor antagonist.

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Connaughton, S. et al. (1988), Br. J. Pharmacol. Suppl. 94, 433P.
Connaughton, S. et al. (1988), Br. J. Pharmacol. Suppl. (in press).
Daly, R.N. et al. (1988), J. Pharmacol. Exp. Ther. 247, 122-128.
Ruffolo, R.R. et al. (1987), Naunyn-Schmiedebergs Arch. Pharmacol. 336, 415-418.

ISOLATION OF POST-JUNCTIONAL α_2-ADRENOCEPTORS IN RABBIT ISOLATED DISTAL SAPHENOUS ARTERY: INFLUENCE OF ANGIOTENSIN-II

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Under normal experimental conditions, responses to UK-14304 are mediated via postjunctional α_1-adrenoceptors in the isolated rabbit distal saphenous artery (DSA). However in the presence of angiotensin-II (A II) a component of the response to this agonist, which is mediated via postjunctional α_2-adrenoceptors, is revealed (Dunn *et al.*, 1989). We have now attempted to isolate a population of postjunctional α_2-adrenoceptors in this preparation using a protocol similar to that employed in the rabbit lateral saphenous vein by Daly *et al.*, (1988).

3-4mm segments of DSA were placed in Krebs solution containing 1μM propranolol and 10μM cocaine, gassed with 95% O_2: 5% CO_2 at 37°C, under a resting tension of 0.6-0.8 g.wt. After obtaining a control cumulative concentration response curve (CRC) to noradrenaline (NA) attempts were made to isolate a population of postjunctional α_2-adrenoceptors by receptor protection. Briefly, tissues were exposed to 1μM rauwolscine 5 min before addition of 0.3 μM phenoxybenzamine. Both antagonists were allowed to remain in contact with the tissue for 60 min. Subsequently the tissues were washed a minimum of 7 times over the period of 1 hour. 1μM rauwolscine was present during the 1st two washes to maximise receptor protection. Thereafter, responses to NA, UK-14304 or phenylephrine (PE) were obtained both in the absence and presence of A II (0.05μM). In experiments involving the use of A II, this compound was added 15 min prior to the α-adrenoceptor agonists. In some experiments prazosin or rauwolscine were added 45 min before the onset of a CRC to NA in the presence of A II.

In the absence of A II no responses were observed to either NA, PE or UK-14304 after attempting to isolate a population of postjunctional α_2-adrenoceptors. However in the presence of A II (0.05μM), a concentration producing a transient contraction which returned to baseline after 6-11 min, all three agonists produced concentration dependent contractions. For NA and UK-14304, under these conditions, the maximum response observed increased from 0 to 45 ± 8% of the initial control maximum response to NA, prior to the protection protocol. The order of potency for these agonists was : UK-14304 > NA > PE. In addition, prazosin (0.1μM) did not antagonise responses to NA under these conditions but rauwolscine (1μM) produced an approximately 2 log unit shift to the right in the CRC to NA after attempted isolation of postjunctional α_2-adrenoceptors in the presence of A II.

In conclusion, attempted isolation of a discrete population of postjunctional α_2-adrenoceptors in the rabbit isolated distal saphenous artery was not possible under normal experimental conditions. If however the tissues were exposed to an appropriate physiological stimulus, in this case A II, concentration-dependent contractions to α-adrenoceptor agonists were revealed. The relative potencies of the agonists UK-14304 > NA > PE and of the antagonists rauwolscine > prazosin against responses to NA indicate that in the presence of A II a quiescent population of postjunctional α_2-adrenoceptors become functional. These results are consistent with the necessary presence of A II in demonstrating responses to UK-14304 mediated via postjunctional α_2-adrenoceptors in the DSA (Dunn *et al..*, 1989) and the enhancement of α_2-adrenoceptor mediated function with A II in the rabbit saphenous vein (Schümman & Lues. 1983).

Daly, C. J., Dunn, W. R., McGrath, J. C. & Wilson, V. G. (1988) Br.J.Pharmacol. **95**, 501-511.
Dunn, W. R., McGrath, J. C. & Wilson, V. G. (1989) Br.J.Pharmacol. **96**, 259-261.
Schümman, H. & Lues, I. (1983) Naunyn.Schmiedebergs.Arch.Pharmacol. **323**, 328-334.

REVERSIBILITY OF β-ADRENOCEPTOR DESENSITIZATION IN GUINEA-PIG RIGHT ATRIA AND THE EFFECT OF PERSISTENT AGONIST

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Desensitization of β-adrenoceptors has been demonstrated in vitro in isolated respiratory (Omni et al. 1985; Goldie et al. 1986) and cardiac tissues (Kaumann & Birnbaumer 1976; Broadley & Herepath 1987). However, little attention has been paid to monitoring the removal of agonist from the tissue after incubation and before challenge to test for sensitivity. In this study we use right atrial rate to measure β-adrenoceptor-mediated responses and to follow the adequacy of agonist removal following incubation with isoprenaline.

Guinea-pig right atria were set up in Krebs-bicarbonate solution containing ascorbic acid (1mM) at 37.5°C and gassed with 5% CO_2 in O_2. Spontaneous rate of contraction was recorded. A cumulative concentration-response curve (CRC) was constructed for isoprenaline (ISO) and after reaching either 10^{-6} or 10^{-5}M, this concentration was left in contact with the tissue for 2 or 4h. The tissue was then washed twice and again at regular intervals for 0.5, 1, 1.5 or 3h, before repeating a CRC for ISO. Control experiments, performed identically except that ISO was washed out BEFORE commencing the sham incubation period, were used to correct the pre-incubation CRCs of test experiments. Increases in rate were plotted as % of the pre-incubation maximum and EC50 values and dose-ratios (DR) determined. $n \geq 4$ throughout.

After 2h incubation with 10^{-6}M ISO and 0.5h washout, the ISO curve was significantly displaced to the right (DR, 10.6±2.1) indicating desensitization. However, extending the washout to 1h almost completely reversed this desensitization (DR, 3.8±0.9). After 4h incubation with 10^{-6}M ISO and 1h washout, the CRC was still displaced (DR, 17.4±6.2) indicating poorly reversible desensitization.

Increasing the concentration to 10^{-5}M (4h incubation, 1h wash) showed an apparent increase in the rightwards shift and a depression of the maximum response to 58.3±10.2%. However, the resting rate (291.7±5.9 bpm) had failed to return to the preincubation level (272.8±9.0 bpm, corrected), resulting in the artefactually depressed curves. The 1h washout was thus inadequate for removal of ISO at 10^{-5}M. Longer washout times were therefore used after 4h incubations and the removal monitored from the spontaneous rate of contraction. The resting rates fell to 291.0±7.6, 264.2±9.7 and 254.2±11.0 bpm respectively after 1.5h (5 washes), 1.5h (10 washes) and 3h (10 washes) compared with the corresponding corrected preincubation values of 265.5±9.2, 244.2±8.7 and 231.6±13.5bpm. They were all therefore still significantly elevated and exerted artefactual effects upon postincubation CRCs. Thus even 10 washes in 3h failed to remove the isoprenaline. In contrast, when 10^{-5}M ISO was immediately removed from the bath in control experiments (no incubation), 3h wash returned the rate (280.0±5.5bpm) to the resting level (282.5±6.6 bpm). After 10^{-6}M ISO, the resting rate was restored within 0.5h, whether control or tests (4h incubation) were examined.

This study therefore serves as a warning when using concentrations of ISO in excess of 10^{-6}M for β-adrenoceptor desensitization, in that inadequate removal of agonist may interfere with the subsequent assessment of sensitivity.

Broadley, K.J. & Herepath, M.L. (1987) Br. J. Pharmac. 90, 197P
Goldie, R.G. et al. (1986) J. Pharmac. exp. Ther. 237, 275-282
Kaumann, A.J. & Birnbaumer, L. (1976) N.S. Arch. Pharmac. 293, 199-202
Omini, C. et al. (1985) Eur. J. Pharmac. 106, 601-606

ABSENCE OF FUNCTIONAL 'DOWN-REGULATION' OF CARDIAC β-ADRENOCEPTORS IN LOW-OUTPUT FAILURE IN THE RABBIT

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Bristow et al. (1982) proposed a decrease in ß-adrenoceptor density in failing human hearts leading to subsensitivity to ß-adreneroceptor stimulation, i.e. 'down-regulation' of cardiac ß-adrenoceptors. We have investigated whether functional 'down-regulation' of cardiac ß-adrenoceptors occurs in a rabbit model of chronic low-output failure (Wanless et al., 1987). Rabbits were dosed for seven weeks with adriamycin (AD) then left either two (AD (7+2)) or six (AD (7+6)) weeks before haemodynamic assessment of the conscious animal. Animals were killed 1-2 days later; left and right papillary muscles were removed, suspended in modified Krebs-Henseleit solution at 32°C under approximately 1.2g resting tension and electrically stimulated (0.5Hz, 5 ms, suprathreshold voltage). Cumulative concentration-response curves to isoprenaline and the $ß_1$-adrenoceptor 'partial' agonist, Ro363 (McPherson et al., 1984), were constructed. Similar studies were carried out on papillary muscles taken from untreated rabbits, implanted with either saline-filled or isoprenaline-filled mini-osmotic pumps (SMOP and IMOP, respectively). Pumps delivered isoprenaline at 0.1mg/kg/hr for 7 days. All data is expressed as arithmetic mean ± s.e.m. and was analysed by Student's unpaired "t"-test.

Compared with control rabbits, AD (7+2) rabbits had a significantly ($p<0.05$) reduced cardiac index (219±13 vs 336±40 ml/min/kg, respectively) which showed a further significant reduction after six weeks recovery (179±13 vs 255±10 ml/min/kg, respectively). Additionally after six weeks recovery there was a significant increase in total peripheral resistance index (0.549±0.028 vs 0.380±0.014 TPR units.kg, respectively). AD-treated rabbits were judged to be in moderate heart failure which was progressive. Receptor 'down- regulation' should lead to a parallel rightwards displacement of the concentration-response curve to a 'full' agonist and to a depression of the maximum of that to a 'partial' agonist (Kenakin & Ferris 1983). In IMOP rabbits, sensitivity to isoprenaline and the maximum response to Ro363 were significantly reduced compared with SMOP rabbits. In contrast, there was no significant difference in sensitivity to isoprenaline or maximum response to Ro363 in the other four groups of rabbits, nor were they different from the results obtained in SMOP rabbits (Table 1).

Table 1: Sensitivity to isoprenaline and Ro363 in control (C), adriamycin-, saline- and isoprenaline-pretreated rabbits.

TREATMENT GROUP	SMOP	IMOP	C (7+2)	AD (7+2)	C (7+6)	AD (7+6)
Isoprenaline pD_2	7.78 ±0.14	6.95* ±0.06	7.51 ±0.08	7.37 ±0.08	7.52 ±0.09	7.49 ±0.10
Ro363 as % Isoprenaline max.	66 ±9	9* ±1	56 ±6	48 ±4	55 ±5	57 ±7
n	4	4	8	8	6	9

* significantly different from SMOP group.

Thus although cardiac ß-adrenoceptors can be functionally 'down-regulated' in the rabbit, this does not happen in adriamycin-induced heart failure. This confirms and extends the work of Woodcock et al. (1988) which showed that the cardiac ß-adrenoceptor density and adenylate cyclase activity in the AD-treated rabbit were unaltered.

Bristow, M.R. et al. (1982), N. Engl. J. Med., 307 (4), 205-211.
Kenakin, T.P. and Ferris, R.M. (1983), J. Cardiovasc. Pharmacol., 5(1), 90-97.
McPherson, G.A. et al. (1984), Br. J. Pharmac., 82, 897-904.
Wanless, R.B. et al. (1987), Cardiovasc. Res., 21, 7-13.
Woodcock, E.A. et al. (1988), J. Mol. Cell. Cardiol., 20, 771-777.

β-ADRENOCEPTOR RESPONSIVENESS OF ISOLATED VENTRICULAR MYOCYTES FROM ISOPRENALINE- OR ADRIAMYCIN-TREATED RABBITS

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In order to study beta-adrenoceptor desensitisation in cardiac failure, we have been examining the contractile responses of single isolated myocytes from human and animal hearts. The present experiments were performed in order to determine whether altered contractile function of single cells reflected changes that had been observed in vivo or in intact muscle (Gurden et al, 1989). A model of chronic low-output failure was used in which rabbits were dosed for seven weeks with adriamycin, then left two weeks to recover (AD(7+2)) before removal of the heart for cell isolation (Wanless et al 1987). A separate group of rabbits had either saline-filled or isoprenaline-filled mini-osmotic pumps implanted (SMOP and IMOP, respectively). Pumps delivered isoprenaline at 0.1mg/kg/hr for 7 days. Myocytes were isolated from the hearts of these animals by perfusion with a low calcium solution (9uM), followed by treatment with collagenase and either protease or hyaluronidase. Isolated cells were superfused with a modified Krebs-Henseleit solution at 32^{o}C and electrically stimulated (0.5Hz, 5ms, suprathreshold voltage). Contraction amplitude was measured with a video camera/edge detection system linked to an inverted microscope (Harding et al., 1988), and is expressed as percentage change in cell length. Cumulative concentration-response curves to isoprenaline were constructed. All data is expressed as arithmetic mean ± s.e.mean. and was analysed by Student' unpaired t-test. When results were obtained from several cells from one animal, the data was pooled.

Cells from AD(7+2) (n=11) rabbits had a significantly lower maximum contraction amplitude when maximally activated with calcium than cells from controls (n=14) (11.1 ± 0.9% v 13.6 ± 0.5%, $p<0.05$). There was no evidence of specific beta-adrenoceptor desensitisation in the treated animals. Depression of the isoprenaline-induced contractile maximum was no greater than that observed in the presence of maximum calcium (11.6 ± 0.6% v 13.1 ± 0.4%, $p<0.05$). There was no difference in sensitivity to isoprenaline between AD(7+2) and controls (Table 1). In contrast, in IMOP rabbits (n=6), the sensitivity to isoprenaline (Table 1) and the maximum contraction amplitude during beta-adrenoceptor stimulation was significantly reduced compared to SMOP (n=5) (5.4 ± 1.1% v 12.0 ± 1.9%, $p<0.02$). Between-animal variation was significantly greater than between-cell variation for individual animals (eg EC50 for isoprenaline in AD(7+2) plus control (C), $p<0.002$, ANOVA).

Table 1. Sensitivity to isoprenaline in control, adriamycin-, saline- and isoprenaline-pretreated rabbits. Number of animals in brackets.

TREATMENT GROUP	SMOP	IMOP	C	AD(7+2)
Isoprenaline pD_2	8.10 ± 0.10(5)	6.65* ±0.47(5)	8.12 ±0.08(11)	8.14 ±0.10(11)

*significantly different from SMOP group, $p<0.05$.

Changes in beta-adrenoceptor sensitivity in single cells reflected those in papillary muscle; desensitisation was demonstrated in isoprenaline-treated but not in adriamycin-treated rabbits.

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Gurden, J.M., Drew, G.M., Sullivan, A.T., & Poole-Wilson, P.A. (1989) British Pharmacological Society, this meeting.
Harding, S.E., Vescovo, G., Jones, S.M., Gurden, J. & Poole-Wilson, P.A.. (1988), J. Mol. Cell. Cardiol. 20, 635-647
Wanless, R.B., Anand, I.S., Poole-Wilson, P.A. & Harris, P. (1987), Cardiovasc. Res., 21, 7-13.

EFFECTS OF CO-ADMINISTRATION OF XAMOTEROL AND ISOPRENALINE ON RAT VENTRICULAR β-ADRENOCEPTOR DOWN-REGULATION

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Xamoterol (X) is a β1 adrenoceptor (AR) selective partial agonist recently introduced for the treatment of cardiac failure. Chronic therapy with this agent in rats does not lead to down-regulation of cardiac βAR in contrast to treatment with the full agonist isoprenaline (I) (Barnett & Maguire, 1986). We have studied the effect of co-administration of X and I on β AR down-regulation and recovery in order to simulate the situation of the use of X in chronic cardiac failure which is associated with reduced β AR density (Bristow et al, 1982).

Using subcutaneous osmotic minipumps I (40ug/kg/hr) and X (600ug/kg/hr) were administered separately, simultaneously or successively for various time periods to groups of AS rats (200-220g). Rats were sacrificed after 72hrs treatment with I or X alone and after 24hrs and 72hrs treatment with I plus X to assess effects on β AR down-regulation. For recovery experiments I loaded pumps were removed after 72 hrs infusion and rats were either simply resutured (control) or given further treatment with X. Animals were finally sacrificed at 24hrs or 96hrs after 2nd treatment. All X treated animals received a single IP loading dose of X (10mg/100g). Control animals for each group were sham operated.

Ventricular membranes were prepared using previously described methods (Baker et al, 1980). Maximal binding capacities (Bmax) of ventricular β1 and β2 AR were obtained by saturation analysis of 125I-pindolol binding using CGP 20712A as previously described (Lü & Barnett, 1988). Blood samples for X levels were taken by cardiac puncture under anaesthesia prior to sacrifice in representative animals from each group of experiments.

Compared to control total Bmax was reduced by approximately 30% by 24hrs and 50% by 72hrs I treatment but was unaffected by X alone (Table). Co-administration of X did not affect the rate or extent of down-regulation by I on either βAR subtype.

Bmax (fmol/mg protein) of 125I-pindolol binding to rat ventricular membranes after 72hrs subcutaneous infusions (mean ± SEM)

	Control n = 9	Xamoterol n = 3	Isoprenaline n = 3	Isoprenaline & Xamoterol n = 3
Total	20.4 ± 1.2	19.6 ± 0.9	10.0 ± 0.5*	11.7 ± 0.8*
β1	15.0 ± 1.1	13.5 ± 0.9	8.2 ± 0.5*	9.6 ± 0.9*
β2	5.3 ± 0.2	6.2 ± 0.2	1.8 ± 0.1*	2.0 ± 0.2*

* significantly different from control, $P < 0.05$

Following removal of the minipumps recovery of β1 AR in control animals was slower than β2 AR but was complete for both subtypes by 96hrs. The rate or extent of recovery of both subtypes was not significantly affected by co-administration of X.

Plasma X levels measured at the end of all treatment periods (range 100-300 nM) were similar to those producing maximal functional response in rat heart (in vitro) suggesting full receptor occupancy by the partial agonist during the infusion experiments. These results indicate that X does not enhance βAR down-regulation induced by I or impede recovery to the control state following treatment. It seems likely therefore that chronic X therapy in cardiac failure would similarly not adversely effect AR regulation in the human myocardium.

Bristow M, Ginsberg R et al 1982 New Engl J Med, 307, 205-211
Baker SP, Boyd HM, Potter LT 1980 Brit J Pharmacol, 68, 57-63
Lü X, Barnett DB 1988 Brit J Clin Pharmacol, 95, 820P

EFFECTS OF FLOSEQUINAN, A NOVEL ARTERIOVENOUS DILATING AGENT, AND ITS METABOLITE ON BOVINE HEART PHOSPHODIESTERASE ACTIVITY

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Flosequinan, a novel hypotensive agent (Sim et al 1988), is currently under clinical trial for use in both hypertension and heart failure. The vasorelaxant activity of flosequinan has been shown to be associated with increases in cGMP (Allcock et al 1988) and cAMP (unpublished) in rat aortic strips. In isolated guinea-pig atria flosequinan has weak inotropic activity (Yates et al 1988) and in guinea-pig ventricle 6.5 x 10^{-4}M flosequinan produced a 16% and a 24% increase in cAMP and cGMP respectively (unpublished). The sulphone metabolite of flosequinan, BTS 53 554 has a similar pharmacological profile and may be responsible for a major part of the clinical response to flosequinan. In this study the possibility that flosequinan and BTS 53 554 cause an inhibition of cyclic nucleotide phosphodiesterase (PDE) activity was investigated.

A commercial preparation (Sigma) containing all three isoenzymes of PDE from bovine heart was used and activity measured by the method of Thompson et al (1979) over a 10 minute incubation period at 1 μM substrate concentration pH 8.0 and 30°C. Drugs were dissolved in DMSO giving a final assay DMSO concentration of 2.5%. This concentration of DMSO inhibited enzyme activity by 10%. Each drug was tested over the range 1 x 10^{-7}M to 5 x 10^{-3}M and its effect upon both cAMP PDE and cGMP PDE activity measured.

Table 1 Concentration causing 10% inhibition of cAMP and cGMP PDE (IC10) (n=6).

Drug	cAMP PDE		cGMP PDE	
	IC10	Potency	IC10	Potency
Flosequinan	1.0 x 10^{-4}M	1	8.5 x 10^{-5}M	1
BTS 53 554	2.0 x 10^{-4}M	0.5	2.4 x 10^{-4}M	0.3
IBMX	2.3 x 10^{-7}M	434	3.0 x 10^{-7}M	283
Milrinone	8.0 x 10^{-7}M	125	1.1 x 10^{-5}M	8
M & B 22948	5.0 x 10^{-6}M	20	7.5 x 10^{-7}M	113

At IC10 values, IBMX, a non selective PDE inhibitor, and both flosequinan and BTS 53 554 were equipotent against cAMP PDE and cGMP PDE but milrinone, a selective PDE III inhibitor and M&B 22 948 a selective PDE I inhibitor showed selectivity towards cAMP PDE and cGMP PDE respectively (Table 1).

BTS 53 554 was a slightly less potent PDE inhibitor than flosequinan but both compounds were very much less potent than IBMX or milrinone as cAMP PDE inhibitors and IBMX or M&B 22 948 as cGMP PDE inhibitors.

Flosequinan and its major metabolite thus appear to be non selective PDE inhibitors with low potency in this enzyme system. The implication of these findings in the mode of action of flosequinan are difficult to interpret from the use of a mixed PDE preparation. Moreover at concentrations similar to those seen in the clinic, flosequinan (10^{-5}M) had little or no effect on cAMP PDE or cGMP PDE whereas milrinone (10^{-6}M) caused a significant inhibition of cAMP PDE ($p<0.001$). The effects of flosequinan and BTS 53 554 will be further investigated using isolated isoenzymes from cardiac and smooth muscle.

Sim, M.F. et al (1988) Br. J. Pharmacol., 94 371-380.
Allcock, A.A. et al (1988) Br. J. Pharmacol. 94 430P.
Yates, D.B. et al (1988) Br. J. Pharmacol. 95 711P.
Thompson, W.J. et al (1979) Adv. Cyc. Nuc. Res., 10 69-92.

FLOSEQUINAN-INDUCED VASODILATATION OF HUMAN RESISTANCE VESSELS IS ASSOCIATED WITH DECREASED CALCIUM SENSITIVITY

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Flosequinan (7-Fluoro-1-Methyl-3-Methylsulphinyl-4-Quinolone) is a new vasodilator for use in heart failure and hypertension. Its exact mechanism of action is uncertain but it is associated with increased levels of cyclic GMP in rat aorta. Resistance vessels (mean internal diameter 217 $\pm$ 9 um, n=41) were isolated from biopsies of subcutaneous fat from 26 normotensive subjects undergoing routine surgery (mean blood pressure 132/83 $\pm$ 3/2 mmHg, mean age 47 $\pm$ 2 years, 20 females) and mounted as ring preparations on a myograph capable of measuring isometric tension.

Cumulative dose response characteristics were obtained for flosequinan (10^{-5}-10^{-2} mol/l) against contractions produced by noradrenaline (3 umol/l) in the presence and absence of the soluble guanylate cyclase inhibitor methylene blue (10^{-5} mol/l). Flosequinan produced dose dependent relaxation in vessels contracted by noradrenaline, the pD_2 (-log[ED50(mol/l)]) for flosequinan being 3.91 $\pm$ 0.11. The sensitivity of the vessels to flosequinan increased with vessel size ($r=0.622$, $P<0.002$, n=19). The soluble guanylate cyclase inhibitor methylene blue did not inhibit flosequinan induced relaxation (pD2 3.91 $\pm$ 0.2 P NS, n=7).

Vessels were depleted of calcium by repeated stimulation with either noradrenaline 5 umol/l or potassium 125 mmol/l in the presence of EGTA 0.1 mmol/l and zero calcium buffer. Dose response characteristics were then obtained for calcium in vessels activated with either noradrenaline or potassium alone and then in the presence of flosequinan 10^{-5}, 10^{-4} and 10^{-3} mol/l. The pD2 for calcium was 4.38 $\pm$ 0.11 during noradrenaline activation and 3.77 $\pm$ 0.08 during potassium activation. Increasing concentrations of flosequinan progressively depressed the calcium sensitivity of both noradrenaline and potassium activated vessels (pD_2 flosequinan 10^{-4} mol/l 4.1 $\pm$ 0.15, $P<0.002$, n=7, noradrenaline activation; 3.47 $\pm$ 0.12, $P<0.001$, n=8, potassium activation).

Thus flosequinan produced dose dependent relaxation of human resistance vessels and the sensitivity of the vessels to flosequinan was related to the vessel diameter. Although the action of flosequinan is associated with elevated levels of cyclic GMP in large vessels, inhibition of soluble guanylate cyclase in these vessels had no effect on the response to flosequinan. We conclude that stimulation of soluble guanylate cyclase does not explain the mechanism of action of flosequinan in these resistance vessels. Flosequinan may however cause relaxation by decreasing calcium sensitivity.

DOSE-RELATED EFFECTS OF COCAINE HYDROCHLORIDE ON CANINE NASAL VASCULAR AND AIRWAY RESISTANCES

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Cocaine users who frequently snort the drug usually suffer from symptoms of rhinitis medicamentosa such as nasal congestion, edema and rhinorrhea (Schwartz et al. 1989). It is not known whether such symptoms are due to the action of the drug on the nasal mucosa or the effects of adulterants which are often added to increase the profit to the cocaine distributor. The work presented here demonstrates the dose-related actions of cocaine in the control of nasal vascular and airway resistances.

In sodium pentobarbitone anaesthetized dogs (n=6), nasal vascular resistance (R_{nv}) was measured by constant-flow perfusion of the terminal internal maxillary artery, the main arterial supply to the nasal mucosa; nasal airway resistance (R_{na}) was assessed by monitoring the transnasal airway pressure with constant airflow through each of the nasal cavities (Lung & Wang, 1984). Cocaine hydrochloride, in doses ranging from 0.05 to 5000 $\mu g\ kg^{-1}$, was infused intra-arterially via the perfusion circuit at the rate of 0.1ml min^{-1}.

Table 1

Doses of cocaine ($\mu g\ kg^{-1}\ min^{-1}$)	R_{nv} (% change)	R_{na} (% change)
0.05	5±2.3	-5±2.5
0.5	20±3.0*	-15±2.4*
5.0	75±4.5*	-30±3.6*
50	85±5.6*	-60±4.5*
500	-20±3.9*	+5±5.5
5000	-60±4.8*	+13± 4.0*

Results are means±S.E.M. of % change from controls. *P<0.05.

Cocaine in low doses (0.05 to 50 $\mu g\ kg^{-1}\ min^{-1}$) increased R_{nv} and decreased R_{na} whereas in high doses (500 to 5000 $\mu g\ kg^{-1}\ min^{-1}$) decreased R_{nv} and increased R_{na}. The results suggest that cocaine in low doses causes constriction of both resistance and capacitance vessels; however, in high doses, the drug causes dilatation of both resistance and capacitance vessels. Since nasal blood vessels are under predominant sympathetic control (Lung and Wang, unpublished data), the vasoconstriction effect is most likely to be due to potentiation of response to circulatory catecholamines (Ritchie and Greene, 1985). The vasodilatation effect is probably the result of its well-known local anaesthetic action.

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Lung, M.A. and Wang, J.C.C. (1984). J. Pharmacol. Exp. Ther. 228, 215-219.
Ritchie, J.M. and Greene, N.M. (1985). In: The Pharmacological Basis of Therapeutics, ed. Gilman A.G. et al., pp.302-322, New York: Macmillan.
Schwartz, R.H. et al (1989). Arch. Otolaryngol. 115, 63-64.

DOSE-RELATED EFFECTS OF ADENOSINE 5'-TRIPHOSPHATE ON CANINE NASAL VASCULAR AND AIRWAY RESISTANCES

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Sympathetic nervous stimulation causes nasal mucosal vasoconstriction and a decrease in nasal airway resistance whereas parasympathetic nervous stimulation causes nasal mucosal vasodilatation and an increase in nasal airway resistance. The sympathetic response is mediated via the alpha-adrenergic receptors for the resistance vessels but via some non-adrenergic and non-cholinergic mechanism(s) for the capacitance vessels. The parasympathetic response is mediated via non-adrenergic and non-cholinergic mechanism(s) for both resistance and capacitance vessels (Lung and Wang, 1987). In 1972, Burnstock proposed that ATP was released as the principal neurotransmitter from some non-adrenergic and non-cholinergic nerves. Recently, ATP has been shown to act as a co-transmitter in sympathetic and parasympathetic neurotransmission (Burnstock, 1986). Work presented here demonstrates the action of ATP in the control of nasal vascular and airway resistances.

In sodium pentobarbitone anaesthetized dogs (n=6), nasal vascular resistance (R_{nv}) was measured by constant-flow perfusion of the terminal internal maxillary artery, the main arterial supply to the nasal mucosa; nasal airway resistance (R_{na}) was assessed by monitoring the transnasal airway pressure with constant airflow through each of the nasal cavities (Lung & Wang, 1984). ATP, in doses of 0.005, 0.05, 0.5, 5 and 50 $\mu g\ kg^{-1}\ min^{-1}$, was infused intra-arterially via the perfusion circuit at the rate of 0.1 $ml\ min^{-1}$.

Table 1

Doses of ATP ($\mu g\ kg^{-1}\ min^{-1}$)	R_{nv} (% change)	R_{na} (% change)
0.005	-2±1.4	0
0.05	-12±2.8*	2±1.6
0.5	-26±2.9*	6± 1.8*
5	-36±3.1*	10±2.1*
50	-49±3.5*	-9±2.0*

Results are means±S.E.M. of % change from controls. *P<0.05.

ATP in low doses (0.005 to 5 $\mu g\ kg^{-1}\ min^{-1}$) decreased R_{nv} but increased R_{na} whereas the highest dose (50 $\mu g\ kg^{-1}\ min^{-1}$) decreased both R_{nv} and R_{na}. The results suggest that ATP in low doses causes dilatation of both resistance and capacitance vessels (i.e. the venous sinusoidal vessels); however, the purine in high dose causes, in addition to the above actions, dilatation of the outflow venous vessels. The vasodilatatory action of ATP may be due to activation of inhibitory purinoceptors. However, the role of ATP in the autonomic control of nasal blood vessels requires further investigation.

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Lung, M.A. and Wang, J.C.C. (1984). J. Pharmacol. Exp. Ther. 228, 215-219.
Lung, M.A. and Wang, J.C.C. (1987). J. Physiol. 394, 61P.
Burnstock, G. (1972). Pharmacol. Rev. 24, 509-581.

LACK OF SELECTIVITY OF α,β–METHYLENE ATP IN REDUCING SYMPATHETIC PRESSOR RESPONSES OF THE PITHED RAT

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The stable analogue of ATP, α,β-methylene ATP (mATP), reduces selective ly responses of various isolated smooth muscle preparations to exogenously applied ATP *in vitro* by desensitising the P_{2x}-purinoceptors (Kasakov & Burnstock, 1983; Meldrum & Burnstock, 1983). Thus mATP has been used extensively to examine cotransmission by noradrenaline (NA) and ATP in a variety of sympathetically innervated tissues, including vascular smooth muscle (Machaly et al., 1988). It has been reported recently that a novel administration regime of mATP in the pithed rat was able to reduce selectively the portion of the sympathetic pressor response to spinal stimulation which was resistant to α-adrenoceptor antagonists (Bulloch & McGrath, 1988). In the present study the selectivity of this *in vivo* desensitisation procedure was tested using a variety of pressor agents.

Sprague-Dawley rats (262±6g, n=35) were pithed under ether anaesthesia and ventilated with O_2 (48 strokes/min, 1ml/100g). The pithing rod was arranged for electrical stimulation of the T6-T8 spinal outflows (Gillespie *et al.*, 1970). The left carotid artery was cannulated for monitoring of blood pressure and the external jugular veins for administration of drugs. Temperature was maintained at 37°C. Gallamine (10mg/kg+10mg.kg^{-1}.hr^{-1}) and propranolol (1mg/kg) were then administered. Pressor responses to NA (0.1μg/kg), angiotensin II (0.1μg/kg), vasopressin ($7x10^{-5}$i.u./kg), and spinal stimulation (5Hz, 0.1ms pulse duration, 50V for 5s) were obtained at 10min intervals, before and after the mATP desensitisation procedure. The mATP was administered i.v. as 3 doses of 0.05mg/kg followed by 5 doses of 0.5mg/kg, each dose being separated by 1min. In some experiments the pressor response to NA and spinal stimulation were examined in the same preparation. The doses of the pressor agents were chosen to produce a pressor response similar in magnitude to that produced by spinal stimulation.

The magnitude of the pressor response to spinal stimulation at 2min post-desensitisation with mATP was reduced to 21.2±3.2% of the control value ($P<0.01$, Students paired t-test, n=6), confirming the findings of Bulloch & McGrath (1988). The pressor response to NA (0.1μg/kg), at the same time point, was significantly reduced, to 27.2±3.4% of control ($P<0.01$, Students paired t-test, n=6). In preparations where responses to both spinal stimulation and NA were examined, the mATP desensitisation procedure produced a similar reduction of both responses. The mATP desensitisation procedure also reduced the magnitude of responses to angiotensin II and vasopressin to a similar degree. The pressor responses to all stimuli recovered in parallel towards control values and the third and consecutive responses to NA, post mATP desensitisation (22min & 32min), were not significantly different from the control response.

Thus, although mATP selectively reduces responses to ATP *in vitro*, the desensitisation procedure produced a non-selective depression of pressor responses *in vivo*.

Bulloch J.M. and McGrath J.C. (1988) Br. J. Pharmacol. 95 695-700
Gillespie, J.S., MacLaren, A. and Pollock, D. (1970) Br. J. Pharmacol. 40 257-267
Kasakov, L. and Burnstock, G. (1983) European J. Pharmacol. 86 291-294
Machaly, M., Dalziel, H.H. and Sneddon, P. (1988) European J. Pharmacol. 147 83-92
Meldrum, L.A. and Burnstock, G. (1983) European J. Pharmacol. 92 161-163
H.H.D. is an S.E.R.C. Research Student. G.A.G. was an M.R.C. Research Student. This work was supported by S.H.E.R.T. and the Wellcome Trust.

DPCPX AND PD 115,199 AS DISCRIMINATORY TOOLS FOR DIFFERENTIATING ADENOSINE RECEPTORS IN GUINEA-PIG TISSUES

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Three classes of extracellular adenosine receptor (A_1-, A_{2a}- and A_{2b}) have been delineated from studies of adenylate cyclase activity in striatal membranes and of cyclic AMP accumulation in cerebral cortical slices (Daly, 1985). The xanthine-derived compounds 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and PD 115,199 have both been shown to be high affinity adenosine receptor antagonists, with DPCPX able to distinguish between the A_1- and A_{2a}- classes of adenosine receptors (Bruns et al., 1987a,b). In this communication we report the antagonist potencies of these two compounds on the A_{2b}-receptor in guinea-pig cerebral cortex, the A_1- receptor in guinea-pig ileum (Paton, 1981) and A_2-receptor in guinea-pig trachea (Farmer et al.,1988).

The effect of DPCPX and PD 115,199 on the cyclic AMP response to 2-chloroadenosine or 5'-N-ethylcarboxamidoadenosine (NECA) was measured in guinea-pig cerebral cortical slices as described previously (Alexander et al., 1989). Antagonist equilibrium dissociation constants were also estimated from antagonism of the NECA-induced relaxation of pre-contracted (histamine, 0.1mM) tracheal rings (Farmer et al., 1988), or the adenosine-stimulated inhibition (in the presence of 1μM dipyridamole) of the transmural response (stimulus parameters: 0.1 Hz, 1 msec pulse width, 1-25 V) of guinea-pig ileum.

The apparent equilibrium dissociation constants (K_D nM) determined for DPCPX and PD 115,199 were as follows:

	DPCPX	PD 115,199
Ileum	0.30 ± 0.02 (9)	21.1 ± 1.5 (6)
Trachea	3100 ± 400 (7)	28.8 ± 3.9 (9)
Cerebral Cortex	163 ± 35 (6)	395 ± 76 (5)

DPCPX was approximately four orders of magnitude more potent at the ileal A_1-receptors than the tracheal A_2-receptor, but showed intermediate affinity for the A_{2b}-receptor of cerebral cortex. In contrast, PD 115,199 showed high affinity for both peripheral adenosine receptors, with a K_D value similar to that reported for the A_{2a}-receptor (16nM; Bruns et al. 1987b), while displaying a relatively lower affinity for the cortical A_{2b}-receptor. These data indicate that the tracheal receptor may be similar to the brain A_{2a} subclass. We conclude that DPCPX retains the ability to discriminate between adenosine receptor classes in the periphery, while PD 115,199 may prove useful as a discriminatory tool for the differentiation of A_2 receptor subclasses in both central and peripheral tissues.

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Alexander, S.P. et al. (1989) Br. J. Pharmacol. 96, 135P.
Bruns, R.F. et al. (1987a) Naun. Schm. Arch. Pharmacol. 335, 59-63
Bruns, R.F. et al. (1987a) Naun. Schm. Arch. Pharmacol. 335, 64-69
Daly, J.W. (1985) Adv. Cycl. Nucl. Prot. Phos. Res. 19, 29-46.
Farmer, S.G. et al. (1988) Br. J. Pharmacol. 95, 371-378.
Paton, D.M. (1981) J. Auton. Pharmacol. 1, 287-290.

IONIC MODULATION OF ADENOSINE HYPERPOLARISATION OF RAT SYMPATHETIC GANGLIA

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Adenosine hyperpolarises isolated rat superior cervical ganglia (Brown et al, 1979; Connolly & Stone, 1988) and inhibits calcium dependent action potentials recorded from postganglionic neurones. We have now examined the effects of altering extracellular calcium, magnesium and potassium levels on the hyperpolarising responses to adenosine (AD) and 2-chloroadenosine (2CA). DC potentials were recorded from the internal carotid nerves of desheathed ganglia in vitro by a modification of the method of Bowery and Tulett (1975), employing a grease gap. Ganglia were superfused (2-2.5 ml/min) with medium at 25°C pre-equilibrated with 5% CO_2 in oxygen. The composition of the normal medium was (mM): NaCl 125, KCl 5, KH_2PO_4 1, $CaCl_2$ 2.5, $MgSO_4$ 1, $NaHCO_3$ 25, D-glucose 10. For nominally potassium-free medium KCl was omitted and KH_2PO_4 replaced by NaH_2PO_4. A 40 minute interval was allowed between 2 minute applications of AD (10-1000 μM) or 2CA (0.3-10 μM).

Table 1 Amplitude of hyperpolarising responses to AD, 10 μM (μV)

	Calcium (mM)			
Magnesium (mM)	5	2.5	0.1	0
0		55 ± 6 (16)	61 ± 6 (8)	144 ± 36 (6)**
1	20 ± 4 (10)*	46 ± 3 (43)	86 ± 10 (12)*	93 ± 15 (12)*
10	35 ± 5 (8)	37 ± 9 (3)	60 ± 11 (4)**	60 ± 11 (6)**

* P<0.01 compared with 2.5 mM calcium
** P<0.01 compared with 1 mM magnesium
All values are mean ± s.e.m. (M)

In low calcium media (1 mM Mg) the responses to both AD and 2CA were enhanced and prolonged. Conversely in 5 mM calcium the responses were significantly reduced compared with controls. Changing the concentration of magnesium ions from 1 to 10 mM in low calcium media reduced significantly the sensitivity to adenosine. There was a tendency for AD responses to be reduced in high 12 mM) potassium (38 ± 11 μV, n = 5, for AD 100 μM) compared with controls (58 ± 9 μV, n = 8) though this was not significant. In potassium free medium the response to AD was increased to 130 ± 21 μV (n = 4, P<0.01).

The results do not reveal the same dependence on magnesium ions of postsynaptic AD responses which has been noted for presynaptic actions (Bartrup & Stone, 1988), and they also suggest that calcium ions are not important in the hyperpolarising actions of AD on this ganglion. The changes seen with alterations of potassium content however would be consistent with an increase of potassium conductance by AD.

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Bartrup, J.T. & Stone, T.W. (1988) Brain Res. 463, 374-379
Bowery, N.G. & Tulett, D. (1975) J.Physiol. 246, 20P
Brown, D.A. et al (1979) J.Physiol. 290, 441-451
Connolly, G.P. & Stone, T.W. (1988) Br.J.Pharmac. 95, 841P

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EXAMINATION OF WHETHER ADENOSINE INHIBITS THE POSITIVE CHRONOTROPIC RESPONSE TO FIELD-STIMULATED NORADRENALINE RELEASE OF GUINEA-PIG ISOLATED ATRIA

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Exogenously added adenosine has been shown to inhibit the release of noradrenaline from isolated perfused whole hearts (Wakade & Wakade, 1982; Richardt et al. 1987), presumably by a presynaptic action on sympathetic neurones. Adenosine released endogenously by ischaemia may also be inhibitory (Richardt et al. 1987). It is possible that the measurement of released noradrenaline from whole hearts is influenced by adenosine's concomitant effects on coronary flow. To overcome this possible limitation, we have used isolated atria to examine the possible effects of exogenous and endogenous adenosine on the chronotropic responses to neurotransmitter released by field stimulation.

Spontaneously beating paired guinea-pig atria were set up between platinum electrodes in 25ml organ baths. Tissues were constantly perfused (7ml min^{-1}) with Krebs bicarbonate solution containing atropine (1μM) at 38°C and gassed with 5% CO_2 in O_2. Field stimulation (2 x threshold voltage, 1ms, 1-10Hz) for 10-30s alone or alternating with bolus doses of noradrenaline (NA, 1μg) were applied at regular intervals. Rate of contraction was recorded from the isometric tension signal. After obtaining reproducible control responses, adenosine or antagonists were added to the perfusion solution.

Immediately after field stimulation (S) biphasic positive chronotropic responses were obtained. The initial peak was propranolol-sensitive (1.2μM) and therefore due to release of NA onto atrial β-adrenoceptors. The secondary plateau was removed by morphine (12.2μM) and attributed to release of an unidentified transmitter (Wilson, 1981). After 35 min perfusion with adenosine (3.75μM), the peak rate response to S (1Hz, 20s) was inhibited by only 5.6±0.1% and that to NA by 32.1±22.4%. The same result was obtained with a higher concentration (11.25μM) which was just above threshold for direct negative chronotropy. In the presence of morphine to examine only the noradrenaline-mediated response, adenosine (3.75 and 11.25μM) inhibited the responses to S by 11.1±3.2 and 9.4±9.3% and the responses to NA by 51.3±7.4 and 53.4±5.8% respectively. Since NA was also affected, the small inhibitions of S are due entirely to POST-synaptic effects of adenosine. The response to S in the presence of propranolol was unaffected by adenosine (3.75μM).

Adenosine deaminase (0.3 U ml^{-1}) did not affect responses to S (1 or 10Hz) or NA. Therefore neither exogenous nor endogenous adenosine appear to exert an inhibitory effect on the atrial responses to field-stimulated noradrenaline release. This disagrees with results from whole hearts but confirms results on noradrenaline release from mouse atria (Johnson et al. 1987).

Possible means for the lack of effect of adenosine were examined briefly. Neither neuronal uptake of NA nor autoregulatory feedback via α-adrenoceptors were responsible since adenosine was also ineffective in the presence of DMI (1.13μM) alone or with idazoxan (96nM). The technique was capable of detecting pre-synaptic inhibition since clonidine (0.13μM) abolished the response to S.

Johnson, H. et al. (1987) Br. J. Pharmac. 91, 773-781
Richardt, G. et al. (1987) Circ. Res. 61, 117-123
Wakade, A.R. & Wakade, T.D. (1982) Neuroscience 7, 2267-2276
Wilson, A.N.A. (1981) Br. J. Pharmac. 73, 294P.

EFFECT OF 8-PHENYLTHEOPHYLLINE AND ENPROFYLLINE ON THE HYPERAEMIC RESPONSE TO EXERCISE IN THE GRACILIS MUSCLE.

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The release of adenosine has been detected from the skeletal muscle of some species during exercise (Bockman et al., 1975; Ballard et al., 1987; Bockman & MacKenzie, 1983). In the present study we have investigated the contribution of adenosine to exercise hyperaemia by examining the effects of 8-phenyltheophylline (8PT), an adenosine receptor antagonist, on this vasodilator response. The effects of another xanthine (enprofylline) which has low affinity for adenosine receptors were also studied as a control.

Cats(2.3-3.0kg) were anaesthetised with sodium pentobarbital (45mg.kg^{-1} intra thoracic), maintained under anaesthesia by a continuous pentobarbital infusion (approximately 0.3mg.kg^{-1}min^{-1}) and artificially ventilated. Systemic blood pressure was measured in the right common carotid artery. The right gracilis muscle was vascularly isolated, connected to an isometric force transducer and blood flow measured with an electromagnetic flow probe. The obturator nerve was stimulated at 5 volts, 0.1 ms duration at 1.0 Hz for 20 mins and the muscle blood flow and tension measured. This was performed twice in each cat, once with vehicle (50% P.E.G./50% 0.1M NaOH, 0.1ml.min^{-1}) and repeated 40 mins into an infusion of either 8PT (n=5) or enprofylline (n=4) at 2.7x10^{-7} mol. kg^{-1}min^{-1}(i.v.).

8PT significantly reduced the hypotensive response to 2 chloroadenosine (10μg.kg^{-1}) from 37.0±0.8 to 3.4±1.4mmHg (P<0.001), whilst the response was unaffected by enprofylline (30.0±4.5 to 31.2±3.7mmHg).

The active muscle tension produced at 20 mins was unchanged at 6.5±0.6 and 7.0±0.8kg.100g muscle weight^{-1} pre and during 8PT respectively, and 8.0±1.1 and 8.1±1.3kg.100g muscle weight^{-1} pre and during enprofylline respectively.

Table 1 Gracilis muscle vascular conductance at rest and 20 mins exercise (units ml.min^{-1} 100g^{-1}.mmHg^{-1})

	VEHICLE	8PT	VEHICLE	ENPROFYLLINE
REST	0.160±0.026	0.147±0.042	0.212±0.064	0.201±0.007
EXERCISE	0.628±0.110	0.317±0.032	0.605±0.104	0.678±0.067
	P<0.05		N.S.	

All values quoted are mean ± s.e. of mean.

Conductances were not significantly different at rest (Table 1). During all exercise periods muscle conductance increased (P<0.05). However during 8PT infusion the conductance at 20 mins of exercise increased to only 46±6% of that recorded during vehicle infusion (P<0.002) whilst enprofylline had no significant effect (17±17% higher).

These results indicate that adenosine can account for approximately 50% of the vasodilatation during 1.0 Hz twitch contractions of the cat gracilis muscle.

Ballard, H.J. Cotterell, D. & Karim, F. (1987) J. Physiol. 387, 401-403.
Bockman, E.L. Berne, R.M. & Rubio, R. (1975) Pflug Arch. 355, 299-241.
Bockman, E.L. & MacKenzie, J.E. (1983) Am. J. Physiol. 244, H552-H559.
Fuchs, B.D., Gorman,M.W. & Sparks, H.V. (1986) Proc.Soc.Exp.Biol.Med. 181, 364-370.

EFFECTS OF PROPOFOL ON MEMBRANE CURRENTS AND CONTRACTION IN SINGLE MYOCYTES ISOLATED FROM GUINEA-PIG VENTRICLE

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Propofol anaesthesia has been shown to produce myocardial depression *in vivo* (Stephan *et al.*, 1986). Action potentials, membrane currents and contractions of guinea-pig ventricular myocytes were recorded in order to investigate possible mechanisms of action of propofol.

Cells were isolated by enzymic dispersion and superfused in an oxygenated balanced salt solution (36 °C; pH 7.4) containing 2.5M $CaCl_2$. Cells were impaled with glass microelectrodes containing 0.5M K_2SO_4. Resting membrane potentials were -70 to -90mV.

Propofol, in a dose-dependent fashion, depressed the plateaux and shortened the durations of action potentials induced by brief current stimuli. 50 μM propofol reduced the time taken for 20% repolarisation of the action potential by 16.3 ± 4.7% ($p<0.05$ compared with control, n=9). Cells were voltage-clamped with a single microelectrode system at a holding potential of -40mV, to inactivate the sodium current, and step depolarisations to 0mV were applied. A 200 ms depolarisation elicited currents carried by calcium. These were measured as the difference between the peak inward current and the current at the end of the step depolarisation. The associated contractions were measured using an optical method. The calcium activated current was recorded as a slow 'tail' of inward current on repolarisation, following a brief 20ms depolarisation. Student's paired t test was applied to the data. The amplitude of the membrane currents and associated contractions were depressed by propofol in a dose-dependent fashion (Table 1).

TABLE 1. Percent depression of membrane currents and contraction induced by propofol. Values are means ± s.e.m., n is the number of cells (n=6 for tail current values), *$p<0.05$ and **$p<0.005$ compared with control.

Concentration (μM)	25	50	100
n	17	19	15
Calcium current (nA)	4.5 ± 1.8*	25.0 ± 2.7**	45.6 ± 3.3**
Tail current (nA)	22.4 ± 4.6*	25.3 ± 5.4**	51.7 ± 7.4*
Contraction	23.2 ± 4.6*	37.0 ± 5.1*	57.0 ± 2.6**

The magnitude of the calcium current is thought to reflect calcium entry into the cell and the tail current may indicate the calcium transient (Mitchell *et al.*, 1987). The reduction of calcium entry by propofol and the possible influence on the cytosolic calcium transients may contribute to the observed shortening of the action potential duration and the negative inotropic effect of this anaesthetic. The actions of propofol on calcium currents appear to be similar to those of halothane (Terrar & Victory, 1988), but further investigations are required to determine whether there are any additional actions of propofol on intracellular calcium release.

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Mitchell, M. R., Powell, T., Terrar, D. A. & Twist, V. W. (1987) J. Physiol. (Lond). 391, 545-560.
Stephan, H., Sonntag, H., Schenk, H. D., Kettler, D. & Khambatta, H. J. (1986) Br. J. Anaesth. 58, 969-975.
Terrar, D. A. & Victory, J.G.G. (1988) Br. J. Pharmac. 94, 500-508.

EFFECTS OF AMIODARONE ON CALCIUM CURRENTS IN VENTRICULAR CELLS ISOLATED FROM GUINEA-PIG HEART

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Preliminary observations of acute effects of amiodarone have been reported (Nishimura et al, 1986). This has been further investigated in single muscle cells isolated from guinea-pig ventricle. Cells were isolated using collagenase and calcium currents were recorded from voltage-clamped cells in response to step depolarizations to 0 mV from a holding potential of -40 mV (Powell, Terrar & Twist, 1980; Mitchell, Powell, Terrar & Twist, 1987). Amiodarone at a final concentration of 20 μM was prepared in plasma-containing solution (following the procedure of Polster & Broekhuysen, 1976).

Figure 1A shows gradual development of the effects of amiodarone (filled circles) on the amplitude of calcium currents. Superimposed records of calcium currents before and during amiodarone are shown in Figure 1B. In five cells the decrease in amplitude of calcium currents amounted to 29 ± 8% (mean ± s.e.m.) after 2 min of exposure to amiodarone; decreases were observed in a further five cells exposed for shorter periods. Any reversibility of these effects was small and slow under the conditions of our experiments (total recording time less than 30 min). The effects of plasma alone (in equivalent concentrations to those used in the amiodarone experiments) are also shown in Figure 1A (open squares). After 2 min exposure to plasma, the amplitude of calcium currents was decreased in one cell and increased in five; the mean change was an increase of 19 ± 8%. The observed effect of amiodarone on calcium currents may contribute to its acute actions in patients.

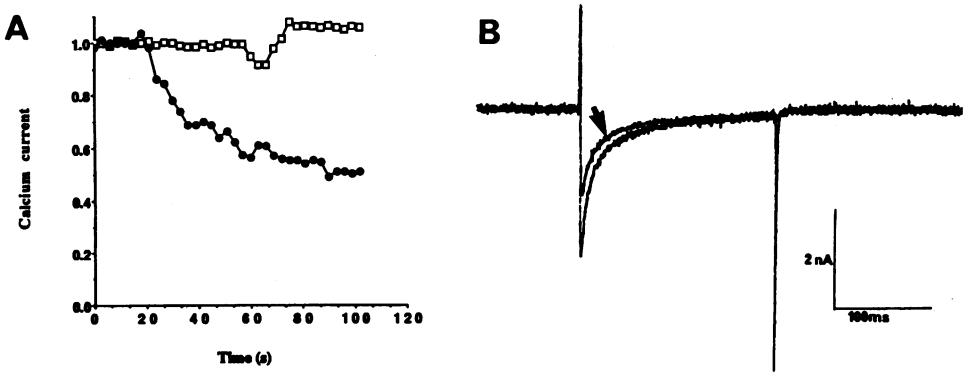


Figure 1. *A*: Development of the effect of amiodarone (20 μM; filled circles); effects of plasma alone in another cell are superimposed (open squares). The first six currents (0 to 18s) were recorded before switching solution, and current amplitudes over the whole period of recording were normalised to the mean over this period. *B*: Superimposed records of calcium currents in response to a step depolarization from -40 to 0 mV before and after two min exposure to amiodarone (arrow).

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Mitchell, M.R., Powell, T., Terrar, D.A. & Twist, V.W. (1987) J. Physiol. 391, 527-544.
Nishimura, M., Follmer, C.H., Cigan, A.L., Yeh, J.Z. & Singer, D.H. (1986) Circulation 74, Suppl. II, II-169.
Polster, P & Broekhuysen, J. (1976) Biochem. Pharmacol. 25, 131-134.
Powell, T. Terrar, D.A. & Twist, V.W. (1980) J. Physiol. 302, 131-153.

PATHWAYS FOR CALCIUM ENTRY DURING CALCIUM OVERLOAD IN CARDIAC MYOCYTES

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Calcium overload can be induced in ventricular myocytes by treatment with veratrine or veratridine (Ver Donk *et al.*,1986; Patmore, 1987), ouabain (Patmore *et al.*, 1989) or subjection to calcium paradox (Chapman and Rodrigo, 1988). These three methods depend on increasing sodium channel conductance, inhibiting sodium efflux and increasing sodium permeability (possibly through calcium channels) respectively. Thus it appears that raised levels of intracellular sodium precede calcium overload. It has been shown that certain calcium antagonists such as nicardipine and flunarizine can protect against calcium overload and that this property is not related to normal calcium channel blocking activity (Patmore *et al*, 1988). It has therefore been suggested that compounds which protect against ouabain-induced calcium overload do so by blocking sodium entry and thus prevent subsequent calcium entry : if nicardipine is applied after ouabain treatment (in the absence of extracellular calcium) and thus after sodium levels have risen on readmission of calcium, a rise in tonic tension (calcium overload) occurs (Patmore *et al.*,1989). We have examined whether this calcium entry occurs through voltage-activated calcium channels.

Embryonic chick myocytes were prepared as described by Patmore *et al.*,(1989) and calcium channel currents were recorded as described by Anderson *et al.*,(1988) We followed the experimental protocol described in fig 9 of Patmore *et al.*,(1989). Throughout this procedure calcium channel currents were evoked on depolarization from a holding potential of -80 mV to 0 mV. In untreated myocytes calcium channel currents of around 100 pA were observed. Myocytes were superfused with salt solution containing 0.5 μM TTX and 10 μM diltiazem to block normal sodium and calcium channels and to inhibit twitch contractures. They were subsequently treated with 10 μM ouabain in the absence of calcium. After 10 min, 5 μM nicardipine (a concentration which if applied before ouabain prevented calcium overload) was added to the superfusate, during this period there was no calcium channel current . After a further 10 min calcium (or barium) was readmitted, which causes a rapidly developing contracture (Patmore ,(1989). However, at this point in time the voltage-activated calcium channel current was blocked.

These data indicate that calcium entry causing calcium overload in cardiac myocytes is not via voltage-activated calcium channels but may be via non-specific cation channels or a modification of the activity of the sodium-calcium exchanger.

Anderson, A. J. *et al.*, (1988) J.Physiol., **407**, 103P
Chapman, R. A. & Rodrigo, G. C. (1988) J.Physiol., **401**, 26P
Patmore, L. (1987) J.Physiol., **381**, 90P
Patmore, L. *et al.*, (1988) Br. J. Pharmac., **93**, 71P
Patmore, L. *et al.*, (1989) Br. J. Pharmac., *(in press)*
Ver Donk, L. *et al.*, (1986) Life Sci., **38**, 765-772

EFFECTS OF ANTIOXIDANTS AND VERAPAMIL ON NORADRENALINE RELEASE AND CONTRACTURE IN THE ISCHAEMIC/REPERFUSED RAT HEART

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Myocardial ischaemia and reperfusion are associated with the development of arrhythmias and other forms of cardiac damage. The precise mechanisms contributing to this damage are poorly understood but local catecholamine release may have a role to play,possibly by enhancing the influx of calcium into cells. Recently a number of reports have shown that antioxidants and free radical scavenging drugs such as glutathione, superoxide dismutase(SOD), catalase(CAT) and mannitol(MAN) can protect the heart against ischaemic / reperfusion induced injury. This raises the possibility that these drugs may be working by reducing the local release of catecholamines from the heart. In order to examine this the effects of glutathione,and a combination of SOD/CAT/MAN ($10u.ml^{-1}$,$300u.ml^{-1}$,50mM respectively) on catecholamine release from the globally ischaemic/ reperfused rat heart have been studied. The effects of these agents on the contracture which develops following the onset of ischaemia have also been studied. Hearts from male Wistar rats were paced at 300bpm and perfused at 37°by the Langendorff technique at a flow rate of $10ml.min^{-1}$. Developed tension was measured by apex displacement at a resting tension of 2g. After a 20min. equilibration period ischaemia was induced for 60min. by reducing the flow rate to $1ml.min^{-1}$ and superfusing the heart with anoxic perfusate at$9ml.min^{-1}$. Reperfusion with oxygenated perfusate was performed for 5min. All drugs were added 10min. before the onset of ischaemia. Perfusate samples coming from the heart were assayed for noradrenaline(NA) by HPLC using electrochemical detection. Perfusion pressure,heart rate and developed tension were measured continuously. As glutathione(1mM) produced coronary dilation and a slight fall in developed tension a comparison was made with verapamil at a concentration (15nM) which produced similar changes in these parameters.

Table 1 Effects of drugs on the onset and time to maximal rise in the ischaemically induced contracture. mean+sem, *p<0.05cw control.

	n	Onset	Maximal
Control	6	7min40s±12s	15min20s±12s
GSH 0.1mM	4	9min±0s *	15min40s±20s
GSH 1.0mM	8	10min7s±21s *	18min7s±30s *
SOD/CAT/MAN	8	6min26s±17s *	13min53s±23s *
VERAPAMIL 15nM	4	11min30s±38s *	22min±1min13s *

From Table 1 it can be seen that GSH and verapamil delayed the onset and time to maximal contracture,in contrast the SOD/CAT/MAN mixture reduced these times. In control hearts basal levels of NA release (10.8+0.9pmol/min/g) were increased during ischaemia to a peak of 18+1.1pmol/min/g. During reperfusion peak release was 26.6+0.9pmol/min/g,this declined to the basal level within 5min. At the concentrations used none of the drugs had any significant effect on NA release during the ischaemic or reperfusion periods. This data indicates that the protective action of antioxidant drugs in the isolated rat heart which has been previously reported is not due to a reduction in the release of NA.

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A PHARMACOLOGICAL ACTION OF VITAMIN E UNRELATED TO ANTI-OXIDANT ACTIVITY

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The current view of vitamin E (alpha-tocopherol) as an in vivo anti-oxidant, that protects membranes from attack by free radicals generated within the cell, seems generally accepted (Lucy, 1978) and the biological potencies of tocopherols appear to be proportional to their anti-oxidant activity (Century & Horwitt, 1965).

In guinea-pig isolated hypoxic colon the vitamin was able to elicit dose-dependent contractions and restoration of mechanical activity (Kelly, 1986) and this model was used to test the hypothesis that the mechanism of action of vitamin E in hypoxic colon is related to its anti-oxidant potency. Segments of guinea-pig colon (2 cm long) were suspended vertically in tissue baths containing Krebs-Henseleit solution bubbled with a 95% O_2/5% CO_2 gas mixture. After a period of equilibration in this 'normoxic' environment the gas mixture was switched to 95% N_2/5% CO_2 and contractile activity was measured. The oxygen content of the suspending medium was also recorded. After 15 min the oxygen tension had fallen to less than 0.1% of its original level and during this 'hypoxic' period drugs were added to the bath and subsequent contractile activity was observed.

The tocopherols, alpha-, beta-, gamma- and delta-, differ in the number and position of methyl groups in the chromanol ring structure and this feature gives them different anti-oxidant capacities. Their potencies in eliciting contractions in 'hypoxic' colon were measured. Mean EC_{50} values (± s.e.mean) for (±)-alpha-tocopherol, (±)-gamma-tocopherol and (±)-delta-tocopherol were 71.7 ± 14.0, 12.7 ± 4.0 and 6.8 ± 2.7 μM respectively for 10 animals. This order of potency differs markedly from and indeed reverses the order for anti-oxidant capacities (Burton et al, 1983) and their biological potency using the normal bioassay method of foetal resorption.

In addition the anti-oxidant capacity of the chroman ring structure of the vitamin, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), is about 40% of that of vitamin E itself (Burton et al, 1983) but when tested using the colon model it was ineffective. Phytyl side chain structures, such as phytol, structurally resembling that of the vitamin but without apparent anti-oxidant capacity were potent stimulants of contractile activity. The results suggest that the stimulant action of vitamin E in the colon during hypoxia is unrelated to the vitamin's known anti-oxidant property.

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Burton, G.W., Cheeseman, K.H., Doba, T., Ingold, K.U. and Slater, T.F. (1983) Ciba Found. Symp. 101, 4-14.
Century, B. & Horwitt, M.K. (1965) Fed. Proc. 24, 906-911.
Kelly, M.J. (1986) J. Pharm. Pharmacol. 38, 66-68.
Lucy, J.A. (1978) in 'Tocopherol, Oxygen and Biomembrane': de Duve, E. & Hayaishi, O. (eds), pp 109-119. New York, Elsevier.

INCREASED RENAL RESPONSE TO FENOLDOPAM IN SPONTANEOUSLY HYPERTENSIVE RATS

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A defect in the renal dopaminergic system has been suggested to play a role in the pathogenesis of hypertension (Lee, 1986). We therefore studied the renal effects of fenoldopam (F), a selective DA-1 receptor agonist known to induce renal vasodilation and natriuresis, in both spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar-Kyoto rats (WKY). F was infused during 1 hour (1μg.kg-1.min-1) at an infusion rate of 12 ml.min-1, preceeded and followed by infusion of saline (12ml.min-1) during 30 min. Four timed 30 min urine collections were done to assess the diuretic and natriuretic effects. Renal blood flow (RBF) was measured with an electromagnetic flow meter (SKALAR MDL 1401) using perivascular flow sensors. Renal vascular resistance (RVR) was defined as blood pressure/renal blood flow. Statistical significance was assessed by two way analysis of variance. (* significantly different from baseline ($p<0.05$), + significant difference between SHR and WKY ($p<0.05$)).

Table1 Mean BP (mmHg), RVR (mmHg.min.ml-1) and urinary sodium excretion (UNa)(μmol.min-1) before and during infusion of F during 30 and 60 min.

	WKY (n=7)			SHR (n=6)		
	before	30'	60'	before	30'	60'
mean BP	129.1	118.3 *	125.4	178.6 +	160.8 *+	149.8 *+
RVR	83.74	65.90 *	60.73 *	71.19	39.83 *+	41.31 *+
UNa	0.58	0.82 *	1.08 *	0.90 +	1.81 *+	2.49 *+

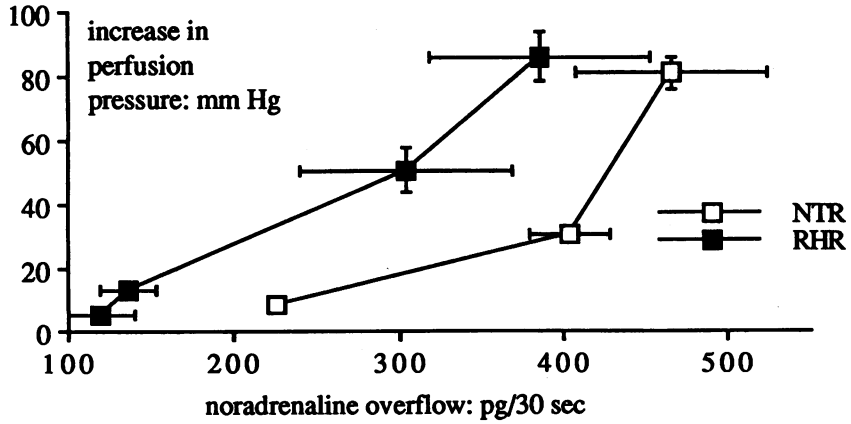
Baseline levels of mean BP, RBF and UNa were higher in SHR. F reduced BP and RVR, and enhanced UNa in both WKY and SHR. However the hypotensive effect of F, the renal vasodilatory and natriuretic response to F were more pronounced in SHR. It is concluded that the renal response to DA-1 receptor stimulation is enhanced in SHR as compared to their normotensive controls. This could be related to up-regulation or changes in affinity of renal DA-1 receptors.

Lee, M.R. (1982) Clinical Science **62**, 439-448.

VASCULAR NORADRENERGIC TRANSMISSION IN RENOVASCULAR HYPERTENSIVE RATS

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Renovascular hypertension is associated with a decrease in vasoconstrictor responses to electrical stimulation in vitro (Fouda *et al.*, 1987). This could be due to decreased release of noradrenaline and/or decreased postsynaptic sensitivity. We investigated changes in noradrenaline (determined by HPLC) content, overflow and contraction induced by electrical stimulation, in normotensive (NTR) and renovascular hypertensive (RHR, 2 kidney, 1 clip) rat tail arteries (n = 7 per group). Tail arteries were perfused and superfused at a flow rate of 2 ml/min; vasoconstriction induced by field stimulation (Fouda *et al.*, 1987) was estimated from the increase in perfusion pressure. Noradrenaline content decreased in RHR (5.8 ± 0.6 vs 8.1 ± 1.0 ng/mg; $P < 0.05$). There was no change in spontaneous noradrenaline overflow before electrical stimulation (NTR = 47 ± 5 vs RHR = 68 ± 10 pg/min). There was a decrease in vasoconstriction and noradrenaline overflow in RHR tail arteries at each frequency of electrical stimulation, e.g. 0.3 Hz, NTR = 30 ± 5 and 404 ± 58 vs RHR = 13 ± 2 mm Hg and 136 ± 17 pg/30 sec.; $P < 0.01$). A plot of vasoconstriction against noradrenaline overflow showed an increase in sensitivity to endogenous noradrenaline in RHR (see figure). Cocaïne (4×10^{-6} M) increased both contraction and noradrenaline overflow but did not alter sensitivity (i.e. contraction vs noradrenaline overflow).



These results show an increase in vascular receptor sensitivity and/or in receptor coupling efficiency, associated with a decrease in noradrenaline overflow following stimulation of sympathetic nerves, in renovascular hypertension.

Fouda A.K., Marazzi A., Boillat N., Sonnay M., Guillain H. & Atkinson J. (1987). Blood Vessels 24, 63-75.

NAFTIDROFURYL ATTENUATES VASOCONSTRICTION INDUCED BY 5-HT IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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As naftidrofuryl antagonizes the vasoconstriction induced by 5-hydroxytryptamine (5HT) (Maloteaux *et al.*, 1986) and it has been used in the chronic treatment of arterial disease (Vanhoutte & Zander, 1988), we have investigated the effects of chronic treatment of SHR with naftidrofuryl on vascular responses to 5HT in vitro. Naftidrofuryl was given as the fumarate salt (base: 30 mg/kg, ip, per day); controls received fumaric acid (7 mg/kg, ip, per day). Treatment started at 1 month of age and continued for 3 months until the SHR reached an age of 4 months. Vascular responses to 5HT were tested in tail arteries removed from the SHR following measurement of systolic arterial pressure. Proximal portions of the tail artery were perfused/superfused at a constant flow rate of 2 ml/min. A concentration-response curve was determined by injecting 0.1 ml bolus of 10^{-8} - 10^{-3} M 5HT into the perfusion circuit. A second curve was then constructed in the presence of 10^{-6} M naftidrofuryl. In preliminary experiments, 2 concentration-response curves for 5HT in the absence of naftidrofuryl gave similar results. Chronic treatment with naftidrofuryl produced the following changes: a decrease in the maximum vasoconstrictor response (240 ±18 vs. 305 ±13 mm Hg, $P < 0.05$ versus controls) and the sensitivity to 5HT (DE_{100} mm Hg: 1.8 ± 0.2 vs. 0.8 ± 0.2 x 10^{-6} M 5HT, $P < 0.05$). The in vitro sensitivity to naftidrofuryl was unchanged -log K_B 7.4 ± 0.2 vs. 7.9 ± 0.2(NS).
Naftidrofuryl did not modify systolic arterial pressure; values after 3 months' treatment were 214 ± 9 (n = 6, controls 203 ± 9, n = 6).
In conclusion chronic treatment with a dose of naftidrofuryl which does not lower blood pressure, attenuates vascular responses to 5HT.

Maloteaux J.M., Haeich J., De Campeneere D., Berta P. & Vidal N. (1986). Arzneim.-Forsch./Drug Res. 36, 1194-1198.
Vanhoutte P.M. & Zander J.F. (1988). in 'Vasodilatation: Vascular Smooth Muscle, Peptides, Autonomic Nerves, and Endothelium': Vanhoutte, P.M. (ed.), pp 281-288. New York, Raven Press.

CHARACTERISING THE 5-HT RECEPTOR MEDIATING CORONARY VASODILATATION IN THE DOG USING TRYPTAMINE ANALOGUES

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Using a series of tryptamine analogues, previous studies identified pharmacologically distinct $5HT_1$-like receptors located on the vascular smooth muscle and endothelium, which mediate vasorelaxant responses to 5HT (Martin et al, 1987). Extending this approach to the intact animals, we concluded that the systemic vasodilator response to 5HT in the anaesthetised rat was primarily mediated by the receptor located on the vascular smooth muscle (Martin et al, 1987). In the anaesthetised dog we have also observed that 5HT causes coronary vasodilatation, and therefore we have utilised these tryptamine analogues to characterise the 5HT receptor mediating this coronary vasodilatation response in vivo.

Beagle dogs (10-17kg, either sex), were anaesthetised with pentobarbitone (60mg/kg i.v.), artificially ventilated, and instrumented to measure left circumflex coronary blood flow (CBF), cardiac contractility (LVdP/dt) and rate (HR), and systemic blood pressure (BP). Coronary conductance (CC) was calculated as the reciprocal of coronary resistance x 1000 and expressed as conductance units . Each animal was pretreated with ketanserin (0.1 mg/kg i.v) and MDL-72222 (0.5 mg/kg i.v.), to block $5HT_2$ and $5HT_3$ receptors respectively, prior to generating a dose response curve to one of the following tryptamine analogues: 5-carboxamidotryptamine (5CT), 5HT, α-methyl 5HT (αT), N,N dipropyl 5-carboxamidotryptamine (DT), or GR43175 (GR, synthesised at Wellcome Research Laboratories). These analogues were administered as bolus injections via a left atrial cannula to avoid pulmonary degradation.

Table 1 Maximum haemodynamic change (mean +/- sem) and dose to produce this change for each tryptamine analogue.

Analogue (ug/kg)	CBF (ml/min)	CC (cond. units)	LVdP/dt (mmHg/sec)	HR (bpm)	BP (mmHg)	n
5CT (1)	49 ± 4	97 ± 5	400 ± 306	10 ± 3	-45 ± 5	6
5HT (100)	71 ± 14	77 ± 16	1333 ± 217	-30* ± 12	-33 ± 5	3
αT (1000)	65 ± 8	28 ± 8	11733 ± 933	70 ± 18	82 ± 10	3
DT (300)	6 ± 2	11 ± 2	-1000 ± 200	-55* ± 20	-28 ± 3	3
GR (300)	-1 ± 1	-3 ± 2	-250 ± 144	-15* ± 3	-3 ± 6	3

* transient response

As some of these tryptamine analogues produced systemic and cardiac effects, the true coronary vascular responses are indicated by the changes in CC, hence the rank order of potency for coronary vasodilatation was 5CT > 5HT> DT > αT > GR. These findings also confirm that the small increase in CC associated with the large increase in CBF (and BP), produced by αT, reflects its marked cardiac stimulant action. The poor coronary vasodilator activity of αT, relative to 5CT and 5HT, is therefore consistent with coronary vasodilatation mediated by the vascular smooth muscle $5HT_1$-like receptor (Martin et al, 1987). Furthermore, the weak activity of DT and GR suggest that this receptor is unlikely to be of the $5HT_{1A}$ subtype, for which DT has a high affinity (Markstein et al, 1986), nor does it resemble the recently identified vascular $5HT_1$ receptor subtype, for which GR is a selective agonist (Humphrey et al, 1988).

Humphrey, P.P.A. et al (1988) Br.J.Pharmacol. 94, 1123-1132
Markstein, R. et al (1986) N.S.Arch.Pharmacol. 333, 335-341,
Martin, G.R. et al (1987) N.S.Arch.Pharmacol.336, 365-373.

THE EFFECT OF ALKYLXANTHINES ON POST-ISCHAEMIC ACUTE RENAL FAILURE IN THE RAT

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Lin et al (1986) have shown that theophylline reduces the severity of post-ischaemic acute renal failure (ARF) that follows from clamping the renal artery. In this study we have compared the effect of theophylline and 8-phenyltheophylline (8-PT), a more potent adenosine antagonist, on the development of post-ischaemic ARF.

Rats were anaesthetised with thiobutabarbitone (120 mg kg^{-1} i.p.) and cannulae inserted into the jugular bein and carotid artery for infusion of saline (0.12 ml min^{-1} containing inulin 4 mg ml^{-1} and [^{3}H]-inulin 0.5 μCi ml^{-1}) and for the collection of blood. Both ureters were also cannulated. Two hours later a control 45 min urine collection was made and then the left renal artery was clamped for 45 min. One hour prior to clamping, infusions of either theophylline (20 mg kg^{-1} prime, 0.05 mg min^{-1}), 8-PT (28 mg kg^{-1}, 0.08 mg min^{-1}) or vehicle for 8-PT (2.8 ml kg^{-1}, 0.08 ml min^{-1}; 50% v/v 0.1M NaOH/polyethylene glycol) were started and continued throughout the experiment. One hour after the release of the clamp, urine was collected for three 45 min periods for the determination of [^{3}H]-inulin clearance (C_{IN}).

Table 1 The effect of theophylline and 8-PT on post-ischaemic renal function in the left kidney

	Untreated	Theophylline	Vehicle	8-PT
C_{IN} (ml min^{-1})				
Pre-ischaemia	1.17±0.13	1.09±0.05	1.31±0.05	1.21±0.08
[a]Post-ischaemia	0.06±0.02	0.15±0.02*	0.05±0.01	0.05±0.01
Urine Flow (μl min^{-1})				
Pre-ischaemia	29.5 ±5.0	34.8 ±5.0	27.9 ±1.9	17.9 ±2.5
[a]Post-ischaemia	14.5 ±4.0	37.3 ±5.5*	13.9 ±3.7	11.7 ±2.7
Left kidney weight (g)	1.70±0.03	1.36±0.04*	1.59±0.08	1.83 ±0.08

*P<0.001 relative to untreated group (t-test). [a] Post ischaemia period taken 1h 45 min after release of clamp.

Clamping the renal artery led to a severe diminution in left kidney function characterised by reductions in C_{IN} and urine flow and an increase in kidney weight (Table 1). Treatment with theophylline significantly improved these parameters but, by contrast, 8-PT had no effect. These findings suggest that the beneficial effect of theophylline on post-ischaemic renal function is not a consequence of adenosine antagonism.

Lin, J-J., et al. (1986) J. Lab. Clin. Med., 108, 150-154.

AN *IN VITRO* STUDY OF CARDIAC RESPONSES IN RATS WITH ACUTE RENAL FAILURE

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Reduced cardiac reponses to isoprenaline have been noted in experimental models of acute renal failure (ARF) (Yates et al., 1985; Mann et al., 1986). The mechanism underlying these changes is unclear. The aim of this study was to determine the basis of these reduced responses by investigating the response of isolated cardiac tissue to a range of stimulants which act at different biochemical levels within the myocardium.

ARF was induced in male Wistar albino rats (250-400g) by i.m. injection of glycerol (10ml kg^{-1}). Control rats received i.m. saline. 48 hours later, rats were killed, the heart removed and right atria and right ventricle were mounted for isometric recording in Krebs' solution at 35°C gassed with 5%CO_2 in 95%O_2. Chronotropic responses were measured with the spontaneously beating right atria and the right ventricle was stimulated (1.6Hz, 5ms, 5V) for measurement of inotropic responses. Cumulative concentration-response curves were obtained to isoprenaline (ISO), 3-isobutyl - 1-methylxanthine (IBMX), the calcium agonist BAY K 8644 and Ca^{2+}. Results are summarised in Table 1.

Table 1. The effect of ARF on chronotropic and inotropic responses of isolated cardiac preparations.

	RIGHT ATRIA				RIGHT VENTRICLE			
	Basal rate ($Beats\ min^{-1}$)		Max.↑ rate ($Beats\ min^{-1}$)		Basal tension (mg)		Max.↑ tension (%)	
	C	ARF	C	ARF	C	ARF	C	ARF
ISO	251 ± 7	281* ± 6	186 ± 11	146** ± 6	168 ± 31	194 ± 44	152 ± 30	69* ± 13
IBMX	237 ± 10	283** ± 6	211 ± 11	172** ± 7	267 ± 75	420 ± 80	80 ± 13	32* ± 8
BAY K 8644	-	-	-	-	200 ± 49	222 ± 76	142 ± 37	35* ± 8
Ca^{2+}	-	-	-	-	74 ± 18	108 ± 27	611 ± 41	187*** ± 29

Results are mean ± s.e. mean (n = 8 in each group). C = control, ARF = acute renal failure, Max.↑ rate = maximum increase in rate, Max.↑ tension = maximum increase in tension. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ relative to respective control group (Student's t-test).

Basal rate was significantly elevated in rats with ARF, but there was no significant change in basal tension. The maximum increase in rate to ISO and IBMX was reduced in ARF as was the maximum increase in tension in response to all four inotropes.

The reduced maximum increase in rate of right atria from rats with ARF may be related to the elevated basal rate in these preparations. However, reduced inotropic responses to ISO, IBMX, BAY K 8644 and Ca^{2+} suggest the existence of a defect in the myocardium which lies at a point on the stimulatory pathway common to the action of all agents tested. A possible site is that of energy production within the cell and this is at present under investigation.

Mann, J.F.E., et al. (1986). Am. J. Physiol., 250, H846-H852.
Yates, M.S., et al. (1985). J. Pharm. Pharmacol., 37, 175-179.

CHARACTERISATION AND COMPARISON OF THE GLYCEROL-INDUCED AND 5/6 NEPHRECTOMY MODELS OF RENAL FAILURE

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Renal disease has been shown to affect the metabolic clearance of many drugs (Terner et al, 1978), reduce cytochrome P450 content in rat liver (van Peer & Belpaire, 1977) and significantly alter certain pharmacokinetic parameters (Barber et al, 1989). The clinical importance of renal disease has resulted in the development of several animal models used during research into the condition. The present study was designed to fully characterise two such models and compare their features.

Male Sprague-Dawley rats (200-300 gms) were randomly assigned to glycerol or nephrectomy groups. Renal failure in the glycerol group was induced by i.m. injection of glycerol in normal saline (50% v/v) with a control group injected with saline only. All animals were used 48 hours after treatment. Animals in the nephrectomy group had one kidney removed and 2 of the 3 branches of the contralateral renal artery were ligated. Controls were sham-operated and all animals were used 1 week after surgery. All the animals were placed in metabowls overnight and food, water, urine and blood samples collected for analyses, after 48 hours of acclimatisation in the metabowls. A summary of measured and calculated parameters is shown below.

Table 1 Metabowl data for the two models of renal failure

Measurement	Glycerol (n=6)	Control (n=5)	p-value
UFR (ml/hr/kg)	5.46 ± 3.30	1.75 ± 0.56	$p < 0.05$
Cr.Cl (ml/hr/kg)	41.50 ± 45.90	287.20 ± 35.91	$p < 0.01$
NAG (nmol released/ml/hr)	789.02 ± 315.41	72.61 ± 6.36	$p < 0.01$
Urea (mmol/l)	64.7 ± 25.5	5.52 ± 1.3	$p < 0.01$
	Nephrectomy (n=6)	**Control (n=6)**	**p-value**
UFR (ml/hr/kg)	4.35 ± 0.85	1.46 ± 0.24	$p < 0.01$
Cr.Cl (ml/hr/kg)	146.61 ± 39.54	196.23 ± 45.04	N.S.
NAG (nmol released/ml/hr)	21.46 ± 7.15	28.79 ± 6.63	N.S.
Urea (mmol/l)	34.80 ± 26.30	7.90 ± 2.31	$p < 0.05$

Results are given as mean ± standard deviation
Legend: Cr.Cl = creatinine clearance, UFR = urine flow rate, NAG = N-acetyl glucosaminidase, N.S. = not significant.

The highly significant differences in creatinine clearance, urinary NAG and serum urea in glycerol rats is contrasted by much smaller changes for nephrectomised rats. These values reflect the severity of the glycerol model compared to the surgical method and suggest that the former may be superior in reliability in producing the desired effect of renal failure.

Barber H.E. et al. (1989) Br. J. Pharmac. 96 : 291P
Terner U.K. et al. (1978) Clin. Biochem. 11, 156.
van Peer A.P. & Belpaire F.M. (1977) Arch Int. Pharmacodyn, 288, 180-183

MECHANISM OF DOPAMINE-INDUCED DIURESIS IN THE ANAESTHETISED CAT

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Intravenous infusion of dopamine increases urine output in the anaesthetised cat without altering renal haemodynamics or resting blood pressure (Wassermann et al., 1980). We have re-examined the renal effects of dopamine in this model and have attempted to identify the receptor type(s) that mediates the diuresis.

Pentobarbitone anaesthetised, artificially respired cats were infused with sodium bicarbonate (0.03mmol/kg/min i.v) to prevent metabolic acidosis. Aortic blood pressure and left renal artery blood flow (RBF) were measured, and both ureters cannulated for collection of urine. Glomerular filtration rate (GFR) was calculated from creatinine clearance. Saline (30ml/kg i.v.) was infused over 2h followed by a maintenance infusion (0.1ml/kg/min i.v.). Each dose of dopamine was infused sequentially for a period of 10 minutes.

In control animals receiving saline alone (0.25ml/min i.v.) in place of dopamine little variation (<10%) occurred in measured parameters. In contrast, dopamine (10–100μg/kg/min i.v) caused a dose-related increase in mean blood pressure (MBP, 6–56%), urine output (U_V, 28–46%), and fractional sodium excretion (FE_{Na}, 5–38%) from both kidneys, and a decrease in renal vascular conductance (RVC, 20–50%). No increase in GFR was associated with the diuresis. The increase in U_V (Figure 1), MBP and FE_{Na}, and decrease in RVC caused by dopamine, were not blocked in separate cats pretreated with either the selective dopamine DA_1 or DA_2 receptor antagonists SCH23390 (30μg/kg i.v.) or domperidone (1mg/kg i.v.), respectively. However, in cats treated with the non-selective α-adrenoceptor antagonist, phentolamine (1mg/kg i.v.) dopamine (10–100μg/kg/min i.v.) caused decreases in U_V (Figure 1), GFR, MBP and FE_{Na}. Dopamine-induced decreases in RVC were only slightly attenuated in phentolamine treated cats.

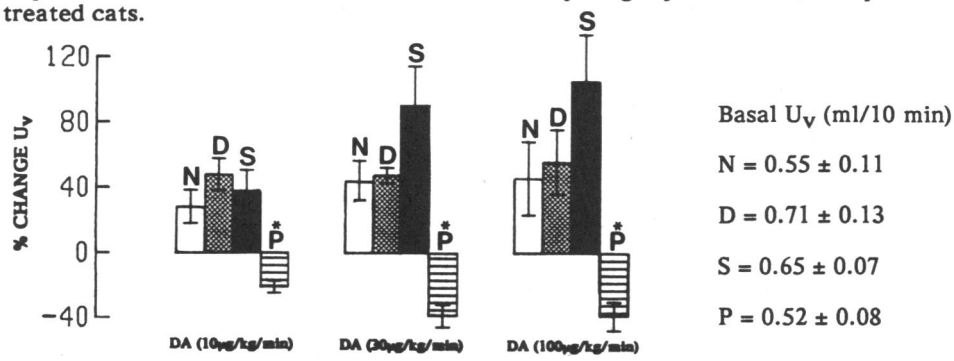


Figure 1: Basal values and effects of dopamine (DA) on Uv from the left kidney in cats with no pretreatment (N, n=6), and in cats pretreated with SCH23390 (S, n=5), domperidone (D, n=4), or phentolamine (P, n=4). Values are mean change ± s.e.m. from the post-antagonist basal value. *p<0.05, Student's t-test, antagonist treated group (D, S or P) compared to untreated group (N).

The present study has confirmed the finding of Wassermann et al. (1980) that intravenous infusion of dopamine causes diuresis in the anaesthetised cat. However, in contrast to these workers we found no convincing evidence for an involvement of dopamine receptors in this effect; rather the diuretic effect of dopamine appears to be linked to stimulation of α-adrenoceptors.

Wassermann, K. et al., (1980). Naunyn-Schmiedeberg's Arch. Pharmac., 312, 77–83

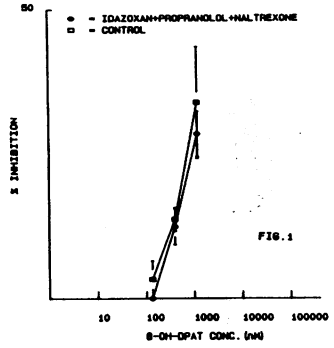
DOPAMINE AGONIST ACTIVITY OF 8-OH-DPAT

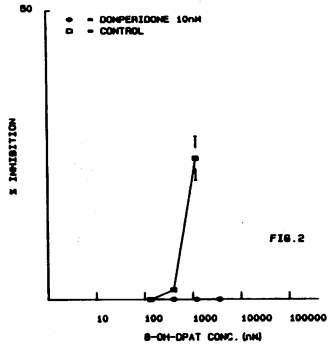
C.F.C. Smith and Susan D. Cutts (introduced by P.W. Dettmar), Reckitt & Colman plc, Dansom Lane, Hull, HU8 7DS, N. Humberside, England.

8-OH-DPAT was first identified as a 5-HT agonist by Hjorth et al. (1982) who investigated the compound in rats and reported it to produce a marked behavioural syndrome which was antagonised by methiothepin. These workers failed to see any significant dopaminergic effects and concluded that the compound was devoid of dopamine agonist activity. A short time later Middlemiss and Fozard (1983) designated the compound as a selective 5-HT_{1A} agonist on the basis of binding studies performed using rat frontal cortex. Since then the compound has been widely used as a prototype 5-HT_{1A} agonist.

While investigating this compound in a range of isolated tissue preparations in order to determine whether we could identify the existence of a population of 5-HT_{1A} receptors in a peripheral tissue, we discovered it possessed a biphasic dose response curve (initial inhibition followed by potentiation at higher doses) in the mouse isolated electrically stimulated vas deferens preparation (MVD). This communication reports the results of the investigation to determine the nature of the receptor system producing the initial inhibitory phase of the DRC.

Vasa deferentia from mice of strain MF1/OLA and weight <30 grams were set up in Krebs solution in a 50 ml organ bath and stimulated with 3 msec rectilinear pulses at 0.1 Hz. DRC's to 8-OH-DPAT were constructed every 50 mins and the effects of the antagonists determined by introducing the test antagonist into the Krebs solution immediately after the second DRC. A minimum of four DRC's were constructed on each preparation.





A combination of propranolol (300 nM), idazoxan (300 nM) and naltrexone (300 nM) had no effect on the response of the MVD to 8-OH-DPAT (Figure 1) indicating that the effect of the compound is not mediated via β, α_2 or opioid receptors. Domperidone (10 nM) (Figure 2), sulpiride (300 nM) and fluphenazine (30 nM), on the other hand, all produced a marked antagonism of the effects of 8-OH-DPAT. This is consistent with the inhibitory effect of 8-OH-DPAT being due to agonist activity at the dopamine receptors present in the MVD (Smith and Rowland, 1988).

A comparison of 8-OH-DPAT and apomorphine in the same preparations showed 8-OH-DPAT to possess 0.01 the potency of apomorphine.

Hjorth, S. et al. (1982), J. Neural Transmission, 55, 169-188
Middlemiss, D.N. and Fozard, J.R. (1983), Eur. J. Pharmacol., 90, 151-153.
Smith, C.F.C. and Rowland P.B. (1988), Br. J. Pharmac. Proc. Suppl., 96, 259P.

THE GUINEA-PIG ILEUM AS A MODEL FOR 5-HT$_{1A}$ AGONISTS: ANOMALOUS EFFECTS WITH RS-30199

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5HT$_{1A}$ receptors in the guinea-pig ileum myenteric plexus preparation have been shown to inhibit acetylcholine release following field stimulation and 8-hydroxy-n-dipropyl-aminotetralin (8-OH-DPAT) inhibits the contractile responses to field stimulation (Fozard & Kilbinger, 1985). We have modified this assay by using the more potent and selective agonist dipropyl-5-carboxyamido-tryptamine (DP-5CT) to investigate the roles of agonists and antagonists at 5HT$_{1A}$ receptors.

Guinea-pig ileum preparations were set up in 30ml isolated organ baths filled with Tyrode solution (0.9mM Ca^{2+}_o, 37°C, gassed with 100%O_2) and stimulated via two parallel platinum electrodes, one of which passed through the lumen, at 0.1Hz (1msec pulse duration, supramaximal voltage). Cumulative concentration-response curves were obtained to 8-OH-DPAT (threshold 10nM) and DP-5CT (threshold 0.1nM, maximal 30nM) only once in any tissue to avoid desensitisation; antagonists were preincubated for 40min. The concentration-response curve to 8-OH-DPAT was biphasic and only the first phase (10-300nM, 20-30% inhibition) was sensitive to spiperone (100nM). In contrast the concentration-response curve to DP-5CT (1-300nM) was monophasic with a maximal inhibition of 35-50% of the response to field stimulation; the responses were antagonised by spiperone and resistant to idazoxan (1μM).

Buspirone and ipsapirone caused inhibition of the response to field stimulation (1μM 19%±1%, n=14, & 16%±1%, n=6, respectively) and the inhibition faded over 30min after which the preparations were less sensitive to 8-OH-DPAT and DP-5CT. The inhibitory responses to buspirone and ipsapirone (1μM) were resistant to idazoxan (1μM) and abolished by pretreatment with a threshold dose of 8-OH-DPAT (10nM). These results are consistant with cross-desensitisation occuring between buspirone and 8-OH-DPAT.

Although RS-30199-193 (Brown et al, this meeting) and buspirone have equivalent affinity for the 5HT$_{1A}$ site in rat cerebral cortex, RS-30199-193 (0.1-10μM) caused a slight augmentation of the reponses to field stimulation (1μM, 15±4%, n=8) and this response was resistant to pretreatment with 8-OH-DPAT (10nM). RS-30199-193 (1μM for 20min) did not affect the sensitivity of the tissues to 8-OH-DPAT or to DP-5CT, indicating that RS-30199-193 may interact with a different subset of 5HT$_1$ receptors than buspirone. These findings indicate that binding to 5HT$_{1A}$ sites may not always correlate with functional effects.

Fozard, J.R. & Kilbinger, H. (1985). Br.J.Pharmacol., 86, 601P.

THE PUTATIVE 5-HT$_{1A}$ RECEPTOR IN GUINEA-PIG ILEUM: DIFFERENTIAL CLASSIFICATION USING TRYPTAMINES

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Fingerprints comprising tryptamine affinity and efficacy estimates provide a quantitative and economical means of identifying positively, and at the same time differentiating 5-HT receptor subtypes (Leff & Martin, 1988). Here we consider the classification of the 5-HT receptor mediating inhibition of electrically-evoked twitch in the guinea-pig isolated ileum (GPI), using tryptamine fingerprints to compare and contrast this receptor with four other 5-HT receptors currently allocated to the 5-HT$_1$-like, 5-HT$_2$ and so-called 'orphan' receptor classes.

Segments of proximal ileum were obtained from male Dunkin-Hartley guinea-pigs (250-450g). Changes in isometric force were recorded from tissues mounted for transmural stimulation (0.5msec, supramaximal voltage, 0.1Hz) in Krebs solution (37°C) containing 0.63mM Ca^{++}. Experiments were performed in the presence of ketanserin (0.1μM) and BRL24924A (30μM).

5-HT-induced inhibitions of twitch (p[A_{50}] = 6.6, max = 68%) were mimicked by 5-carboxamidotryptamine (5-CT) with higher potency (p[A_{50}] = 8.2, max = 71%). Ketanserin (0.1μM) and BRL24924A (30μM) were without effect on these responses, but methiothepin (0.03μM) caused surmountable antagonism (Δ p[A_{50}] = 0.5). Spiperone behaved as a simple competitive antagonist. Estimates of the antagonist dissociation constant (pK_B) were agonist-independent (vs 5-HT : 7.94 ± 0.07, 16df; vs 5-CT : 8.21 ± 0.11 , 24df) and were unchanged when experiments were performed in the presence of 2.5mM Ca++ (vs 5-CT: 7.91 ± 0.07, 16df).

Affinity (pK_A) and relative efficacy (τ) estimates were obtained for 5-HT, 5-CT, (±) α-methyl-5-HT (α-Me-5-HT) and N,N-di-n-propyl-5-CT (N-DP-5-CT) using direct model-fitting methods as described elsewhere (Black *et al*, 1985). The results, shown below, are compared with estimates obtained at other 5-HT receptor types previously differentiated using tryptamines (Leff and Martin, 1988).

ASSAY	Receptor		5-HT	5-CT	a-Me-5-HT	N-DP-5CT
GPI	5-HT$_1$-like	pK_A	6.39	7.90	<5.00	7.36
		τ	*1.00*	*1.10*	-	*0.38*
RbSV	5-HT$_1$-like	pK_A	7.12	7.53	5.68	6.22
		τ	*1.00*	*0.98*	*0.93*	*0.30*
DCA	5-HT$_1$-like	PK$_A$	6.20	6.66	<4.50	5.56
		τ	*1.00*	*4.70*	-	*0.55*
RbJV+E	'orphan'	pK_A	8.36	7.51	8.14	5.17
		τ	*1.00*	*0.95*	*1.04*	*0.32*
RbA	5-HT$_2$	pK_A	6.92	5.90	6.59	5.09
		τ	*1.00*	*0.35*	*0.79*	*0.26*

Key : RbA, rabbit aorta : RbSV, rabbit saphenous vein ; RbJV + E, endothelium-intact rabbit jugular vein ; DCA, beagle dog coronary artery.

These results show that the receptor in GPI is 5-HT$_1$-like, consistent with its proposed 5-HT$_{1A}$ classification (Fozard & Kilbinger, 1985). Tryptamine fingerprints identify this receptor positively and enable it to be differentiated quantitatively from other types of peripheral 5-HT receptor regardless of their current appellation.

Black, J.W. *et al* (1985) Br. J. Pharmacol., 84, 561-571.
Leff, P. & Martin, G.R. (1988) Med. Res. Rev., 8, 187-202.
Fozard, J.R. & Kilbinger, H. (1985) Br. J. Pharmacol., 86, 601P.

THE EFFECT OF VASOACTIVE DRUGS ON THE CIRRHOTIC RAT PORTAL VEIN

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Although beta blockers and serotonin antagonists have been tested in the treatment of portal hypertension in man, little is known of their direct effect on the portal vein. Beta$_2$ receptors in the normal rat hepatic vasculature mediate vasodilatation (Burns et al, 1989a). In man blockade of such receptors may be undesirable when the aim of treatment is to lower portal venous pressure (Ppv). We have examined the effects of propranolol (Pro), salbutamol (S), ketanserin (K) and prazosin (Pra) on Ppv in the portal vein of the cirrhotic rat using a constant flow isolated perfused rat liver preparation (IPRL).

The livers (mean weight (sd) 12.2 (2.2)g) of 46 cirrhotic male Wistar rats were isolated and perfused in situ as described previously (Burns et al, 1989). The animals were rendered cirrhotic by the administration of carbon tetrachloride and phenobarbitone (Burns et al, 1989b). Ppv was monitored continuously; flow 10 ml/min; perfusate volume 100ml. After a 30 min stabilisation period a noradrenaline (NA) infusion (200ng/min) was started and 30 min later the test drug was added to the reservoir of the perfusion apparatus. The initial perfusate concentrations are shown in the table with the mean (sd) changes in Ppv produced by the drugs.

Drug	n	Conc. (uM)	Pretreatment Ppv (cm water)	% rise in Ppv with NA	Change in Ppv after drug (% of Ppv after NA) 4min	10min
NA	6		11.8 (1.9)	41 (30)	1 (1)$_{*}$	1 (1)$_{**}$
Pro	7	100	11.5 (2.4)	67 (11)	-4 (4)$_{***}$	-4 (4)$_{***}$
S	7	100	11.7 (4.2)	45 (24)	-9 (2)$_{**}$	-9 (4)$_{**}$
S	6	10	10.6 (3.4)	32 (38)	-4 (2)$_{**}$	-5 (3)$_{**}$
S	8	1	9.7 (4.3)	23 (14)	-6 (4)$_{***}$	-5 (5)$_{***}$
K	6	13	11.6 (2.8)	39 (20)	-18 (7)$_{***}$	-19 (6)$_{***}$
Pra	6	0.2	10.4 (2.6)	37 (21)	-16 (7)	-17 (7)

*p<0.02, **p<0.01, ***p=0.001 for differences between NA alone and NA+Pro, NA+S, NA+K or NA+Pra.

Analysis of variance did not show any difference between the pretreatment pressures or the rise in Ppv with the NA infusion. The pressure changes with S are consistent with the findings in the normal rat liver. Pro has no effect on Ppv in the normal rat liver (Burns et al, 1989) but lowered Ppv in the cirrhotic liver. The concentrations of K and Pra were selected as they are equipotent in their systemic effects in portal hypertensive rats (Cummings et al, 1988). K and Pra produced similar changes in Ppv. It has been suggested that K acts by lowering cardiac output (Cummings et al, 1988), but in the constant flow IPRL preparation changes in Ppv reflect changes in resistance. Thus, the results of the present study suggest K may have a direct effect on the hepatic vasculature. It is possible that the effect of these agents may be useful in augmenting the portal hypotensive effect of drugs that lower portal inflow.

Burns et al (1989a) Br J Pharmac in press
Burns E (1989b) Clin Sci in press
Cummings et al (1988) Hepatology 8, 1112

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AGE-RELATED ALTERATIONS IN THE INHIBITORY ACTIONS OF 5-HT$_1$ RECEPTOR AGONISTS IN THE RAT VAS DEFERENS

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Prejunctional inhibitory 5-HT receptors in rat brain cortex (Engel et al., 1986) have been reported to resemble the 5-HT-1B binding site. Although we have previously failed to identify the inhibitory prejunctional 5-HT receptor of rat vas deferens (Docherty & Warnock, 1986), it has recently been reported that these prejunctional inhibitory 5-HT receptors of rat vas deferens resemble 5-HT-1B binding sites (Feniuk & Pickles, 1988). As a result, we have re-investigated the prejunctional effects of 5-HT receptor agonists in the rat vas deferens.

Epididymal portions of rat vas deferens were obtained from male Wistar rats of 4 age groups (1.5, 3, 6 and 22 month), and were treated with nifedipine (10 μM) to eliminate postjunctional actions of 5-HT receptor agonists. Tissues were stimulated with a single electrical pulse (supramaximal voltage, 0.5 ms pulses) every 5 min, and agonist concentration-response curves (0.5 log unit increments) were obtained in the absence or presence of antagonist

In epididymal portions of rat vas deferens, 5-carboxamidotryptamine (5-CT) and flesinoxan produced concentration-dependent inhibition of stimulation-evoked contractions. The IC_{50} (concentration producing 50% inhibition of stimulation-evoked contractions) of flesinoxan did not differ between 1.5 and 3 month rats, with values of 0.69 μM (n=4) and 1.20 μM (n=14), respectively. Effects of flesinoxan were not antagonised by cyanopindolol (1 μM) (5-HT-1B ligand)nor by yohimbine (0.3 μM) (alpha-2 adrenoceptor antagonist).

For the agonist 5-CT, analysis of data revealed that potency of 5-CT divided into two distinct ranges: IC_{50} lower than 0.1 μM and IC_{50} higher than 1 μM. In 1.5 month rats 5-CT had high potency in 7 from 9 experiments, but in 3 month rats 5-CT had high potency in 4 from 10 experiments (with 1 intermediate value). In 6 month and 22 month rats, 5-CT showed only low potency. The IC_{50} of 5-CT was 0.060μM (n=9), 0.89 μM (n=10), 3.89 μM (n=6) and 17.8 μM (n=3) in 1.5, 3, 6 and 22 month rats, respectively. Since IC_{50} values in 1.5 and 3 month animals did not follow a normal distribution, data were assessed employing the non-parametric Mann- Whitney U-test, and 5-CT was significantly more potent in 1.5 month than in 3, 6 and 22 month old animals ($P<0.05$ in each case).

Cyanopindolol (1 μM) shifted the potency of 5-CT only in experiments in which 5-CT showed high potency, so that cyanopindolol was ineffective in all 6 and 22 month animals. In 1.5 and 3 month animals, cyanopindolol shifted the potency of 5-CT with an apparent pA_2 of 7.29 (using only experiments in which 5-CT showed high potency in vehicle controls).

In conclusion, the 5-HT-1A ligand flesinoxan produces inhibition of contractions of rat vas deferens resistant to cyanopindolol, whereas the 5-HT-1 agonist 5-CT had two components to its inhibition, a high potency component antagonised by cyanopindolol and present only in 1.5-3 month rats, and a low potency component resistant to cyanopindolol. Hence, the component of the response to 5-CT which resembles a 5-HT-1B response is lost in maturation.

Docherty, J.R. & Warnock, P. (1986). Br. J. Pharmacol., 89, 843P.
Engel, G. et al. (1986). Naunyn-Schmiedeberg's Arch. Pharmacol., 332, 1-7.
Feniuk, W & Pickles, R.J. (1988). Proc. Cong. Cardiovasc. Pharmacol of 5-HT

THE METABOLISM OF LEUKOTRIENES AND OF REACTIVE OXYGEN SPECIES IN SENSITISED GUINEA-PIG LUNGS

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The cysteinyl leukotrienes (LT), C_4, D_4 and E_4, are major candidates for the mediation of bronchial asthma, and are released on allergen challenge of sensitised human and animal lungs. LTs are not stored intracellularly and their release depends on de novo synthesis from arachidonic acid. Before the role of LTs in asthma can be fully understood it is important to assess their synthesis and metabolism in this abnormal physiological state. We have, therefore, measured the metabolism of LTC_4 and LTD_4 in subcellular fractions prepared from sensitised guinea pig lungs. In addition, the efficiency with which sensitised lung tissue can detoxify reactive oxygen species has been determined.

Male Dunkin-Hartley guinea pigs (250-300g) were injected intraperitoneally with 10 μg ovalbumin in 20% (w/v) $ALOH_3$ as adjuvant and used for experiments after 14 days. Control animals received adjuvant alone. Subcellular fractions were prepared by ultracentrifugation and LT metabolism quantified by HPLC analysis. The activities of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase, the enzymes involved in detoxification of reactive oxygen species, were measured in the cytosol.

The recovery of microsomes was 3.88±0.28 mg protein/g lung from control animals and 4.21±0.34 mg protein/g lung from sensitised animals. The conversion of 16 μM LT C_4 to LTD_4 by microsomes from sensitised guinea pig lungs was significantly increased, whilst the metabolism of 20 μM LTD_4 to both LTC_4 and LTE_4 by lung homogenate was significantly decreased in the sensitised animals as shown in Table 1. The overall result of these three alterations is an increase in LTD_4 synthesis and a reduction in its catabolism. This may lead to an increase in the concentration of LTD_4 in sensitised guinea pig lungs.

Table 1. Leukotriene metabolism in guinea pig lungs

Reaction	Control	Sensitised
C_4 to D_4	68.2±5.4(6)	93.1±7.8(6)*
D_4 to C_4	21.8±2.8(8)	14.5±0.8(6)*
D_4 to E_4	61.5±3.4(8)	50.8±0.9(6)*

Values are mean ± S.E.M., with the number of animals used in parentheses. Reaction rates are pmol/min/g lung. *P<0.05, by non-paired Student's t test.

The activities of catalase and glutathione reductase were not significantly altered in sensitised guinea pig lungs. In contrast, the activity of cytosolic superoxide dismutase was decreased in sensitised guinea pig lungs (583.7±76.9 units/g lung in control animals (n=6) compared with 368.6±39.1 units/g lung in sensitised animals (n=6; P<0.05)). The activity of selenium-dependent glutathione peroxidase was unaltered by sensitisation, whereas that of the selenium-independent peroxidase was significantly increased (2.49±0.30 μmol/min/g lung in control animals (n=5) compared with 3.55±0.26 μmol/min/g lung in sensitised animals (n=5, P<0.05)). Thus, sensitised lungs may be more susceptible to the effects of superoxide, although they can detoxify organic hydroperoxides more efficiently.

EFFECTS OF EP092 ON CONTRACTIONS TO U46619, 5-HT AND OXYGEN IN THE HUMAN UMBILICAL ARTERY

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The human umbilical artery will contract to an increase in oxygen tension above 16mmHg (low) oxygen (McGrath *et al* ., 1986). This contraction is blocked by the action of the cyclo-oxygenase inhibitors indomethacin, flurbiprofen and aspirin (McLennan *et al* ., 1988). Also contractions to 5-hydroxytryptamine (5-HT) show a biphasic concentration response curve in high oxygen (pO_2 = 120mmHg) (McGrath et al 1988). The lower part of the curve is blocked by the cyclo-oxygenase inhibitors, is absent in low oxygen and is thus dependent on high oxygen and cyclo-oxygenase. In 16mmHg oxygen the 5-HT curve can be competitively inhibited by the $5\text{-}HT_2$ antagonist ketanserin. In 120mmHg the lower part of the curve which is absent in low oxygen is resistant to ketanserin and suggests a $5\text{-}HT_1$-like "receptor" MacLennan *et al* . (in press). This current study was carried out to determine the role, if any, of thromboxane in the contraction to oxygen and the biphasic response to 5-HT by using the specific thromboxane antagonist EP092.

Umbilical cords collected directly after birth were stored in ice-cold Krebs for up to 24 hrs. Isometric tension was recorded from longitudinal strips of artery in Krebs at 37°C. To mimic physiological blood gas tensions *in utero*, the Krebs was bubbled with 2.5% O_2, 8% CO_2, balance N_2 to give pO_2 16mmHg ± 3mmHg. pO_2 was measured continuously by an IL1302 O_2 electrode placed in one of the organ baths. Cumulative concentration response curves (CCRC) to U46619 were carried out in controls or in the presence of EPO92; 10nM, 0.1μM or 1μM . Alternatively a contraction to an increase in oxygen tension to 20% (~120mmHg) was obtained, then repeated in the presence of one of the three EPO92 concentrations or alone as a time control. Thirdly a CCRC to 5-HT was carried out in both low 16mmHg and high 120mmHg oxygen either alone or in the presence of one of the three concentrations of EPO92 .

At physiological pO_2 (16mmHg) U46619 contracted the artery over the range 0.3nM-0.3μM (pD_2 8.82 ± 0.04). EPO92 10nM had little effect on the contraction, with 0.1μM shifting the curve to the right (pD_2 8.1± 0.08). EPO92(1μM) almost abolished the response to U46619 with the maximum response reduced from 2,920 ± 560mg to 785 ± 200mg. EPO92 10nM & 0.1μM reduced the contraction to 20% oxygen with 1μM completely abolishing the response. Finally this concentration of antagonist also abolished the increase in sensitivity to 5-HT normally seen in high oxygen (20%) when compared with low oxygen (2.5%) (McGrath *et al* ., 1988). However EP092 did not affect the contractions to 5-HT in low pO_2, demonstrating that it was selective against U46619 and the responses dependent on high oxygen.

In conclusion these experiments would seem to suggest that the contraction to oxygen is almost entirely if not exclusively due to thromboxane release. This is also borne out by the results with 5-HT where the antagonist inhibits the initial component of the contraction which only becomes apparent in high oxygen.

McGrath, J.C., MacLennan, S.J., Mann, A.C., Stuart-Smith, K. & Whittle, M.J. (1986) J.Physiol., 380; 513-519.
McGrath, J.C., MacLennan, S.J. & Whittle, M.J.(1988) Quart.J.Exp.Physiol., 73; 547-559.
MacLennan, S.J., McGrath, J.C. & Whittle, M.J. (1988) Prostaglandins, 36 (5); 711-729.
MacLennan, S.J., McGrath, J.C. & Whittle, M.J. (1989) Br.J.Pharmacol., (in press).

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PROSTAGLANDIN E_2 RECEPTORS IN RAT UTERUS

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Prostaglandin E_2 causes contraction of the rat uterus *in vitro*. This effect may be mediated via a prostaglandin E_2-sensitive (EP) receptor. EP-receptors can be divided into EP_1, EP_2 and EP_3-receptor subtypes (Coleman et al., 1987). The purpose of this study was to characterise the EP receptors present in rat uterus.

Uterine horns were obtained from Sprague-Dawley rats which had been pretreated with diethylstilboestrol (1 mg kg^{-1}, s.c.) 18h previously. Both horns were bissected longitudinally providing 2.5 cm strips which were set up as described by Massele & Senior 1981 and superfused at a rate of 2 ml min^{-1} with oxygenated (95% O_2/5% CO_2) Krebs solution at 37° and which contained indomethacin (2.8 μM).

Uterine strips initially showed spontaneous activity but this usually disappeared within one hour of setting up the tissue. PGE_2 caused contraction of uterine strips (ED_{50} (range) 9.6 (2.29-29.12) nmoles) and the EP_1/EP_3-receptor selective agonist, sulprostone (Coleman et al., 1987) also caused contraction (ED_{50} (range) 7.23 (2.15-14.62) nmoles). Responses to sulprostone were qualitatively similar to those obtained with PGE_2.

The EP_1/IP-receptor selective agonist, iloprost (Coleman, 1987) also caused contraction in 30% of all preparations but the contraction appeared non-dose related and was variable in its action.

In order to study the effects of agonists on the EP_2-receptor which normally causes relaxation of the tissues in response to stimulation some tissue activity had to be induced in the rat uterus preparation. This preparation has no tone and to induce rythmic activity similar to the spontaneous activity seen at the beginning of the experiment, oxytocin (2.5 milliunits ml^{-1}) was added to the superfusate. The EP_2-receptor selective agonist, butaprost (Gardiner, 1986) produced no inhibition in the activity of these oxytocin-driven uterine strips.

The EP_1-receptor blocking drug, AH6809 (10 μM) (Coleman et al., 1985) did not affect responses to either PGE_2 (ED_{50} (range) 13.41 (4.76-28.28) nmoles) or sulprostone (ED_{50} (range) 7.26 (3.01-11.61) nmoles).

In conclusion it appears that there are very few, if any, EP_1-receptors and no EP_2-receptors present in this tissue. Our results support the presence of contractile EP_3-receptors in the rat uterus.

Coleman, R.A. (1987) Prostaglandins and Related Substances, a Practical Approach, ed, C. Benedetto et al., IRL Press, Oxford 267-303
Coleman, R.A. et al. (1985) Br. J. Pharmac. 85, 273P
Coleman, R.A. et al. (1987) Br. J. Pharmac. 91, 407P
Gardiner, P. (1986) Br. J. Pharmac. 87, 45-56
Massele, A.Y. & Senior, J. (1981) Br. J. Clin. Pharmac. 12, 285P.

PROSTANOID RECEPTORS IN GUINEA-PIG UTERUS

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Guinea-pig uterus (GPU) is highly sensitive to the contractile effects of various natural and synthetic prostanoids, particularly those of the E class (Whalley & White 1980a&b; Elger et al., 1981; Eglen et al., 1988). Eglen et al (1988) attempted to characterise the receptors mediating contraction, and demonstrated that GPU is sensitive to TP-, EP-, and FP-receptor agonists but not to those most active at the IP- and DP-receptors. In the present study we have examined the actions of a range of natural and synthetic prostanoids, and have attempted to characterise the receptors present.

Specimens of GPU were obtained from virgin Dunkin-Hartley guinea-pigs (600-800g) pre-treated with diethylstilboestrol ($1mkg^{-1}$ day^{-1} i.p. for two days) to synchronise the stage of the oestrous cycle. Two 1.5cm muscle strips were obtained from each uterine horn and mounted under an initial tension of 2g. Preparations were superfused at a rate of $2ml\ min^{-1}$ with oxygenated (95% 0_2/5% $c0_2$) Krebs solution at 37°C containing indomethacin (2.8μM).

The order of potency of the natural prostaglandins and the stable thromboxane mimetic U-46619 (11,9-epoxymethano PGH_2) was as follows; PGE_2 > U-46619 > PGI_2 = PGD_2 = $PGF_2\alpha$ (ED_{50} (95% C.L.) nmoles = 0.040 (0.034-0.048, n=6), 0.70 (0.51-0.97, n=5), 5.1 (2.4-10.8, n=4), 8.0 (2.5-25.6, n=5), and 10.7 (3.1-37.2, n=5), respectively). At a concentration of 1μM, the TP-receptor blocking drug GR32191 antagonised responses to U-46619 (dose ratio ~ 1000), and in all subsequent experiments, GR32191 (1μM) was included in the superfusion fluid to prevent TP-receptor involvement. Responses obtained to PGE_2, $PGF_2\alpha$, PGD_2 or PGI_2 in the presence of GR32191 were not significantly different from those obtained in its absence. To investigate the involvement of FP-, DP-, and EP-receptors, a selection of synthetic receptor-selective agonists were tested. Whilst both the FP-receptor agonist ICI81008, and the DP-receptor agonist BW245C (Coleman 1987) were inactive, the EP-receptor selective compounds sulprostone (EP_1/EP_3) and rioprostil (EP_2/EP_3) (Coleman et al 1988) and the IP/EP_1-selective compound iloprost (Coleman et al 1987) were all contractile agonists (ED_{50} (95% C.L.) nmoles = 0.43 (0.05-3.89, n=6) 0.10 (0.03-0.30, n=6), 4.7 (0.7-31.3, n=5), respectively), although substantial variability in potency was observed. The EP_1-receptor blocking drug AH6809 (10μM) had no effect against contractions to PGE_2 and sulprostone but did exhibit some antagonism of responses to iloprost, particularly at the lower end of the iloprost concentration-effect curve.

These data support the presence in GPU of both TP- and EP-receptors mediating contraction. Furthermore, the results suggest that the EP-receptors comprise a mixed popultion of EP_1 and EP_3-receptor subtypes, but that the EP_3-receptors appear to predominate.

Coleman, R.A. et al. (1988). Br. J. Pharmac. 95, 724P.
Coleman, R.A. (1987) in Prostaglandins and Related Substances, A Practical Approach. ed. C. Benedetto et al. IRL Press, Oxford, 267-303.
Eglen, R.M. et al (1988) FASEB J. 2, 1572.
Elger, W. et al (1981). Prostaglandins 21, 2, 259-266.
Whalley, E.T. & White, S.K. (1980a). Br. J. Pharmac. 68, 150p-151p.
Whalley, E.T. & White, S.K. (1980b). Br. J. Pharmac. 69, 309p.

DUAL ACTIVITY OF THE THROMBOXANE A_2 ANALOGUE, STA_2, ON HUMAN PLATELETS

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In characterising thromboxane (TP-) receptors, it would appear prudent to estimate the agonist potencies of stable analogues of both PGH_2 and TXA_2. We wish to point out a potential problem with the use of STA_2 (9,11-thia-11a-carba TXA_2) (Katsura et al, 1983), a potent full agonist on thromboxane-sensitive smooth muscle preparations (Jones et al, 1989).

On washed human, rat and rabbit platelets, STA_2 is between 3 and 6 times more potent as an aggregator than the PGH_2 analogue U-46619 (Jones et al, 1987). However we have observed reduced responses to STA_2 on human platelets at concentrations above that required for maximal aggregation (Fig.1). This effect was not seen with either U-46619 on human platelets or STA_2 on rat and rabbit platelets. In the presence of a high concentration of EP 092 (6μM), STA_2 inhibited PAF(100nM)-induced aggregation of human platelets (Fig.1). U-46619 up to 3000nM did not affect PAF action. STA_2 had little effect (inhibition <20%) on ADP(1μM)-induced aggregation of rat platelets or PAF(10nM)-induced aggregation of rabbit platelets.

pA_{10} values for block of platelet TP-receptors by EP 092 using U-46619, STA_2 and PGH_2 (synthetic, 80μM dazoxiben present) as agonists were as follows: human 6.77, 7.85, 6.61; rat 6.84, 6.86, 6.58; rabbit 5.75, 6.04, 6.02. The high pA_{10} value for EP 092 vs STA_2 on human platelets may be due to an inhibitory activity of STA_2 itself at the higher concentrations required to overcome the receptor block.

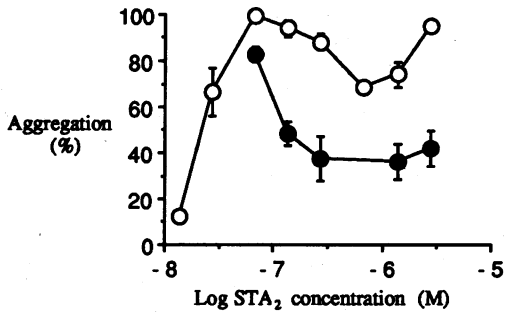


Figure 1. Aggregation of washed human platelets by STA_2 (○) and the effect of STA_2 on PAF aggregation in the presence of 6μM EP 092 (●). Means ± s.e.m (n = 4).

We have previously shown that two other analogues of TXA_2, CTA_2 and PTA_2, have inhibitory activity against non-thromboxane agents on human platelets (Armstrong et al, 1985). However, in the case of STA_2 acting alone, the inhibitory effect may be less obvious since it is opposing a full agonist action rather than a partial agonist action. This dual effect on human platelets may contribute to the low aggregating potency reported of STA_2 in some studies; for example, on human PRP the STA_2 EC_{50} = 1.1μM, 2.3 times greater than that of U-46619 (Mais et al 1985).

Armstrong, R.A. et al (1985) Br.J.Pharmacol., 84, 595-607
Jones, R.L. et al (1987) Br.J.Pharmacol., 90, 226P
Jones, R.L. et al (1989) Br.J.Pharmacol., 96, 875-887
Katsura, M. et al (1983) Adv.Prost.Thromb.Leuk.Res., 11, 351-357
Mais, D.E. et al (1985) J.Pharmacol.Exp.Ther., 233, 418-424

IS TOLFENAMIC ACID A DUAL INHIBITOR OF CYCLO-OXYGENASE AND 5-LIPOXYGENASE?

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Arachidonic acid metabolites derived from both cyclo-oxygenase (CO) and 5-lipoxygenase (5LO) pathways are mediators of inflammmation. Inhibitors of CO are clinically effective anti-inflammatory agents and dual inhibitors of 5LO and CO may have greater therapeutic efficacy in inflammatory diseases. Recently, therapeutic concentrations of tolfenamic acid, a non steroidal anti-inflammatory drug, were reported to inhibit 5LO and CO in plasma-free leukocyte suspensions in vitro (Moilanen et al, 1988). We have investigated the effects of tolfenamic acid on CO and 5LO in vitro and ex vivo and we conclude that in the presence of physiological concentrations of plasma proteins the drug is a selective CO inhibitor.

Release of arachidonic acid metabolites from human leukocytes and human or rat blood was induced by calcium ionophore A23187 (McMillan et al, 1985; McMillan et al, 1986). In some experiments studies with leukocytes were performed in the presence and absence of plasma. Release of the 5LO product, LTB_4, and the CO product, TXB_2, was measured by radio-immunoassay.

In plasma-free suspensions of human leukocytes, tolfenamic acid potently inhibited A23187-induced release of the CO product TXB_2 (IC_{50} = 0.05uM) and at higher concentrations in this system the drug also inhibited release of the 5LO product LTB_4 (IC_{50} = 10uM). In human blood, tolfenamic acid was less potent at inhibiting release of TXB_2 (IC_{50} = 6.5uM) and no significant inhibition of release of LTB_4 was observed at concentrations up to 100uM. In studies with rat blood in vitro, tolfenamic acid also inhibited release of TXB_2 (IC_{50} = 5uM) but not LTB_4. In addition, oral administration of tolfenamic acid (100mg/Kg, p.o.) inhibited ex vivo synthesis of TXB_2 in rat blood (97%; $P<0.001$) but did not significantly inhibit release of LTB_4.

In accord with previous reports, these data demonstrate that tolfenamic acid inhibits LTB_4 release from human leukocytes (Moilanen et al, 1988) but this is observed at more than 100 times the concentrations required to inhibit synthesis of CO products. Tolfenamic acid is extensively bound to plasma proteins, as demonstrated by the marked decrease in CO inhibitory potency in whole blood, and in whole blood studies we found no evidence for inhibition of 5LO. We conclude that at therapeutic blood levels (7-20 uM; Pentikainen et al, 1981) tolfenamic acid is a selective CO inhibitor and that neither its therapeutic efficacy nor its low gastric irritancy is likely to be a consequence of 5LO inhibition.

McMillan, R.M., Masters, D.J., Sterling, W.W. and Bernstein, P.R. (1985). IN "Prostaglandins, Leukotrienes and Lipoxins" (Ed. J.M.Bailey), pp655-668, Plenum Press: New York.
McMillan, R.M., Millest, A.J., Proudman, K.E. and Taylor, K.B. (1986). Br. J. Pharmacol. 87, 53P
Moilanen, E., Alanko, J., Seppala, E. and Vapaatalo, H. (1988). Agents and Actions, 24, 387-394.
Petikainen, P.J., Neuvonen, P.J. and Backman, C. (1981). Eur. J. Clin. Pharmacol., 19, 359-365.

PROBENECID INCREASES URINARY PGE_2 EXCRETION IN THE ANAESTHETISED RAT

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Probenecid, an inhibitor of organic acid transport in the proximal tubule, is well known to inhibit the tubular secretion of prostaglandins (Bito & Baroody 1978). Probenecid however is also a uricosuric agent inhibiting both the tubular reabsorption and the secretion of urate (Frankfurt & Weinman 1977). More recently, experiments using probenecid in man have identified a pathway for the tubular reabsorption of the eicosanoid,TXB2 (Zipser & Smorlesi 1984). Since considerable controversy exists over the importance of the tubular reabsorption of PGE2 in determining its urinary excretion rate, we have investigated the effect of probenecid on urinary PGE2 excretion in the anaesthetised rat.

Male Wistar rats (300-350G) were anaesthetised with thiobutabarbitone (175mg/kg) and infused with 0.154M sodium chloride at 6ml/h through a cannula placed in the jugular vein for a 6 hour period. Urine was collected from a catheter placed in the bladder and assayed for PGE2 as described previously (Haylor et al 1984). Probenecid was administered by an I.V. bolus injection(100mg/kg) followed by an I.V. infusion at 100mg/kg/hr. The dose of probenecid employed was the minimal dose required to produce the maximal inhibition of proximal PAH secretion. PAH secretion was measured by the local renal injection of isotopically labelled PAH and inulin in a separate series of experiments. Urine measurements were compared in the 1-hour periods before and after probenecid administration, probenecid being administered after 5h of saline infusion.

In a control series (n=5) receiving the drug vehicle, urinary PGE excretion remained unchanged at 9.6 ± 3.5 compared to 9.7 ± 3.8 pmol/h. In the experimental series (n=16) however, urinary PGE excretion increased ($P<0.01$) from 13.1 ± 1.3 to 105.0 ± 23.2 pmol/h during probenecid administration. Probenecid administration also produced a significant fall in urine flow from 4.7 ± 0.4 to 1.6 ± 0.2ml/h ($P<0.001$) while urine pH remained unchanged. Systemic blood pressure fell by a mean of 23 mmHg. However, in a separate series of experiments the fall in systemic blood pressure induced by probenecid remained unaffected by the administration of indomethacin 10mg/kg I.V..

The large increase in urinary PGE excretion following probenecid is consistent with a high degree of tubular reabsorption for PGE2 of some 85%. However, a number of other explanations are equally plausible including the possibility that probenecid could increase the renal delivery of PGE2 by reducing its systemic metabolism.

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Bito,L.Z. & Baroody,R.A. (1978) Am. J. Physiol. 234 F80-F88.
Frankfurt & Weinman (1977) Proc. Soc. Exp. Biol. Med. 155 554-557.
Haylor,J., et al (1984) Clin. Sci. 66 675-681.
Zipser,R.D. & Smorlesi,C. (1984) Prostaglandins 27 257-267.

PHARMACOLOGICAL REACTIVITY OF MYOFIBROBLAST CONTAINING TISSUES FROM A RANGE OF HUMAN PATHOLOGICAL CONDITIONS

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Myofibroblasts are unique mesenchymal cells capable of both contraction and collagen production. The morphological, histochemical and functional evidence first reported by Gabbiani et al. (1971) identified these cells as being responsible for the force behind wound contracture. Unfortunately, situations arise when myofibroblast accumulation and proliferation leads to excessive contraction, collagen production and scar formation often resulting in functional impairment and deformity. Conditions caused or exacerbated by myofibroblast accumulation include keloid formation and hypertrophic scarring, Dupuytren's disease and contraction of capsules surrounding mammary implants (for review see Lipper et al., 1980). This study investigated the pharmacological reactivity of myofibroblast containing tissues from these conditions together with those from actively granulating human wounds. Normal skin and 'mature' scar being used as controls.

Tissue samples (Keloid n = 6, hypertrophic scar n = 2, 'mature' scar n = 5, palmar nodules of Dupuytren's disease n = 14, breast expander capsules (<6 months since insertion) n = 28, granulation tissue n = 9, and normal dermis (post auricular n = 9, palmar n = 2) were obtained from patients undergoing surgery at the Plastic Surgery Department of St Luke's Hospital, Bradford. Each tissue was used within 60 minutes of excision, cleared of extraneous material and cut into strips (approx 25 mm x 2 mm x 2 mm), along, where recognisable, the longitudinal axis. All tissues were superfused with Krebs Henseleit solution (37°C and flow rate = 2ml min^{-1}) for isometric (1g preload) recording (Grass FT03C). The effects of a range of agonists were studied by comparing dose/concentration-effect relationships, considering (a) the magnitude of the maximum tension generated (mg) and (b) the duration of the effect (mins). Agonists administered in bolus doses (<100μl) were potassium chloride (1-8 mg), barium chloride (1-8 mg), mepyramine (100μg-8mg) and on a limited number of tissues 5-hydroxytryptamine (5-HT) (1-20μg).

Only 3 of the granulating wound samples showed any responsiveness to potassium or barium ions, all other tissues were insensitive at all doses. Mepyramine produced contractile, dose-dependent, reproducible and reversible responses in all myofibroblast containing tissues. Normal skin and mature scar samples were insensitive to this agent. 5-HT failed to produce responses in any of the samples in which it was tested (breast capsule (n = 14), Dupuytren's nodules (n = 4) and granulation tissue (n = 1)).

This study demonstrates that although the myofibroblast containing tissues were obtained from such a wide range of pathological conditions they all exhibited a similar pharmacological profile. The responsiveness to the agonists used agrees with predictions from studies employing other myofibroblast containing tissues including human placenta (Cross et al 1988a), Wharton's jelly (Cross et al. 1988b) and rat testicular capsule (Chander et al. 1985). The study also clearly demonstrates that myofibroblasts do not behave pharmacologically the same as smooth muscle cells, a proposal made on the basis of the very limited experimental evidence of Gabbiani et al. (1971).

Chander, C.L. & Naylor, I.L. (1985) Br. J. Pharmacol. 86, 517P
Cross, S.E. & Naylor, I.L. (1988a) Br. J. Pharmacol. 95, 632P
Cross, S.E. & Naylor, I.L. (1988b) Br. J. Pharmacol. 95, 831P
Gabbiani, G., Ryan, G.B. and Majno, G. (1971) Experientia 27, 549-50
Lipper, S., Kahn, L.B. & Reddick, R.L. (1980) Path. Ann. Pt(1), 409-441

INEFFECTIVENESS OF β-ADRENOCEPTOR ANTAGONISTS IN RAT ADJUVANT-INDUCED ARTHRITIS

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It has recently been reported that the mixed β1/β2 adrenergic antagonist propranolol, and the β2 antagonists ICI 118,551 and butoxamine can attenuate rat adjuvant arthritis when assessed radiologically at 28 days (Levine et al 1988). These findings are of importance since they suggest that β antagonists may find a role in the therapy of rheumatic diseases. We have therefore examined the effects of propranolol, ICI 118,551, metoprolol, the β2 agonist salbutamol and the NSAID indomethacin on adjuvant arthritis in the Alderley Park (AP) Wistar-derived rat.

Adjuvant arthritis was induced in male AP rats by subplantar injection of 500μg Mycobacterium tuberculosis in mineral oil into the right hind foot. Animals were treated from two days before adjuvant administration to twenty one days post-injection with metoprolol (50mg/kg ip), ICI 118,551 (25mg/kg ip), propranolol (20mg/kg ip), or salbutamol (5mg/kg ip) given three times daily or indomethacin (3mg/kg po) given once daily. Arthritis was monitored by measurement of hind paw thickness, cumulative weight gain, serum acute phase protein levels (alpha 1 glycoprotein), clinical score, radiological evaluation of the hind paws on days 14 and 21, and terminal histology of the hind paws.

In contrast to the finding of Levine et al, propranolol and ICI 118,551 had no effect on radiological damage in the hind paws on days 14 or 21.Treatment with metoprolol and salbutamol also had no effect on radiological damage at either time. Indomethacin, however, inhibited radiological damage at both timepoints ($p<0.01$). None of the β antagonists had any effect on right foot swelling, weight gain, serum alpha 1 glycoprotein, clinical score or histological damage. Propranolol,unlike ICI 118,551 or metoprolol, inhibited left foot swelling on days 14 and 21 ($p<0.05$). Interestingly, salbutamol, a β2 agonist, also inhibited left foot swelling and histological damage but had no effect on the other disease parameters. Indomethacin, as the positive control, inhibited left and right foot swelling ($p<0.05$), clinical score ($p<0.01$), lowered serum alpha 1 glycoprotein levels ($p<0.01$), and increased weight gain relative to controls ($p<0.05$), but had no effect on histological damage.

In conclusion, we were unable to demonstrate, using a multi-parameter assessment, that β adrenoreceptor antagonists have a biologically significant inhibitory effect on adjuvant arthritis in the AP rat. Further studies are required to identify the reasons for the differences between these data and those of Levine et al.

Levine et al PNAS (1988) 85, 4553-4556.

THE EFFECT OF α_1-ACID GLYCOPROTEIN ON QUININE DISPOSITION IN THE ISOLATED PERFUSED RAT LIVER PREPARATION

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α_1-Acid glycoprotein (AAG) is known to increase non-specifically in response to certain pathophysiological changes (Kremer *et al* 1988). During malaria, plasma AAG is elevated with a compensatory fall in albumin (Silamut *et al* 1985). Quinine binds to AAG rather than albumin *in vitro* (Mihaly *et al* 1987) so changes in AAG concentrations may influence the disposition of quinine. Malaria infection alters quinine disposition in the isolated perfused rat liver (IPRL) preparation (Mansor *et al* 1989).

We have studied the effect of 2 different concentrations of AAG on quinine disposition in livers isolated from 3 groups of male Wistar rats (200-250g); (A) Control, without the addition of bovine AAG (N=6), (B) Addition of AAG of 50 mg/100ml; N=4 (normal concentration) and (C) Addition of AAG of 200 mg/100ml; N=4 (the concentration observed during malaria infection (Voulgari *et al* 1982)). Following bolus administration of quinine (1mg), perfusate was sampled at intervals to 4 h in each group and perfusate plasma assayed by HPLC. Additional samples were taken hourly to measure protein binding by ultrafiltration.

Pharmacokinetic parameters are tabulated below

Parameter	Control	50 mg/100ml	200 mg/100ml
AUC (nghr/ml)	1856 ± 460	2006 ± 659	6035 ± 2374**
CL (ml/min)	9.57 ± 2.87	8.89 ± 2.38	3.10 ± 1.17**
Vd (ml)	1198 ± 416	1690 ± 330	466 ± 95**
T½ (hr)	1.47 ± 0.39	2.27 ± 0.44	1.88 ± 0.64

Table 1: Pharmacokinetic parameters (mean ± s.d.) for quinine in the IPRL. (**P < 0.01 compared with control, ANOVA, accepting P < 0.05 as significant).

Significantly higher perfusate concentration of quinine, as evidenced by an increased AUC were observed in perfusate from Group C (200 mg/100ml) compared to controls. No significant difference in AUC was observed between controls and Group B (50 mg/100ml). The significant difference in AUC (P < 0.05) of Group B and C is related to change in Vd (P < 0.005) and Cl (P < 0.01) as there is no difference in T½ between 50 and 200 mg/100ml; AAG. Higher concentrations of AAG (200 mg/100ml) resulted in a significant decrease in Cl (68%) and Vd (61%) but not T½ compared to control. At lower concentrations (50 mg/100ml) compared to control, there was no significant difference in Cl, Vd and T½. Percentage unbound quinine in control and experimental perfused livers 1h after drug administration was 64 ± 3% (control), and 51 ± 4% (50 mg/100ml; AAG), and 20 ± 5% (200 mg/100ml; AAG). Over 4h, the percentage of unbound drug increased in all groups.

These data indicate that changes in AAG can affect the disposition of quinine in the IPRL and emphasises the importance of alterations in unbound drug concentrations in disease and their relation to the pharmacological response.

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Kremer J.M.H. *et al.* (1988) Pharmac. Rev. 40, 1-47.
Silamut K. *et al.* (1985) Am. J. Trop. Med. Hyg. 3, 681-686.
Mihaly G.W. *et al.* (1987) Br. J. clin. Pharmac. 24, 769-774.
Mansor S.M. *et al.* (1989) Br. J. Pharmac. 96, 296P.
Voulgari F. *et al.* (1982) Br. Heart J. 48, 352-356.

TIME-COURSE CHANGES IN LIVER CYTOCHROMES P-450 FOLLOWING INJECTION OF EMULSIFIED PERFLUOROCHEMICALS IN RATS

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Emulsified perfluorochemicals (PFCs) are attracting interest as potential physiological gas transport fluids and have been tested for oxygenation of ischaemic tissues and isolated organs (Lowe, 1987). Components of such emulsions are known to induce cytochromes P-450 (P-450) activity in the liver (Obraztsov et al., 1985) although the time course of such changes, possible differences between male and female animals, and effects of emulsion composition have not been examined in detail. We have therefore studied the effects of low doses of different PFC emulsions on this enzyme system in rats.

Male or female Wistar rats (body weight (b.w.): 180-310g; n = 143) were used. They were injected intravenously (i.v.) via a tail vein with 10 ml/kg b.w. of one of the following: (i) saline (0.9% w/v NaCl); (ii) Fluosol-DA 20% (F-DA; Green Cross, Japan); (iii) Oxypherol (FC-43; Green Cross); and (iv) a novel 20% (w/v) perfluorodecalin (FDC) emulsion containing 1% (w/v) of a C-16 oil additive and 4% (w/v) Pluronic F-68 (Sharma et al., 1987). At 24hr, 72hr and 7 days after injection, groups of animals were killed and their livers removed and weighed. Tissues were homogenized in 1.15% (w/v) Tris/HCl buffered KCl solution and hepatic microsomal protein and P-450 concentrations measured using standard assays (Omura & Sato, 1964).

Mean liver weights in male rats were increased by 27% and 32% ($P < 0.01$) at 72hr and 7 days respectively following injection of the novel emulsion. Liver weight was similarly increased by 17% at 7 days after injection of F-DA ($P<0.05$); no comparable changes occurred following injection of FC-43. In female rats, liver weight was also increased by 10% at 72 hr following injection of novel emulsion ($P<0.05$) but was unaffected by injection of either F-DA or FC-43. Mean liver P-450 concentrations in male rats were increased up to 3 fold at 72 hr following injection of either F-DA ($P<0.05$) or the novel emulsion ($P<0.02$); P-450 was up to 65% greater ($P<0.01$) than the corresponding control mean value (0.96 ± 0.12 nmol/mg protein; n = 4) at 7 days after injection of novel emulsion in male rats. P-450 concentrations in female rats did not change in response to injection of F-DA, FC-43 or the novel emulsion; overall mean liver P-450 in the 3 groups of control female rats was 0.64 + 0.09 nmol/mg protein (n = 12).

These results show that emulsion composition is an important determinant of liver microsomal P-450 induction by emulsified PFCs in rats. The increase in P-450 following injection of F-DA or novel emulsion was consistent with previous findings that FDC is the active principle involved (Obratzsov et al., 1985). We speculate that the marked variation in response between male and female rats is due, at least in part, to differences in circulating androgens which are known to affect liver P-450 activity in this species (Kato & Onoda, 1970).

Kato, R. & Onoda, K. (1970) Clin. Pharmac. 19, 1649-1660.
Lowe, K.C. (1987) Comp. Biochem. Physiol. 87A, 825-838.
Obratzsov, V.V., Shekhtman, D.G., Sologub, G.R. & Beloyartsev, F.F. (1985) Biochem. U.S.S.R. 50, 1041-1048.
Omura, T. & Sato, R. (1964) J. Biol. Chem. 239, 2370-2385.
Sharma, S.K., Bollands, A.D., Davis, S.S. & Lowe, K.C. (1987) In Oxygen Transport to Tissue, Vol. IX (eds. Silver, I.A. & Silver, A.), pp. 97-108. London, Plenum.

EMULSIFIED PERFLUOROCHEMICALS AS ADJUNCTS TO PHOTODYNAMIC THERAPY IN MICE

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Photodynamic therapy (PDT) is being evaluated for the treatment of tumours in man. It depends on the use of tumour-localising porphyrins that are activated by visible light to produce tumour destruction (Fingar et al., 1987). PDT is oxygen dependent and recent attention has focussed on the value of emulsified perfluorochemicals (PFCs) for improving tumour oxygenation and thereby increasing the effectiveness of PDT. We have therefore studied the effects of PDT on skin and tumours in mice previously injected with a novel perfluorodecalin (FDC) emulsion.

Inbred BALB/c female mice with subcutaneous implants of a plasma cell tumour (Berenbaum et al., 1986) were given various doses of a new, potent photosensitizer, meta-tetra(hydroxyphenyl)porphyrin (m-THPP) (Berenbaum et al., 1986). 24h later, tumours and skin were exposed to 10 J cm ^{2} of light at the activating wavelength (648 nm) from a dye laser. Some mice were given, at 2.5 hr before PDT, 0.5 ml/10g b.w. of a 20% (w/v) emulsion of FDC stablized with 1% (w/v) of a C-16 oil additive, perfluoroperhydrofluoranthrene (Sharma et al., 1987). The next day, mice were injected i.v. with 0.2 ml of 1% (w/v) Evans Blue in saline and killed 1 hr later. The depth of necrosis was measured in tumour slices as reported previously (Berenbaum et al., 1986). Additional groups of animals were injected with m-THPP either 24 hr or 48 hr before light and emulsion either 2.5 hr or 24 hr before light. 4 hr after illumination, they were killed and a 1 cm disc of skin was removed and weighed; skin Evans Blue concentration was measured by absorbance at 625 nm following sodium sulphate-acetone extraction (Berenbaum et al., 1986).

The mean depth of tumour necrosis 24 hr after PDT was 2.5 ± 0.1 mm (n = 7) in mice given FDC emulsion and 2.14 ± 0.6 mm (n = 7) in controls; this difference was not significant ($P > 0.05$). However, PDT-induced skin damage was significantly less in mice injected with FDC emulsion, as shown by shifts in the dose-response curves for skin disc weight (oedema) and uptake of Evans blue dye (vascular permeability). For example, in mice injected with 12.5 μm/kg m-THPP 24 hr before light and emulsion 2.5 hr before light, mean skin disc weight was up to 60% lower than control. Moreover, in animals injected with 12.5-50 μm/kg m-THPP 48 hr before light and emulsion 24 hr before light, skin Evans Blue concentration was up to 44% lower than control.

These results show that, while pretreatment with emulsified fluorocarbons does not alter PDT-induced tumour necrosis in mice, it nevertheless provides protection against photodynamic skin damage. The protective effects of the emulsion may be due to inhibition of leucocyte-mediated inflammatory responses in accord with previous observations (Lowe, 1987). Alternatively, the apparent skin protection may simply reflect haemodilution effects of the emulsion. Further studies are in progress to distinguish between these possibilities.

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Berenbaum, M.C., Akande, S.L., Bonnett, R., Kaur, H., Ioannou, S., White, R.D. & Winfield, U.-J. (1986) Br. J. Cancer 54, 717-725.

Fingar, V.H., Mang, T.S. & Henderson, B.W. (1988) Cancer Res. 48, 3350-3354.

Lowe, K.C. (1987) Comp. Biochem. Physiol. 87A, 825-838.

Sharma, S.K., Bollands, A.D., Davis, S.S. & Lowe, K.C. (1987) In Oxygen Transport to Tissue, Vol. IX (eds Silver, I.A. & Silver, A.), pp. 97-108. London, Plenum.

589C80, A POTENT LONG-ACTING LEFT-SHIFTER OF THE OXYGEN SATURATION CURVE OF HAEMOGLOBIN *IN VIVO* IN THE RAT

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A compound which left-shifts the oxygen saturation curve of haemoglobin is of potential utility in the prophylaxis of sickle cell disease (Dean & Schechter, 1978) and may also be useful in cancer therapy (Adams et al, 1986). 12C79 (5-(2-formyl-3-hydroxyphenoxy)pentanoic acid) was designed to produce such a left-shift by a preferential binding between the terminal amino groups of the α-chains in the oxy conformation of haemoglobin (Beddell et al, 1984). 589C80 (4-(2-formyl-3-hydroxyphenoxymethyl)benzoic acid) is a close analogue in which the tetramethylene chain of 12C79 is replaced by a benzyl group. This change results in an increased potency of interaction with haemoglobin and a much longer duration of action in vivo.

Oxygen saturation curves were measured at 37°C for dilute human haemoglobin solutions (23μM) using a Hemox Analyzer (TCS Medical Products) and for whole human blood using a Hem-O-Scan (Aminco). In both solution and whole blood a fully left-shifted curve was obtained with 589C80 to haemoglobin ratios of 4 to 1 (100μM in solution and 10 mM in whole blood). The solution curve was devoid of cooperativity with a Hill Coefficient of 1 and an oxygen affinity equivalent to that of the oxy conformation of the haemoglobin tetramer (P50 ca 1 mm Hg). This suggests that 589C80 binds more tightly to oxy haemoglobin than does 12C79 since the fully shifted curve elicited by 12C79 still displays cooperativity. At lower concentrations of 589C80 the oxygen saturation curves were markedly biphasic which enabled estimation of the amount of haemoglobin reacted (%MOD) as described previously for 12C79 (FitzHarris et al, 1985).

Groups of 5 male rats were treated i.v. with either 12C79 (87.5 mg/Kg) or 589C80 (100 mg/Kg) (i.e. mole equivalent) and blood samples taken by tail bleed at various times. The %MOD values were estimated from the measured oxygen saturation curves and plotted against time. Peak %MOD levels were achieved rapidly (< 15 min) for both compounds but there was a very marked difference in duration of effect. The $t_{\frac{1}{2}}$ was estimated as 2.8 h for 12C79 and 16 h for 589C80. An even longer duration of action with 589C80 was observed after oral dosing but in this case the peak %MOD level was attained only after several hours. Thus, 589C80 is a potentially useful compound for assessing the effects on disease states of a long term left-shift of the oxygen saturation curve.

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Adams, G.D. et al. (1986) Int. J. Radiation Oncology Biol. Phys. 12, 1299 - 1302.
Beddell, C.R. et. al. (1984) Br. J. Pharmac. 82, 397 - 407.
Dean, J. & Schechter, A.N. (1978) New Eng. J. Med. 299, 752 - 763, 804 - 811 & 863 - 870.
FitzHarris, P. et. al. (1985) Br.J. Clin. Pharm. 19, 471 - 481.

5-HT_3 RECEPTORS MEDIATE INHIBITION OF [^{3}H]ACETYLCHOLINE RELEASE FROM RAT ENTORHINAL CORTEX *IN VITRO*

LOCAL APPLICATION OF ω-CONOTOXIN GVIA TO SYMPATHETIC NERVE TERMINALS IN THE GUINEA-PIG ISOLATED VAS DEFERENS

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A novel peptide, ω-conotoxin first isolated from the fish hunting mollusc *Conus geographus,* has been shown to inhibit voltage sensitive calcium channels in nerves but not in muscle (McCleskey *et al.*1987). In contrast to many other calcium-entry blockers, electrically evoked transmitter release in a variety of smooth muscle preparations is potently inhibited by this peptide (Maggi *et al.* 1988). In the present study the effects of synthetic ω-conotoxin GVIA on impulse propagation and transmitter release from sympathetic nerve terminals have been investigated using focal extracellular recording techniques (see Brock & Cunnane, 1988). Briefly, a small suction electrode was applied to the surface of the tissue to record concomitantly the arrival of the nerve impulse in the terminals and spontaneous and evoked release of transmitter, presumably adenosine 5'-triphosphate . The recording electrode was internally perfused with either Krebs solution or Krebs solution containing 10 nM ω-conotoxin GVIA (Fig. 1.)

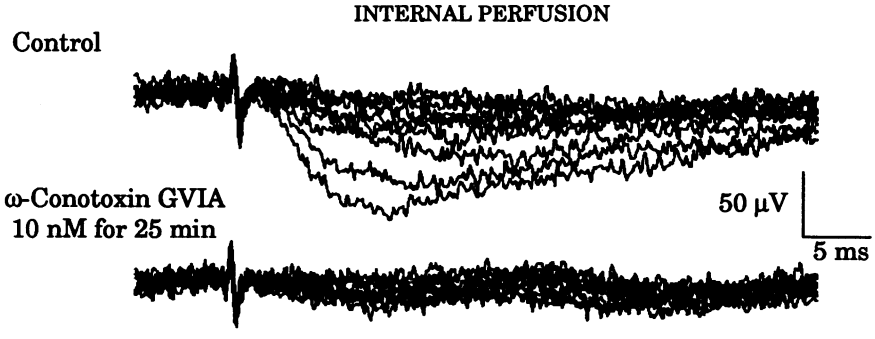


Fig 1. Inhibition of transmitter release by locally applied ω-conotoxin GVIA in the guinea-pig vas deferens. Vasa deferentia were pinned out in a 2 ml organ bath and superfused at 2 ml/min with Krebs solution at 36°C. The hypogastric nerve trunk was stimulated through a Ag/AgCl suction electrode. Electrical activity was evoked by trains of 10 stimuli at 1 Hz.

ω-Conotoxin GVIA blocked electrically evoked transmitter release irreversibly without blocking impulse propagation. Spontaneous transmitter release was apparently unaffected. In experiments following bath application of the peptide the nerve terminal impulse was often reduced in size perhaps reflecting the loss of a calcium component.

REFERENCES

McCleskey, E. W. et al., (1987) Proc. Natl. Acad. Sci. USA. **84**, 4327-4331
Maggi, C. A. et al., (1988) Naunyn-Schmiedeberg's Arch. Pharmacol. **338**, 107-113
Brock, J. A. & Cunnane, T. C. (1988). J. Physiol. **399**, 607-632

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DO PHORBOL MYRISTATE AND PHORBOL DIBUTYRATE ENHANCE NORADRENALINE RELEASE BY THE SAME MECHANISMS?

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Protein Kinase C is present in sympathetic nerves (Malhotra et al, 1988) and has been suggested to be involved in the modulation of action potential-induced noradrenaline release (Malhotra et al, 1988). The role of protein kinase C has been mainly studied using phorbol esters which activate protein kinase C. The purpose of this present study was to compare the effects of phorbol dibutyrate (PDB) and phorbol myristate acetate (PMA) on noradrenaline release from sympathetic nerves.

Mouse isolated atria were incubated with (^{3}H)-noradrenaline and the overflow of radioactivity induced by electrical field stimulation (5 Hz, 60 s) was used as an index of noradrenaline release.

Both PDB (0.001-1.0 μM) and PMA (0.001-1.0 μM) increased stimulation-induced (S-I) overflow of radioactivity in a concentration-dependent manner when in contact with the atria for 15 min, and this enhancement reached a maximum plateau level for both PDB (at 0.1 μM) and PMA (at 0.1 μM). The maximal facilitatory effect of PDB (0.1 μM) was 277 ± 9% of control (mean ± s.e.mean), n=9,and this was significantly higher than than that produced by PMA (0.1 μM, 193 ± 16%, n=8). The smaller effect of PMA was not simply due to PMA requiring a longer time to produce an effect, as the facilitation produced by PMA (0.1 μM) after 15 min of contact with the atria was identical to that produced after 45 and 75 min of contact. When PDB (0.1 μM) was added to the maximally effective concentration of PMA (0.1 μM) the S-I overflow was significantly enhanced (297 ± 33, n=6) and this was not significantly different to that produced by PDB (0.1 μM) alone (277 ± 8, n=9). These results suggest that PMA and PDB have quantitatively different effects on transmitter release, with PMA producing less facilitation. Paradoxically, in purified protein kinase C preparations PMA is more potent than PDB (Castagna et al, 1982) and which suggests that PDB may also act through pathways other than activation of protein kinase C to enhance noradrenaline release. However, the effects of both PDB (0.01 μM) and PMA (0.03 μM) were abolished by the protein kinase inhibitor KT 252a ((8*R**,9*S**,11*S**)-(-)-9-hydroxy-9-methoxycarbonyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1H,8H,11H-2,7b,11a-triazadibenzo[a,g]cycloocta [cde]trinden-1-one; 1.0 μM). It may be that sympathetic nerves posses multiple forms of PKC which are differentially activated by PMA and PDB.

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Castagna, M. et al (1982) J Biol. Chem. 257, 7847-7851.
Malhotra, R.K. et al (1988) J.Neurochem. 51, 967-974.

EVIDENCE FOR PRE-JUNCTIONAL MUSCARINIC RECEPTORS ON PULMONARY SYMPATHETIC NERVES IN THE GUINEA-PIG

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In the airways, the noradrenergic and cholinergic autonomic nerves have opposing effects on airway smooth muscle and also interact at the prejunctional level, so that sympathetic nerves release noradrenaline onto inhibitory adrenoceptors situated prejunctionally on parasympathetic nerve terminals (McCaig 1987). Acetylcholine release is also inhibited by prejunctional M_2 muscarinic autoreceptors (Fryer and Maclagan, 1984). Very little is known however, about modulation of sympathetic neurotransmission by the adjacent parasympathetic nerves and the presence of muscarinic receptors on pulmonary sympathetic nerves has not yet been demonstrated.

In the present experiments the effect of muscarinic agonists on sympathetic nerve-induced relaxations of the isolated, innervated guinea-pig trachea were investigated. The trachea, with the sympathetic nerve trunk, vagi and recurrent laryngeal nerves attached (Blackman and McCaig 1983), was mounted in oxygenated Kreb's solution containing indomethacin ($5x10^{-6}$M) and maintained at 37°C. In order to record relaxations of the trachealis muscle, the intraluminal pressure (ILP) was raised using spasmogens such as acetylcholine (10^{-7} - $3x10^{-5}$ M) or the stable thromboxane analogue U46619 (10^{-8} - 10^{-6} M). Under these conditions stimulation of the sympathetic nerve trunk (40Hz, V_{max}, 0.2ms, 5s) evoked a relaxation response, measured as a decrease in ILP, which was abolished by propranolol (10^{-5} M) but unaffected by hexamethonium ($5x10^{-5}$ M), indicating stimulation of sympathetic post-ganglionic noradrenergic fibres. These nerve-induced relaxations increased as the ILP was raised and therefore all comparisons were made at identical ILPs.

Sympathetic nerve-induced relaxations were reduced in the presence of acetylcholine in a dose-dependent manner, when compared to those obtained at the same ILP in the presence of U46619. When the ILP was raised to 200mm$H_2$0 with acetylcholine, the sympathetic nerve-induced relaxation was 13 ± 3 mm$H_2$0, whereas in the presence of U46619 the relaxation was 80 ± 12 mm$H_2$0. At a higher ILP level (300 mm$H_2$0) the values were 17 ± 7 and 129 ± 20 mm$H_2$0 respectively ($\bar{x}$ ± s.e.m $n \geq 5$). The post-junctional action of noradrenaline was similar in tissues contracted to identical ILPs with either acetylcholine or U46619.

These results suggest a prejunctional inhibitory action of acetylcholine on sympathetic neurotransmission. This inhibitory effect could be blocked with the muscarinic antagonists atropine (10^{-7} M), hexahydrosiladifenidol (10^{-5} M) and pirenzepine (10^{-5} M) at doses which also blocked the postjunctional contractile action of acetylcholine on airway smooth muscle mediated via M_3 muscarinic receptors. The M_2 selective antagonists, gallamine (10^{-5} M) and methoctramine (10^{-5} M), depressed neither the postjunctional action of acetylcholine nor its inhibitory action on sympathetic nerve-induced relaxations.

These results suggest that in the trachea, acetylcholine inhibits transmitter release from sympathetic nerve terminals via pre-junctional muscarinic receptors. These hetero-receptors appear to be similar to the airway smooth muscle M_3 receptors and differ from the M_2 autoreceptors on parasympathetic nerve terminals in the lung.

Blackman J.G. & McCaig D.J. (1983). Br.J. Pharmacol. *80*, 703-710.
Fryer A.D. & Maclagan J. (1984). Br. J. Pharmacol. *83*, 973-978.
McCaig D.J. (1987) Br. J. Pharmacol. *91*, 385-394.

VARIATION IN ESTIMATES OF SELECTIVITY AT MUSCARINIC RECEPTORS IN GUINEA-PIG ILEUM AND ATRIA

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The selectivity of an antagonist, i.e. the extent to which it blocks one type of receptor rather than another, can be estimated from measurements of its affinity for the receptors, either in dose-ratio experiments with functional tissues or in radio-ligand binding experiments with cell fragments or receptor protein. The selectivity is indicated by the difference between the values for different preparations, i.e. as differences in the log. equilibrium constant ($Log.K_B$, $log.K_d$ or pA_2). Though these may look bigger if expressed on an arithmetic scale as the ratio of dissociation constants or of values of (dose-ratio - 1), the variation appears to be log. normal and differences in log.K indicate differences in the free energy of binding, ΔG.

The confidence which can be placed in estimates of selectivity depends on the variation to be expected between two means, always supposing that there are no systematic differences in the way the experiments have been made. Measurements of dose-ratio are made with matching responses and should be independent of the agonist or changes in the sensitivity of the tissue to the agonist but might be affected by changes in the composition of the physiological salt solution, because these might alter the structure of the antagonist binding site (Birdsall et al., 1979). To investigate this, dose-ratios were measured with 4DAMP (4-diphenylacetoxy-N-methyl-piperidine) methobromide and with AFDX-116 on guinea-pig ileum and atria with Krebs' solutions which contained altered concentrations of calcium and magnesium ions.

The first results with atria and ileum suggested that decreased calcium or increased magnesium increased the dose-ratios obtained with 4DAMP methobromide but with a wider range (Ca^{++}:Mg^{++} ratios ranging from 0.5 to 6.4) it was not possible to establish the existence of any trend statistically (even for p=0.1) because of the unexpectedly large variation between groups of experiments. Although the standard error of any one group is usually less than 0.1 log unit, results differing by nearly 0.3 log units were obtained with 4DAMP methobromide on ileum when experiments were later repeated in what should be identical conditions. The sensitivity of the ileum to the agonist, carbachol, varied over a period of months (up to 6-fold at 37°C, 4.5-fold at 30°C) but different results with the antagonists were obtained with ileum which had similar sensitivity to the agonist.

The large between-group variation in dose-ratio may indicate changes in receptor structure or in the ratio of receptor subtypes with time. Whatever the reason, it could explain why estimates of selectivity vary from one laboratory to another. In this particular set of experiments the results for 4DAMP methobromide (4-fold ileal selective) and for AFDX-116 (6-fold atrial selective) show much less selectivity than previously reported (e.g. Barlow & Shepherd, 1986).

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Birdsall, N.J.M., Burgen, A.S.V., Hulme, E.C. & Wells, J.W. (1979). Br. J. Pharmac., 67, 371-377.

Barlow, R.B. & Shepherd, M.K. (1986). Br. J. Pharmac., 89, 837-843.

MUSCARINIC AGONISTS AND GUINEA-PIG PERFUSED TRACHEAL TUBES: EFFECTS OF EPITHELIAL REMOVAL AND ROUTE OF ADMINISTRATION

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Tested in isolated, opened segments of guinea-pig trachea, acetic acid cholinesters (acetylcholine, ACh; methacholine) are potentiated (2-3 fold) by epithelial removal whereas carbamic acid cholinesters (carbachol, CCh; bethanechol) are not. This difference does not result from loss of cholinesterase activity caused by epithelial removal (Dixon et al., 1989). In experiments of this kind, the muscarinic agonist reaches the trachealis by diffusion both from the luminal and adventitial surfaces of the tissue. In order to test whether the effects of epithelial removal are attenuated by agonist penetration from the adventitial surface, we have now examined the effects of epithelial removal on responses of indomethacin (2.8 μm)-treated trachea to ACh, CCh and oxotremorine (Ox) in a tubular preparation (Pavlovic et al., 1988) which allows selective administration of agonist either to the luminal or to the adventitial surface of the tissue.

When agonists were applied to the adventitial surface of the trachea, epithelial removal slightly reduced the potency of CCh and Ox, but did not affect the potency of ACh (Table 1). Maximal responses to these agonists were unchanged by epithelial removal.

When agonists were applied to the luminal surface, epithelial removal markedly potentiated both ACh (approximately 40 fold) and CCh (approximately 10 fold). Ox, too, was potentiated though less markedly (approximately two fold; Table 1). Maximal responses to the agonists were unchanged by epithelial removal.

	Adventitial administration		Luminal administration	
	EP-	EP+	EP-	EP+
ACh	4.96 ± 0.09	5.04 ± 0.02	5.81 ± 0.06	4.18 ± 0.07*
CCh	6.49 ± 0.01	6.84 ± 0.03*	6.67 ± 0.08	5.67 ± 0.08*
Ox	6.66 ± 0.08	7.08 ± 0.10*	7.28 ± 0.07	6.98 ± 0.03*

Table 1. -log EC50 values (mean ± s.e. mean) for muscarinic agonists. Ep- and Ep+ = epithelium-denuded and -intact tissues respectively. * indicates a significant ($p<0.05$) difference from Ep- tissue.

These data suggest that the epithelium modulates the potency of muscarinic agonists most greatly when the agonist is diffusing towards the trachealis only from the luminal surface. The differing degrees of potentiation of the muscarinic agonists may reflect their differing abilities to penetrate a diffusion barrier provided by the epithelium or their differential release of an epithelium-derived relaxant factor.

D.M. Good is an SERC CASE scholar.

Dixon, J.S., Good, D.M., Gosling, J.A. & Small, R.C. (1989). Br. J. Pharmacol. In press.
Pavlovic, O., Fournier, M., Aubier, M. & Pariente, R. (1988). Am. Rev. Respir. Dis. 137 (suppl.) 243.

SENSORY NERVE DEPLETION POTENTIATES NON-ADRENERGIC, NON-CHOLINERGIC INHIBITORY NERVES IN GUINEA-PIG TRACHEA

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Neurogenic responses to electrical field stimulation (EFS) in the guinea-pig trachea are biphasic with an initial atropine-sensitive contraction followed by a prolonged relaxation. The relaxation response is only slightly reduced by propranolol and is therefore predominantly non-adrenergic, non-cholinergic (i-NANC) (Coburn and Tomita, 1973). The neurotransmitter of these i-NANC nerves, while not certain, is likely to be vasoactive intestinal peptide (VIP) or a related peptide (Ellis and Farmer, 1989). Capsaicin pretreatment in guinea-pigs leads to selective degeneration of afferent nerves containing sensory neuropeptides such as substance P (SP), while cholinergic nerves are reported to be unaffected (Jansco et al., 1977). We have studied the effect of sensory nerve depletion on i-NANC responses to EFS and responses to exogenous VIP in guinea-pig trachea.

Upper tracheal segments (immediately adjacent to the larynx) from guinea-pigs pretreated with capsaicin or vehicle and from untreated animals were suspended between platinum field electrodes in 10ml organ baths containing Krebs-Henseleit solution at 37°C, bubbled with 95% O_2/5%CO_2, pH 7.4. In the presence of atropine and propranolol (both 10^{-6}M), the effects of sensory nerve depletion on i-NANC responses to EFS (40V, 0.5ms, 1-50Hz for 30s) and on responses to exogenously applied VIP (10^{-9}-$3x10^{-7}$M) and isoprenaline (Iso) (10^{-11}-$3x10^{-6}$M) were studied. Relaxation responses were expressed as % maximal relaxation of the tissue to papaverine (10^{-4}M). All i-NANC responses were blocked by tetrodotoxin (10^{-6}M).

In animals pretreated with capsaicin, the maximal relaxation response to EFS at 20Hz was 52.6 $\pm$ 5.0% compared with 26.4 $\pm$ 2.9% in vehicle-treated animals ($p < 0.001$, n=10). Relaxation responses to VIP were significantly augmented in capsaicin-treated animals, with a mean EC_{50} value of 31.5nM compared with 47.3nM in controls ($p < 0.001$, n=8). Concentration-response relationships constructed to Iso were not significantly different in tissues from all animal test groups (EC_{50} values of 9.5nM in capsaicin-treated tissues compared with 10.2nM in controls).

In conclusion, our results demonstrate that the i-NANC response elicited to EFS in guinea-pig trachea is significantly enhanced after depletion of neuropeptides from sensory nerves, suggesting that these neuropeptides may modulate i-NANC nerves. The relaxant response to VIP, the putative transmitter for i-NANC nerves was also enhanced, whereas relaxation responses to Iso were unaffected by capsaicin-pretreatment. This suggests that facilitation of i-NANC responses may be, at least in part, due to a selective effect on postjunctional VIP receptors. The mechanism by which neuropeptide depletion from sensory nerves leads to specific increase in VIP-responsiveness is uncertain but may involve either VIP receptors or their coupling to adenylate cyclase.

Coburn and Tomita (1973), Am. J. Physiol., **224**; 1072-1080.
Ellis and Farmer (1989), Br. J. Pharmacol., **96**; 513-520.
Jansco et al., (1977), Nature, **270**; 741-743.

NEUROPEPTIDE Y MODULATES NON-ADRENERGIC, NON-CHOLINERGIC NEURAL BRONCHOCONSTRICTION *IN VIVO* AND *IN VITRO*

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Neuropeptide Y (NPY), is a 36 amino acid peptide localised to sympathetic nerves associated with vessels and smooth muscle within the guinea-pig trachea (Sheppard et al.,1984). NPY has been shown to modulate cholinergic neurotransmission in the guinea-pig trachea by a mechanism which is unaffected by adrenergic blockade, suggesting a prejunctional receptor on postganglionic cholinergic nerves (Stretton and Barnes, 1988). Non-adrenergic, non-cholinergic (NANC) bronchoconstrictor responses in guinea-pig are due to the release of neuropeptides such as substance P (SP) and neurokinins from sensory nerves. We have investigated the effect of NPY on the NANC bronchoconstrictor response elicited both in vivo (via bilateral vagal nerve stimulation) and in vitro (via electrical field stimulation, EFS).

Male Dunkin-Hartley guinea-pigs were anaesthetised with urethane (i.p.). The trachea was cannulated and the animal ventilated with a constant volume pump. Overflow pressure was measured using a modified Konzett and Rossler procedure. Both cervical vagus nerves were sectioned and placed over platinum electrodes for electrical stimulation (5V, 5ms, 3 or 5Hz for 30s). Atropine, propranolol (each 1mg kg^{-1}) and phentolamine (2.5mg kg^{-1}) were administered 30min prior to commencing the experiment. All drugs were administered intravenously. Guinea-pig main and hilar bronchi were dissected and mounted between platinum field electrodes in 10ml organ baths containing Krebs-Henseleit solution at 37°C, bubbled with 95% O_2/5% CO_2 at pH7.4. EFS (40V, 0.5ms, 8Hz for 20s) produced a long lasting NANC contractile response in the presence of atropine and propranolol (10^{-6}M), indomethacin (10^{-5}M) and phentolamine ($2x10^{-6}$M).

NPY inhibited the NANC bronchoconstrictor response elicited to vagal nerve stimulation in vivo in a dose-dependent manner (90.0 $\pm$ 3.4% inhibition at 500ug kg^{-1}), but NPY failed to alter the bronchoconstrictor responses to exogenous SP. NPY also inhibited the NANC bronchoconstrictor responses in both main and hilar bronchi in vitro (90.1 $\pm$ 5.5% in main bronchi and 46.34 $\pm$ 7.5% in hilar bronchi at 10^{-6}M), whilst having no effect on the contractile responses to SP.

These results suggest that NPY modulates the release of transmitter from excitatory NANC nerves. These experiments were conducted in the presence of alpha- and beta-adrenoceptor antagonists and therefore the inhibitory effect of NPY is likely to be mediated by a direct action on its prejunctional receptor on sensory nerves. Adrenergic nerves may therefore modulate NANC bronchoconstriction via NPY receptors in addition to previously demonstrated $alpha_2$-receptors (Grundstrom and Andersson, 1985).

Grundstrom, N. and Andersson, R.G.G. (1985), Naunyn-Schmied. Arch. Pharmacol., **328**; 236-240.
Sheppard, M.N. et al., (1984), Thorax, **39**; 326-330.
Stretton, C.D. and Barnes, P.J. (1988), Br. J. Pharmacol., **93**; 672-678.

THE EFFECT OF SENSORY NERVE DEPLETION ON CHOLINERGIC NEUROTRANSMISSION IN GUINEA-PIG AIRWAYS

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Sensory C-fibre primary afferent nerves, containing neuropeptides such as substance P (SP) and neurokinin A, have been localised in the guinea-pig respiratory tract and appear to mediate non-adrenergic, non-cholinergic (e-NANC) bronchoconstrictor responses. Systemic capsaicin pretreatment in guinea-pigs causes selective degeneration of these nerves (Jansco et al., 1977) with the abolition of NANC bronchoconstrictor responses (Lundberg et al., 1983). Cholinergic nerves are reported to be unaffected (Martling et al., 1984). However, exogenous tachykinins markedly potentiate cholinergic neural bronchoconstriction in the guinea-pig trachea by an effect on NK-2 receptors on postganglionic nerve terminals (Hall et al., 1989). Since the distribution of capsaicin-sensitive sensory nerves is not uniform within the respiratory tract, we have studied the effect of sensory nerve depletion on cholinergic responses at different airway levels in the guinea-pig in vitro.

Upper trachea (adjacent to the larynx), lower trachea (above the carina), main bronchi and hilar bronchi from guinea-pigs either pretreated with capsaicin or vehicle or untreated were suspended between platinum field electrodes in 10ml organ baths containing Krebs-Henseleit solution at 37^{o}C, bubbled with 95% O_2/5% CO_2, pH 7.4. The effect of sensory nerve depletion on both cholinergic contractile responses elicited to electrical field stimulation (EFS) (40V, 0.5ms, 0.5-64Hz, 15s for trachea; 40V, 0.5ms, 8Hz, 20s for bronchi) and contractile responses to exogenous SP and acetylcholine (ACh) were studied. Contractile responses to EFS were expressed as % maximal contraction to 10^{-2}M ACh. All responses were abolished by atropine and tetrodotoxin (10^{-6}M).

Sensory nerve depletion completely abolished the e-NANC component to EFS and significantly reduced the cholinergic responses to EFS at all airway levels when compared with tissues from vehicle-treated and untreated animals (33% reduction in upper trachea, 57% in lower trachea, 29% in main bronchi and 37% in hilar bronchi, at 8Hz). At all airway levels, sensory nerve depletion had no effect on responses produced to either ACh or SP and EC_{50} values were not significantly different in tissues from all test groups.

In conclusion, our results indicate that sensory nerve depletion completely abolishes the e-NANC bronchoconstrictor response and also produces a reduction in cholinergic neurotransmission, suggesting that release of sensory neuropeptides from sensory nerves in airways may facilitate cholinergic neurotransmission at postganglionic nerve terminals.

Hall et al., (1989) Br. J. Pharmacol., 97; 274-280.
Jansco et al., (1977) Nature, 270; 741.
Lundberg et al., (1983) Acta. Physiol. Scand., 119; 243.
Martling et al., (1984) Naunyn-Schmied. Arch Pharmacol., 325; 343.

MODULATION OF RAT GASTRIC BLOOD FLOW DURING PAF CHALLENGE BY OPIATE-SENSITIVE AFFERENT NEURONES

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Intravenous administration of PAF in the rat induces a substantial decrease in gastric mucosal blood flow, which is considered to underlie the subsequent mucosal damage (Whittle et al, 1986). PAF-induced gastric damage is augmented by morphine and the peripherally acting, N-methyl morphine, these effects being reversed by both naloxone and N-methyl nalorphine suggesting actions on peripheral neurones (Esplugues et al, 1989). Likewise, pretreatment with capsaicin, which destroys primary sensory neurones (see Holzer, 1988), augments the gastric damage induced by PAF (Esplugues et al, 1989). We have now determined the effects of capsaicin pretreatment or morphine administration on gastric mucosal blood flow (MBF), estimated by hydrogen gas clearance, following intravenous challenge with PAF.

Male rats (250-320g) fasted for 18h, were anaesthetised with ketamine HCl (100 mg kg^{-1}, i.m.), the stomach exposed and a platinum electrode placed in contact with the mucosa through an incision in the gastric wall, with a reference electrode placed in the peritoneal cavity, for the measurement of hydrogen gas clearance (Pique et al, 1988). In some experiments, 2 day-old rats were anaesthetised with ether, capsaicin (50 mg kg^{-1} s.c.) or vehicle was administered, and the rats studied 3-4 months later. Gastric damage was assessed by histological examination of 4µm wax-embedded sections of corpus mucosa, processed by routine techniques and stained with haematoxylin and eosin.

Intravenous infusion of PAF (25 and 50 ng kg^{-1} min^{-1} for 30 min) induced dose-related damage to the gastric mucosa, with 17 ± 4% and 25 ± 2% (n=8 and 6 respectively) of the length of the histological sections exhibiting glandular disruption, which was significantly greater in capsaicin-pretreated rats (38 ± 3%, n=8 and 54 ± 6%, n=7 of the section length respectively, $P<0.05$). Resting arterial blood pressure (BP) and MBF, and the fall in BP induced by intravenous infusion of PAF was not significantly different in vehicle or capsaicin-pretreated rats. However, capsaicin-pretreatment significantly augmented the fall in MBF following PAF infusion (25 and 50 ng kg^{-1} min^{-1}) from 68±5 to 39± 6% and 41±8 to 17±9% of basal respectively ($P<0.05$). Morphine (1.5mg kg^{-1} i.v.; 5 min prior to challenge) did not alter resting BP or MBF but significantly augmented the fall in MBF induced by PAF (25 ng kg^{-1} min^{-1}) to 42 ± 4% of basal (n=6; $P<0.05$). Mucosal damage induced by this dose of PAF was likewise augmented by morphine (to 30 ± 8% of the section length, n=6, $P<0.05$), while both the increase in PAF-induced damage and the enhanced fall in MBF following morphine was abolished by concurrent administration of naloxone (1mg kg^{-1} i.v.; n=5).

These findings indicate that capsaicin pretreatment or morphine administration augments gastric mucosal damage and accentuates the reduction in MBF induced by PAF. These effects were not a consequence of changes in systemic BP. Thus, activation of opioid-sensitive sensory neurones in the stomach appear to be involved in the regulation of local protective microvascular responses to noxious challenge, perhaps by the release of vasoactive neuropeptides.

Esplugues,J.V., Whittle,B.J.R. & Moncada S.(1989) Br. J. Pharmac., 97, 579-585
Holzer, P. (1988) Neuroscience 24, 739-768
Pique, J.M. et al (1988) Gastroenterology, 95, 642-650
Whittle, B.J.R. et al (1986) Am. J. Physiol. 251, G772-778

EPITHELIUM REMOVAL INCREASES AIRWAY SMOOTH MUCLE SENSITIVITY TO VASOACTIVE INTESTINAL PEPTIDE: EFFECTS OF PEPTIDASE INHIBITORS

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Epithelium removal modulates airway responsiveness to agonists (Farmer, 1987), and there is evidence that the epithelium releases a smooth muscle inhibitory factor(s) (Hay *et al* 1987; Fernandes *et al* 1989). In contrast, augmentation of guinea-pig tracheal relaxation to isoprenaline following epithelium removal appears to be due solely to loss of catecholamine uptake and degradation (Farmer *et al* 1986). Similarly, the increase in responsiveness to contractile effects of substance P and related tachykinins may be due largely to loss of epithelial neutral endopeptidase (NEP) (EC 3.4.24.11), which normally degrades these peptides in guinea-pig trachea (Frossard *et al* 1989; Devillier *et al* 1988). Vasoactive intestinal peptide (VIP) may be a non-adrenergic, non-cholinergic inhibitory neurotransmitter in trachea (Ellis & Farmer, 1989), and although the mechanisms underlying VIP degradation in the airway are unclear, an endopeptidase cleaves VIP in rat spinal tissue *in vitro* (Barbato *et al* 1988). Thus, we have assessed the effects of epithelium removal on tracheal sensitivity to VIP, and the influence of inhibitors of NEP or kininase II.

Transverse strips of guinea-pig trachea were suspended in tissue baths containing oxygenated Krebs-Henseleit solution (37°C), and isometric responses were measured. Epithelium was gently removed from alternate strips using cotton applicators. Relaxations induced by VIP, added cumulatively, were expressed as a percentage of the maximum response to sodium nitroprusside (30 μM). pD_2 Values were determined from regression analyses of logit-transformed concentration-response curves. Where appropriate, enzyme inhibitors were added to the bath 20 min prior to VIP. We also determined the effect of epithelium removal and the inhibitors on sensitivity to isoprenaline. Differences between group pD_2 values were compared by paired *t*-test, and considered significant at $P < 0.05$.

Epithelium removal caused a leftward shift of 3.6 $\pm$ 0.4 fold (n=14) in the VIP curve. The pD_2 values of 7.74 $\pm$ 0.05 and 8.25 $\pm$ 0.06 in intact and denuded trachea, respectively, were significantly different. In the presence of captopril (10 μM), epithelium removal still caused a leftward shift (4.7 $\pm$ 2.3, n=8) in the VIP curve. In these experiments, the pD_2 for VIP in intact tissues, 7.64 $\pm$ 0.12, was significantly different from 8.10 $\pm$ 0.12 in denuded tissues. Captopril, therefore, did not affect responses to VIP. Phosphoramidon (10 μM), increased sensitivity to VIP in intact trachea only. In the presence of phosphoramidon, the pD_2 value for VIP in intact tissues (8.24 $\pm$ 0.06; n=8), was significantly different from control, and represented a leftward shift of 3.2-fold. Phosphoramidon abolished the effect of epithelium removal on sensitivity to VIP (mean shift 1.6 $\pm$ 0.3). Thiorphan (10 μM) had a similar effect to phosphoramidon (n=7). Epithelium removal increased sensitivity to isoprenaline ($P < 0.05$), pD_2 values in intact and denuded tissues being 8.35 $\pm$ 0.06 and 8.68 $\pm$ 0.14 (n=7) respectively. This was unaffected by a cocktail of the enzyme inhibitors. Mean shifts for isoprenaline were 2.5 $\pm$ 0.6 and 6.4 $\pm$ 3.2 in the absence and presence respectively of inhibitors. These data suggest that the increased sensitivity to VIP in guinea-pig trachea following epithelium removal is due to loss of NEP. That the NEP inhibitors had no effect in epithelium-denuded tissues indicate that, in this tissue, the principal source of degradation of VIP by NEP exists in the epithelial layer.

Barbato, G.F. *et al* (1988) *Ann.NY Acad.Sci.* **527**, 582-585
Ellis, J.L. & Farmer, S.G. (1989) *Br.J.Pharmacol.* **96**, 513-520 & 521-526
Farmer, S.G. *et al* (1986) *Br.J.Pharmacol.* **89**, 407-414
Farmer, S.G. (1987) *Trends Pharmacol.Sci.* **8**, 8-10
Fernandes, L.B. *et al* (1989) *Br.J.Pharmacol.* **96**, 117-124
Frossard, N. *et al* (1989) *J.Pharmacol.Exp.Ther.* **248**, 292-298
Hay, D.W.P. *et al* (1987) *Eur.J.Pharmacol.* **136**, 247-250

DO PHORBOL ESTERS PRODUCE RELAXATION OF TRACHEAL MUSCLE BY GENERATION OF ARACHIDONIC ACID FROM AIRWAY EPITHELIUM?

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There is some debate as to whether epithelium removal or cyclooxygenase inhibition can influence responses to phorbol esters in airway smooth muscle in vitro. Menkes et al. (1986) and Hirst et al. (1989) found no effects whereas Souhrada & Souhrada (1989) demonstrated an epithelium-dependent relaxation which was converted to contraction following indomethacin treatment. The latter effects resemble those of exogenously applied arachidonic acid (AA) which is metabolized by the epithelium to a relaxant prostanoid (Nijkamp & Folkerts, 1986). Phorbol esters have been suggested to be able to liberate AA (Menkes et al., 1986) although on the other hand AA may activate C-kinase (Dreher & Hanley, 1988) . The purpose of the present study was to examine these possibilities by comparing the effects of epithelium removal and cyclooxygenase inhibition on responses to phorbol 12,13 dibutyrate (PDB) and AA in the guinea-pig trachea.

Tracheas were removed from male, Dunkin-Hartley guinea pigs (250-350 g), cut into transverse strips and suspended under a load of 2 g in Krebs-Henseleit solution (37 ^{o} C, gassed with 5 % CO_2 in oxygen). Every second strip had its epithelium removed with a cotton swab. Changes in the force of contraction were recorded by conventional means. Responses to PDB and AA were examined under basal conditions and in tissues precontracted with KCl-depolarizing solution(30mM) When used, indomethacin (3 uM) was added to the bath 15 min before the agonist.

PDB relaxed intact tissues (+EPI) in a concentration-dependent manner to 670 $\pm$ 100 mg at 3 uM but contracted the denuded tissues (-EPI) to 360 $\pm$ 100 mg at the same maximum concentration. Epithelium removal increased tissue sensitivity to PDB (pD_2: +EPI, 6.27 $\pm$ 0.11: -EPI, 6.68 $\pm$ 0.12) by 2.6-fold. AA showed a similar profile of action relaxing +EPI tissues by 305 $\pm$ 37 mg but contracting the -EPI to 380 $\pm$ 37 mg at the maximum concentration of 100 uM. After indomethacin only contractile responses were seen to PDB (+EPI, 325 $\pm$ 87 mg; -EPI, 520 $\pm$ 118 mg) and AA (+EPI, 374 $\pm$ 75 mg; -EPI, 425 $\pm$ 60 mg). Indomethacin did not affect tissue sensitivity to PDB or AA. In KCl-depolarized tissues only contractile responses were seen to PDB (+EPI, 410 $\pm$ 80 mg; -EPI, 340 $\pm$ 40 mg) whereas AA still relaxed +EPI (225 $\pm$ 55 mg) and contracted -EPI (260 $\pm$ 50 mg). As before, indomethacin converted the relaxant response to AA to contraction.

These results indicate that 1) responses to PDB and AA are influenced by the epithelium, 2) the epithelium-dependent relaxation is mediated by a cyclooxygenase product and 3) depolarization inhibits the epithelium-dependent relaxation to PDB but not AA. This suggests that under basal conditions PDB like AA may produce relaxation by the generation of a dilator prostanoid by the epithelium. However, in tissues stimulated with KCl the effects of PDB cannot be explained by prostanoid generation and only reponses due to activation of C-kinase are seen. It is possible that PDB acts via AA release in the presence of the epithelium under basal conditions.

Dreher, M.L. & Hanley, M.R. (1988) Trends Pharmac. Sci. 9, 114-115
Hirst, S.J., Smith, E.A., Beesley, J.E. & Dale, M.M. (1989) Proc. B.P.S., Bristol, 1989.
Menkes, H., Baraban, J.M. & Snyder, S.H. (1986) Eur. J. Pharmac. 122, 19-27.
Nijkamp, F.P. & Folkerts, G. (1986) Eur. J. Pharmac. 131, 315-316.
Souhrada, M. & Souhrada, J.F. (1989) Am. Rev. Resp. Dis. 139, A354.

EFFECT OF A BRADYKININ B_2 ANTAGONIST ON RESPONSES OF INTACT AND RUBBED GUINEA-PIG TRACHEA *IN VITRO*

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Bradykinin (Bk) has been shown to cause dose-dependent relaxations of guinea-pig trachea (GPT) in organ bath *in vitro* (Mizrahi et al 1982). Recently we have demonstrated that removal of the epithelium alters the response of Bk from a relaxation to a contraction. In addition the response of the intact GPT to Bk is converted to a contraction by indomethacin (Bramley et al 1988).

Since the availability of new peptide analogues of Bk which are selective for either the B_1 and B_2 receptors (Vavrek and Stewart 1985) the responses of many tissues to Bk have been characterised. However the receptor type mediating the responses of the GPT to Bk has not been established, and both B_1 and B_2 antagonists have been found to be inactive on the GPT.
We demonstrate here that the bradykinin B_2 antagonist D-Arg-(Hyp^3-Thi ^{5,8}-D-Phe^7)-Bk possesses antagonist activity on the Bk-induced contraction of the rubbed GPT, and also alters the response of Bk on the intact GPT.

Tracheae from male Dunkin-Hartley guinea pigs (500-600g) were cut longitudinally opposite the band of smooth muscle. Two strips of GPT, 1cm long, were used; one was kept intact while the other was denuded of epithelium by gentle rubbing with a cotton swab. Finally GPT were cut in a zig-zag fashion and suspended in 10 ml organ baths containing Krebs-Henseleit solution gassed with 95%O_2/5%CO_2 at 37°C. All drugs were given as sequential bolus doses except indomethacin (1.4µM) which, when used, was added to the buffer.

The responses to Bk were calculated as a percentage of the response to either salbutamol (10nM) or acetylcholine (ACh) (1µM), which were used as standard agonists for the intact and rubbed GPT respectively. Responses to Bk (0.01-1µM) were obtained both before and after treatment with the Bk antagonist D-Arg-(Hyp^3-Thi^{5,8}-D-Phe^7)-Bk (0.3-3µM).

The contractile response of the rubbed GPT to Bk, but not to ACh, was significantly blocked by treatment with the B_2 antagonist (1µM and 3µM) ($P<0.05$, n=6-8). However in intact GPT, relaxations induced by 0.1µM and 1µM of Bk were significantly potentiated in the presence of the B_2 antagonist ($P<0.05$, n=4-6), whereas those due to salbutamol were unaffected. In some experiments the initial transitory peak obtained prior to the relaxation due to Bk was antagonised. The B_2 antagonist also reduced the contractions induced by Bk on the indomethacin-treated intact GPT.

The above results suggest that the contractile responses of the rubbed GPT due to Bk, and those of the intact GPT in the presence of indomethacin are mediated via a B_2 receptor population. The fact that the B_2 antagonist seems to also potentiate Bk-induced relaxations of the intact GPT, suggests that the antagonist may act as a partial agonist at other Bk receptors. This observation requires additional studies using B_1 and B_2 agonists.

Further investigation into the type of receptors involved in the actions of Bk in the airways should determine the relevance of Bk in respiratory diseases where epithelial damage occurs.

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Bramley, A.M *et al.* (1988) *Br. J. Pharmacol., 95:* 774P.
Mizrahi *et al.* (1982) *Pharmacology., 25:* 320-326
Vavrek, R.J and Stewart, J.M. (1985) *Peptides., 6:* 161-164

CHARACTERISATION OF STEREOSPECIFIC BINDING SITES FOR [^{3}H]-INOSITOL (1,4,5)-TRISPHOSPHATE IN AIRWAY SMOOTH MUSCLE

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Inositol(1,4,5)-trisphosphate (Ins(1,4,5)P3), produced by receptor-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate, is a major intracellular second messenger causing an elevation in cytosolic free [Ca^{2+}] and cell activation (Berridge, 1987). Although muscarinic-receptor stimulation in airway smooth muscle (ASM) induces a rapid accumulation of Ins(1,4,5)P3 (Chilvers et al. 1989) which releases Ca^{2+} from internal stores and initiates contraction (Hashimoto et al. 1985) the mechanism of Ins(1,4,5)P3-induced Ca^{2+} release in this tissue remains uncertain. We report the identification and characterisation of [^{3}H]Ins(1,4,5)P3 binding sites in ASM which are thought to represent the functional Ins(1,4,5)P3 receptor for Ca^{2+} release.

Bovine trachealis muscle was homogenised in 20 mM $NaHCO_3$, 1 mM dithiothreitol and a 'P_2' membrane fraction obtained (Challiss et al. 1988). [^{3}H]Ins(1,4,5)P3 binding assays contained 200 µg protein freshly prepared ASM membranes, 2 nM [^{3}H]D-Ins(1,4,5)P3 (NEN) and 25 mM Tris-HCl, 1 mM EDTA (pH 7.8) in a final volume of 120 µl. Non-specific binding was determined using 10 µM DL-Ins(1,4,5)P3. Incubations were performed for 30 min. at 4°C with separation of bound and free [^{3}H]Ins(1,4,5)P3 achieved by filtration (GF/B). For studies examining the effect of Ca^{2+} on [^{3}H]Ins(1,4,5)P3 binding, EDTA was omitted, and assays performed in the absence (1-5 µM Ca^{2+}) or presence of 1 mM Ca^{2+}. Preliminary experiments established <2% metabolism of the [^{3}H]Ins(1,4,5)P3 under the above conditions.

Scatchard analysis of [^{3}H]Ins(1,4,5)P3 displacement by D-Ins(1,4,5)P3 indicated a single binding site with a K_D of 3.8 ± 0.2 nM (mean ± SEM) and B_{max} of 1003 ± 170 fmol/mg protein. Binding was rapid ($t_{½}$ 2 min) and reversible with a $t_{½}$ off, determined by adding 10 µM DL-Ins(1,4,5)P3, of 5 min. [^{3}H]Ins(1,4,5)P3 binding also displayed strict stereo- and positional specificity with IC_{50} values for L-Ins(1,4,5)P3, DL-Ins(1,3,4,5)P4 and D-Ins(1,3,4)P3 of 29, 0.49 and 3.4 µM respectively. In addition, heparin displaced [^{3}H]Ins(1,4,5)P3 binding with an IC_{50} of 7.6 ± 1 µg/ml. Specific [^{3}H]Ins(1,4,5)P3 binding was markedly enhanced under alkaline conditions (maximal specific binding pH 7.75) and in distinct contrast to [^{3}H]Ins(1,4,5)P3 binding in rat cerebellum membranes, prepared in an identical manner, was not inhibited significantly by 5 µM or 1 mM Ca^{2+}. This latter observation may indicate a distinct difference in the way Ins(1,4,5)P3-induced Ca^{2+} release is regulated in neuronal and peripheral tissues and the data provides further support for the proposed role for Ins(1,4,5)P3 in regulating ASM contraction.

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Berridge, M.J. Ann. Rev. Biochem. 1987; 56:159-193.
Challiss, R.A.J. et al. Biochem. Biophys. Res. Commun. 1988; 157:684-691.
Chilvers, E.R. et al. Eur. J. Pharmacol. (in press).
Hashimoto, T. et al. Br. J. Pharmac. 1985; 86:191-199.

INHIBITION BY SALBUTAMOL, FORSKOLIN AND IBMX OF ALF_4^--INDUCED INOSITOL PHOSPHATE FORMATION IN BOVINE AIRWAY SMOOTH MUSCLE

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Airway smooth muscle spasmogens are thought to induce contractile responses through the formation of the intracellular second messengers inositol 1,4,5 trisphosphate and diacylglycerol (Takuwa et al., 1987). We have previously shown that inositol phospholipid hydrolysis induced by histamine and 5-hydroxytryptamine (5-HT) in bovine tracheal smooth muscle can be inhibited by a range of agents which elevate tissue cyclic AMP levels (Hall et al., 1989; Hall & Hill 1989). In order to examine the regulation of inositol phosphate formation at the post-receptor level we have investigated the effect of aluminium fluoride (AlF_4^-), which stimulates phospholipase C activity in smooth muscle via its action on a regulatory G protein (Marc et al., 1988), on inositol phospholipid hydrolysis in bovine tracheal smooth muscle.

Slices of bovine tracheal smooth muscle labelled with [^{3}H]-inositol were prepared as previously described (Hall et al., 1989). Following stimulation with agonist for 45 minutes, total [^{3}H]-inositol phosphate formation was assayed by anion exchange chromatography (Hall et al., 1989). Where used, 3-isobutyl-1-methylxanthine (IBMX) was added 20 minutes prior to AlF_4^- administration.

AlF_4^- (NaF + 10μM $AlCl_3$) stimulated inositol phosphate formation at concentrations of NaF above 1mM. The maximum response (20mM NaF + 10μM $AlCl_3$) was 187 ± 31 % (n=9) of that produced by histamine (1mM). When both histamine and AlF_4^- were added simultaneously the observed response was significantly ($P < 0.05$, wilcoxon signed rank) less than additive, being 210 ± 37% (n=9) of the response to histamine alone. As has been observed with other agents which stimulate inositol phospholipid hydrolysis in this tissue (Hall et al., 1989; Hall & Hill, 1989), 20mM AlF_4^- (20mM NaF + 10μM $AlCl_3$) increased the incorporation of [^{3}H]-inositol into the tissue slices by 189 ± 37% (n=3) compared to basal (unstimulated, =100%). The β_2 adrenoreceptor agonist salbutamol (1 μM) inihibited the inositol phosphate response to 20 mM AlF_4^- (IC_{50}= 0.08 ± 0.01 μM, n=3) by 60 ± 6% (n=8, $p<0.05$). In addition, IBMX and forskolin inhibited the fluoroaluminate response (20mM AlF_4^-) by 73 ±2% and 46 ±5% respectively (both n=3).

These results show that AlF_4^- can produce an inositol phosphate response in slices of bovine tracheal smooth muscle which is subject to regulation by agents which have previously been shown to alter receptor-mediated inositol phosphate responses. These data suggest that the regulation of histamine- and 5-HT-induced inositol phosphate accumulation by agents which raise cyclic AMP levels (Hall et al., 1989; Hall & Hill, 1989) is mediated at the G protein/ phospholipase C level.

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Hall, I.P. & Hill, S.J. (1989). Br. J. Pharmac. in press
Hall, I.P. et al., (1989). Br. J. Pharmac. 97, 603-613.
Marc, S. et al., (1988). Biochem. J. 255, 705-713.
Takuwa, Y. et al., (1987). Am. J. Physiol. 253, C817-C827.

HUMAN RECOMBINANT NEUTROPHIL-ACTIVATING FACTOR/INTERLEUKIN-8 IS A SPASMOGEN OF AIRWAY SMOOOTH MUSCLE *IN VITRO*

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Neutrophil-activating factor/interleukin-8 (NAF/IL-8) is a peptide of 72 amino acids derived from endotoxin-stimulated monocytes and IL-1-stimulated fibroblasts, endothelial cells and alveolar macrophages (for refs, see Westwick *et al.*, 1989). The human recombinant (hr) NAF/IL-8 is biologically equivalent to the naturally produced peptide, with respect to neutrophil activation and chemotaxis of lymphocytes (Lindley *et al.*,1988; Bacon *et al.*, 1989). We have examined the effect of hrNAF/IL-8 on preparations of airways and vascular smooth muscle *in vitro*.

The preparations used were guinea-pig trachea (GPT) cut in a zigzag fashion and suspended in organ bath (Emerson & Mackay 1979) and in a separate series of experiments, guinea-pig lung parenchymal strips (GPP), GPT and spirally-cut rabbit aorta (RA) superfused with Tyrode (Piper & Samhoun, 1982; Piper & Vane, 1969). In some experiments the effect of a combination of antagonists containing hyoscine (340 nM), mepyramine (350 nM) and methysergide (550 nM) was investigated whilst in others indomethacin (2.8 μM) was used. HrNAF caused a dose related contraction of GPT in organ bath; concentrations of 0.3, 3 and 300 pM produced contractions of 14.25 ± 3.16, 19.8 ± 3.3 and 23.4 ± 3.9mm/100 mV (n=5) respectively. By comparison, LTD_4 at 3, 10 and 30 nM produced contractions of 15.9 ± 2.5, 29.2 ± 4.18 and 41.95 ± 6.6 mm/100 mV respectively (n=10-13).

In contrast, superfused GPT contracted to LTD_4 (10-100 pmol) and to U44069 a thromboxane A_2 mimetic, (100-1000 pmol) but not to hrNAF (3-300 pmol). Superfused RA contracted to U44069 (100-1000 pmol) but not to either LTD_4 (10-300pmol) or hrNAF (0.3-300pmol). HrNAF at 30 and 100 pmol produced contractions of superfused GPP of 9.3 ± 5.5 and 35.5 ± 8.8 mm/20 mV (n=3) respectively, compared to contractions of 19.9 ± 11.5, 32 ± 7.5 and 40.4 ± 13.8 mm/20 mV (n=3) produced by 1, 3 and 10 pmol respectively of LTD_4. Repeated application of hrNAF exhibited marked tachyphylaxis, although no cross-tachyphylaxis was observed with LTD_4 or U44069. A combination of mepyramine, methysergide and hyoscine did not alter hrNAF-induced responses of GPT or GPP in organ bath or in superfusion n=3-9. Contractions of GPT in organ bath and GPP in superfusion were virtually abolished by pretreatment with indomethacin (2.8μM, n=3-6). Conversely, indomethacin caused a significant potentiation of the LTD_4-induced contraction of GPT in organ bath and a 50% inhibition ($p < 0.05$) of LTD_4-induced contraction of superfused GPP.

These experiments show that hrNAF contracts preparations of guinea-pig lung *in vitro*. The above results combined with its reported pro-inflammatory effect and its generation by alveolar macrophages indicate that this cytokine may have a role in inflammatory lung disease.

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Bacon, K.B. *et al.* (1989) Unpublished
Emerson, J. & Mackay (1979) *J.Pharm.Pharmacol.*, 31, 798
Lindley, I. *et al.* (1988) *PNAS.USA*, 85, 9199-9203.
Piper, P.J. & Samhoun, M.N. (1982) *Br.J.Pharmacol.*,77, 267-275.
Piper, P.J. & Vane, J. (1969) *Nature*, 223, 29-35.
Westwick, J. *et al* (1989) *Immunol.Today*, 10, 146-147.

ASCORBIC ACID PREVENTS OZONE-INDUCED BRONCHIAL HYPERREACTIVITY IN GUINEA-PIGS

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A characteristic feature of asthma is bronchial hyperreactivity (BHR). It is well established that exposure of guinea-pigs to ozone (O_3) produces BHR to bronchoconstrictor agonists administered i.v. but the degree of BHR seen is usually small (Murlas & Roum, 1985). Using an aerosol agonist dosing technique we have recently shown that a much greater degree of non-specific BHR can be detected in this species after O_3 exposure (Yeadon & Payne, 1989). We now report that the primary lesion in O_3-induced BHR is oxidant damage and is not dependent on either arachidonic acid 5-lipoxygenase (5-LO) or cyclooxygenase (CO) products. Conscious male guinea-pigs (450g-700g) were exposed to O_3 (3±0.5ppm) for 30min; control animals breathed laboratory air. Under pentobarbitone (60mg/kg i.p.) anaesthesia, the trachea was cannulated and the animal artificially ventilated. Aerosols were generated by a nebuliser in the afferent arm of the ventilator circuit, and changes in pulmonary inflation pressure (PIP) were measured from a side-arm in the tracheal cannula. Bronchial reactivity to inhaled histamine (H) was assessed by nebulising solutions of H (1.25-320ug/ml base) for 10s and measuring the increase in PIP. The concentration of H producing a 20cmH_2O rise in PIP (H PC_{20}) was determined by interpolation. The H PC_{20} in control animals was 80.7±11.4ug/ml. After O_3 a parallel leftward shift in the H dose-response curve was observed, with H PC_{20} falling to 10.4±1.4ug/ml, an 8-fold enhancement of bronchial reactivity ($p<0.001$, n=9). In addition, the basal PIP prior to dosing with H was elevated. In animals pretreated with the antioxidant ascorbic acid (0.1-1.0g/kg i.p.) 1hr prior to O_3 both the development of BHR and increase in basal PIP were reduced in a dose-related manner (Table 1).

Table 1: Effect of treatments on O_3-induced BHR

	Air control	O_3 alone	+Ascorbic acid (g/kg) 0.1	0.3	1.0	+Indo (30mg/kg)	+BW755C (100mg/kg)
H PC_{20} (ug/ml)	80.7± 11.4	10.4± 1.4	22.7± 9.4	*40.0± 10.3	**54.3± 8.3	15.9± 3.4	8.5± 1.7
Basal PIP	9.7± 0.4	14.2± 0.9	12.8± 2.0	*10.3± 0.3	*9.5± 0.2	14.8± 2.2	14.7± 2.9

Values are mean ± SEM, n = 4-9. Units of PIP are cmH_2O.
* $p<0.05$; ** $p<0.001$ vs O_3 alone.

Ascorbic acid (1g/kg) did not influence control responses to aerosolised H, while acetic acid (1.2M) used as a control for ascorbic acid did not prevent the onset of O_3-induced BHR. Neither 1hr pretreatment with the CO inhibitor indomethacin (30mg/kg i.p.) nor the 5-LO/CO inhibitor BW755C (30-100mg/kg i.p.) blocked the development of BHR after O_3 (Table 1). Thus, we have shown that O_3-induced BHR in guinea-pigs can be prevented by pretreatment with ascorbic acid, but not by inhibitors of either 5-LO or CO. This suggests that the primary lesion responsible for BHR in this model is oxidant damage rather than resulting from the synthesis and release of arachidonic acid metabolites.

Murlas, C.G. & Roum, J.H. (1985) Am. Rev. Resp. Dis., 131, 314-320.
Yeadon, M. & Payne, A.N. (1989) Eur. Resp. J. (in press).

CALCIUM PERMEABILITY OF PIG AORTIC MICROSOMES IS INCREASED BY HISTAMINE AND A GUANYL NUCLEOTIDE

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Agonist-induced smooth muscle contraction involves mobilization of intracellular calcium and stimulation of influx, the molecular mechanism of which is unclear. To study this, microsomes were prepared from the tunica media of pig aorta (Suemetsu et. al., 1984). The acyl phosphate derivatives of the Ca^{2+}ATPases were prepared and resolved by SDS gel electrophoresis (Wuytack et. al., 1982). Vesicles were sonicated for 10s with histamine to allow access to internally located receptor sites and then incubated with GppNHp to activate putative guanyl nucleotide transducing proteins. Steady-state, ATP-dependent accumulation of 5μM (final concentration) of $^{45}Ca^{2+}$ was measured after 4 min by filtration. Release of Ca^{2+} from vesicles passively loaded with 5mM $^{45}Ca^{2+}$ was measured by filtration 10s after dilution 100-fold (Meissner, 1984). ATPase activity was measured in the presence and absence of 5μM Ca^{2+} (Wuytack and Casteels, 1980) and the difference taken to represent Ca^{2+}ATPase activity.

Microsomes contained acyl phosphate derivatives of 130 and 100 kDa Ca^{2+}-ATPases characteristic of plasmalemma and endoplasmic reticulum respectively. In vesicles sonicated with 500μM histamine, 1mM GppNHp reduced steady state Ca^{2+} accumulation by 20±4(s.e. mean) %, ($p<0.001$, paired t-test, n=10). This effect was not mimicked by 10μM-inositol-1,4,5-trisphosphate (5±4%, n=10). In vesicles incubated with 1mM GppNHp, sonication with 500μM histamine reduced Ca^{2+} accumulation 19% from 64±12 to 52±11 nmol/mg protein compared to sonication without histamine ($p<0.03$, paired t-test, n=15). The effect of histamine was dose-related and was abolished 8±4% (NS, n=12) by 5μM mepyramine (an H_1 antagonist) but not affected 25±8% ($p<0.04$, paired t-test, n=12) by 5μM cimetidine (an H_2 antagonist). In vesicles sonicated with 500μM histamine, 1mM GppNHp increased Ca^{2+} ATPase activity from 6.1±2.5 to 10.1±2.6 nmol/min/mg protein ($p<0.02$, paired t-test, n=15). In similar vesicles, 1mM GppNHp also increased the rate of Ca^{2+} efflux from passively loaded vesicles. Ca^{2+} remaining at 10s was decreased by GppNHp from 141±14 to 128±13 nmol/mg protein ($p<0.04$, paired t-test, n=20). GppNHp was without effect on Ca^{2+} efflux from vesicles sonicated without histamine (154±11 vs 161±17 nmol/mg protein, n=20).

The microsomal preparation contained plasmalemma (only inverted vesicles accumulate Ca^{2+}) and endoplasmic reticulum vesicles. The reduction by histamine and GppNHp of steady-state Ca^{2+} accumulation was not by inhibition of Ca^{2+} ATPase suggesting instead an increase in vesicle permeability. This was confirmed by measurement of calcium release. The increase in permeability was unlikely to be from endoplasmic reticulum since it was not mimicked by inositol trisphosphate. The requirement for both histamine H_1 receptor (cf Matsumoto et. al., 1986) and a guanyl nucleotide suggest that it occurred by the receptor operated calcium influx mechanism of intact cells.

Matsumoto, T., Kanade, H., Nishimura, J., Shogakiuchi, Y., Kobayashi, S., & Nakamura, M. (1986) Biochem. Biophys. Res. Comm. 135, 172-177.
Meissner, G., (1984). J. Biol. Chem. 259, 2365-2374.
Suematsu, E., Hirata, M., Hashimoto, T., & Kuriyama, H. (1984) Biochem. Biophys. Res. Comm. 120, 481-485.
Wuytack, F., Casteels, R., (1980) Biochem. Biophys. Acta 595, 257-263.
Wuytack, F., Remaeker, L., Dr Shutter, G., & Casteels, R. S(1982) Biochem. Biophys. Acta, 693, 45-52.

MECHANICAL RESPONSES TO INOSITOL (1,4,5)-TRISPHOSPHATE IN PERMEABILISED STRIPS OF GUINEA-PIG ILEUM LONGITUDINAL SMOOTH MUSCLE

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Inositol 1,4,5-trisphosphate (IP3) has been reported to act as a second messenger by releasing calcium from stores in a wide range of cells including rat dispersed duodenal and ileal cells (van Corven et al., 1987). Mechanical responses have also been measured in permeabilised guinea-pig stomach muscle cells (Bitar et al., 1986) and taenia-coli cells (Hashimoto et al., 1986). In this study responses to exogenous IP3 were measured in permeabilised strips of guinea-pig ileum longitudinal smooth muscle.

Small strips (0.3 mm in width and 4 mm in length) were dissected from the longitudinal smooth muscle layer of the ileum, and set up in organ baths (0.3 ml, room temperature) using fine silk thread. Tension was recorded by way of a Shinkoh UL-2GR strain gauge. The tissues were permeabilised with saponin (50 µg/ml, 20 minutes) in standard relaxing solution (130 mmol/l KCl, 20 mmol/l Trizma-maleate, 3.3 mmol/l Na_2ATP, 1 mmol/l $MgCl_2$, pH 6.8), and loaded with calcium by incubating in an EGTA buffered medium containing 3 µmol/l free Ca^{2+}. This was then replaced with a calcium-free medium and the tissues left for 10 min before responses to IP3 were measured. Only one concentration of IP3 was applied to each tissue.

Magnesium concentration in the standard relaxing solution was lower than had been used in other studies, because these higher concentrations caused a slow development of tension. Saida & Nonomura (1978) reported that magnesium only caused tension to develop in permeabilised taenia caecum at concentrations of 4 mmol/l and above. In our study this effect occured with magnesium concentrations of over 1 mmol/l.

Tension development in response to Ca^{2+} was concentration dependent with a pD_2 of 5.78 ± 0.15. Tension development was rapid - 3 µM Ca^{2+} produced peak tension in 90 - 150 sec. However, incubation of the tissue in 3 µmol/l Ca^{2+} for 10 minutes was found to produce "latch tension" - tissues failed to relax even after all the calcium was removed by an EGTA-containing medium. When the incubation time was reduced to 3 min, however, tissues relaxed completely within 3 minutes in an EGTA-free solution after a brief wash (30 sec) with EGTA-containing solution.

IP3 elicited concentration-dependent tension development with an EC50 of 7.4×10^{-6} mol/l with maximal responses to 10^{-4} mol/l. At the maximum the response was 28.1% that produced by the loading dose of calcium (3 µmol/l).

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Bitar, K.N., Bradford, P.G., Putney, J.W.Jr & Makhlouf, G. (1986) J.Biol.Chem. 261, 16591-16596.
Hashimoto, T., Hirata, M., Itoh, T., Kanmura, Y. & Kuriyama, H. (1986) J.Physiol. 370, 605-618.
Saida, K. & Nonomura, Y. (1978) J.Gen.Physiol. 72, 1-14.
van Corven, E.J., Verbost, P.M., de Jong, M.D. & van Os, C.H. (1987) Cell Calcium 8, 197-206.

CHARACTERISATION OF THE INTRACELLULAR CALCIUM RESPONSE TO VASOPRESSIN IN A7R5 SMOOTH MUSCLE CELLS

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Vasopressin is known to produce an increase in intracellular calcium (Ca) and the breakdown of phosphatidyl inositol (PI) in cultured vascular smooth muscle cells (Capponi *et al.*, 1985; Nabika *et al.*, 1985). The present study was designed to characterize the nature of this intracellular Ca response in smooth muscle cells and the role of PI breakdown. The experiments were carried out using A7r5 cells, an established smooth muscle cell line derived from rat aorta (Kimes & Brandt, 1976). A7r5 cells were grown to confluence in culture flasks. For measurement of intracellular Ca using fura 2, cells were harvested using trypsin in the presence of EDTA. The resulting suspension was washed and resuspended in HEPES-buffered physiological salt solution (PSS). After loading for 45 min with fura 2-AM (4 μM), intracellular Ca was measured using a fluorimeter. For PI studies, cells were plated out in 3 cm culture dishes and labelled to equilibrium with ^{3}H-myo-inositol.

Vasopressin (1-100 nM) produced an initial rapid rise in intracellular Ca which was apparent within 5 sec and reached a peak within 15 sec. Thereafter the Ca concentration fell to a lower level which was maintained in the presence of vasopressin. Both the initial peak height and the level of the maintained phase were dose- dependent. In the absence of extracellular Ca, the initial peak of the response to vasopressin (10 nM) was reduced (30%) but the maintained phase was almost completely abolished. Vasopressin (0.1-100 nM) produced a concentration dependent increase in total inositol phosphate turnover and time-course studies of inositol trisphosphate (IP3) production showed an increase in IP3 concentration within 5 sec after the addition of 10 nM vasopressin. Pre-incubation with nifedipine (1 μM) had no effect on the initial peak but attenuated the maintained phase of the calcium response to vasopressin (1 nM, 10 nM), whereas cromakalim (10 μM) had no effect on either part of the response. The addition of vasopressin (10 nM) in sodium (Na)-free PSS (in which N-methyl-D-glucamine was substituted for Na) resulted in an increase in the initial peak height (21%) and an inhibition (60%) of the maintained phase.

It is concluded from these results that the intracellular Ca response to vasopressin in A7r5 smooth muscle cells is biphasic, with an initial phase mainly dependent on intracellular Ca release and a secondary maintained phase dependent on entry of extracellular Ca. The time course studies of IP_3 production suggest that it may mediate the release of intracellular Ca which generates the initial peak response. The influx of extracellular Ca during the maintained phase is partially inhibited by nifedipine and a component of this may therefore occur through L-type calcium channels. The failure of cromakalim to inhibit the Ca response to vasopressin suggests the absence of K channels which respond to this agent. However, removal of extracellular Na inhibits the entry of extracellular Ca which is responsible for the maintained phase of the vasopressin response.

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Capponi, A.M., Lew, P.D. & Vallotton. (1985). J. Biol. Chem., 260, 7836-78742.
Kimes, B.W. & Brandt, B.L. (1976). Exper. Cell. Res. 98, 349-366.
Nabika, T., Velletri, P.A., Lovenberg, W. & Beaven, M.A. (1985). J. Biol. Chem., 260, 4661-4670.

ADP-β-F IS NOT A SELECTIVE P_2Y-RECEPTOR AGONIST IN RABBIT JUGULAR VEIN

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The classification of P_2-purinoceptors is limited by the absence of selective, competitive antagonists. In addition, few agonists have been described which are both pharmacologically selective and resistant to ectonucleotidase activity. However, a recent report (Hourani et al., 1988) identifies adenosine 5'-(2-fluorodiphosphate) (ADP-β-F) as a specific and relatively stable P_2y-receptor agonist in guinea-pig taenia coli. ADP-β-F is the first compound of this type and we have attempted to confirm its selectivity using a vascular preparation. Comparison has been made with adenosine 5'-(2-thiodiphosphate) (ADP-β-S) a stable structural analogue also reported to have P_2y-receptor agonist properties (Burnstock et al., 1984).

Rings of external jugular vein from male NZW rabbits were prepared for organ bath experiments as described elsewhere (Leff et al., 1987). Some tissues were denuded of endothelium by gentle abrasion with a scored cannula. Rings were contracted with the thromboxane mimetic U46619 and a single cumulative agonist-concentration effect (E/[A]) curve was constructed for either ADP-β-F or ADP-β-S. Relaxant responses to each compound were determined in endothelial-intact or denuded preparations in the presence or absence of the selective P_1-purinoceptor antagonist 8-sulphophenyltheophylline (8-SPT) ($3x10^{-4}$M). Responses were expressed as a percentage of the U46619 contraction.

In endothelially-intact tissues ADP-β-F produced an E/[A] curve which appeared to consist of two vasorelaxant components, one in the concentration range 10^{-7} - $3x10^{-5}$M, the other in the range 10^{-4} - 10^{-3}M. In the presence of 8-SPT the higher potency phase was abolished leaving the apparently monophasic lower potency component. The converse result was obtained upon endothelial denudation. These data demonstrated that ADP-β-F activates a P_1-receptor on the smooth muscle of the rabbit jugular vein at lower concentrations than those at which it activates an endothelial, presumably P_2y-receptor. Theoretical analysis showed that the results were quantitatively consistent with the one-agonist:two-receptor model of Furchgott (1981). In contrast, the E/[A] curve for ADP-β-S in intact tissues appeared to be monophasic. However, 8-SPT treatment and endothelial denudation revealed both endothelial- and P_1-induced components of relaxation, the latter occurring over a higher concentration range. Again, these data accorded with Furchgott's model. The potency ($p[A_{50}]$) and maximal effect (α) of each compound at P_1-receptors (denuded) and P_2y-receptors (intact, +8-SPT) are shown in the Table as mean values ± s.e., n = 4-6.

	P_1		P_2y	
	$p[A_{50}]$	α	$p[A_{50}]$	α
ADP-β-F	5.58 ± 0.13	0.36 ± 0.09	3.98 ± 0.07*	0.91 ± 0.17*
ADP-β-S	5.73 ± 0.20	0.56 ± 0.10	6.86 ± 0.12	0.83 ± 0.06

* Calculated using only those tissues in which maximum effect was achieved (n = 4)

By using vascular tissue which, unlike visceral smooth muscle, allows ready separation of P_1- and (putatively) P_2y-mediated effects, we have shown that ADP-β-F and ADP-β-S possess agonist properties at both receptors. Indeed, ADP-β-F was selective for P_1-receptors in the jugular vein. As such, this agonist cannot be regarded as a selective probe for P_2y-receptors. If used in the classification of P_2-receptors appropriate precautions should be taken to eliminate its P_1-receptor properties.

Burnstock, G., Cusack, N.J. and Meldrum, L.A. (1984) Br. J. Pharmac. 82, 369-374
Furchgott, R.F. (1981) Proc. 4th Meeting on Adrenergic Mechanisms, 10-29
Hourani, S.M.O., Welford, L.A., Loizou, G.D. and Cusack, N.J. (1988) Eur. J. Pharmac. 147, 131-136
Leff, P., Martin, G.R. and Morse, J.M. (1987) Br. J. Pharmac. 91, 321-331

COMPARISON OF P_2 RECEPTORS OF AORTIC ENDOTHELIAL CELLS WITH THOSE OF ADRENAL MEDULLA NON-CHROMAFFIN CELLS

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The chromaffin cells of the adrenal medulla release large amounts of ATP, along with catecholamines. The microvascular endothelial cells of the adrenal medulla are therefore exposed to an unusual environment with respect to ATP. Aortic endothelial cells have been shown to respond to ATP by action at P_2 purinoceptors. Here we have compared aortic endothelial cells with cells from the adrenal medulla with respect to synthesis of inositol phosphates in response to ATP and various analogues.

We used endothelial cells from bovine thoracic aorta (AG 4762 cells) and bovine adrenal non-chromaffin (BANC) cells as prepared by Banerjee et al. (1985). These BANC cells have been characterised as endothelial and shown to respond to ATP with an enhanced synthesis of inositol phosphates (Banerjee et al. 1985; Forsberg et al. 1987). Cells were prelabelled with [^{3}H]myo-inositol for 48 h, and stimulated with 10 mM lithium (measurements of total labelled inositol phosphates) or without lithium when individual inositol phosphates were separated on small anion exchange columns or by anion exchange high performance liquid chromatography (HPLC).

While ATP was found to stimulate dose dependent increases in the level of total inositol phosphates in both cell types, the profile of agonist potency, using ATP analogues, differed. In aortic cells 2-methylthio ATP was the most potent analogue tested (EC_{50} 0.9 ± 0.4 μM), ATP-γ-S was more potent than ATP while β, γ-methylene ATP was ineffective. In BANC cells ATP-γ-S had an EC_{50} of (2.81 ± 0.22 μM), while 2-methylthio ATP and β, γ-methylene ATP were ineffective.

In BANC cells stimulated with 30 μM ATP there was a steady rise in labelled $InsP_3$, $InsP_2$ and $InsP_1$ over a period of 2 min, which did not subsequently decline. The response in aortic cells to 30 μM ATP consisted of a rapid transient increase in labelled $InsP_3$ formation which decreased over 1 min, with similar slightly delayed patterns of change in labelled $InsP_2$ and $InsP_1$. HPLC separation of $InsP_3$ isomers at 15 sec stimulation showed the BANC cells to respond with a rise in both labelled Ins(1,4,5)P_3 and Ins(1,3,4)P_3 while in stimulated aortic cells the only labelled trisphosphate detectable was Ins(1,4,5)P_3. The labelled $InsP_2$ eluted in the position of the 1,4-isomer.

One interpretation of these observations is the presence of a P_{2y} receptor on aortic endothelial cells with an 'atypical' purinoceptor, i.e. neither P_{2x} nor P_{2y}, on BANC cells. The two cell types also show a different pattern of inositol phosphates produced in response to stimulation for 15 sec, presumably reflecting different functional consequences of P_2 receptor activation.

Banerjee, D.K., Ornberg, R.L., Youdim, M.B.H., Holdman, E. and Pollard, H. (1985) Proc Natl Acad Sci (USA) 82, 4702-4706

Forsberg, E.J., Feverstein, G., Shoham, E. and Pollard, H. (1987) Proc Natl Acad Sci (USA) 84, 5630-5634

RELAXANT POTENCIES OF PROSTAGLANDIN E ANALOGUES ON RABBIT JUGULAR VEIN

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The characterisation of subtypes of the EP- (prostaglandin E) receptor should be facilitated by the use of isolated preparations which are highly sensitive to PGE agonist action. In terms of EP_2-receptors mediating relaxation of smooth muscle, cat and guinea-pig tracheal rings or spirals have been used. However IC_{50} values for PGE_2 are rarely less than 10nM. We wish to report the rabbit jugular vein as a highly sensitive EP_2(?)-receptor preparation and to compare its structure-activity relationships for PGE analogues with those previously reported for the cat trachea.

Ring preparations from male NZW rabbits were suspended in Krebs-Henseleit solution, gassed with 95%O_2/5%CO_2, at 37°C; 1μM indomethacin was present. Tension changes were recorded with Grass FT03 transducers. Histamine, usually 1μM, was used to generate tone (50-60% of histamine maximum).

Table 1 Potencies of PGE analogues as relaxant agents

Prostanoid	Equi-effective molar ratio Rabbit jugular vein (n = 5-8)	Cat trachea(n = 4-8)
PGE_2	1.0 IC_{50} (nM) = 0.61 ± 0.11	1.0 IC_{30} = 40* IC_{50} = 20**/***
16,16-Dimethyl PGE_2	2.1 ± 0.5	9.4*, 27**
ICI 80205	83 ± 15	70*
17-Phenyl-ω-trinor PGE_2	200 ± 53	--
Sulprostone	>3000	>7000**
11-Deoxy-PGE_2 1-alcohol	4.6 ± 1.0	--
11-Deoxy PGE_1	1.4 ± 0.59	13*
Misoprostol	8.3 ± 1.0	3.7**
Butaprost	690 ± 190	27***

* Dong *et al*, 1986; ** Coleman *et al*, 1988; *** Gardiner, 1986.

The low potency of butaprost, a compound claimed to be a specific EP_2-receptor agonist (Gardiner, 1986), is surprising. We can confirm to some extent the reported activity for butaprost on guinea-pig trachea: Gardiner IC_{50} = 300nM versus histamine; our IC_{50} = 73nM (EMR = 4.0) versus iloprost. However it must be remembered that PGE_2 has contractile activity on guinea-pig trachea mediated via EP_1-receptors and this could offset its relaxant effect. The question is - to what extent?

The upper four PGE analogues have potent agonist activity at EP_1- (ICI 80205 > 16,16-dimethyl PGE_2 > 17-phenyl-ω-trinor PGE_2 = PGE_2 > sulprostone) and EP_3- (sulprostone = 16,16-dimethyl PGE_2 > ICI 80205 = PGE_2 > 17-phenyl-ω-trinor PGE_2) receptors. In terms of selectivity for EP_1- receptors, we would recommend 17-phenyl-ω-trinor PGE_2 (Cayman) as a standard agonist. It shows no evidence of relaxant activity on guinea-pig trachea.

Dong, Y.J. , Jones, R.L. & Wilson, N.H. (1986) Br.J.Pharmacol. 87, 97.
Coleman, R.A., Humphray, J.M., Sheldrick, R. & White, B.(1988) Br.J.Pharmacol. 95, 724P.
Gardiner, P.J. (1986) Br.J.Pharmacol. 87, 45.

VASCULAR ACTIONS OF 8-OH-DPAT MEDIATED *VIA* ACTIVATION OF NON-5-HT$_{1A}$ RECEPTORS

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Sumatriptan (GR43175) contracts the dog isolated saphenous vein and selectively constricts the carotid arterial circulation of anaesthetised dogs by activation of 5-HT$_1$-like receptors (Humphrey et al, 1988; Feniuk et al, 1989). The 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (8-hydroxy-2-[di-n-propyl-amino]-tetralin) also causes carotid arterial vasoconstriction in anaesthetised pigs (Bom et al, 1989), but whether this action involves stimulation of 5-HT$_{1A}$ receptors is not known. The present study compares the actions of sumatriptan and 8-OH-DPAT in the dog isolated saphenous vein and carotid arterial circulation of anaesthetised dogs.

Dog saphenous vein strips were prepared for measurement of contractile responses and agonist potencies determined as previously described, (Humphrey et al, 1988). Cumulative concentration-response curves to 5-HT, sumatriptan or 8-OH-DPAT were constructed in the absence and presence of a single concentration of either spiperone (1μM) or methiothepin (0.1μM). These experiments were carried out in the continuous presence of ketanserin (1μM).

Anaesthetised dogs (7-9kg; ♂ or ♀) were prepared to record arterial blood pressure, carotid arterial blood flow and vascular resistance (CVR) as previously described (Feniuk et al, 1989). Dogs were ganglion blocked (mecamylamine, 5mgkg^{-1} i.v. and atropine, 0.5mgkg^{-1} i.v.) and agonists (sumatriptan, 0.1-1μgkg^{-1} or 8-OH-DPAT, 0.1-3μgkg^{-1}) administered directly into the carotid artery via the cranial thyroid artery. Agonist dose-response curves were obtained before and after spiperone (1mgkg^{-1} i.v.) and subsequently after methiothepin, (1mgkg^{-1}i.v.).

Sumatriptan and 8-OH-DPAT caused concentration-dependent contractions of dog saphenous vein (EC_{50} 0.3 (0.1-0.8) and 3.9 (2.0-7.5)μM respectively) and increases in CVR (69±4 and 76±6% at 1μgkg^{-1} i.a. respectively) in anaesthetised dogs.

The non-selective 5-HT$_1$-like receptor antagonist methiothepin caused a similar, marked attenuation of contractile responses elicited by sumatriptan and 8-OH-DPAT in dog isolated saphenous vein and of carotid vasoconstrictor responses elicited by either agonist in anaesthetised dogs. In marked contrast the effects of either agonist were unaffected by the 5-HT$_{1A}$ antagonist spiperone. These results are shown in Table 1.

Table 1 — Agonist concentration- or dose-ratio

	Dog saphenous vein				Anaesthetised dog		
	spiperone	n	methiothepin	n	spiperone	methiothepin	n
5-HT	1.1 (0.8 - 1.4)	4	11 (2.5 - 50)	4	-	-	
Sumatriptan	1.0 (0.3 - 3.0)	4	66 (33 - 133)	4	1.1 (0.8 - 1.3)	15 (8 - 27)	4
8-OH-DPAT	0.8 (0.4 - 1.6)	4	16 (6 - 40)	4	1.1 (0.8 - 1.5)	17 (5 - 54)	4

We conclude that 8-OH-DPAT causes contraction of dog isolated saphenous vein and carotid arterial vasoconstriction in anaesthetised dogs by activation of 5-HT$_1$-like receptors which are not of the 5-HT$_{1A}$ subtype, but are apparently the same as those mediating the effects of sumatriptan in the two systems.

Bom, A.H. et al, (1989) Br. J. Pharmacol., 96, 125-132.
Feniuk, W. et al, (1989) Br. J. Pharmacol., 96, 83-90.
Humphrey, P.P.A. et al, (1988) Br. J. Pharmacol., 94, 1123-1132.

CHARACTERISATION OF [^{3}H]-SENKTIDE BINDING TO NK_3 TACHYKININ RECEPTORS IN GUINEA-PIG ILEUM AND CEREBRAL CORTEX

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Tachykinin receptors can be sub-divided into three main types (NK_1, NK_2 and NK_3). The NK_3 receptor exhibits preferential affinity for neurokinin B (NKB) in bioassays such as the rat portal vein (Mastrengelo et al, 1986) and the longitudinal muscle-myenteric plexus (LM/MP) preparation of the guinea-pig (Laufer et al, 1985). Radioligand binding studies in the CNS, have shown that NKB displaces ^{125}I-BH-eledoisin, ^{125}I-BH-senktide and ^{3}H-NKB binding more potently than neurokinin A or substance P (Mantyh et al, 1989). There have been no reports of the binding of these radiolabels to peripheral NK_3 sites. We have used ^{3}H-senktide, (^{3}H-succ-[Asp^6,MePhe^8]SP_{6-11}) a potent and selective NK_3 receptor agonist (Wormser et al, 1986), to characterise both peripheral (guinea-pig LM/MP) and central (cerebral cortex) NK_3 receptors.

Tissue homogenates were prepared and ^{3}H-senktide binding assayed as previously described (Laufer et al, 1986; Guard et al, 1988). Saturation analysis revealed that specific binding of ^{3}H-senktide (defined with 1μM senktide) was to a single site in both LM/MP and cortical membranes (LM/MP K_D = 2.21 ± 0.65 nM, Bmax = 13.49 ± 0.04 fmol/mg protein; cortex K_D = 8.52 ± 0.45nM, Bmax = 76.31 ± 1.6 fmol/mg protein (n = 2, means ± ranges). A range of naturally-occurring tachykinins and synthetic analogues displaced specific binding of ^{3}H-senktide (1.5nM) with the selective NK_3 agonists [MePhe^7]NKB and senktide being approximately three orders of magnitude more potent than the NK_1-selective agonists [Sar^9,Met(O_2)^{11}]SP and substance P methyl ester (Table 1), consistent with ^{3}H-senktide binding to NK_3 sites. A good correlation was obtained between pIC_{50} values (-log of the concentration of peptide required to inhibit 50% of the binding) for these peptides at LM/MP and cortex NK_3 sites (r = 0.98). It is concluded that ^{3}H-senktide labels a similar site in LM/MP and cortex membranes with a pharmacological profile indicative of an NK_3 receptor.

Table 1. Inhibition of ^{3}H-senktide binding by tachykinins and analogues (mean pIC_{50} ± S.E.M., n = 3-5)

	LM/MP	Cortex
[MePhe^7]NKB	9.05 ± 0.05	8.71 ± 0.10
SENKTIDE	8.44 ± 0.03	8.06 ± 0.02
NEUROKININ B	8.43 ± 0.05	8.35 ± 0.03
NEUROKININ A	6.53 ± 0.07	6.85 ± 0.07
SUBSTANCE P	7.01 ± 0.05	6.54 ± 0.24
[Sar^9,Met(O_2)^{11}]SP	5.68 ± 0.05	5.42 ± 0.03
SUBSTANCE P METHYL ESTER	< 5.00	< 5.00

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Mastrengelo, D. et al (1986) Eur. J. Pharmacol. 134, 321-326.
Laufer, R. et al (1985) Proc. Nat. Acad. Sci. 82, 7444-7448.
Mantyh, P.W. et al (1989) J. Neurosci. 9, 258-279.
Wormser, U. et al (1986) EMBO J. 5, 2805-2808.
Laufer, R. et al (1986) J. Biol. Chem. 261, 10257-10263.
Guard, S. et al (1988) Reg. Peptides 22, 75.

PHARMACOLOGICAL EFFECTS OF HAEM BIOSYNTHETIC INTERMEDIATES: ACTIONS OF Δ-AMINOLAEVULINIC ACID IN THE RAT JEJUNUM

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It has been suggested from in vitro studies that the porphyrin precursor, delta-aminolaevulinic acid (ALA), may precipitate attacks of acute porphyria by inhibiting the stimulated release of GABA from nerve endings (Brennan & Cantrill, 1979; 1981). This effect, occurring at a concentration of 1 μM ALA, is blocked by picrotoxin and bicuculline. Furthermore, ALA has also been found in vivo to act as a partial GABA agonist (Wehner & Marley, 1986). During acute porphyric attacks blood concentrations of ALA are raised to values which range from 9 to 12 μM (Gorchein & Webber, 1987).

The present studies assess the effects of pretreatment with bicuculline (10 μM) on the response to ALA by isolated preparations of rat jejunum. Effects have been examined in preparations depolarized with 100 mM potassium chloride as well as in non-depolarized preparations. Preparations of rat jejunum were bathed in oxygenated Krebs solution at 37 °C. Contractions of the preparations were recorded by an isotonic transducer and displayed on a calibrated Washington 400 MD2R oscillograph. In preparations that had not been depolarized, ALA (150-6500 μM) increased the amplitude of contractions and dose-dependently reduced tone, whereas at a concentration of 5 μM it increased tone. Pretreatment with the competitive $GABA_A$ antagonist, bicuculline (10^{-5} M) significantly attenuated the alteration in tone and contraction amplitude evoked by ALA (150 μM). Atropine (10^{-6}M) significantly reduced the effects upon tone produced by ALA at 150 μM, but enhanced the increase of tone produced by ALA at 5 μM. In depolarized preparations, ALA reduced tone at 0.05-10 μM concentrations. The effects of ALA (10 μM) in depolarized preparations were significantly attenuated by bicuculline whereas the $GABA_B$ receptor antagonist, delta-aminovaleric acid (10^{-3}M) was without significant effect.

In rat jejunum, $GABA_A$ receptors mediate cholinergic contraction along with a non-adrenergic non-cholinergic relaxation (Krantis & Harding, 1987). Additionally, M_1 muscarinic receptors, associated with the presynaptic control of GABA release, mediate relaxation of the rat intestine (Micheletti et al, 1988). The present results provide support for the hypothesis that interference with $GABA_A$-mediated functions by ALA at the concentrations occurring in acute porphyric attacks may account for some of the gastrointestinal disturbances.

Brennan, M.J.W. & Cantrill, R.C. (1979) Nature, 280, 514-515.

Brennan, M.J.W. & Cantrill, R.C. (1981) Mol. Cell. Biochem. 38, 49-58.

Gorchein, A. & Webber R. (1987) Clin. Science 72, 103-112.

Krantis, A. & Harding R.K. (1987) Europ. J. Pharmac. 141, 291-298.

Micheletti, R. et al. (1988) J. Pharm. exp. Therap. 224, 680-684.

Wehner, J.M. & Marley, R.J. (1986) Exp. Neurol. 94, 280-291.

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FURTHER CHARACTERISATION OF THE 5-HT RECEPTOR MEDIATING SMOOTH MUSCLE RELAXATION IN RAT OESOPHAGUS

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It has been reported that 5-hydroxytryptamine (5-HT) produces relaxation of rat isolated oesophageal muscularis mucosa precontracted with muscarinic agonists (Bieger & Triggle, 1985). This effect is apparently not mediated by 5-HT_1-like or 5-HT_2 receptors since metergoline, methysergide and ketanserin failed to inhibit this response. Additionally an involvement of neuronal 5-HT_3 receptors seems unlikely because tetrodotoxin had no effect on the 5-HT-induced relaxation. However, more recently it has been found that certain 5-HT_3 receptor antagonists (ICS205-930, MDL72222, BRL43694, metoclopramide) inhibit the effect of 5-HT in this preparation (Triggle et al., 1988). In the present study we have investigated further the receptor type mediating the 5-HT-induced relaxation of the rat oesophagus.

Isolated segments of rat distal oesophagus were suspended in the longitudinal plane in Krebs-Henseleit solution at 32°C and gassed with 95% O_2/5% CO_2. All layers, muscle and mucosa, of the oesophagus were intact and a basal tension of 0.5g was applied. The preparations were contracted with a submaximal concentration of carbachol (0.5μM) which produced a well maintained contraction for at least 20 minutes. 5-HT or other 5-HT receptor agonists were added in a sequential manner with washouts between dosing. All antagonists (one per preparation) were equilibrated for 30 minutes prior to dosing with an agonist.

5-HT (0.03-10μM) caused reproducible concentration-related relaxations of the oesophagus with a mean EC_{50} value of 0.22 (0.18-0.27)μM and a maximum reduction of the carbachol-induced tone of 69.6±2.7% (n=20). This effect was mimicked (equipotent concentration ratio, 5-HT =1) by α-methyl-5-HT (28.5), 5-carboxamidotryptamine (17.8) and 5-methoxytryptamine (108.5) whereas 2-methyl-5-HT, and GR43175 (Humphrey et al., 1988) were weak or inactive at concentrations up to 100μM. The relaxation to 5-HT was not antagonised by the 5-HT_1-like receptor antagonists methiothepin (1μM), metergoline (1μM) or methysergide (10μM) or the 5-HT_2 receptor antagonist, ketanserin (1μM). The 5-HT_3 receptor antagonists BRL43694 (10μM), MDL72222 (1μM) and GR38032 (1μM) were also without effect and the absence of any neuronal involvement was confirmed by the lack of effect of tetrodotoxin (0.3μM). In contrast high concentrations of the 5-HT_3 receptor antagonist ICS205-930 (1 & 10μM) produced parallel dose-related shifts of the 5-HT concentration-response curve, from which a pK_B value of 6.1 (5.9-6.4) was calculated. Additionally three benzamide structures, BRL24924 (0.01-10μM), cisapride (0.01-1μM) and metoclopramide (1-10μM) produced dose-related unsurmountable inhibition of the 5-HT responses. Neither ICS205-930 (10μM), nor the benzamide compounds (1-10μM) had any inhibitory effect on the relaxant responses to isoprenaline (0.001-3μM).

These data provide evidence that the rat oesophagus contains a 5-HT receptor mediating relaxation, which is selectively blocked by ICS205-930, cisapride, BRL24924 and metoclopramide.

Bieger, D., & Triggle, C.R. (1985). Br. J. Pharmac. 84, 93-106.
Humphrey, P.P.A. et al. (1988). Br. J. Pharmac. 94, 1123-1132.
Triggle, C.R. et al. (1988). Pharmacologist 30, A126.

ATYPICAL 5-HT RECEPTORS IN GUINEA-PIG ISOLATED ILEUM

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In guinea-pig ileum, low concentrations of 5-HT (10^{-14}-10^{-7}M) evoke two responses which may not be mediated by 5-$HT_{1,2,3}$ receptors. These are a well maintained cholinergically-mediated contraction (Buchheit et al, 1985) and a potentiation of cholinergically-mediated, electrically-evoked contractions (Sanger, 1987). Furthermore, in myenteric neurones 5-HT evokes a slow depolarization, resistant to classical 5-HT receptor antagonists, mediated by "5-HT_{1p}" receptors, mimicked by 5- and 6-OH-indalpine and antagonised by N-acetyl- or N-hexanoyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (5-HTP-DP or 5-HTP-DP-hex) and by the gut motility stimulant BRL 24924 (Mawe et al, 1986; 1989). We have investigated the possibility that either of the atypical responses to 5-HT in the guinea-pig ileum is mediated by the 5-HT_{1p} receptor.

Tissues were dissected as described by Sanger (1987); 10^{-6}M methiothepin was present in experiments involving electrical stimulation. Methysergide (2 x 10^{-6}M) was present in resting tissues. Results are given as mean ± sem. $P<0.05$ = significance (Student's t-test).

In the resting ileum neither 5-HTP-DP nor 5-HTP-DP-hex (10^{-7}-10^{-5}M, n=7-8) reduced the concentration-response curve to 5-HT. 5- and 6-OH-indalpine (4 x 10^{-12}-4 x 10^{-4}M and 3.5 x 10^{-12}-3.5 x 10^{-4}M respectively, n=6) did not mimic the atypical response to 5-HT; 5-OH-indalpine evoked contractions at high concentrations (4 x 10^{-7}M), blocked by the 5-HT_3 receptor antagonist BRL 43694 (5 x 10^{-7}M).

Concentration-dependent increases in electrically-evoked contractions were obtained with 5-HTP-DP and 5-HTP-DP-hex (10^{-7}-10^{-5}M, n=7, max. increases of 108 ± 21% and 41 ± 8% at 10^{-5}M and 10^{-6}M respectively), BRL 24924 (10^{-14}-10^{-4}M, n=8, max. increase of 65 ± 11% at 10^{-7}M) and 5-HT (2.6 x 10^{-14}-2.6 x 10^{-4}M, n=15, max. increase of 102 ± 27% at 10^{-8}M). Each curve was bell-shaped and unaffected by methiothepin (10^{-7}M), (-) propranolol (10^{-6}M), mianserin (10^{-7}M), ketanserin (10^{-6}M) and BRL 43694 (10^{-7}M) ($P>0.1$, n=8 each), suggesting that 5-HT_{1-3} receptors were not involved. Craig and Clark (1989) reported that high doses of ICS 205-930 (0.3-3 x 10^{-6}M) antagonises the potentiating effects of 5-HT and BRL 24924. For 5-HT, this result was confirmed, but a non-competitive antagonism is suggested.

Both 5-OH-indalpine (4 x 10^{-11}-4 x 10^{-3}M, n=9, max. increase of 25 ± 11% at 4 x 10^{-9}M) and 6-OH-indalpine (2.8 x 10^{-11}-2.8 x 10^{-4}M, n=6, max. increase of 23 ± 8% at 2.8 x 10^{-8}M) caused small, consistent increases in the electrically-evoked contractions. Dose-response curves were again bell-shaped.

Conclusion: compounds defined as 5-HT_{1p} antagonists or agonists both increase the response to electrical stimulation. Since 5-HT_{1p} receptors may be easily desensitised (Mawe et al, 1986), agonists could behave like antagonists. Alternatively, since the increase in electrically-evoked contractions caused by both 5-HT and by BRL 24924 are blocked by high concentrations of ICS 205-930, the receptor involved may have more in common with '5-HT_4' (Dumuis et al, 1989) than 5-HT_{1p}.

Buchheit et al, 1985. Naunyn-Schmiedeberg's Arch. Pharmacol. 329, 36-41.
Craig & Clark, 1989. Br. J. Pharmac. 96, 247P.
Dumuis et al, 1989. Mol. Pharmac. 34, 880-887.
Mawe et al, 1986. Proc. Natl. Acad. Sci. USA. 83, 9799-9803.
Mawe et al, 1989. Am. J. Physiol., in press.
Sanger, 1987. Br. J. Pharmac. 51, 77-87.

A COMPARISON OF THE INOTROPIC ACTIVITY OF ORG 30029, IBMX, MILRINONE AND ROLIPRAM IN RAT PAPILLARY MUSCLES

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It is now established that there are several cyclic nucleotide phosphodiesterase (PDE) isoenzymes in mammalian tissue cells (Beavo, 1988). In the ventricular myocardium of guinea-pig, rabbit, dog, rat and man four isoenzymes with distinct regulatory and kinetic properties have been identified (Reeves et al., 1987; Weishaar et al., 1987; Shahid et al., 1989; Nicholson et al., 1989). Selective inhibitors of PDE III, a low Km cyclic AMP, Ca^{++} calmodulin independent, cyclic GMP inhibited isoenzyme, increase cardiac contractility in species such as dog and monkey but not in rat (Weishaar et al., 1987). This has been attributed to the necessity of inhibiting membrane-bound PDE III, which is absent in rat heart, to induce positive inotropism (Weishaar et al., 1987). The present study has further examined this hypothesis. The ability of selective inhibitors of PDE III, milrinone (Weishaar et al., 1987) and PDE IV, rolipram (PDE IV is a further low Km cyclic AMP, Ca^{++}/calmodulin independent PDE in myocardial cells; Reeves et al., 1987) to modulate force of contraction of rat papillary muscles was compared with that of a non selective PDE inhibitor, IBMX (Nicholson et al., 1989) and a dual inhibitor of PDE III and PDE IV, Org 30029 (Shahid et al., 1989).

Experiments were performed with papillary muscles from rat right ventricle suspended in Krebs - bicarbonate buffer containing propranolol ($1x10^{-7}$ mol/l) at 37°C (pH 7.4). Preparations were electrically stimulated (1Hz) with square wave pulses (1.0 ms) at a supramaximal voltage. The maximum response to isoprenaline was established in all preparations and results are expressed as a % of this maximum (means±s.e.mean; $n \geq 4$).

Org 30029 (1-300µmol/l) and IBMX (1-100µmol/l) produced concentration-dependent increases in developed tension, up to 76.5±15% and 74±11%, respectively. Milrinone (1-100 µmol/l) and rolipram (1-100µmol/l) were without effect when given alone. However, in the presence of milrinone (100µmol/l), rolipram (1-100µmol/l) did increase developed tension, up to 67.4±9.3%, whilst in the presence of rolipram (100µmol/l), milrinone (1-100µmol/l) increased developed tension, up to 63.7±11%.

The results show that despite the absence of a membrane-bound PDE III activity, it is possible to increase the force of contraction of rat papillary muscle with PDE inhibitors. Inotropic activity is, however, only observed following administration of non-selective PDE inhibitors such as IBMX, dual inhibitors of PDE III and PDE IV such as Org 30029 or combinations of selective PDE III or IV inhibitors such as rolipram and milrinone. In order to produce positive inotropism in rat papillary muscles it is necessary to inhibit at least PDE III and IV. This result is not compatable with the hypothesis that inotropic activity is induced, in all species, only by selective inhibition of a membrane-bound PDE III. An alternative explanation is that it is necessary to inhibit a sufficient proportion of total PDE activity in a relevant intracellular locale in order to induce positive inotropism.

Beavo, J.A. (1988) in 'Advances in Second Messenger and Phosphoprotein Research vol 22': Greengard, P. & Robison, G.A. (eds), pp 1-38. New York, Raven Press.
Nicholson, C.D. et al. (1989) Br. J. Pharmac., in press.
Reeves, M.L. et al. (1987) Biochem. J. 241, 535-541.
Shahid, M. et al. (1989) Br. J. Pharmac. 96, 196P.
Weishaar, R.E. et al. (1987) Circ. Res. 61, 539-547.

BRONCHODILATOR AND TRACHEAL RELAXANT EFFECTS OF POTASSIUM CHANNEL OPENERS IN THE GUINEA-PIG

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Several structurally distinct K^+-channel openers have now been identified and their therapeutic potential as bronchodilator agents is currently under investigation. The prototype cromakalim (CRK) relaxes airway as well as vascular smooth muscle and prevents bronchospasm in the guinea-pig (Arch et al, 1988). We have compared the relaxant effects of CRK, RP49356, pinacidil (PIN) and the pinacidil analogue P1060 on isolated guinea-pig trachea with their actions on 5HT-induced bronchospasm and resting blood pressure in the anaesthetised guinea-pig.

For tissue bath studies, strips of guinea-pig trachea were suspended under initial tension of 1.5-2g in Krebs solution containing indomethacin (3uM). Tissues were contracted with KCl (17.5mM) and relaxant agents added at 10-fold concentration increments once tension reached a stable level. In the anaesthetised guinea-pig, changes in lung resistance (R_L) and dynamic compliance (C_{dyn}) were monitored according to the method described previously (Alabaster & Keir 1987). A submaximal dose of 5HT (5-15ug/kg i.v.), which produced an increase in R_L of approximately 300% and a decrease in C_{dyn} of approximately 50%, was determined and repeated at regular time intervals until consistent responses were observed. Compounds under investigation were administered cumulatively (i.v) until the 5HT response was abolished.

P1060,CRK, PIN and RP49356 caused relaxation of KCl-contracted guinea-pig trachea with IC_{50} values of 0.6 ± 0.2μM, 1.2 ± 0.2μM, 2.9 ± 0.2μuM and 2.6 ± 0.07μM respectively (mean ± s.e.m, n=6 CRK n=8). In contrast to relaxation induced by isoprenaline and aminophylline, the effects of these agents were antagonised by the sulphonylurea glibenclamide (1uM). The doses of compounds (ug/kg) which caused a 50% inhibition of 5HT responses (IC_{50}) and a 25% fall in diastolic blood pressure (BP) in the anaesthetised guinea-pig are shown in the table (mean ± s.e.m., n=4).

Effect of K-channel openers on 5HT-induced bronchoconstriction and resting blood pressure.

COMPOUND	IC_{50}		BP
	R_L	C_{dyn}	
P1060	23.0 ± 5.8	24.3 ± 3.7	14.8 ± 7.3
CRK	80.5 ± 31.0	115.0 ± 47.0	37.3 ± 4.4
PIN	480.0 ± 21.6	590.0 ± 54.4	58.8 ± 10.5
RP49356	362.0 ± 104.0	370.0 ± 82.0	57.0 ± 17.0

The results from tissue bath studies extend earlier findings which indicated that this class of compound evokes glibenclamide-sensitive relaxation of tracheal smooth muscle (Gater 1989). In the anaesthetised guinea-pig, all four compounds produced falls in blood pressure at doses lower than those required for bronchodilation. In agreement with Weston et al (1988), we have found the analogue P1060 to be a more potent spasmolytic than pinacidil itself. We conclude that the tracheal relaxant potency of these compounds shows good correlation with their bronchodilator activity in vivo :- P1060 > CRK > PIN = RP49356.

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Alabaster VA & Keir RF (1987) Br.J.Pharmac. 90 145P
Arch JRS, Buckle DR, Bumstead J, Clarke GD, Taylor JF & Taylor SG (1988) Brit.J.Pharmac. 95 763-770
Gater PR (1989) BPS (Leeds meeting) C63
Weston AH, Southerton JS, Bray KM, Newgreen DT & Taylor SG (1988) J.Cardiovasc.Pharmacol. 12 (Suppl 2) S10-S16

EFFECTS OF CROMAKALIM AND GLIBENCLAMIDE ON ISOLATED STRIPS OF BOVINE TRACHEAL SMOOTH MUSCLE

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It is known that potassium (K) channel opening drugs such as cromakalim can relax airways smooth muscle (Allen et al., 1986). However, the type of K channel involved has not yet been characterized. In the present study we have examined the effects of cromakalim and glibenclamide (a selective blocker of ATP-sensitive K channels found in pancreatic ß-cells; de Weille et al., 1988) on smooth muscle isolated from bovine trachea.

Strips of bovine tracheal smooth muscle were mounted on a manifold for simultaneous ^{42}K and ^{86}Rb efflux studies (Edwards & Weston, 1989) or in a tissue bath for recording of either isometric tension or membrane potential. The basal efflux rate coefficients for ^{42}K and for ^{86}Rb were 0.41 ± 0.01 $\%min^{-1}$ and 0.36 ± 0.01 $\%min^{-1}$ (mean ± s.e. mean; n = 5) respectively. Glibenclamide (1µM) alone had no effect on these values, whereas cromakalim (10µM) alone caused 3.5 and 2.5 fold increases in ^{42}K and ^{86}Rb efflux, respectively. In the presence of glibenclamide (1µM) the stimulatory effect of cromakalim (10µM) on ^{42}K efflux and on ^{86}Rb efflux was inhibited by 48% and 63% respectively. Tissue bath studies showed that cromakalim inhibited KCl (25mM)-induced contractions. In 4 to 6 experiments this inhibition was antagonised by glibenclamide which altered IC_{50} values for cromakalim from 0.19µM (control) to 0.28, 0.61, 1.34 and 3.67µM (glibenclamide 0.03, 0.1, 0.3 and 1.0µM respectively). Electrophysiological studies showed that the resting membrane potential of tracheal smooth muscle cells ranged from -44mV to -77mV (mean ± s.e. mean -60.7 ± 2.0mV; n = 20 cells from eight different tissues). Cromakalim (1µM) caused membrane hyperpolarization, the magnitude of which was greater the lower the resting membrane potential (range -5.7mV to -35.0mV; mean ± s.e. mean -18.6 ± 3.9 mV; n = 7 cells from five different tissues). This hyperpolarization was reversed by glibenclamide (1-10µM).

The results show that the actions of cromakalim on tracheal smooth muscle are consistent with the opening of K channels. Glibenclamide was a more effective inhibitor of cromakalim stimulated ^{86}Rb efflux than ^{42}K efflux. This suggests that cromakalim may open more than one type of K channel and that one of these channels is permeable to ^{86}Rb and more sensitive to the effects of glibenclamide. Furthermore, higher concentrations of glibenclamide were required to inhibit the effects of cromakalim compared to those required to exert effects in pancreatic ß-cells (de Weille et al., 1988). Therefore, it seems unlikely that the K channels involved in the action of cromakalim in bovine tracheal smooth cells are identical to the the ATP-sensitive K channel found in pancreatic ß-cells.

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Allen, S.L., Boyle, J.P., Cortijo, J., Foster, R.W., Morgan, G.P. & Small, R.C. (1986) Br. J. Pharmac., 89, 395-405.
Edwards, G. & Weston, A.H. (1989) Br. J. Pharmac. Proc. Suppl., (This meeting).
de Weille, J., Schmid-Antomarchi, H., Fossett, M. & Lazdunski, M. (1988) Proc. Natl. Acad. Sci., (USA), 85, 1312-1316.

EVALUATION OF INHALED CROMAKALIM AND ITS ACTIVE ENANTIOMER BRL 38277 AS BRONCHODILATORS IN THE GUINEA-PIG

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The potassium channnel activator cromakalim and its active enantiomer BRL 38227 have been shown to relax guinea-pig tracheal spirals in vitro and to protect conscious guinea-pigs from histamine or antigen-induced bronchospasm when given p.o. (Arch et al., 1988; Taylor et al., 1988; J.F. Taylor unpublished). Orally administered cromakalim also protects healthy volunteers from histamine-induced bronchoconstriction (Baird et al., 1988) and shows potential in the treatment of nocturnal asthma (Williams et al., 1988). In the present study cromakalim and BRL 38227 have been compared with salbutamol in guinea-pigs using the inhaled route of administration.

Salbutamol was dissolved in water and cromakalim and BRL 38227 in 5 to 20% aqueous dimethylsulphoxide. Conscious male guinea-pigs (300-350g) were exposed in an 8 litre chamber to nebulized bronchodilators for a total of 105s. 15s later they were exposed to nebulized histamine diphosphate (5mM) until collapse or for 240s.Freely respiring anaesthetized (1.25g kg^{-1}, i.p. urethane) guinea-pigs (500-800g) were prepared by cannulating the trachea, oesophagus (intraoesophageal pressure), carotid artery and jugular vein. Airway resistance and dynamic lung compliance were calculated by the method of Dennis et al. (1969) using an analogue computer of our design. The bronchodilators were given by inhalation through a T-piece connected to the pneumotachograph for 2 min immediately prior to challenge with histamine diphosphate (2-4µg kg^{-1},i.v.). Mean blood pressure was measured immediately prior to challenge.

Inhaled cromakalim (0.125 to 2mg ml^{-1} solutions) prolonged the time before collapse in conscious guinea-pigs. The concentration required to protect half of the animals for 240s was 0.5 to 1mg ml^{-1}.BRL 38227 was twice as potent as cromakalim. In anaesthetized guinea-pigs inhaled BRL 38227 (63 to 1000µg ml^{-1} solutions) caused similar percentage inhibitions of histamine -induced changes in resistance and compliance (e.g. 74±10, 78±4% respectively, n=6, at 250µg ml^{-1}), whereas salbutamol had more effect on resistance than compliance (65±4%, 43±5% at 10µg ml^{-1}). This difference may reflect a relatively marked effect of BRL 38227 on small airways. Inhaled BRL 38227 had no effect on blood pressure at dose levels that had a marked bronchodilator effect (e.g. 4±2% reduction in blood pressure at 250µg ml^{-1}). These results demonstrate the bronchodilator potential of inhaled cromakalim and BRL 38227, and indicate a difference from salbutamol in their effects on airways dynamics.

Arch, J.R.S. et al. (1988) Br. J. Pharmac. 95, 763-770.
Baird, A. et al. (1988) Br. J. Clin. Pharmac. 25, 114P.
Dennis, M.W. et al. (1969) J. Appl. Physiol. 26, 248-252.
Taylor, S.G. et al. (1988) Br. J. Pharmac. 95, Proc. Suppl., 795P.
Williams, A.J. et al. (1988) Allergy Proc. 9, 249.

EFFECTS OF GLIBENCLAMIDE ON CROMAKALIM-INDUCED RESPONSES IN RABBIT ISOLATED AORTA

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We have recently suggested that in rabbit aorta the K^+ - channel opener cromakalim (CK) may inhibit the ability of the noradrenaline (NA)- sensitive intracellular calcium (Ca^{2+}) store to load Ca^{2+} (Bray et al., 1988). In the present study the effects of glibenclamide on CK - induced responses in rabbit aorta have been investigated. Glibenclamide is a specific inhibitor of the ATP - dependent K^+ - channel in pancreatic ß - cells (Sturgess et al., 1985) and antagonises the relaxant effects of CK in a variety of vascular tissues (Newgreen, Longmore & Weston, 1989; Winquist et al., 1989).

Endothelium - free strips of rabbit aorta were prepared from the thoracic aorta of male Half - lop rabbits (2 - 3Kg). Tissues were mounted for isometric tension recordings in MOPS - buffered physiological salt solution (PSS) at 37°C, pH 7.4, under an initial tension of 2g. For efflux studies tissues were preloaded with $^{86}Rb^+$ ($5\mu Ci.ml^{-1}$) or $^{42}K^+$ ($1.57\mu Ci.ml^{-1}$) for 90 and 180min, respectively.

In tissues precontracted with NA (0.1µM) CK - induced relaxations were inhibited by prior incubation with glibenclamide (0.1 - 1µM) in a concentration - dependent manner. In the presence of the highest concentration of glibenclamide used (1µM) the maximum relaxation to CK was inhibited by 52 ± 4% (mean ± s. e. mean, n = 6). In Ca^{2+} - free PSS containing EGTA (1mM), $MgCl_2$ (10mM) and nifedipine (0.3µM), NA produced a phasic mechanical contraction taken as an index of Ca^{2+} release from intracellular stores. The NA - sensitive Ca^{2+} store could be refilled by exposure to PSS containing Ca^{2+}(1mM) for 25min (Bray et al., 1988). Inclusion of CK (10µM) during exposure to PSS containing Ca^{2+} (1mM) inhibited the refilling of the NA - sensitive Ca^{2+} store, an effect which was antagonised by glibenclamide (1µM) ($p< 0.05$, n = 4). After the Ca^{2+} store had been refilled, the addition of CK produced only a small reduction in NA responses elicited subsequently in the presence of CK. This effect of CK was not significantly antagonised by glibenclamide (1µM, $p > 0.05$, n = 4).

CK (1 - 10µM) produced a concentration dependent increase in both $^{86}Rb^+$ and $^{42}K^+$ efflux from rabbit aortic strips. Prior incubation of tissues with glibenclamide (1µM) significantly inhibited the $^{86}Rb^+$ - efflux produced by CK ($p< 0.05$, n = 5). In contrast, the mean $^{42}K^+$ efflux during exposure to cromakalim (10µM) was reduced but not significantly antagonised by glibenclamide (mean efflux during period of drug exposure: glibenclamide control 0.36 ± 0.01%.min^{-1}; CK 1.95 ± 0.10%. min^{-1}; glibenclamide + CK 1.69 ± 0.31%. min^{-1}, n = 5).

It is concluded that cromakalim inhibits the refilling of the NA - sensitive Ca^{2+} store , an effect antagonised by glibenclamide and associated with the opening of $^{86}Rb^+$ - permeable K^+ - channels. The ability of cromakalim to inhibit the release of Ca^{2+} from intracellular stores may involve the opening of a glibenclamide - insensitive K^+ - channel .

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Bray, K. M., Weston, A. H., McHarg, A. D., Newgreen, D.T., Southerton, J. S. & Duty, S. (1988). Pflugers Arch.,411, R202.
Newgreen, D. T., Longmore, J. & Weston, A. H. (1989). Br. J. Pharmacol., 96, 116P.
Sturgess, N. C., Ashford, M. L. J., Cook, D. L. & Hales, C. N. (1985). Lancet 2, 474 - 475.
Winquist, R. J., Heaney, L. A., Wallace, A. A., Baskin, E. P., Stein, R. B., Garcia, M., L. & Kaczorowski, G. J. (1989). J. Pharmacol. Exp. Ther., 248, 149 -156.

CROMAKALIM, RP49356, PINACIDIL AND MINOXIDIL SULPHATE IN THE RAT UTERUS AND THEIR ANTAGONISM BY GLIBENCLAMIDE

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Cromakalim (CRK) is a relaxant of uterine smooth muscle, an effect suggested to be due to the opening of K^+ channels (Hollingsworth et al., 1987). However, the relaxation is not associated with ^{86}Rb efflux or marked hyperpolarisation as seen in other smooth muscles. In vascular smooth muscle CRK is antagonised by the sulphonylurea glibenclamide (Quast & Cook 1988), a blocker of ATP dependent K^+ channels in pancreatic B-cells (Schmid-Antomarchi et al., 1987). RP49356, pinacidil (PIN) and minoxidil sulphate (M^XSO_4) are all vasorelaxant drugs whose purported mechanism of action is via K^+ channel opening (Weston 1988). In this study we have compared the effects of the above K^+ channel openers and a relaxant drug of different mechanism, the β-adrenoceptor agonist salbutamol, on both oxytocin and K^+ spasms and their susceptibility to antagonism by glibenclamide.

Uterine strips from Sprague Dawley rats (200-250g; pretreated 24h earlier with 17-β oestradiol benzoate 100 $\mu g\ kg^{-1}$ s.c.) were mounted for isometric recording in Krebs' solution at 37°C and gassed with 95% O_2, 5% CO_2. CRK, RP49356, PIN, M^XSO_4 and salbutamol added cumulatively relaxed phasic spasms induced by 0.2 nM oxytocin (mean IC_{50}: 407nM, 933nM, 631nM, 16.6μM and 1.3nM respectively). Glibenclamide (0.3 - 10 μM) antagonised CRK, RP49356 and PIN with the interactions consistent with competitive antagonism (mean pA_2 ± s.e. mean [n]: 6.57 ± 0.18 [9]; 7.00 ± 0.11 [6]; 6.22 ± 0.12 [8] respectively; mean slope ± s.e. mean: -1.19 ± 0.11; -1.08 ± 0.12; -1.33 ± 0.08 respectively). M^XSO_4 was not antagonised by glibenclamide (10 μM), pD_2 in presence of glibenclamide 4.7 ± 0.17 (n=6) c.f. 4.8 ± 0.18 (n=6) in the absence of glibenclamide. The selectivity of glibenclamide was shown by its lack of antagonism of salbutamol. Glibenclamide (0.3 - 10 μM) had no effect on the spasm to 0.2 nM oxytocin.

Studies of the effects of these compounds on mechanical response to KCl showed that CRK (1-10 μM), RP49356 (1-10 μM) and M^XSO_4 (100 μM) were all able to inhibit spasms to low (<40 mM) but not high (≥40 mM) K^+. PIN at high concentrations (10 and 100 μM) and salbutamol (0.01-1μM) inhibited spasms to all concentrations of K^+ (10 - 80 mM).

In conclusion, the relaxant actions of CRK, RP49356 and PIN in the uterus are consistent with K^+ channel opening and appear to be via a channel(s) sensitive to glibenclamide. However, PIN at high concentrations would appear to have an additional mechanism(s) of action. M^XSO_4 showed low potency and insensitivity to glibenclamide, results which contrast with those found in vascular smooth muscle (Newgreen et al, 1989). Possible explanations are that the site of interaction(s) of M^XSO_4 present in vascular smooth muscle is absent in uterus or that the channel(s) opened by M^XSO_4 is not active in uterus. Whilst M^XSO_4 does show selective inhibition of low K^+ spasms its mechanism of action in the uterus remains unclear.

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Hollingsworth, M. Amédée, T., Edwards, D., Mironneau, J., Savineau, J.P., Small, R.C. & Weston, A.H. (1987) Br. J. Pharmacol. 91, 803-813.
Newgreen, D.T., Longmore, J. & Weston, A.H. (1989) Br. J. Pharmacol. 96, Proc. Suppl., 116P.
Quast, U. & Cook, N.S. (1988) Br. J. Pharmacol. 93, Proc. Suppl. 204P.
Schmid-Antomarchi, H., DeWeille, J., Fossett, M. & Lazdunski, M. (1987) J. Biol. Chem. 262, 15840-15844.
Weston, A.H. (1988) Drugs 36, Suppl. 7, 1-3.

GLIBENCLAMIDE AND PROCAINE, BUT NOT APAMIN, INHIBIT THE RELAXANT RESPONSES TO RO 31-6930 IN GUINEA-PIG *TAENIA CAECI*

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Ro 31-6930, 2-(6-cyano-2,2-dimethyl-2H-1-benzopyran-4-yl)pyridine-1-oxide, is a novel K^+ channel opener with smooth muscle relaxant properties in vitro and antihypertensive activity in vivo (Paciorek et al, 1989). To investigate the type of K^+ channel activated by this compound, we have assessed the inhibitory effects of glibenclamide (Buckingham et al, 1989), apamin (Habermann, 1984) and procaine (Yamanaka et al, 1985) against the relaxant responses to Ro 31-6930 and, for comparison, cromakalim, noradrenaline (NA) and ATP in the guinea pig taenia caeci.

Segments of taenia caeci were removed from female guinea pigs and suspended in a MOPS-buffer (37°C, 95% O_2/5% CO_2). Tissue length changes were measured isotonically under a 1 g load. Test agents were administered cumulatively and relaxation was expressed as a percentage of the maximal relaxation to 0.1 mM papaverine. Ro 31-6930 (0.01-0.1 µM), cromakalim (0.1-10 µM), NA (0.01-30 µM) and ATP (0.1-300 µM) each produced a concentration-dependent relaxation with IC_{50} values of 0.045 ± 0.003, 0.35 ± 0.04, 0.24 ± 0.06 and 3.6 ± 0.6 µM respectively (mean ± s.e.mean n = 4). ATP did not produce a full inhibition of spontaneous tone but the maximal relaxant effects of all the other compounds were identical to that of papaverine.

Glibenclamide (1 µM) did not modify the spontaneous tone of the taenia caeci, nor did it affect the relaxant actions of either ATP or NA. However, glibenclamide shifted the log concentration/effect curves of Ro 31-6930 and cromakalim 60- and 10-fold to the right respectively without depressing their maximal effects. Apamin (0.1 µM) caused an approximately 30% increase in the spontaneous tone of the taenia caeci but did not affect the inhibitory responses to Ro 31-6930 or cromakalim. Responses to NA were abolished by apamin (0.1 µM) as were responses to ATP except for at high concentrations of ATP (>300 µM). Procaine (5 mM) substantially increased the tone of the taenia caeci and evoked spike activity. Procaine shifted the log concentration/effect curves of Ro 31-6930, cromakalim, NA and ATP 3000-, 400-, 20- and 10-fold to the right respectively.

This data shows that Ro 31-6930, like cromakalim (Buckingham et al, 1989), evokes relaxation via activation of a glibenclamide-sensitive K^+ channel but, unlike NA and ATP (Weir & Weston, 1986), Ro 31-6930 does not act via the apamin-sensitive K^+ channel in the guinea pig taenia caeci.

Buckingham, R.E. et al (1989) Br.J.Pharmacol. 97, 57-64.
Habermann, E. (1984) Pharmacol.Ther. 25, 255-270.
Paciorek, P.M. et al (1989) Br.J.Pharmacol. (in press).
Weir, S.W. & Weston, A.H. (1986) Br.J.Pharmacol. 88, 113-120.
Yamanaka, K. et al (1985) Naunyn-Schmiedebergs Arch.Pharmacol. 331, 96-103.

5-HT_{1A} RECEPTORS MEDIATE A HYPERPOLARISATION OF RAT VENTROMEDIAL HYPOTHALAMIC NEURONES *IN VITRO*

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5-hydroxytryptamine (5-HT)-containing cells innervate the rat ventromedial hypothalamus (Willoughby & Blessing, 1987) but little is known of the electrophysiological actions of 5-HT in this region. Preliminary evidence (Newberry & Priestley, 1988) indicated that 5-HT inhibited ventromedial hypothalamic neurones in vitro through a 5-HT_1-like receptor and a pertussis toxin sensitive pathway. The present study sought to extend those observations.

As previously described (Newberry & Priestley, 1988), 350 µm transverse slices of the mediobasal hypothalamus, taken from male Sprague-Dawley rats, were superfused with artificial cerebrospinal fluid (aCSF) at 30°C. For extracellular recordings, using aCSF-filled glass microelectrodes, 1 µM quisqualate was added to the superfusing aCSF to increase and stabilise the neuronal firing rates (to ca. 5 Hz). For intracellular recordings, with 3M KCl-filled electrodes and an Axoclamp 2A amplifier, quisqualate was not added to the aCSF. Agonists were superfused for 1 min periods and antagonists were preincubated for 30 min before reapplying the agonist.

5-HT, 5-CT (5-carboxyamidotryptamine) and 8-OH-DPAT completely inhibited the extracellularly recorded firing rate of these neurones in a concentration-dependent manner with mean pIC_{50} (mean ± s.e.m.) values of 5.61 ± 0.07 (n = 11), 7.73 ± 0.08 (n = 20) and 6.33 ± 0.15 (n = 6) respectively. The putative selective 5-HT_3 agonist 2-methyl-5-HT was ca. 7000 times weaker than 5-CT (n = 7). Buspirone, like 8-OH DPAT, produced a slow, long lasting inhibition of these cells with a half maximal inhibition at 1-3 µM. On 2 cells the maximum inhibition was 100%, but on 3 others it was ca. 50%. The 5-HT_3 antagonist MDL 72222 (10 µM, n = 3) and the 5-HT_2 antagonist ketanserin (1 µM, n = 3) did not antagonise the 5-HT or 5-CT-induced inhibition. However, the 5-HT_{1A} antagonists spiperone (1 µM, n = 3) and (±)cyanopindolol (1 µM, n = 3) antagonised 5-CT with mean log dose-ratios of 1.7 and 1.1, respectively . Intracellular recordings revealed that both 5-HT (10 µM) and 5-CT (0.1 µM) evoked a tetrodotoxin (1 µM)-resistant hyperpolarisation associated with a reduction in the apparent neuronal input resistance. Voltage clamp experiments on 3 cells indicated that these compounds activated an outward current with a reversal potential of -93, -94 and -97 mV.

Taken together, this evidence indicates that, as in hippocampal CA1 cells (Andrade & Nicoll, 1987; Colino & Halliwell, 1987), 5-HT hyperpolarises ventromedial hypothalamic neurones via 5-HT_{1A} receptors which activate a potassium conductance. This is consistent with the autoradiographic localisation of 5-HT_{1A} binding sites in this region (Verge et al, 1986).

Andrade, R. & Nicoll, R.A. (1987) J. Physiol., 394, p 99.
Colino, A. & Halliwell, J.V. (1987) Nature, 328, p 73.
Newberry, N.R. & Priestley, T. (1988) Br. J. Pharm, 95, p 6
Verge, D. et al (1986), J Neuroscience, 6, p 3474
Willoughby, J.O. & Blessing W.W. (1987) Brain Res., 418, p 170.

CARDIOVASCULAR EFFECTS FOLLOWING INJECTION OF 8-OH-DPAT AND FLESINOXAN INTO THE DORSAL RAPHE NUCLEUS OF CONSCIOUS RATS

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We have examined the cardiovascular effects of the 5-HT_{1A} receptor agonists 8-hydroxy-2 (di-n-propylamino) tetralin (8-OHDPAT) and flesinoxan (Wouters et al, 1988) injected into the dorsal raphe nucleus (DRN) of conscious rats. Male Lister Hooded rats (270±30g) were chronically implanted with a guide cannula above the DRN as described by Higgins et al (1988). At least 7 days later, rats were anaesthetised (enflurane) and the right carotid artery was cannulated for measurement of blood pressure (BP) and heart rate (HR). Experiments were performed 24-48 hours later; drug or vehicle (0.9% saline) was injected (0.5µl over 1 min) into the DRN. Injection sites were verified histologically and results were used only where this was within the DRN.

The effects following injection of 8-OHDPAT or flesinoxan into the DRN are shown in Table 1; both agonists caused marked decreases in BP and HR. Effects were immediate in onset and maximum changes were seen about 10 min after injection. 8-OHDPAT and flesinoxan also caused a dose-dependent flattening of body posture (FBP, Table 1).

Table 1: Effects of DRN injection of 8-OH-DPAT or flesinoxan.

DRN Injection		% change from resting levels BP	HR	Incidence of FBP	n
Vehicle	0.5µl	-3 ± 2 (109 ± 11)	-3 ± 3 (408 ± 46)	0/4	4
8-OHDPAT	100ng	-5 ± 5 (113 ± 9)	-1 ± 2 (359 ± 33)	0/4	3
	500ng	**-25 ± 2 (131 ± 3)	*-20 ± 4 (447 ± 16)	4/5	5
	2.5µg	**-26 ± 4 (125 ± 9)	**-28 ± 3 (421 ± 31)	4/4	4
Flesinoxan	2.5µg	-9 ± 2 (119 ± 4)	-10 ± 2 (405 ± 28)	1/5	5
	5.0µg	*-26 ± 7 (117 ± 5)	-21 ± 7 (426 ± 25)	4/4	4

Changes measured 10 min after injection; resting levels (mean BP, mmHg and HR, bpm) shown in brackets. Values are means ± S.E.M. *$P<0.05$, **$P<0.01$ Student's t-test.

In separate experiments, 8-OHDPAT (500ng DRN) markedly decreased BP and HR (-26 ± 4% and -24 ± 3% respectively, n = 4) when measured 15 min after saline (0.5µl DRN) pretreatment, given 5 min before 8-OH-DPAT. Pretreatment with the 5-HT_{1A} receptor antagonist (-)pindolol (500ng DRN; Schoeffter and Hoyer, 1988), followed by saline (0.5µl DRN 5 min later) slightly reduced BP but caused little change in HR (-10 ± 5% and -1 ± 6% respectively, n = 3). Administration of 8-OH-DPAT after (-)pindolol changed BP and HR by -11 ± 1% and 3 ± 3% respectively (n = 4). Thus, (-)pindolol significantly ($P<0.05$) reduced the cardiovascular effects of 8-OH-DPAT. Furthermore, (-)pindolol reduced the incidence of FBP following 8-OH-DPAT (1/4 compared with 4/4 in saline-pretreated animals).

In conclusion, these results suggest that activation of 5-HT_{1A} receptors in the DRN mediates hypotension and bradycardia in conscious rats. This may reflect inhibition of DRN cell firing since the GABA agonist muscimol which, like 8-OHDPAT inhibits raphe unit activity (Gallager et al, 1978; Sprouse & Aghajanian, 1987), produces similar cardiovascular changes following DRN injection (Robinson et al, 1986).

Gallager, D.W. (1978) Eur. J. Pharmacol., 49, 133-143
Higgins, G.A. et al (1988) Neuropharmacol., 27, 993-1001
Robinson, S.E. et al (1986) Neuropharmacol., 25, 611-615
Schoeffter, P. and Hoyer, D. (1988) Br. J. Pharmacol., 95, 975-985
Sprouse, J.S. and Aghajanian, G.K. (1987) Synapse, 1, 3-9
Wouters, W. et al., (1988) Eur. J. Pharmacol., 149, 213-223

EVIDENCE THAT THE REFLEX VAGAL BRADYCARDIA CAUSED BY I.V. PHENYLBIGUANIDE IS MEDIATED BY CENTRAL 5-HT_{1A} RECEPTORS

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There is anatomical and pharmacological evidence for an excitatory 5-HT-containing pathway which innervates cardiac vagal motoneurones and involves the activation of 5-HT_{1A} receptors (Steinbusch, 1981; Ramage & Fozard, 1987; Izzo et al., 1988; Sporton et al. 1989). Excitatory inputs to cardiac vagal motoneurones arise from cardiopulmonary afferents, arterial baroreceptors, carotid body afferents and from receptors in the airways involved in the diving response (see Daly, 1986). Hence it was decided to examine if any of these excitatory inputs may be mediated by this 5-HT pathway, starting with the cardiopulmonary reflex (Bezold-Jarisch reflex) which can be activated by i.v. phenylbiguanide (Dawes & Mott, 1950).

Experiments were performed on anaesthetised spontaneously breathing adult male Sprague-Dawley rats. Anaesthesia was induced with halothane and maintained with α-chloralose (70mg kg^{-1}; i.v.). The femoral artery was cannulated for measurement of blood pressure and heart rate and the jugular vein was cannulated for administration of drugs. The animals were then placed in a head holder and a cannula was placed through the atlanto-occipital membrane so that drugs could be administered intracisternally (i.c.). The rats were then pretreated with atenolol (1 mg kg^{-1}; i.v.). Once BP and HR had stabilised a Bezold-Jarisch reflex was induced with phenylbiguanide (10 μg kg^{-1}; i.v.) and repeated at 15 min intervals. The test drug was given intracisternally (in 10 μl over a period of 20 s) 10 min after the third injection of phenylbiguanide and the reflex was tested 5 min later and again every 15 min. All drugs were dissolved in saline except spiperone and (-)sulpiride. Spiperone was dissolved in 0.4M lactic acid while (-)sulpiride was dissolved in a few drops of 1N HCl and then diluted in saline.

The effect of i.c. application of spiperone, lactic acid, methiothepin, BW 501C, alfuzosin, and (-)sulpiride on the Bezold-Jarisch reflex is shown in Table 1.

Table 1 Change in Heart Rate (beats min^{-1}) Evoked by Phenylbiguanide

Drug	Control	5 min	20 min	35 min
Lactic acid (n=5)	-117±7	-78±10	-100±15	---
Spiperone(100 μg kg^{-1};n=5)	-159±19	-32±11**	-52±14*	76±17
Methiothepin(200 μg kg^{-1};n=3)	-115±23	-28±9*	-33±10*	47±19
BW 501C (100 μg kg^{-1};n=3)	-165±6	-130±15	-150±15	---
Alfuzosin (100 μg kg^{-1};n=3)	-102±10	-125±28	-117±26	---
(-)Sulpiride(100 μg kg^{-1};n=3)	-152±14	-132±17	---	---

Results are mean ± s.e. mean * $p<0.05$ ** $p<0.01$. Student's paired t-test.

Spiperone (n=4; 100μg kg^{-1}) given i.v. and, in another two experiments, ICS 205-930 (0.5 μg kg^{-1}) given i.c. had no effect on the Bezold-Jarisch reflex.

The failure of the selective 5-HT_2 antagonist BW 501C, the dopamine antagonist (-)sulpiride and the α_1-antagonist alfuzosin to attenuate the vagally induced bradycardia caused by phenylbiguanide suggests that the ability of spiperone and methiothepin to attenuate this part of Bezold-Jarisch reflex when applied centrally was due to their ability to block 5-HT_{1A} receptors.

Daly, M de B. (1986) In: Handbook of Physiology, section 3, The Respiratory System. Vol. 2. Part II. pp 529-594.
Dawes, G.S. and Mott, J.C. (1950) Br. J. Pharmac. 5, 65-76.
Izzo, P.N., Jordan, D. and Ramage, A.G. (1988) J. Physiol. 406, 19P.
Ramage, A.G. and Fozard, J.R. (1987) Eur. J. Pharmacol. 138, 170-191.
Sporton, S.C.E., Shepheard, S.L., Jordan, D. and Ramage, A.G. (1989) Br. J. Pharmac. 97, 409P.
Steinbusch, H.W.M. (1981) Neuroscience 6, 557-618.

BRAIN 5-HT UPTAKE SITES, LABELLED WITH [^{3}H]-PAROXETINE, IN POST-MORTEM SAMPLES FROM DEPRESSED SUICIDE VICTIMS

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A defect in serotonergic (5-HT) neurotransmission has long been implicated in depression and suicide. An abnormality of 5-HT uptake is suggested by the lower platelet 5-HT uptake and lower [^{3}H]imipramine binding in depressed patients compared to controls. Reduced [^{3}H]imipramine binding has also been reported in some post-mortem studies (Stanley et al, 1982; Perry et al, 1983), but not others (Owen et al, 1986).

We now report the binding of [^{3}H]paroxetine ([^{3}H]PAR, a potent and selective 5-HT uptake inhibitor) in six brain regions from suicides (15M, 7F; mean age ± SEM, 42 ± 3 years; post-mortem delay, 35 ± 4 h), in whom a firm retrospective diagnosis of depression was established and who were not receiving antidepressant drugs, and matched controls (15M, 5F; 43 ± 3 years; 40 ± 3h). Well-washed membranes were incubated with [^{3}H]PAR (0.009-0.5 nM) for 90 min in 50 mM Tris-HCl pH 7.5, 120 mM NaCl, 5 mM KCl. Specific binding was defined with 1 μM citalopram.

[^{3}H]PAR binding was of high and uniform affinity (Kd values: controls, 21-38 pM; suicides, 21-41 pM) and Kd values did not differ significantly between suicides and controls in the brain regions studied. [^{3}H]PAR Bmax varied widely, being lowest in cortex and highest in substantia nigra (Table 1). [^{3}H]PAR Bmax did not differ significantly between depressed suicides and controls (Table 1). No significant relationship was found between [^{3}H]PAR Bmax and post-mortem delay in suicides, controls or when both groups were combined.

Table 1. [^{3}H]PAR Bmax in depressed suicides and controls

	Controls	Suicides
Frontal cortex (Brodmann area 10)	51 ± 2	50 ± 3
Occipital cortex (Brodmann area 17/18)	49 ± 3	49 ± 3
Hippocampus	104 ± 10	96 ± 9
Caudate	181 ± 10	170 ± 10
Putamen	215 ± 15	197 ± 13
S. Nigra	394 ± 20	411 ± 20

Means ± SEM, fmol/mg protein. n=20-22 for suicides, n=20 for controls

Kd and Bmax did not differ significantly when suicides who died by violent means (hanging, jumping or stab wound, n=12) or by non-violent means (drug overdose, carbon monoxide poisoning, n=10) were compared to matched controls except in putamen. [^{3}H]PAR Bmax in putamen was significantly lower in non-violent suicides than controls (177 ± 12 v 232 ± 21, $p<0.05$) but did not differ significantly in violent suicides compared to controls (218 ± 21 v 201 ± 27).

The present results in a moderately large group of depressed suicides who were not receiving antidepressant drugs, do not support the view of abnormal 5-HT uptake sites in depression and suicide.

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Owen, F. et al (1986) Brain Res 362, 185-188.
Stanley, M. et al (1982) Science 216, 1337-1339.
Perry, E.K. et al (1983) Br J Psychiatry 142, 188-192.

EFFECTS OF CLOZAPINE AND OTHER NEUROLEPTICS ON BINDING OF [^{3}H] -Q ICS 205-930 TO CENTRAL 5-HT$_3$ RECOGNITION SITES

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Behavioural data have indicated that GR38032F, a potent antagonist at peripheral 5-HT$_3$ receptors, can induce antidopamine-like activity in rodents and primates (Costall et al, 1987). These data imply the existence of central 5-HT$_3$ receptors and suggest that these sites may represent a novel target for neuroleptic drugs. We have recently used the [^{3}H]-quaternised derivative of ICS 205-930, a potent 5-HT$_3$ antagonist, to label 5-HT$_3$ recognition sites in rat brain (Watling et al, 1988). In the present study, the effects of various neuroleptics have been examined on the binding of [^{3}H]-quaternised ICS 205-930 ([^{3}H]-Q ICS 205-930) to 5-HT$_3$ recognition sites in rat cortex.

Binding of [^{3}H]-Q ICS 205-930 (0.5-0.7nM) to rat cortical membranes (pKd 9.05 $\pm$ 0.18nM and Bmax 74.9 $\pm$ 18.4 fmol/mg protein; mean $\pm$ SEM, n = 4) was performed as described previously (Watling et al, 1988). The selective 5-HT$_3$ receptor antagonist MDL 72222 (10μM) was used to define specific binding which accounted for 60-70% of total binding. All drugs were dissolved in dimethylsulphoxide which at a final assay concentration of 1% had no significant effects on the levels of either total or non-specific binding.

When examined at a final concentration of 10μM in at least three separate experiments, several neuroleptics from various chemical classes, including spiperone, haloperidol, bromperidol, benperidol, (-)sulpiride, sultopride, α-flupenthixol, pimozide and molindone failed to lower specific [^{3}H]-Q ICS 205-930 binding by 50% or more. Accordingly, these neuroleptics displayed pIC$_{50}$ values ($-\log_{10}$ concentration of drug inducing 50% inhibition of specific binding) of < 5.0. In contrast, the dibenzodiazepine derivative clozapine (10μM), an atypical neuroleptic devoid of extrapyramidal side effects (Matz et al, 1984), induced an almost complete inhibition of specific [^{3}H]-Q ICS 205-930 binding. Subsequent inhibition curves performed over the concentration range 0.001-10μM yielded a pIC$_{50}$ for clozapine of 6.74 $\pm$ 0.05 and a Hill slope of 1.07 $\pm$ 0.06 (mean $\pm$ SEM, n = 3). Only two other neuroleptics examined, chlorpromazine and thioridazine, lowered specific [^{3}H]-Q ICS 205-930 binding to 50% or less, displaying pIC$_{50}$ values of 5.41 $\pm$ 0.05 (mean $\pm$ SEM, n = 4) and 4.99 $\pm$ 0.09 (mean $\pm$ SEM, n = 3), respectively.

In summary, these results indicate that in addition to its known activity at central dopamine D$_2$, 5-HT$_2$, α-adrenergic, histamine H$_1$ and muscarinic receptors (Miller and Hiley, 1974; Peroutka and Snyder, 1980), clozapine is a relatively potent inhibitor of binding to central 5-HT$_3$ recognition sites, possessing a Ki value of approximately 100nM. In contrast, a variety of other neuroleptics were essentially devoid of this activity. The possibility exists, therefore, that the atypical neuroleptic profile of clozapine may be mediated, at least in part, via its interaction with central 5-HT$_3$ recognition sites.

Costall, B. et al (1987) Br. J. Pharmacol. 92, 881-894.
Matz, R. et al (1974) Curr. Therap. Res. 16, 687-695.
Miller, R. and Hiley, C.R. (1974) Nature 248, 596-597.
Peroutka, S.J. and Snyder, S. (1980) Am. J. Psychiatry 137, 1518-1522.
Watling, K.J. et al (1988) Eur. J. Pharmacol. 149, 397-398.

BEHAVIOURAL INDICES OF THE INTERACTION OF CLOZAPINE WITH D_1 AND D_2 DOPAMINE RECEPTORS

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Clozapine is a well researched but atypical neuroleptic with low potency to induce catalepsy or to block apomorphine stereotypy. While in vitro binding techniques show it to have similar affinity for both D-1 and D-2 dopamine receptor sites, recent studies have suggested greater antagonist activity at the D-1 receptor, at the level of adenylate cyclase (Andersen & Braestrup, 1986), and in vivo (Chipkin & Latranyi,1987). We have now investigated clozapine in recently-developed behavioural models (Murray & Waddington, 1989a,b) with the potential to distinguish preferential D-1 from D-2 antagonist activity.

Male Sprague-Dawley rats were injected s.c. with the selective D-1 agonist SK&F 77434 (Murray & Waddington, 1989a) or the selective D-2 agonist LY 163502 (Murray & Waddington, 1989b), after treatment with vehicle or clozapine. Behavioural responses were assessed using a behavioural check list procedure that generates counts for each individual behaviour, and the in vitro affinity of clozapine for D-1 and D-2 sites was determined by displacement of ^{3}H-SCH 23390 and ^{3}H-spiperone, respectively (Murray & Waddington, 1989a).

	mg/kg	Gr_i		mg/kg	L
Vehicle		0.5±0.4	Vehicle		1.5±0.7
SK&F 77434	0.75	6.0±1.1	LY 163502	0.05	5.9±1.1
+ clozapine	2.5	1.6±0.5*	+ clozapine	2.5	1.1±0.5*
	10.0	0.4±0.4*		10.0	4.0±2.4
	25.0	0±0 *		25.0	0.3±0.2*

Means ± s.e. mean, n=8. * $P<0.05$

The D-1 agonist SK&F 77434 induced intense grooming (Gr_i) that was blocked by as little as 2.5 mg/kg clozapine; no vacuous chewing emerged during this blockade. The D-2 agonist LY 163502 induced locomotion (L) that was blocked by as little as 2.5 mg/kg clozapine; there was some emergence of atypical limb/body jerking during this blockade ($P<0.05$). K_i values for displacement of ^{3}H-SCH 23390 and ^{3}H-spiperone were similar (geometric means: 542nM and 449nM, respectively; n=3).

Selective blockade of D-2 receptors not only attenuates typical D-1 agonist responses, through co-operative D-1:D-2 interactions (Waddington & O'Boyle, 1987), but also releases atypical vacuous chewing; selective blockade of D-1 receptors not only attenuates typical D-2 agonist responses similarly, but also releases atypical limb/body jerking (Murray & Waddington, 1989a,b). The present results therefore indicate that clozapine potently antagonises typical behavioural responses induced by both selective D-1 and D-2 agonists, while the overall pattern of emergence of atypical responses suggests it may exert some preferential, but non-selective, activity to antagonise D-1 receptor-mediated function.

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Andersen, P.H. & Braestrup,C. (1986) J. Neurochem. 47, 1822-1831.
Chipkin, R.E. & Latranyi, M.B. (1987) Eur. J. Pharmacol. 136, 371-375.
Murray, A.M. & Waddington, J.L. (1989a) Eur. J. Pharmacol. 160, 377-384.
Murray, A.M. & Waddington, J.L. (1989b) Psychopharmacology (in press).
Waddington, J.L. & O'Boyle, K.M. (1987) Rev. Neurosci. 1, 157-184.

D_1 OR D_2 RECEPTOR-INDUCED PURPOSELESS CHEWING IN RATS IS DIFFERENTIALLY MODULATED BY CHOLINERGIC DRUGS

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Administration of D-1 or D-2 agonist or antagonist drugs appears to induce an identical syndrome of purposeless chewing in rats (Collins et al. 1989). Cholinergic agonists also induce purposeless chewing and alter that produced by neuroleptic drugs (Rupniak et al. 1986). We have examined the effect of combined cholinergic manipulation and altered D-1 and D-2 dopamine receptor function on purposeless chewing.

Male Wistar rats (225-250 g) were acclimatised overnight in a quiet room. Rats were assessed individually for purposeless chewing in single cages for 5 min periods. The effect on purposeless chewing of i.p. administration of SKF 38393, sulpiride or quinpirole alone and in combination with pilocarpine, physostigmine or scopolamine was assessed. Results are presented as the number of purposeless chewing movements occurring in a 5 min period at the time of maximum drug effect.

Administration of pilocarpine (0.5-4.0 mg/kg) or physostigmine (0.05-0.2 mg/kg) caused a dose dependent increase in the frequency of purposeless chewing. Similarly, administration of sulpiride (100 mg/kg), SKF 38393 (20 mg/kg) or quinpirole (0.1 mg/kg) enhanced the incidence of purposeless chewing. Sulpiride (100 mg/kg) in combination with either pilocarpine (0.5 mg/kg) or physostigmine (0.1 mg/kg) increased in an additive manner the frequency of purposeless chewing compared to either drug alone. SKF 38393 (20 mg/kg) in combination with pilocarpine (0.5 mg/kg), but not physostigmine (0.1 mg/kg) increased the frequency of purposeless chewing above that produced by either drug alone. Combined administration of quinpirole (0.1 mg/kg) with either pilocarpine (0.5 mg/kg) or physostigmine (0.1 mg/kg) did not increase the purposeless chewing frequency above that produced by either drug alone.

Table 1 Effect of cholinergic stimulation on purposeless chewing in rats

Treatment	Control	Pilocarpine	Control	Physostigmine
Control	5.8 ± 1.4	31.0 ± 4.8*	6.8 ± 1.6	18.5 ± 3.1*
Sulpiride	30.5 ± 2.5*	52.8 ± 4.2+	24.7 ± 3.5*	60.0 ± 12.6+
Control	4.8 ± 2.0	29.7 ± 5.2*	-	-
SKF 38393	21.5 ± 4.7*	54.3 ± 8.1+	27.2 ± 6.3*	18.0 ± 5.3
Control	7.5 ± 1.8	24.8 ± 5.4*	-	31.0 ± 7.6*
Quinpirole	43.8 ± 9.4*	36.5 ± 4.5*	-	47.5 ± 7.3

All results expressed as mean ± S.E.M.; * $p < 0.05$, Mann Whitney U test compared to the corresponding control; + compared to the corresponding treated groups.

Scopolamine (0.1 mg/kg) completely blocked the purposeless chewing induced by pilocarpine (0.5 mg/kg), sulpiride (100 mg/kg) or SKF 38393 (20 mg/kg), but had no effect on purposeless chewing induced by quinpirole (0.1 mg/kg).

Although identical purposeless chewing is induced by D-1 or D-2 agonists or antagonists, the interaction with cholinergic drugs suggests different underlying mechanisms.

Collins, P. et al. (1989) Leeds Meeting BPS.
Rupniak, N.M.J. et al. (1986) Psychopharmacology 88, 403-419.

FLUCTUATIONS IN RESPONSE TO L-DOPA IN PARKINSON'S DISEASE MAY BE EXPLAINED BY CHANGES IN STRIATAL DOPAMINE TURNOVER

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Long-term treatment of Parkinson's disease with L-DOPA leads to fluctuations in drug response (Marsden et al. 1982). These may result from changes in the pharmacokinetic handling and metabolism of L-DOPA, or from altered pharmacodynamics of dopamine function in the brain. We report the effect of 1 years administration of L-DOPA plus carbidopa on specific ^{3}H-spiperone and ^{3}H-SCH 23390 binding, and the uptake and metabolism of orally administered L-DOPA in the striatum of normal rats.

Male Wistar rats (125 ± 2g) received L-DOPA (191-220 mg/kg/day) plus carbidopa (24-26 mg/kg/day), or carbidopa (24-26 mg/kg/day) via their daily drinking water for 1 year. Control rats received normal drinking water. Animals were withdrawn from drug treatment for 4 days and fasted for 16 hours prior to testing. Striatal ^{3}H-spiperone and ^{3}H-SCH 23390 binding was measured using 10^{-5}M sulpiride and 10^{-5}M cis-flupenthixol respectively to define specific binding. Dopa decarboxylase (DDC) activity was determined in the supernatant of striatal homogenates using a radioenzymatic assay. Other rats were treated acutely with L-DOPA (200mg/kg po) and were killed at intervals up to 8 hours following L-DOPA administration. Control rats received vehicle orally and were killed immediately. L-DOPA, DA, DOPAC and HVA levels were measured in plasma and striatum by HPLC.

Administration of L-DOPA plus carbidopa or carbidopa alone for 1 year to normal rats did not alter specific ^{3}H-spiperone and ^{3}H-SCH 23390 binding to striatal membranes (Table 1). Chronic administration of L-DOPA plus carbidopa or carbidopa alone did not alter DDC activity in the striatum of rats compared to control animals (Table 1). Following acute oral administration of L-DOPA there was no overall difference in peak plasma L-DOPA level, or the rate of decline of plasma L-DOPA between rats treated for 1 year with L-DOPA plus carbidopa or carbidopa alone compared to control rats. In the striatum, dopamine turnover, measured by the ratio DOPAC+HVA/DA, was increased in all groups. However, peak dopamine turnover was attained earlier, and returned towards control levels earlier in animals treated chronically with L-DOPA plus carbidopa compared to the carbidopa treatment and control groups

Table 1 Striatal D-1 and D-2 receptor binding and DDC activity

	^{3}H-SCH 23390 Bmax (pmol/g)	^{3}H-spiperone Bmax (pmol/g)	DDC activity (nmol L-DOPA/mg/15 min)
Control	76.9 ± 1.0	21.9 ± 1.7	5.1 ± 0.9
Carbidopa	72.4 ± 3.6	23.4 ± 1.1	7.1 ± 0.7
L-DOPA plus carbidopa	79.6 ± 4.9	21.6 ± 3.0	5.1 ± 0.7

Specific ^{3}H-spiperone and ^{3}H-SCH 23390 binding to striatal membranes, and striatal DDC activity were unaltered by chronic administration of L-DOPA plus carbidopa to normal rats. However, the earlier peak and reduced period of raised striatal dopamine turnover after an acute oral challenge with L-DOPA may be related to response fluctuations occurring in Parkinson's disease.

Marsden, C.D. et al. (1982) In: Movement Disorders, ed. Marsden, C.D. and Fahn, S. pp 96-122, Boston, Butterworth Scientific.

CORTICAL MUSCARINIC RECEPTORS IN DEMENTED PATIENTS WITH ALZHEIMER'S DISEASE OR PARKINSON'S DISEASE

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The characteristic clinical and neuropathological features of Alzheimer's disease (AD) and Parkinson's disease (PD) are different. However, patients with Alzheimer-type dementia as well as patients with PD show neuronal loss in the substantia innominata and decreased choline acetyltransferase activity in the cerebral cortex (Price et al., 1986). We have examined whether altered cholinergic function is associated with similar changes in muscarinic receptor binding in the frontal cortex and temporal cortex of demented patients with AD or PD.

Brains were obtained from 7 demented patients with AD and from 7 age-matched control subjects without neurological or psychiatric diseases as well as from 5 demented patients with PD and 7 controls. The Parkinsonian patients had all received L-dopa therapy up to the time of death, in addition one patient had received anticholinergic medication. Using washed membranes saturation analysis was performed for [^{3}H]- quinuclidinylbenzilate binding (QNB; total number of muscarinic receptors; concentrations 9.4 pM - 0.3 nM) and [^{3}H]-pirenzepine binding (PZ; M-1 receptors; concentrations 0.5 - 64 nM). Non-specific binding was defined as the amount of tritiated ligand bound in the presence of 1μM atropine.

Table 1. B_{max} values for muscarinic receptor binding (fmol/mg protein; means ± s.e.mean)

	FRONTAL CORTEX		TEMPORAL CORTEX	
	[^{3}H]-QNB	[^{3}H]-PZ	[^{3}H]-QNB	[^{3}H]-PZ
CONTROLS	664 ± 18	483 ± 28	833 ± 30	581 ± 29
ALZHEIMER	617 ± 29	498 ± 31	787 ± 37	597 ± 17
CONTROLS	651 ± 18	473 ± 21	848 ± 24	651 ± 20
PARKINSON	771 ± 21 *	576 ± 9 *	954 ± 16 *	753 ± 17 *

*) Significantly different from controls ($P < 0.05$, Mann-Whitney U test)

In AD no changes in densities of muscarinic receptors measured by specific binding of [^{3}H]-QNB and [^{3}H]-pirenzepine were observed in the frontal and temporal cortex. By contrast, Parkinsonian subjects had increased concentrations of both the total number of muscarinic receptors and postsynaptic M-1 receptors. Alterations of K_D values were not seen.

The results suggest that muscarinic receptor alterations found in the cortex in AD are not identical to those of PD. The increased cortical muscarinic receptor concentrations in PD may be a compensatory mechanism of the damaged innominato-cortical cholinergic system and may maintain normal cognitive function during the early stages of alterations of central cholinergic transmission (Lange et al., 1989).

Price, D.L., Whitehouse, P.J. & Struble, R.G. (1986) Trends Neurosci. 9, 29-33.
Lange, K.W., Wells, F.R., Jenner, P. & Marsden, C.D. (1989) J. Neurochem. 52, S38.

LOWER CORTICAL β-ADRENOCEPTOR BINDING SITES IN POST-MORTEM SAMPLES FROM DEPRESSED SUICIDE VICITMS

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Down-regulation of cortical β-adrenoceptor binding sites is the most consistent effect of antidepressant treatment in rats. Although a relationship with therapeutic benefit has not been established, significantly higher densities of frontal cortical β-adrenoceptors have been reported in suicide victims compared to controls (Mann et al, 1986; Biegon and Israeli, 1988).

In this study we have quantitated total and β_1-adrenoceptor binding in three cortical areas from suicides (15M, 7F; mean age ± SEM, 42 ± 3 years; post-mortem delay, 35 ± 4 h), in whom a firm retrospective diagnosis of depression was established and who were not receiving antidepressant drugs, and matched controls (15M, 5F; 43 ± 3 years; 40 ± 3h). Saturation binding of [^{3}H]CGP 12177 (6 concentrations, 0.03-1 nM) to well-washed membranes from frontal (Brodmann area 10), occipital (Brodmann area 17/18) and temporal cortex (Brodmann area 38) was performed as previously described (De Paermentier et al, 1989). Specific binding was defined with 200 μM (-)isoprenaline (total β) or 500 nM CGP 20712A (β_1).

Kd values did not differ significantly between controls (0.12-0.16 nM) and suicides (0.11-0.14 nM), whereas Bmax was lower in suicides, significantly for total β-adrenoceptors in occipital and temporal cortex (Table 1) and for β_1-adrenoceptors in occipital cortex (39 ± 2 v 46 ± 2 fmol/mg protein).

Table 1. Bmax of [^{3}H]CGP 12177 binding to total β-adrenoceptors

	Controls	Suicides
Frontal cortex	67 ± 2.5	60 ± 2.5
Occipital cortex	65 ± 3.2	56 ± 2.7*
Temporal cortex	72 ± 3.7	59 ± 2.4**

Means ± SEM, fmol/ mg protein, * $p<0.05$, ** $p<0.01$

Suicides who died violently (hanging, jumping or stab wound, n=12) had significantly lower numbers of frontal cortical β-adrenoceptors (55 ± 2.6 v 71 ± 3.9, $p< 0.01$) and β_1-adrenoceptors (39 ± 2.7 v 52 ± 3.5, $p< 0.01$) compared to matched controls whereas total β and β_1-adrenoceptor density did not differ significantly in occipital or temporal cortex. In contrast, suicides who died by non-violent means (drug overdose, carbon monoxide poisoning, n=10) had significantly lower numbers of β (55 ± 3.5 v 75 ± 5.4, $p<0.01$) and β_1-adrenoceptors (40 ± 3.8 v 52 ± 3.7, $p<0.05$) in temporal cortex and β-adrenoceptors in occipital cortex (49 ± 4.5 v 64 ± 5.1, $p<0.05$) than matched controls, whereas total and β_1-adrenoceptor density did not differ in frontal cortex. No significant relationship between β-adrenoceptor density and post-mortem delay was found within suicides,controls or when the groups were combined.

The present findings, in a moderately large group of depressed suicides who were not receiving antidepressant drugs, contrast markedly with previous reports of increased frontal cortical β-adrenoceptors in suicides (Mann et al, 1986; Biegon and Israeli, 1988).

Biegon, A. and Israeli, M. (1988) Brain Res 442, 199-203.
De Paermentier, F. et al (1989) BPS Bristol meeting, C16.
Mann, J.J. et al (1986) Arch Gen Psychiat 43, 954-959.

EXTRACELLULAR AMINO ACID LEVELS IN THE GLOBUS PALLIDUS OF THE CONSCIOUS PRIMATE STUDIED BY INTRACEREBRAL MICRODIALYSIS

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Intracerebral microdialysis is a novel technique which allows sampling of small molecular weight substances from the extracellular space in vivo. The probes are constructed of stainless steel and cellulose tubing (200um o.d.) which can be introduced into localized regions of the brain. Experiments with rodents indicate that amines and amino acids can be collected using this method, and that experimental manipulation of neurotransmitter release can be detected (Imperato and Di Chiara, 1984; Tossman et al., 1985).

Nuclei of the basal ganglia are intimately involved in the control of movement. In the primate the globus pallidus is divided into medial and lateral segments both of which receive inhibitory GABAergic afferents from the putamen. In addition the pallidal segments also receive an excitatory input (probably glutamatergic) from the subthalamic nucleus. We have developed an experimental protocol which allows us to dialyse the pallidum of the conscious monkey in order to measure amino acids levels in this area, with a view to detecting alterations in their levels in disorders of movement such as parkinsonism.

Dialysis probes were introduced into different sites within the lateral pallidum of a cynomolgus monkey through stereotaxically implanted indwelling cannulae. Artificial cerebrospinal fluid (aCSF) was continuously perfused at a rate of 2ul/min (Carnegie Medicin microinfusion pump). 20ul dialysate samples were thus collected every 10 minutes and were subsequently analyzed for amino acids by HPLC (precolumn derivatization with fluorimetric detection). Probes were tested in a standard solution of amino acids to estimate recovery (c. 10%). GABA and glutamate are routinely measured (1 - 10 pmol/20ul sample). A liquid switch has been employed to allow the instantaneous exchange of the normal perfusion medium of aCSF containing 3mM K^+ to one containing 100mM K^+ for a period of 10 minutes. Dialysates corresponding to these time periods have contained increased (up to 10 fold) levels of GABA, indicating evoked release of the amino acid. We have further manipulated the level of GABA detected in the dialysates by perfusing with aCSF containing 2mM nipecotic acid (specific GABA uptake blocker). In these experiments (n=2) there was a sustained increase (approximately 4 fold) in the GABA levels.

These findings suggest that amino acid levels within the pallidum of the monkey can be studied by microdialysis. They also provide a basis for future work in primate models of movement disorders where there may be altered activity of pathways of the basal ganglia utilizing these amino acids.

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Imperato, A. and Di Chiara, G. (1984) J. Neurosci. 4, 966-977
Tossman, U., Wieloch, T. and Ungerstedt, U. (1985) Neurosci. Letts. 62, 231-235

PRECLINICAL STUDIES ON L-671,152, A TOPICALLY EFFECTIVE OCULAR HYPOTENSIVE CARBONIC ANHYDRASE INHIBITOR

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Systemically administered carbonic anhydrase (CA) inhibitors have been used in the treatment of glaucoma and ocular hypertension for over thirty years. However, patient compliance is poor, due to unwanted side effects resulting from the extraocular inhibition of the enzyme, despite the fact that the drugs are very effective in lowering intraocular pressure (IOP). Previous efforts to obtain a topically active CA inhibitor have been unsuccessful and the quest for such an agent has constituted a tantalizing goal. The instillation of one drop of a 2% solution of MK-927 (R,S-5,6-dihydro-4-(2-methylpropylamino)-4H-thieno-(2,3b)-thiopyran-2-sulfonamide-7,7-dioxide HCl) lowers the IOP of experimental animals (Sugrue et al., 1988) and glaucoma patients (Hennekes et al., 1988). MK-417 is the S-enantiomer of MK-927 and is the more potent isomer in blocking CA II in vitro. This agent is also topically active in glaucoma patients (Diestelhorst et al., 1989; George et al., 1989).

L-671,152 (S,S-5,6-dihydro-4H-4-ethylamine-6-methylthieno-(2,3b)-thiopyran-2-sulfonamide-7,7-dioxide HCl) is closely related structurally to MK-927 and, like the latter, possesses good aqueous solubility. The IC_{50} of L-671,152 against CA II isolated from human red blood cells was 0.2 nM. The corresponding values for MK-417 and MK-927 were 0.54 nM and 1.23 nM. In an ex vivo model, a 1 hr pretreatment (one drop, 50 µl) with 0.02% and 0.1% solutions of L-671,152 blocked CA activity in a homogenate of albino rabbit iris + ciliary body by 87% and 100%, respectively; thus, indicating good ocular penetration.

The acute IOP lowering activities of L-671,152 and MK-927 were compared in two paradigms. In the first, ocular hypertension was induced in ketamine-sedated, cynomolgus monkeys by laser-induced photocoagulation of their trabecular meshwork (Lee et al., 1985). The IOP of these animals was maximally lowered by 8.6, 11.8 and 13.8 mm Hg after the instillation of 0.5%, 1% and 2% solutions of L-671,152, respectively. The decline of 13.8 mm Hg occurred at 5 hr after 2% dosing and this represented a reduction of 37% from the corresponding baseline value of 36.9 mm Hg. Peak falls in IOP were 4.7, 5.9 and 9.6 mm Hg after the instillation of 0.5%, 1% and 2% solutions of MK-927. The duration of action of L-671,152 was longer than that of MK-927. In another study, 2% solutions of L-671,152 and MK-417 were compared at 4 and 8 hr. The declines in IOP were 12.6 and 8.2 mm Hg after 2% L-671,152. The corresponding values for 2% MK-417 were 9.8 and 4.7 mm Hg. The second model used in comparative studies was pigmented rabbits whose IOP was inherently elevated. The instillation of 0.25%, 0.5%, 1% and 2% solutions of L-671,152 maximally lowered IOP by 4.1, 4.8, 4.8 and 6.1 mm Hg, respectively. Corresponding reductions were 2.3, 4.3 and 4.8 mm Hg after 0.5%, 1% and 2% MK-927. The instillation of 0.01%, 0.1% and 0.5% solutions of L-671,152 maximally reduced the elevated IOP of alpha-chymotrypsinized rabbits by 3.1, 6.1 and 9.8 mm Hg, respectively. The unilateral instillation of 0.5% L-671,152 into the left eye had no effect on the elevated IOP of the right eye; thus, indicating a local site of action. A 2% solution of L-671,152 was well tolerated by the eyes of rabbits and monkeys, and no deleterious side effects were observed.

These preclinical findings indicate that L-671,152 is a water-soluble CA inhibitor which, on topical administration, lowers IOP by virtue of an action confined to within the eye and that the agent was more potent than MK-417 and MK-927.

Diestelhorst, M. et al. (1989) Invest. Ophthalmol. Vis. Sci. 30, (Suppl.) 23.
George, J.-L. et al. (1989) Invest. Ophthalmol. Vis. Sci. 30, (Suppl.) 98.
Hennekes, R. et al. (1988) Invest. Ophthalmol. Vis. Sci. 29, (Suppl.) 82.
Lee, P.-Y. et al. (1985) Curr. Eye Res. 4, 775-781.
Sugrue, M.F. et al. (1988) Invest. Ophthalmol. Vis. Sci. 29, (Suppl.) 81.

THE EFFECT OF CROMAKALIM ON KIDNEY FUNCTION IN THE ANAESTHETISED RAT

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Many of the recently developed blood pressure lowering drugs, eg the angiotensin converting enzyme inhibitors and calcium channel blockers which cause dilation of vascular smooth muscle, have been found to be natriuretic and diuretic. The possibility existed that the antihypertensive action of cromakalim (Clapham & Buckingham, 1988) was associated with increased fluid excretion. This investigation examined the effect of cromakalim on blood pressure and renal haemodynamic and tubular function in the rat.

Male Sprague-Dawley rats (300-400 g) were anaesthetised with sodium pentobarbitone and prepared for renal function measurements (Johns, 1988). Cannulae were placed in the carotid artery, to measure blood pressure and removal of blood samples, and the jugular vein, to infuse saline (6 ml/h) and drugs. The left kidney was exposed abdominally, a flow probe placed on the artery and its ureter cannulated and the experiments begun 2 h later. The protocol consisted of a sequence of 15 min clearance periods: 2 baseline clearances, after which a bolus dose of cromakalim (25 µg/kg) was given iv; 15 min later two further clearances were taken; this sequence was repeated to give cumulative doses of 50, 100 and 150 µg/kg. Parallel studies were undertaken in which bolus doses of vehicle (saline) were given.

	BP	RBF	GFR	UV	$U_{Na}V$	FE_{Na}
Control (n=6)	128±4	21.8±1.9	3.02±0.35	81.7±14.4	19.2±3.6	4.4±0.73
25µg/kg (n=6)	126±4	20.8±1.9	2.92±0.20	87.6±16.7	19.0±3.0	4.4±0.63
50µg/kg (n=6)	118±4[1]	19.54±2.6	2.58±0.15	72.6±21.1	14.6±3.7	3.91±0.95
100µg/kg (n=6)	114±5[2]	19.2±2.1	2.49±0.14	57.8±14.3	11.3±2.3	3.31±0.93
150µg/kg (n=6)	105±4[2]	17.6±2.2	2.44±0.21	58.0±14.4	11.2±2.4	3.41±0.97

BP = blood pressure, mmHg; RBF = renal blood flow, ml/min/kg; GFR = glomerular filtration rate, ml/min/kg; UV = urine flow, µl/min/kg; $U_{Na}V$ = absolute sodium excretion, µmol/kg/min; FE_{Na} = fractional sodium excretion, %. [1] = $p<0.05$; [2] = $p<0.01$, control compared to cromakalim (Student's 't' test).

There was a dose related reduction in blood pressure, which was associated with gradual, but not significant, declines in renal blood flow and glomerular filtration rate. Urine flow, absolute and fractional sodium excretion fell but did not reach statistical significance even at the highest dose of drug. In the time control group of rats, blood pressure remained unchanged while there were small falls in renal blood flow and glomerular filtration rate and increases in urine flow, absolute and fractional sodium excretions which became significant ($p<0.05$) at the time equivalent to the final dose of drug.

Administration of cromakalim at doses which reduced blood pressure had only marginal effects on renal haemodynamics comparable to those observed in the time control rats. The gradual fall in fluid output with increasing doses of cromakalim was probably related to the reduced perfusion pressure rather than any direct action on the tubular reabsorptive processes.

Johns, E.J. (1988) Br. J. Pharmac. 93 561-568.
Clapham, J.C. & Buckingham, R.E. (1988) J. Cardiovasc. Pharmac. 12 555-561.

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THE NATRIURETIC AND DIURETIC EFFICACY OF ATRIOPEPTIDASE INHIBITORS FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION

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The intravenous administration of atrial natriuretic factor (ANF) evokes natriuretic and diuretic responses in animals and man. We have recently reported that the selective atriopeptidase (EC 3.4.24.11) inhibitor, UK-69,578, prolongs the half-life of ANF and evokes natriuretic and diuretic responses. (Samuels et al 1989). To investigate the effects of atriopeptidase inhibitors in conditions of raised endogenous levels of ANF we have developed a model in which i.v. administration of 600 µl saline to mice (male, CD1) evokes a natriuretic and diuretic response. This renal response was accompanied by a transient rise in plasma ANF (as measured by RIA) from 78 ± 21 to 160 ± 25 pg/ml 5 mins after administration (n=6). The natriuretic and diuretic response was reduced by 56% and 50% respectively by prior administration of antiserum to ANF (n=6). These findings are consistent with a role for ANF in the response evoked by saline loading.

Following 600 µl of saline UK-69,578 (0.3 - 10 mg/kg i.v.) evoked a dose related increase in both natriuresis and diuresis (increases of 58 ± 10% and 47 ± 8% at 0.3 mg/kg, and 98 ± 10% and 70 ± 8% at 10 mg/kg respectively, $n \geq 14$). A comparison was made of the effect of volume loading on the responses to hydrochlorothiazide (HCTZ 0.3 mg/kg i.v.) and UK-69,578 (3 mg/kg i.v.). The data are presented in the table.

Effect of UK-69,578 and HCTZ in the Conscious mouse ($n \geq 12$)

	Volume Load (µl)	Urine Volume (µl)	Urine Sodium (µEq)
	600	136 ± 11	10.8 ± 1.1
	50	48 ± 5	3.3 ± 0.4
	0	58 ± 6	4.3 ± 0.4
HCTZ	+600	217 ± 18	28.9 ± 2.6
HCTZ	+50	104 ± 11	14.2 ± 1.5
UK-69,578	+600	249 ± 15	21.8 ± 1.5
UK-69,578	+50	68 ± 4	4.8 ± 0.4

Following either 50 µl or 600 µl of saline, HCTZ evoked a marked increase in both parameters ($p<0.05$ t-test). UK-69,578, however, evoked a marked increase only following 600 µl ($p<0.05$, t-test). UK-69,578 administered with 50 µl of saline evoked a small but statistically significant increase in both parameters compared to animals receiving 50 µl of saline alone, but had no effect compared to animals without treatment or saline. The effect of UK-69,578 on plasma ANF levels has been evaluated in anaesthetised mice. Administration of 600 µl of saline evoked a transient rise in plasma ANF of 120% within 5 min (41.8 ± 6 → 91.4 ± 9 pg/ml) which returned to baseline values within 10 minutes. In contrast, following UK-69,578 (3 mg/kg) plasma ANF rose by 154% at 5 min but remained elevated for the duration of the experiment (30 min) when levels were elevated by 191%.

UK-69,578 has no significant effects following oral administration to volume loaded (600 µl saline) mice (22% increase in natriuresis following 30 mg/kg) due to poor absorption. UK-69,578 is a mixture of 2 enantiomers and the indanyl ester of its most active enantiomer (Barclay et al this meeting) is UK-79,300. This compound is an effective pro-drug and on oral administration to volume loaded mice 3 mg/kg evokes an increase in both the diuretic (84%) and natriuretic (71%) responses.

In conclusion, UK-69,578 evokes a natriuretic and diuretic response consistent with a potentiation of the effects of endogenous ANF and the prodrug (UK-79,300) effectively delivers the active moiety by the oral route.

Barclay, P.L. et al (1989) This meeting.

THE EFFECTS OF UK-79,300, AN ORALLY-ABSORBED ATRIOPEPTIDASE INHIBITOR, IN A CONSCIOUS DOG MODEL OF CARDIAC INSUFFICIENCY

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UK-79,300 is the 5-indanyl ester of the (+)-enantiomer of UK-69,578 (3{1-[(cis-4-carboxycyclohexyl)carbamoyl]cyclopentyl}-2-(2-methoxyethoxymethyl)propanoic acid), a potent, selective atriopeptidase (EC 3.4.24.11) inhibitor (Samuels *et al*, 1989). The effects of UK-79,300, as an orally-absorbed prodrug of UK-69,578, were examined in 6 conscious dogs with elevated plasma ANF due to atrioventricular (AV) heart block. Intravenous UK-69,578 has been previously shown to produce natriuresis and diuresis in this model of cardiac insufficiency (Alabaster *et al*, 1989) and in heart failure patients (Northridge *et al*, 1989).

Dogs with chronic surgically-induced AV block were used in which heart rate was controlled at 30 (n=2) or 40 (n=4) beats/min by an implanted pacemaker. Urine was collected for 4h after the administration of UK-79,300 p.o., UK-69,578 i.v. or vehicle p.o. for measurement of urine volume (UV), sodium (UNaV), potassium (UKV) and ANF excretion (UANFV). Blood samples (0h and +2h) were assayed for plasma renin activity (PRA) and plasma aldosterone concentration (PAC).

Table 1 Urinary effects of vehicle, UK-79,300 and UK-69,578.

Treatment (mg/kg)		UV (ml/4h)	UNaV (mEq/4h)	UKV (mEq/4h)	UANFV (pg/4h)
Vehicle	p.o.	39 ± 8	5.1 ± 1.1	2.7 ± 0.6	1659 ± 526
UK-79,300 0.5	p.o.	60 ± 16	8.7 ± 1.7 *	3.3 ± 0.7	1223 + 99
UK-79,300 2.0	p.o.	58 ± 18 *	8.1 ± 2.0 *	3.0 ± 0.8	1615 ± 428
UK-79,300 8.0	p.o.	93 ± 22 *	11.3 ± 2.3 *	3.2 ± 0.8	4639 ± 785 *
UK-69,578 3.0	i.v.	82 ± 16 *	13.2 ± 1.4 *	4.0 ± 0.8 *	6659 ± 1684 *

Data are mean ± s.e.mean. Asterisks indicate $p < 0.05$, Student's t test, following adjustment of means for differences in baseline using analysis of covariance suitable for the randomised block design.

UK-79,300 produced natriuretic and diuretic effects without kaliuresis (Table 1) which were apparent by 1h and maximal at 3.5h after dose. UK-69,578 i.v., at the optimally-effective dose from a previous study (Alabaster *et al*, 1989), produced a similar degree of natriuresis to UK-79,300 8 mg/kg but the onset was faster with a slight kaliuresis. Both UK-79,300 8 mg/kg and UK-69,578 increased UANFV. PAC was significantly reduced (analysis of variance, $p < 0.05$) by both UK-79,300 and UK-69,578 from a pretreatment value of 37 ± 9 pg/ml. PRA was generally unchanged (pretreatment, 0.72 ± 0.15 ngAI/ml/h).

Thus, UK-79,300 is an orally-active natriuretic and diuretic agent which does not activate the renin-angiotensin system but increases UANFV. These actions are consistent with an ability to inhibit ANF breakdown *in vivo* . This unique profile may provide useful therapy in hypertension and heart failure.

Alabaster, C.T. *et al.* (1989) J. Am. Coll. Cardiol. 13(2), 75A
Northridge, D.B. *et al.* (1989) J. Am. Coll. Cardiol. 13(2), 76A
Samuels, G.M.R.S. *et al.* (1989) J. Am. Coll. Cardiol. 13(2), 75A

THE NATRIURETIC AND DIURETIC RESPONSES TO THE ATRIOPEPTIDASE INHIBITOR UK-69,578 IN VOLUME EXPANDED RATS

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ANF is a 28 amino acid peptide which, on intravenous administration, evokes natriuretic and diuretic responses in animals and man. We have recently shown that the atriopeptidase inhibitor UK-69,578 (UK) both elevates circulating ANF and is natriuretic and diuretic in animal models (Samuels, et al, 1989). UK is a potent competitive inhibitor of EC 3.4.24.11 (atriopeptidase) isolated from rat kidney, with a Ki of 36 nM. At concentrations as high as 10^{-4}M, UK is without effect on carboxypeptidase A, leucine aminopeptidase, angiotensin converting enzyme renin, trypsin and chymotrypsin. UK is a racemate, and resolved (+) and (-) enantiomers show a 30 fold difference in enzyme affinity (Ki's of 14 nM and 470 nM respectively).

The purpose of this present study was to investigate the natriuretic and diuretic responses to UK and its resolved enantiomers in volume expanded anaesthetised rats. Anaesthetised rats (Male, Sprague Dawley) received an i.v. infusion of 20 mls 0.9% saline over 1 h. Ten minute urine collections were made throughout, and analysed for volume and electrolytes. Prior to the start of the infusion, animals ($n \geq 5$ per group) received either vehicle or treatment.

Volume expansion in animals receiving vehicle alone resulted in natriuretic and diuretic responses of 0.18 ± 0.03 mEq and 0.86 ± 0.16 ml over the hour of the experiment. Administration of UK (10 mg/kg i.v.) evoked a natriuresis (+172%) and diuresis (+121%) significantly higher ($p<0.05$, Student's independent t test) than that observed in vehicle controls. This response was similar in magnitude to that produced by 3 mg/kg i.v. hydrochlorthiazide (HCTZ, natriuresis +222%, diuresis +208%, $p<0.05$ compared to vehicle).

In a separate experiment, a specific ANF antiserum (Gutkowska et al, 1984) was used to investigate the ANF dependence of the natriuretic response to UK in volume expanded rats. ANF antiserum reduced the natriuretic response to volume expansion by approximately 50% in comparison with animals treated with normal rabbit serum (NRS, see Table). UK significantly enhanced the natriuresis evoked by volume expansion, and this enhanced response could be completely ablated by prior treatment with ANF antiserum, demonstrating the ANF dependence of this response. In contrast, an equinatriuretic dose of HCTZ was unaffected by antiserum.

Effect of ANF antiserum on the natriuretic response to UK in volume expanded rats

	Control(NRS) Natriuresis (mEq)	ANF antiserum Natriuresis (mEq)
Vehicle	0.4 ± 0.1	0.2 ± 0.1
UK (10 mg/kg i.v.)	0.8 ± 0.2*	0.2 ± 0.1+
HCTZ (3 mg/kg i.v.)	0.8 ± 0.1*	0.7 ± 0.1

* Significantly different from vehicle control ($p<0.05$)
\+ " " " NRS ($p<0.05$)

Finally the (+) and (-) enantiomers of UK were evaluated in order to establish that the natriuretic effects of UK correlated with enzyme inhibitory activity. A dose of 3 mg/kg i.v. (+) enantiomer enhanced the natriuretic (+131%) and diuretic (+124%) responses to volume expansion. In contrast, this dose of the (-) enantiomer was without effect, demonstrating that greater natriuretic activity resided in the more potent enzyme inhibitor. Thus, in conclusion specific inhibition of EC 3.4.24.11 by the atriopeptidase inhibitor UK-69,578 enhances natriuretic and diuretic responses in volume expanded rats by a mechanism which is dependent on ANF.

Gutkowska J. et al. (1984) Biochem. Biophys. Res. Commun. 122, 593.
Samuels G.M.R. et al. (1989) J. Am. Coll. Cardiol. 13, 75A.

ELECTRON TRANSPORT AND PROTECTION OF LIVER SLICES IN THE LATE STAGE OF PARACETAMOL INJURY OF THE LIVER

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Paracetamol injury of the liver is characterized by a phase of contact during which paracetamol is metabolised to reactive materials, and metabolites bind to cell macromolecules. This phase is followed many hours later by cell death. In the intervening time liver cells appear essentially normal but are clearly on the path to destruction. We have investigated this second phase of injury using electron acceptors (Devalia et al 1982, McLean & Nuttall 1978).

Liver slices were prepared from rats pretreated with phenobarbital. The tissues were incubated with paracetamol (10mM) in Ringer solution for 2 hours, after which time the medium was replaced by a solution without paracetamol, but containing electron acceptors or donors such as dichlorophenol-indophenol (DCPIP 10μM), phenazinemethosulphate (PMS 20μM) or ethanol (2mM). After 4 hours incubation in the second medium, cell injury was assessed by measurement of leakage of enzymes (expressed as % of total enzyme content of the slice) and by measurement of potassium content of the slice.

Cell Injury in Liver Slices in the 4 hours following 2 hours of exposure to 10mM Paracetamol

Incubation Medium 0-2h	2-6h	LDH leakage % of total in slice	(K^+) content μM/g dry weight
1. Ringer	Ringer	8.5 ± 2.1	153 ± 30
2. + paracetamol	Ringer	36.0 ± 6.1	63 ± 5
3. + paracetamol	+ DCPIP (10mM)	12.0 ± 3.0	134 ± 10
4. + paracetamol	+ DPPD (4μM)	4.0 ± 1.0	160 ± 5
5. + paracetamol	+ PMS (20μM)	75.0 ± 7.0	38 ± 4
6. + paracetamol	+ ethanol (2mM)	12.0 ± 1.0	160 ± 6
7. + paracetamol	+ ethanol (2mM) + pyruvate (20mM)	32.2 ± 4.0	78 ± 9 [mean ±SD]

These results show that one of the electron acceptors (DCPIP) is able to afford powerful protection against liver injury. In contrast PMS causes an increase in injury. The ability of ethanol to protect the slices and the reversal by pyruvate also suggests that there is a link between protection and the NAD/NADH ratio.

Protection against the late phase of paracetamol injury has been found with antioxidant radical scavangers such as DPPD (paraphenylene diamine), the flavone catechin, and powerful reducing agents such as dithiothreitol, and some prostaglandin analogues (Tee et al 1986). Present experiments suggest that it is possible to divert electrons into a pathway not normally available to the cell and so prevent the injury process. This could be related to reduction of sites oxidised by the quinone metabolite of paracetamol.

Devalia, J.L., Gilvie, R.C. & McLean A.E.M. (1982) Biochem Pharmacol 31: 3745-3749.

McLean, A.E.M. & Nuttall L. (1978) Biochem Pharmacol 27: 425-430.

Tee, L.B.G., Boobis A.K., Huggett, A.E. & Davies, D.S. (1986) Toxicol App Pharmacol 83: 294-315.

THE EFFECTS OF L-VESAMICOL ON TRANSMITTER RELEASE FROM RAT MOTOR NERVE TERMINALS AT HIGH FREQUENCIES OF NERVE STIMULATION

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l-Vesamicol (2-(4-phenyl piperidino) cyclohexanol, AH5183) blocks neurotransmission at the skeletal neuromuscular junction through an inhibition of the process by which acetylcholine is actively transported into synaptic vesicles (for review see Marshall & Parsons, 1987). In order to determine the role of vesicular bound acetylcholine in transmitter mobilization and release at high levels of transmitter output we have studied the effect of l-vesamicol on the rundown of end-plate current (EPC) amplitudes at a high frequency of nerve stimulation.

Trains of EPCs elicited at 50Hz for 1 second were recorded at -50mV from the cut rat hemidiaphragm muscle at 32°C using a conventional two-electrode voltage clamp procedure described previously (Gibb & Marshall, 1984). Trains of EPCs were separated by a 20 second period of rest and 2 - 4 separate trains were averaged in each cell to give a clear picture of EPC amplitude rundown. Percentage rundown was calculated over the last ten EPCs in the train. Because the effects of vesamicol on transmitter release are known to be dependent on the frequency of nerve stimulation (Marshall, 1970), EPC rundown in the presence of vesamicol was measured both in the absence of prior nerve stimulation (for at least 20 minutes) and immediately following a 5 minute period of continuous 2 Hz nerve stimulation. To ensure that any post-stimulation effect was not due to the period of nerve stimulation alone, the effect of 5 min of 2Hz nerve stimulation on control trains was also examined.

For controls, the amplitude of the first EPC in the train (EPC_1) was 83 ± 20 nA (n=7) and the rundown was 29.2 ± 3.5% (n=7). These values were not affected by 5 min of 2Hz nerve stimulation in the absence of l-vesamicol: EPC_1, 95 ± 23 nA (n=5); rundown, 29.0 ± 2.1% (n=5). Following the application of 1 μM l-vesamicol, in the absence of prior nerve stimulation, no change was seen in EPC_1: 77 ± 26 nA (n=8). However, the rundown in EPC amplitude was 43.8 ± 2.5% (n=8) which was significantly ($P<0.02$, two-tailed Mann-Whitney U-test) greater than the control rundown. Following a 5 minute period of 2 Hz nerve stimulation rundown was further increased (59.8 ± 4.4% (n=6), $P<0.05$ versus 1 μM l-vesamicol with no stimulation) and there was also a 20% depression in EPC_1 (79.1 ± 3.3% (n=5)). Under all conditions EPC amplitude reached a plateau level within the first 10 - 15 stimuli. None of the above effects of l-vesamicol could be attributed to blockade of end-plate ion channels since the compound had no effect on the decay time constant of EPCs at any point in the high frequency trains.

It is clear that vesamicol has two, possibly unrelated, effects on EPC rundown. The stimulation-dependent component probably reflects a reduction in the number of vesicles available for release following prolonged transmitter release in the absence of vesicle refilling. At present, the nature of the stimulation-independent increase in train rundown is not clear, but its is clearly not due to any post-synaptic end-plate ion channel block.

Marshall, I.G. & Parsons, S.M. (1987). Trends in Neuroscience. 10, 174-177.
Gibb, A.J. & Marshall, I.G. (1984). J. Physiol. 351, 275-297
Marshall, I.G. (1970). Brit. J. Pharmacol. 38, 503-516.

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BRADYKININ ACTIVATION OF PRIMARY AFFERENT TERMINALS IN THE NEONATE RAT SPINAL CORD *IN VITRO*

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The nonapeptide bradykinin (BK) can stimulate sensory neurones through the activation of protein kinase C (PKC) (Dray et al., 1988a). BK when applied to the spinal cord of neonatal rats also produces depolarization of the ventral root (Dray et al., 1988b). However since BK binding sites are located on primary afferents, and in the dorsal horn of the spinal cord (Steranka et al., 1988), the response recorded from the ventral root may be secondary to depolarization of the primary afferent terminals. Experiments have been carried out to examine this possibility, and to see whether these responses are mediated via the activation of PKC

Experiments were carried out on the lumbar sections of hemisected spinal cords from 1 day old rat pups. The section of cord was placed in one compartment of a two chamber bath, and perfused with Hepes buffered physiological salt solution at room temperature (22 - 24°C). The peripheral end of a single dorsal or ventral root was pulled through a grease- sealed partition between the two compartments, and DC potential changes were recorded from the spinal cord with respect to either the dorsal or ventral root, by recording the potential between the two chambers. These potential changes are thought to reflect changes in the membrane potential of motor neurones, and the terminal region of the primary afferent fibres respectively.

Application of BK to the spinal cord evoked concentration dependent depolarization recorded via the ventral root. This response was abolished in Ca^{2+}free solution, and was reduced by the Substance P antagonist Spantide (5μM). BK also evoked a concentration-dependent depolarization of the cord with respect to the dorsal root, with an EC_{50} of 30nM. This response to BK was unaffected by removal of Ca^{2+} from the bathing solution. Capsaicin selectively depolarizes sensory C fibres (Marsh et al., 1987). When capsaicin (10μM) was applied to the cord, a much larger depolarization was recorded via the dorsal root. When BK was applied during a prolonged application of capsaicin, the response was greatly attenuated, suggesting that BK and capsaicin were acting to depolarize the same population of fibres.. The depolarization to BK recorded via the dorsal root was antagonised by D-Arg[Hyp^3,Thi^{5,8},D-Phe^7]-BK with a pA_2 of 6.8±0.6 (n=6), but not by the selective B_1 antagonist Leu^8[des-Arg^9]-BK. The selective B_1 agonist [des-Arg^9]-BK at concentrations up to 3μM failed to produce any depolarization.

Phorbol dibutyrate (1μM) also evoked a depolarization of the cord with respect to the dorsal root. This response was greatly enhanced by removal of Ca^{2+} from the bathing solution, and was greatly reduced by the PKC inhibitor staurosporine (200nM). In contrast, the response to BK was not affected by removal of Ca^{2+}, and was not reduced by staurosporine.

These results indicate that BK, acting on B_2 receptors, can depolarize primary afferent terminals in the spinal cord. This results in the release of neurotransmitters which subsequently gives rise to the depolarization recorded from the ventral root. Although the action of BK on the dorsal root can be mimicked by phorbol dibutyrate, the results are not consistent with the response to BK being mediated through the activation of PKC.

Dray, A., Bettaney, J., Forster, P. & Perkins, M.N., (1988a) Neurosci. Lett., 91, 301-307
Dray, A., Bettaney, J., Forster, P. & Perkins, M.N., (1988b) Br. J. Pharmacol., 95, 1008-1010
Marsh, S.J., Stanfield, C.E., Brown, D.A., Davey, R. & McCarthey, D., (1987) Neuroscience, 23, 275-289
Steranka, L.R., Manning, D.C., DeHaas, C.J., Ferkany, J.W., Borosky, S.A., Connor, J.R., Vavrek, R.J., Stewart, J.M., & Snyder,S.H.,(1988) Proc. Natl. Acad. Sci. 85, 3245-3249

CHANGES IN SPINAL CORD NEUROCHEMICALS FOLLOWING REPEATED INTRATHECAL GALANIN INJECTION IN THE RAT

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Immunohistochemical evidence suggests that the 29 amino acid peptide galanin is present in primary sensory nerve terminals and interneurones in the dorsal horn and also co-exists with 5-hydroxytryptamine (5-HT) in medullary raphe neurones which project to motoneurones in the ventral horn of the spinal cord (Skofitsch & Jacobowitz, 1985). The functional role of galanin in these subsets of spinal neurones and the effects that the peptide has on the co-existent neurotransmitters are, however, unknown and are the subject of the present study.

Indwelling intrathecal cannulae (7.5 cm x 0.5 mm O.D.) were implanted along the spinal subarachnoid space of male Wistar rats (360-390 g) under sodium methohexitone anaesthesia (60 mg kg-1, i.p.). Following a seven day recovery period rats received intrathecal injections (10 µl with a 15 µl saline wash-in) of either saline (n=7) or galanin (1 µg, n=8) twice daily (between 08.30 - 09.30 and 17.00 and 18.30 h) for five days. Rats were decapitated after the ninth intrathecal injection and the thoraco-lumbar spinal cord (divided into dorsal and ventral portions) was removed and stored at -80°C. Blood was collected in non-heparinized vials which were centrifuged (2300 g for 5 min) prior to determining plasma glucose (Clandon, model 23AM glucose analyser) and total free insulin (radioimmunoassay kit) levels. Tissue levels of thyrotrophin-releasing hormone (TRH)-, substance P-, calcitonin gene-related peptide (CGRP)-like immunoreactivity were measured using well established sensitive radioimmunoassays, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) by HPLC with electrochemical detection and choline acetyltransferase (ChAT) activity was determined using a radioenzymatic assay, all previously described in detail (Fone et al., 1988). Results are expressed as mean $\pm$ s.e.mean. and Student's t-test was used for statistical analysis.

Five days repeated intrathecal galanin injection significantly elevated ChAT activity (0.296 $\pm$ 0.027 compared with 0.206 $\pm$ 0.017 µmol^{-1} h^{-1} g^{-1} wet weight in saline treated controls, $P<0.02$), substance P-LI (6.74 $\pm$ 0.67 compared with 4.60 $\pm$ 0.72 ng mg^{-1} protein, $P<0.05$) and 5-HT (3.95 $\pm$ 0.40 and 2.81 $\pm$ 0.29 ng mg^{-1} protein, $P<0.05$) levels in the dorsal, and not the ventral, portion of the spinal cord. In contrast, TRH- and CGRP-LI and 5-HIAA levels were unaltered in either spinal cord region by galanin treatment. Chronic galanin injection did not alter plasma glucose or insulin levels from values in saline treated controls (being 6.3 $\pm$ 0.5 mmol l-1 and 44 $\pm$ 4 mU l-1, respectively.

The absence of any change in plasma glucose or insulin levels following intrathecal galanin suggest that the peptide did not reach the systemic circulation. The present findings are consistent with the proposal that galanin may decrease the release of substance P from primary afferent nerve terminals (Guiliani et al., 1989), without affecting CGRP. Intrathecal galanin also selectively reduced 5-HT turnover in dorsal horn nerve terminals in a similar manner to that already described in raphe neurones projecting to limbic and neocortical regions (Fuxe et al., 1988). Taken together with the lack of effect of galanin on ventral horn neurochemical levels, the present data suggest that galanin is more important in regulating spinal sensory than motor function.

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Fone K.C.F., Bennett G.W. & Marsden C.A. (1988) Brain Res. 460, 22-28.
Fuxe K., Ogren S.O., Jansson A., Cintra A., Harfstrand A. & Agnati L.F. (1988) Acta. Physiol. Scand. 133, 579-581.
Guiliani S., Amann R., Papini A.M., Maggi C.A. & Meli A. (1989) Eur. J. Pharmac. 163, 91-96.
Skofitsch G. & Jacobowitz D.M. (1985) Peptides 6, 509-546.

INTRATHECAL SUBSTANCE P AND GALANIN ATTENUATE TRH ANALOGUE-INDUCED WET-DOG SHAKE BEHAVIOUR

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Intrathecal administration of either 5-HT agonists or thyrotrophin-releasing hormone (TRH) analogues produce well-defined motor behaviours (Fone et al., 1989) such as back muscle contractions (BMC, 5-HT agonists) and wet-dog shakes (WDS, 5-HT and TRH). Immunohistochemical evidence shows that bulbospinal raphe neurones which project to ventral horn motoneurones contain TRH-, substance P- and galanin-like immunoreactivity, in addition to 5-HT (Melander et al., 1986). In order to investigate the possible functional interaction between these co-existent neurotransmitters, in the present study we examined the effect of intrathecal galanin and substance P on the motor behaviours produced by the non-selective 5-HT agonist 5-methoxy-N,N'-dimethyltryptamine (5MeODMT) and the stable TRH analogue RX 77368 (pGln-His-3,3'-dimethyl-Pro-NH_2).

Male Wistar rats (250-300g) were anaesthetized with sodium methohexitone (60 mg kg^{-1}, i.p.) to implant an intrathecal cannula along the spinal subarachnoid space such that the caudal tip was at the thoraco-lumbar junction of the cord. Animals were allowed 7 days to recover from surgery prior to receiving intrathecal injections of 5MeODMT (15 µg) or RX 77368 (50 ng) given either; alone, following pretreatment (-30 min) or co-administered with a) galanin (0.1 µg, n=6) or b) substance P (10 ng, n=5). A third group of rats were given galanin alone (0.1 - 10 µg, n=6). The rats were then placed in a sound proofed chamber to which they had previously been habituated the total number of BMC and WDS were independently counted for 30 min. Results are expressed as mean ± s.e.mean and ANOVA was used for statistical analysis.

Neither intrathecal galanin (0.1-10 µg) nor substance P (10 ng) produced any discernable behaviour when administered alone, neither did these neuropeptides have any effect on the 5MeODMT-induced BMC. In contrast, both pretreatment (19 ± 9 WDS in 30 min) and co-administration (31 ± 13) of galanin (0.1 µg) significantly ($P<0.01$) reduced the number of WDS induced by RX 77368 (88 ± 12). Similarly RX 77368-induced WDS (105 ± 18 WDS in 30min) were significantly reduced by substance P given either as a pretreatment (10 ± 3, $P<0.001$) or in combination with (52 ± 11, $P<0.01$) RX 77368.

As substance P and galanin did not alter the 5-HT agonist-induced BMC, which has previously been demonstrated to be a spinally-mediated motor behaviour (Fone et al., 1989), the present study suggests that neither of these neuropeptides functionally interact with 5-HT in ventral horn nerve terminals. In contrast, the WDS induced by intrathecal TRH analogue injection, which do not appear to involve bulbospinal raphe neurones (Fone et al., 1989) were attenuated by both substance P and galanin. This suggests that non-spinal sites or other neurones such as primary afferents, which contain high levels of substance P and galanin (Ju et al., 1987), may be involved in modulating the TRH analogue-induced WDS behaviour.

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Fone K.C.F., Johnson J.V., Bennett G.W & Marsden C.A. (1989) Br. J. Pharmacol. 96, 599-608.
Ju G., Hokfelt T., Brodin E., Fahrenkrug J., Fischer J.A., Frey P., Elde R.P. & Brown J.C. (1987) Cell Tissue Res. 247, 417-427.
Melander T., Hokfelt T., Rokaeus A., Cuello A.C., Oertel W.H., Verhofstad A. & Goldstein M. (1986) J. Neurosci. 6, 3640-3654.

ACTIONS OF SULFONYLUREAS ON NEURONES OF RAT VENTROMEDIAL HYPOTHALAMUS *IN VITRO*

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Sulfonylureas are a class of drugs which stimulate insulin secretion in pancreatic B-cells (Ashcroft, 1988), where they have been shown to act directly on the ATP-sensitive potassium channel (Sturgess et al. , 1985). Characterization of a [^{3}H] glibenclamide binding site in rat brain with a dissociation constant (kd) of 0.7nM has been reported (Bernardi et al. , 1988) and, recently, we have demonstrated that closure of ATP-sensitive potassium channels underlies glucose-induced excitability of a class of neurones in the rat ventromedial hypothalamic nucleus(VMH)(Ashford et al. , 1989). The purpose of the present study was to examine the effects of the sulfonylureas tolbutamide and glibenclamide on recordings made from VMH neurones in vitro.

Intracellular recordings were made from neurones contained within slices of rat brain containing VMH using conventional techniques (Boden & Hill, 1988). Sulfonylureas were applied dissolved directly in artificial cerebrospinal fluid (ACSF). Neurones were hyperpolarised by substitution of 10mM sucrose for D-glucose in the perfusing ACSF. Tolbutamide (100uM) depolarised all such neurones tested (6.2+/-0.4mV, mean +/-sem, n=8) with an increase in input resistance. Glibenclamide (10-500nM) had no effect on the resting membrane parameters of VMH neurones (n=5). In four experiments we tested the effect of 100uM tolbutamide on glucose-sensitive neurones before and after exposure of the slice to glibenclamide (100nM). In all cases the response to tolbutamide was completely abolished by a one minute application of glibenclamide. No effect of the sulfonylureas could be detected in hypothalamic neurones which were insensitive to changing glucose concentrations (n=4).

These results suggest that tolbutamide acts on glucose-sensitive VMH neurones, presumably by blocking ATP-sensitive potassium channels. Glibenclamide, at concentrations up to 500x the reported kd for binding to rat brain membranes had no effect on VMH neuronal properties, but blocked the response to tolbutamide.

Ashcroft, F. A. (1988). Ann. Rev. Neurosci. ,11, 97-118
Ashford, M.L.J. , Boden, P. & Treherne, J. M. (1989). J. Physiol. (in press).
Bernardi,H. , Fosset,M. & Lazdunski, M.(1988). Proc. Nat. Acad. Sci. , 85, 9816-9820.
Boden, P. & Hill, R. G. (1988). Br. J. Pharmacol.,94, 246-252.
Sturgess, N. C. , Ashford, M. L. J. , Cook, D. L. & Hales, N. (1985). Lancet, 2, 474-475.

PRAVADOLINE AND AMINOALKYLINDOLE (AAI) ANALOGUES: ACTIONS WHICH SUGGEST A RECEPTOR INTERACTION

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Previous studies have demonstrated that pravadoline and aminoalkylindole (AAI) analogs inhibit electrically stimulated contractions of the mouse vas deferens preparation (MVD), and inhibit adenylate cyclase (AC) in rat cerebellar membranes with potencies which parallel antinociceptive potency. (Ward et al., 1988; Pacheco et al., 1988).

The present studies were designed to better define the neurochemical consequences of AAI interactions. The data suggest that pravadoline and structurally related AAIs may interact with a G-protein coupled receptor to produce their effects. Mouse vasa deferentia preparations were bathed in warm (37°C), oxygenated (95%O_2; 5% CO_2) Krebs' solution (devoid of Mg^{2+}) and stimulated by platinum ring electrodes (1 cm apart) at 0.1 Hz with twin pulses at the minimum voltage to produce a maximal effect. AC activity was assayed in crude brain membranes from various regions of rat brain in the presence 100 mM NaCl and 50 μM GTP using [3H]-ATP as the substrate in an HPLC procedure. AAI effects on cAMP level were determined in cultured cerebellar granule cells, using a cAMP binding protein method.

AAI-mediated inhibition of AC in rat cerebellar membranes required GTP, and was blocked by pertussis toxin (100 ng/ml for 24h) in primary cultures of cerebellar granule cells. The potency of AAIs in the MVD was highly correlated with potency in AC assays (r=0.99). The interaction of AAIs in all three systems was stereospecific with the active enantiomer being the same in each instance. An AAI analog which did not inhibit in the MVD and AC assays, demonstrated antagonist activity against pravadoline and other AAIs in the MVD and AC assays. The pA_2 for the interaction of this analog with pravadoline in the MVD was 6.73 (6.53-6.93) with a Schild slope of -0.94 (-0.78 - -1.11). In contrast to its actions on AAI-mediated effects, the antagonist analog (10 μM) did not attenuate the inhibitory actions of normorphine in the MVD (ratio of IC_{50}'s = 2.4). The antagonist analog (10 μM) also did not attenuate the AC inhibitory actions of morphine, GABA or 2-chloroadenosine in rat cerebellar membranes. Prototypic AAIs did not interact with binding sites for over 30 known receptors.

It is concluded that pravadoline and structurally-related AAIs interact with a G-protein coupled receptor which is distinct from several known G-protein coupled receptors.

Ward, S.J. et al. (1988), Neuroscience Abstracts, 14, 324.
Pacheco, M. et al. (1988), Neuroscience Abstracts, 14, 325.

THE 2-CHLOROADENOSINE INHIBITION OF HISTAMINE-INDUCED INOSITOL PHOSPHATES ACCUMULATION IN MOUSE BRAIN AND CALCIUM IONS

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2-Chloroadenosine (2CA) and other adenosine analogues are able to modulate the accumulation of inositol phosphates ($InsP_x$) generated by histamine in cerebral cortical slices of guinea pig, mouse and man (Hill & Kendall, 1987; Kendall & Hill, 1988; Kendall & Firth, 1989). In the guinea pig, 2CA augments the response to histamine, while in the mouse and human the effect is inhibitory. Our previous research has led to the elimination of protein synthesis, polyamines, the eicosanoids, and cyclic AMP as potential mediators of the modulatory effect of 2CA (Hill & Kendall, 1987; Kendall & Hill, 1988; Alexander et al, 1989). Since adenosine analogues have been shown to reduce calcium entry in hippocampal slice preparations (Schubert et al, 1986), we have investigated whether modulation of calcium fluxes might be the means by which 2CA inhibits histamine-stimulated $InsP_x$ accumulation.

[^{3}H]Inositol phosphates accumulation in mouse cerebral cortical slices (350 x 350 μm) was carried out in the presence of 5 mM LiCl and 1 U/ml adenosine deaminase as previously described (Alexander et al, 1989). However, in some experiments calcium ions were omitted from the Krebs-Henseleit medium during the [^{3}H]-inositol incorporation period prior to agonist stimulation.

Basal accumulation of [^{3}H]$InsP_x$ was not altered by varying the added calcium ion concentration over the range 0 - 1.3 mM (n=4). However, the accumulation of [^{3}H]$InsP_x$ generated by 0.3 mM histamine increased from a 2-fold stimulation in the absence of added $CaCl_2$, to a 6.5-fold increase in 1.3 mM $CaCl_2$. 2CA was able to inhibit the histamine response by at least two-thirds at all concentrations of added $CaCl_2$. Addition of 0.2 mM EGTA in the absence of added calcium ions prevented the stimulation of [^{3}H]$InsP_x$ accumulation by histamine.

In a second series of experiments using normal calcium medium (1.3 mM $CaCl_2$), the calcium ionophore A23187 (33 μM) induced a 3.5-fold increase in the accumulation of [^{3}H]$InsP_x$ (basal accumulation = 996 ± 228 dpm, n=4), which was unaffected by the presence of 2CA (10 μM). Histamine (0.3 mM) raised [^{3}H]$InsP_x$ accumulation by 5395 ± 1494 dpm. This accumulation was reduced by 86 % in the presence of 10 μM 2CA. The response to histamine was increased in the presence of A23187 to 8128 ± 2705 dpm, however, 2CA was still able to inhibit the histamine response by 66 %. The inhibitory effect of 2CA in the presence of A23187 was maintained over a range of added calcium ion concentrations.

These results indicate that calcium ions influence the $InsP_x$ response to histamine but the modulation of calcium fluxes is not the means by which 2CA inhibits histamine-stimulated $InsP_x$ accumulation in mouse cerebral cortical slices.

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Alexander,S.P., et al. (1989) Br. J. Pharmacol. 96, 135P.
Hill,S.J. & Kendall,D.A. (1987) Br. J. Pharmacol. 91, 661-669.
Kendall,D.A. & Firth,J.L. (1989) Br. J. Clin. Pharmacol., 27, 109P.
Kendall,D.A. & Hill,S.J. (1988) J. Neurochem., 50, 497-502.
Schubert,P., et al. (1986) Brain Res., 376, 382-386.

MODULATION OF INOSITOL (1,4,5)-TRISPHOSPHATE CONCENTRATION BY CYCLIC AMP IN RAT CEREBRAL CORTEX SLICES

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Inositol(1,4,5)trisphosphate (Ins(1,4,5)P_3) and cyclic AMP are important second messengers of many signal transduction pathways. Although evidence is available to suggest an interaction between the pathways which produce these second messengers in some tissues (e.g. Madison and Brown, 1988), little information exists on this possibility in neural tissues. We present data on changes in Ins-(1,4,5)P_3 concentration in response to the muscarinic agonist carbachol (CCH), the excitatory amino acid analogue quisqualate (QUIS), and K^+ depolarisation in cerebral cortex slices, where the initial basal concentration of cyclic AMP has been increased by preincubation with the cyclic nucleotide phosphodiesterase (PDE) inhibitor Ro 20 1724 (Ro).

Cross-chopped cerebral cortex slices (350 x 350 μm) were incubated for 90 min at 37°C in oxygenated Krebs-Ringer bicarbonate buffer (KRB). Aliquots (50 μl) of gravity-packed slices were added to 250 μl KRB containing 100 μM Ro or DMSO vehicle (final [DMSO] = 0.4%). Samples were incubated at 37°C for 30 min, before additions of CCH, QUIS or K^+ were made. Incubations were terminated by addition of 300 μl of 1 M trichloroacetic acid after 1 min (QUIS) or 5 min (CCH, K^+). After ether extraction, samples were brought to pH 7 with $NaHCO_3$ and EDTA added. Cyclic AMP and Ins(1,4,5)P_3 were determined (Brown et al. 1971; Challiss et al. 1988).

Table 1 Changes in second messenger concentrations ($n \geq 6$ experiments)

		Cyclic AMP (pmol/mg prot)		Ins(1,4,5)P_3 (pmol/mg prot)	
		-Ro	+Ro	-Ro	+Ro
Basal		5.5 ± 0.4	23.7 ± 1.9	20.2 ± 0.7	18.9 ± 0.6
CCH	30 μM	5.3 ± 0.3	23.0 ± 0.4	23.2 ± 1.2	20.5 ± 1.0
	100 μM	6.1 ± 0.4	24.1 ± 1.1	25.9 ± 0.9	22.9 ± 0.5
	1000 μM	6.0 ± 0.5	24.9 ± 1.2	27.5 ± 1.2	26.5 ± 1.5
QUIS	100 μM	8.9 ± 0.5	38.0 ± 1.8	23.2 ± 0.7	23.6 ± 1.0
K^+	30 mM	14.6 ± 1.3	83.8 ± 5.4	31.7 ± 1.1	27.7 ± 0.9

Increasing basal cyclic AMP levels with Ro had no effect on Ins(1,4,5)P_3 concentration. CCH, at a maximal concentration caused 36% and 41% increases in Ins(1,4,5)P_3 concentration in the absence and presence of Ro. However, at submaximal concentrations, CCH was significantly less effective in increasing Ins(1,4,5)P_3 levels in slices with elevated cyclic AMP concentration ($P < 0.05$), resulting in an increase in the EC_{50} for CCH stimulated Ins(1,4,5)P_3 formation. QUIS caused a small increase in Ins(1,4,5)P_3 concentration independent of the change in cyclic AMP induced by PDE inhibition, whereas the increase in Ins(1,4,5)P_3 concentration caused by K^+ depolarisation was significantly attenuated ($P < 0.05$) in the presence of the PDE inhibitor. We conclude that cyclic AMP can differentially affect the phosphoinositide pathway at the level of the second messenger Ins(1,4,5)P_3 and modulate muscarinic-receptor-mediated cellular activation.

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Brown, B.L. et al. (1971) Biochem J 121, 561-562
Challiss, R.A.J. et al. (1988) Biochem Biophys Res Commun 157, 684-691
Madison, J.M. & Brown, J.K. (1988) J Clin Invest 82, 1462-1465

CHRONIC LITHIUM TREATMENT ENHANCES INOSITOL POLYPHOSPHATE FORMATION IN MOUSE BRAIN *IN VIVO*

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Lithium (Li^+) treatment is the most effective therapy for manic-depressive disorders, but its mode of action is unresolved. Recently attention has focussed on the effects of Li^+ on calcium mobilising receptor function. Lithium reduces the production of total inositol phosphates ($InsP_s$) and the generation of inositol polyphosphates in ex vivo and in vitro experiments in response to calcium mobilising receptor stimulation (Kendall and Nahorski, 1987; Batty and Nahorski, 1987; Whitworth and Kendall, 1988). We have now examined the effects of chronic Li^+ treatment on in vivo muscarinic receptor stimulation of inositol phosphates formation in mouse brain.

Male C57/BL/6 mice were maintained on a diet containing 0.4% (w/w) LiCl and were killed by focussed microwave irradiation 14 days later. In experiments involving the measurement of [^{3}H]-$InsP_s$ the mice were given intracerebroventricular (i.c.v.) injections of 4μCi myo [2-^{3}H] inositol 24 hours before being killed. Some animals were treated with the muscarinic agonist pilocarpine (200 mg/kg i.p.) 20 min before death. Brains were removed and the forebrains (containing the cortex and hippocampus) were assayed for [^{3}H]-$InsP_s$ accumulation. Samples were treated with a high speed rat brain supernatant preparation in the absence of Mg^{2+} to remove [^{3}H]-inositol 1,3,4 trisphosphate ([^{3}H]-Ins1,3,4P_3) leaving only [^{3}H]-Ins1,4,5P_3 in the [^{3}H]-$InsP_3$ fraction. Individual [^{3}H]-$InsP_s$ were separated on Dowex AG 1-X8 anion exchange chromatography columns. Mass measurements of endogenous Ins1,4,5P_3 were also made in mouse forebrains, using the IP_3 protein binding assay (Amersham).

Pilocarpine caused a significant increase in the accumulation of [^{3}H]-$InsP_2$ but not of [^{3}H]-$InsP_3$ or [^{3}H]-$InsP_4$. Chronic Li^+ treatment alone had no effect on the higher phosphates, but it significantly enhanced the effects of pilocarpine on [^{3}H]-$InsP_2$, [^{3}H]-$InsP_3$ and [^{3}H]-$InsP_4$ production.

	Control	Lithium	Pilocarpine	Pilo. + Li^+
[^{3}H]-$InsP_2$	2916 ± 134	2690 ± 190	4310 ± 424*	10050 ± 806*
[^{3}H]-$InsP_3$	2866 ± 142	2508 ± 156	3346 ± 274	4812 ± 386*
[^{3}H]-$InsP_4$	1181 ± 86	1157 ± 94	1589 ± 190	3665 ± 533*

Results are expressed in d.p.m. * $p<0.01$ compared to control values (n=10).

In contrast to the effects on [^{3}H]-InsP accumulation, both Li^+ and pilocarpine treatments increased endogenous $InsP_3$ from a control level of 7.4 ± 0.5 pmol/mg protein to 13.3 ± 0.8 and 12.6 ± 0.2 pmol/mg respectively ($p<0.01$ n=5). A combination of Li^+ and pilocarpine treatments provoked an additional increase (16.9 ± 1.0 pmol/mg; $p<0.01$ with respect to Li^+ or pilocarpine alone, n=5).

The results from these in vivo studies are markedly different from previous ex vivo investigations, which reported Li^+-induced decreases in muscarinic receptor function, since they demonstrate that chronic Li^+ treatment significantly enhances both basal and muscarinic receptor stimulated $InsP_3$ formation (and by implication, Ca^{2+} mobilization) in the intact brain.

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Kendall, D.A. and Nahorski, S.R. (1987) J.Pharmacol.Exp.Ther. 241, 1023-1027.
Batty, I. and Nahorski, S.R. (1987) Biochem.J. 247, 797-780.
Whitworth, P. and Kendall, D.A. (1988) Br.J.Pharmac. 95: 550P.

INVOLVEMENT OF EXCITATORY AMINO ACID RECEPTORS IN EPILEPTIFORM ACTIVITY IN THE RAT ENTORHINAL CORTEX AND DENTATE GYRUS *IN VITRO*

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Blockade of synaptic inhibition mediated by $GABA_A$-receptors results in the generation of interictal-like epileptiform discharges in neurones of layer IV/V of the entorhinal cortex (EC; Jones, 1988). The present experiments determined (1) The relationship between these events and those occurring in cells of origin of the perforant path in layer II of the EC; (2) The relationship between events in layer II cells and those in dentate gyrus granule cells (DGC); (3) The role of N-methyl-D-aspartate (NMDA) and non-NMDA receptors in the generation of interictal activity in the EC and its propagation via the perforant path.

Slices consisting of hippocampus and EC were cut from rat brains and maintained in vitro by conventional means. Simultaneous intracellular recordings were made from cells in layers IV/V and II of the EC or from layer II cells and DGCs. Epileptiform activity was induced by perfusion with bicuculline methochloride (5-10 uM). D,L-2-aminophosphonovalerate (2-AP5, 20 uM) and 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX, 0.5-5 uM) were used as antagonists of NMDA and non-NMDA receptors respectively. Interictal events in layer IV/V cells consisted of an initial paroxysmal depolarizing shift (PDS) and a variable series of afterdischarges (see Jones, 1988). Simultaneous paired cell recordings showed that these events were followed on a one-to-one basis by similar events in layer II neurones. The latency between events in different pairs varied from 2 to 11 ms but was invariable in a given pair and events in IV/V always preceded II. In simultaneous layer II-DGC recordings (n=13) the initial PDS in the EC cells was invariably reflected by a large depolarizing wave in the DGCs (latency 2-4 ms). Afterdischarges in layer II cells were followed by smaller depolarizations in DGCs. The depolarizations in the DGCs only rarely (n=3) gave rise to action potentials.

Afterdischarges in layer IV/V cells and layer II cells were rapidly abolished in an all-or-none fashion by perfusion with either 2-AP5 or CNQX. The initial PDS in both layers was reduced in amplitude and duration by 2-AP5 but not abolished. On the other hand, CNQX progressively reduced and eventually blocked the PDSs in both layers (n=9). Occasionally a slow, 2-AP5-sensitive depolarization persisted in CNQX (n=3). The abolition of afterdischarges by bath applied 2-AP5 or CNQX in layer II cells was accompanied by a disappearance of the corresponding small depolarizations in DGCs. The initial large depolarization in DGCs was reduced by 2-AP5 and abolished by CNQX, in parallel with the actions of these drugs on the PDS in layer II neurones. Droplet applications of 2-AP5 (0.2-0.3 ul, 80 uM, n=3) to the dentate gyrus had no effect on the discharges recorded in either layer of the EC, but reduced the amplitude of all depolarizing responses in DGCs (15-30%). Similar results were obtained with droplet applications of CNQX (20 uM, n=3) to the dentate except that the reduction was more pronounced (40-65%).

These results indicate that interictal-like discharges induced in the EC by $GABA_A$-blockers arises in the deeper layers and can recruit follower discharges in the more superficial layers. Events in the latter can propagate via the perforant path and elicit depolarizations in DGCs but the dentate appears to be resistant to the generation of overt discharges. Both NMDA and non-NMDA receptors are involved in epileptogenesis in the EC with a primary role played by the latter. The depolarizing events elicited in the dentate also involve activation of both receptor subtypes but again, non-NMDA receptors play a dominant role.

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Jones, R.S.G. (1988) Brain Res. 457, 113-121

THE EFFECTS OF CALCIUM CHANNEL ANTAGONISTS IN AN *IN VITRO* CORTICAL SLICE MODEL OF EPILEPSY

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Changes in the level of extracellular calcium have been shown to precede the onset of seizures in several animal models (DeLorenzo, 1984). Calcium influx can occur through three identified types of calcium channel, N, L and T, where the N-type channel is sensitive to Ω-conotoxin and inorganic calcium channel antagonists, L to organic calcium channel antagonists and T to phenytoin (Kamiya *et al.*, 1988; Stanfield, 1986). In this study we have investigated the effect of some subtype-selective calcium channel blockers on synaptic transmission in the cortical slice.

Cortical wedges (500μm) comprising cortex and corpus callosum were prepared and mounted in tissue baths using grease-seal techniques as previously described (Harrison & Simmonds, 1985). Silver/silver chloride electrodes were used to detect spotaneous epileptiform activity induced in the absence of Mg^{2+}, which comprised large negative-going potentials with a number of afterpotentials superimposed on the decay phase. Drugs were superfused for 60 min.

At a concentration of 31.6μM the order of potency of the drugs in inhibiting the number of afterpotentials min^{-1} was Cd^{2+} > phenytoin > verapamil > Co^{2+}. Ω-conotoxin at 100nM reduced afterpotentials min^{-1} by 20±2% (mean ± s.e.mean, paired t-test, $P<0.05$, n=4). Nifedipine, in contrast, increased the number of afterpotentials min^{-1} recorded. Bursting activity was reduced only by Cd^{2+} and Co^{2+}, with decreases of 80±11% (mean ± s.e.mean) and 27±5% (mean ± s.e.mean) respectively (paired t-test, $P<0.05$, n=4-5); both verapamil and nifedipine significantly increased bursting activity. Ethanol, the vehicle for the dihydropyridines was present at a final concentration of 0.095% and had no significant effects on the spontaneous epileptiform activity when superfused alone over the slices.

From these results it would appear that N-type calcium channels, sensitive to the inorganic calcium channel antagonists and to Ω-conotoxin, and T-type channels (blocked by phenytoin) are implicated in the generation of spontaneous epileptiform activity in this model. The involvement of L-type channels, although not precluded at this stage, has not been demonstrated.

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DeLorenzo, R.J. (1984) Ann. Neurol., 16, S104-114.
Harrison, N.L. & Simmonds, M.A. (1985) Br. J. Pharmacol., 84, 381-391.
Kamiya, H., Sawada, S. & Yamamoto, C. (1988) Neurosci. Lett., 91, 84-88.
Stanfield, P.R. (1986) Brit. Med. Bull., 42, 359-367.

ARE CHANGES IN GLUTAMATERGIC MECHANISMS RESPONSIBLE FOR KINDLING TO THE BENZODIAZEPINE RECEPTOR INVERSE AGONIST, FG 7142?

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FG 7142 (β-carboline-3-monomethylamide) is a partial inverse agonist at central benzodiazepine (BZ) receptors. Acutely, FG 7142 is proconvulsant, but on repeated administration rats and mice develop seizures, a process known as kindling. Attempts to explain the increased sensitivity to FG 7142 have centred on the GABA/BZ receptor complex, but no consistent changes in the biochemical pharmacology of this complex have been found (Schneider and Stephens, 1988). We (Stephens and Weidmann, 1989) have recently reported that the development of kindling to FG 7142 (40 mg/kg, i.p.) in NMRI female mice (20-25 g) could be prevented by coadministration of anticonvulsants which, while allowing FG 7142 to interact with its receptors, prevented the expression of convulsant events. Since one of these anticonvulsant drugs, MK801, acts at N-methyl-d-aspartate (NMDA) subtypes of excitatory amino acid receptors, we explored interactions with glutamatergic mechanisms further.

2-amino-phosphonoheptanoic acid (2-APH), a competitive antagonist at NMDA receptors, given intracerebroventricularly (icv; 25 nmoles) to mice (group n's = 10) 15 min prior to daily FG 7142 (40 mg/kg, i.p.) administration prevented the development of kindling, and the expression of convulsions in already kindled animals. The putatively selective quisqualate and kainate antagonists, 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX) and -D-glutamylaminomethyl sulphonate (-D-GAMS) (both 25 nmoles in 3 µl) did not antagonize the development of kindled seizures, suggesting some selectivity for the NMDA subtype of receptor.

Despite the protective effect of 2-APH against FG 7142 kindling, there was no change in the sensitivity of kindled mice to seizures induced by icv (1 µl) administration of NMDA (ED50 values and 95% confidence limits: Kindled: 0.32 nmoles (0.28-0.36); Control: 0.32 nmol (0.29-0.37), suggesting that FG7142 kindling is not associated with changes in NMDA receptors.

Nevertheless, in male Wistar rats in which kindling had been induced by administering 15 mg/kg FG 7142 i.p. twice daily, preliminary autoradiographic studies indicate an increased binding of specific ^{3}H-glutamate (in sodium free conditions) in hippocampus (384 vs 208 fmol/mg tissue).

Schneider HH and Stephens DN (1988) Eur. J. Pharmacol. 154:35-45.
Stephens DN and Weidmann R (1989) Brain Research 492:89-98.

ENDOGENOUS MEDIATORS OF CEREBRAL ISCHAEMIA-INDUCED HYPERMETABOLISM IN THE RAT

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Induction of cerebral ischaemia by middle cerebral artery (MCA) occlusion in rats results in a pronounced rise in metabolic rate which is associated with only a modest increase in core temperature. We are currently investigating possible mediators of this hypermetabolism using neuropharmacological intervention in conscious animals. We have previously shown that it is dependent on activation of the peripheral sympathetic nervous system since propranolol completely abolishes the hypermetabolic response (O'Shaughnessy et al, 1989a). Corticotrophin releasing factor (CRF), a known thermogenic peptide which is released in response to stress and endogenous pyrogens (Rothwell, 1989), has also been implicated (O'Shaughnessy et al, 1989b). Fever and hypermetabolism associated with infection are mediated by eicosanoids, particularly prostaglandins, and are prevented by cyclooxygenase inhibitors. In this study we have investigated the effects of cyclooxygenase and lipoxygenase inhibitors on the hypermetabolic responses to cerebral ischaemia.

Male Sprague-Dawley rats were anaesthetised with halothane/nitrous oxide and subjected to craniectomy exposing the left middle cerebral artery (MCA). The dura was pierced, peeled back from over the artery (sham-operation) and the left MCA was occluded by electrocautery and was then bissected (MCA-occluded animals). On recovery of consciousness, resting oxygen consumption ($\dot{V}O_2$) was measured over 8h using indirect calorimetry. The lipoxygenase inhibitor, ICI 207968 (30 µg i.c.v.) and cyclooxygenase inhibitor, ibuprofen (10 mg/kg i.p.) were administered after 5h when the hypermetabolism was established. Animals were killed at the end of each experiment and brain sections stained to evaluate the ischaemic damage.

After 3-5h, resting $\dot{V}O_2$ (ml/min/$kg^{0.75}$) in MCA-occluded rats (15.4±0.8; n=8) was significantly higher than sham-operated rats (13.0±0.5; n=8; $P<0.05$, Student's t-test). ICI 207968 and ibuprofen had no effect on $\dot{V}O_2$ in either sham-operated or MCA-occluded rats up to 3h after administration. All MCA-occluded rats showed ischaemic tissue damage in the cerebral cortex and basal ganglia, whilst sham-operated rats were comparable to unoperated control animals.

Ischaemia-induced hypermetabolism is unlikely, therefore, to be mediated through the lipoxygenase/cyclooxygenase pathways and contrasts with the hypermetabolism associated with bacterial infections.

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O'Shaughnessy, C.T., Shrewsbury-Gee, J. & Rothwell, N.J. (1989a) J.Physiol.Proc. (in press)
O'Shaughnessy, C.T., Shrewsbury-Gee, J. & Rothwell, N.J. (1989b) Neurosci.Letts. (in press).
Rothwell, N.J. (1989) Am.J.Physiol. 256, E111-E115.

ENDOTHELIN-1 CONSTRICTS HUMAN PULMONARY RESISTANCE VESSELS *IN VITRO*

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Endothelin (ET-1) is a 21 amino acid vasoconstrictor peptide originally isolated from the conditioned media of cultured endothelial cells (Yanagisawa et al.,1988). Autoradiographical mapping has demonstrated the existence of endothelin binding sites in a diverse range of human, rat and pig vascular tissues (Davenport et al.,1989). These studies together with functional data suggest the existence of endothelin receptors. Structurally, ET-1 shows regional homologies to some neurotoxins active at voltage-gated ion channels and it has been postulated to be an endogenous agonist for voltage dependent calcium channels (Yanagisawa et al.,1988).

Human pulmonary resistance vessels (200-400um i.d.) were obtained from surgical lobectomy specimens from patients with lung carcinoma and cut into rings (2-3mm i.d.) and mounted over two parallel tungsten wires; one being fixed the other attached to a force-displacement transducer. After 60 mins during which the tissues were washed every 15 mins, the vessels were challenged with repeated doses of KCl (60mM) until a consistent response was attained. ET-1 was added to the baths cumulatively.

ET-1 caused a slowly developing dose-dependent contraction of human pulmonary resistance vessels (E.C.50 16nM). The response to 0.3uM was long lasting and diminished back to baseline slowly (70 mins). This decline in tension was unaffected by washing but was not resistant to isoprenaline (10uM), which fully reversed the contraction. In some tissues the maximum dose of ET-1 caused a relaxation. The possibility that at high concentrations ET-1 acts at a different population of receptors, which mediate relaxation, to those causing contraction was discounted by the lack of relaxation of precontracted tissues ($PGF_{2\alpha}$ 1uM) to ET-1 (0.3uM). Endothelial removal did not significantly affect the response to ET-1 at all concentrations tested. However, both nicardipine and verapamil significantly reduced contractile responses to ET-1. Removal of extracellular calcium attenuated the maximum response to ET-1 by 35% at the concentrations used but did not abolish the contraction.

We have shown that ET-1 causes a dose-dependent, slowly developing and long-lasting contraction of human pulmonary resistance vessels in vitro. Endothelial removal did not affect this contraction suggesting that the receptors responsible are located on vascular smooth muscle. This contraction is partly dependent on a calcium flux through voltage-dependent channels. Since the response to ET-1 was not abolished in the absence of external calcium ions, it is possible that ET-1 mobilises intracellular calcium ions. We suggest that ET-1 induced contraction of human pulmonary resistance vessels may involve opening of voltage-dependent calcium channels and mobilisation of intracellular calcium. ET-1 could be important in pulmonary hypertension, but long duration of action make it an unlikely candidate to explain hypoxic vasoconstriction which is rapid in action.

Davenport et al, (1989). J.Cardiovasc Pharmacol, 13 (suppl 5); S166-170.
Yanagisawa et al, (1988). Nature (Lond), 332; 411-415.

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CONTRACTIONS OF RAT AORTA TO ENDOTHELIN ARE SENSITIVE TO NICKEL AND CADMIUM IONS BUT NOT NICARDIPINE OR ω-CONOTOXIN

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Endothelin is a potent vasoconstrictor peptide isolated from the supernatant of cultured vascular endothelial cells (Yanagisawa et al., 1988). These authors suggested that this peptide may activate voltage-operated calcium channels (VOCs), however contractions of rat aorta to endothelin are only antagonised by high concentrations of calcium antagonists (Auguet et al.,1988; Topouzis et al., 1989). We further investigated the possible mechanism involved in endothelin-induced contraction of rat aortic ring preparations.

Aortic rings, devoid of endothelium, from male Wistar rats (300-350g) were suspended under a resting tension of 2g in modified Krebs solution (composition in mM : NaCl 117, $CaCl_2$ 1.25, NaH_2PO_4 1.0, $MgSO_4.7H_2O$ 1.2, KCl 5.0, glucose 11.5, $NaHCO_3$ 25) maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. After an equilibration period of 60 min, the maximum response to KCl (80mM) was determined. Cumulative concentration-response curves to porcine endothelin (0.1-100nM) were constructed on control preparations or in tissues incubated for 30 min with either nickel chloride (Ni^{2+};0.1-0.8mM), cadmium chloride (Cd^{2+};10-30μM), nicardipine (0.1μM), amiloride (0.1-1.0mM) or ω-conotoxin (1.0μM) or for 60 min with cinnarizine (10μM). The EC_{50} values (concentration of endothelin required to produce 50% maximal response) and E_{max} values (expressed as percentage of maximum response to KCl) were calculated for each endothelin curve and reported as means± s.e.mean.

Endothelin evoked concentration-dependent contractile responses of aortic rings (EC_{50}=1.65±0.22nM; E_{max}=125.8±4.5%, n=20). Cd^{2+} produced a concentration-related attenuation of the maximum response to endothelin (IC_{50}=15.4±1.5μM, n=6). The contractile responses to endothelin were also inhibited by Ni^{2+} (response to 10nM endothelin control=132±8.7%, after 0.2mM Ni^{2+}=90.3+14.8%, after 0.4mM Ni^{2+}=54.7±12.3% and after 0.8mM Ni^{2+}=10.3±4.4%, n=6). The maximum responses to endothelin were not determined in the presence of Ni^{2+} due to limited availability of peptide. In contrast, the endothelin concentration-response curve (control EC_{50}=1.79±0.28nM, E_{max}=128±5.8%, n=8) was not modified by nicardipine (0.1μM;EC_{50}=1.90±0.28nM, E_{max}=143±9.4%, n=6) or ω-conotoxin (1μM;EC_{50}=1.13±0.36nM, E_{max}=132.5±9.0%, n=4). Cinnarizine (10μM), however, significantly attenuated the E_{max} to endothelin (96.9±6.0%, n=4, $p<0.05$), but failed to modify the EC_{50} value (2.5±0.8nM, n=4). The maximum of the endothelin concentration-response curve was also attenuated by amiloride (IC_{50}=0.45±0.05mM, n=4). Finally in Ca^{2+}-free Krebs solution (containing 1mM EGTA) aortic rings failed to contract to endothelin (0.1-30nM).

In conclusion, in rat aorta endothelin produced contractions which were not modified by nicardipine and ω-conotoxin eliminating the involvement of L-type (supporting the findings of Auguet et al.,1988; Topouzis et al.,1989) or N-type VOCs. The attenuation of the endothelin-induced contraction by the divalent cations (Ni^{2+} and Cd^{2+}) or amiloride suggests that influx of extracellular calcium into the cytosol via dihydropyridine-resistant calcium channels and/or the Na/Ca exchange mechanism may be responsible. Although the dependence of the endothelin-induced contractions on extracellular calcium supports this hypothesis, involvement of intracellular mechanisms (e.g. Ca^{2+}-induced Ca^{2+}release) cannot yet be excluded.

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Auguet, M. et al. (1988) Biochem. Biophys. Res. Comm., 156, 186-192.
Topouzis, S. et al. (1989) Br. J. Pharmacol., 96, 101P.
Yanagisawa, M. et al. (1988) Nature 332, 411-415.

ENDOTHELIN IN THE ANAESTHETISED RABBIT: ACTION AND INTERACTION IN THE RENAL MICROCIRCULATION

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Endothelin (ET), a peptide produced by the endothelium, has been shown to be a potent vasoconstrictor (Yanigasawa et al. 1988) in several species . We have previously shown that, in the rabbit isolated perfused kidney, ET has a potent effect on renal vascular resistance and glomerular filtration (Cairns et al 1989). We therefore investigated the renal effects of ET infusion, and its interaction with the renin-angiotensin and eicosanoid systems, in vivo in the same species. NZW rabbits were hydrated, anaesthetized (with pentobarbitone and althesin) and ventilated (O_2/air). Cannulae were placed in the L carotid artery for measurement of systemic blood pressure (MAP) and in the aorta, 1cm above the renal arteries, for infusion. At laparotomy, both ureters were cannulated and an electromagnetic flow probe was placed around the L renal artery (RBF). ^{51}Cr-EDTA was infused for calculation of glomerular filtration rate (GFR) in timed urine samples. ET at 5, 20, and 40pmol/kg/min was infused for 40 mins producing an increase in MAP and a fall in pulse pressure (PP) suggesting peripheral vasoconstriction. RBF fell and there was a reduction in GFR of 23±14, 45±13 and 81±23% by the end of infusion, for each dose respectively. Infusions of 20pmol/kg/min were given subsequently, followed by 70 mins recovery.

Animals were then pretreated with Indomethacin (2mg/kg) or captopril (2mg/kg/2hrs). In the presence of indomethacin, the effect of ET on MAP and RBF was enhanced, whilst GFR fell by the same degree as with ET alone. With captopril, the reduction in GFR and RBF was less, although not significantly different from that with ET alone.

TABLE:
Peak % change during ET infusion(20pmol/kg/min) (Mean±SEM)

	MAP	GFR	RBF	
ETalone	+18±8	-45±13	-30±19	
ET+Indo	+27±4	-45±26	-66±5 *	* p<0.02
ET+Cap	+21±6	-26±8	+18±18	(ET alone)

Thus ET is a potent vasoconstrictor in the rabbit, reducing both GFR and RBF suggesting a reduction in intraglomerular pressure by afferent glomerular arteriolar constriction. ET infusion stimulates the release of vasodilator prostanoids which oppose its actions, and interacts with the RAS in a complex way, probably involving other vasoactive mediators. ET may thus be a modulator of renal vascular resistance with a potential role in the pathophysiology of acute renal failure.

Cairns HS, Rogerson ME, Fairbanks LD, Neild GH, Westwick J. Br J Pharmacol 1989; 98:155-160
Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. Nature 1988; 332:411-415.

COMPARISON OF GR32191, R68070 AND CV-4151 UPON U-46619- AND COLLAGEN-NDUCED PLATELET AGGREGATION *IN VITRO* AND *EX VIVO*

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GR32191 is a potent, specific and orally active thromboxane receptor blocking (TxRB) drug with a long duration of action in man (Thomas et al, 1987) currently under clinical evaluation. It has been proposed that the combination of a TxRB drug with a thromboxane synthase inhibitor (TxSI) should provide a superior anti-thrombotic effect to that of either activity alone (Gresele et al, 1984). R68070 ((E)-5-[[[(3-pyridinyl)[3-(trifluoromethyl) phenyl]-methylen]-amino]oxy]pentanoic acid (De Clerck et al, 1989)) and CV-4151 ((E)-7-phenyl-7-(3-pyridyl)-6-heptenoic acid (Imura et al, 1988)) are two drugs claimed to possess both TxRB and TxSI activities in the same molecule. We have therefore compared the inhibitory profile of GR32191 with these two drugs upon aggregation of human platelets in vitro and dog platelets ex vivo.

TxRB activity was quantified in vitro in human citrated whole blood by determining the ability of compounds to inhibit U-46619- and collagen-induced platelet aggregation utilising a platelet counting technique. TxSI activity was assessed by measuring formation of serum TxB_2, the stable metabolite of TxA_2, in clotted whole blood and expressed as a pIC_{50} (log of the concentration to produce 50% inhibition of serum TxB_2 formation). The magnitude and duration of the TxRB (inhibition of collagen-induced aggregation) or TxSI (serum TxB_2) activities were also measured ex vivo following single dose oral administration to conscious dogs.

In human whole blood GR32191, R68070 and CV-4151 produced concentration-related antagonism of U-46619-induced platelet aggregation with pA_2 values (mean n = 4-6; 95% confidence intervals) of 8.2 (7.9-8.6), 5.4 (5.2-5.6) and 4.7 (4.1-5.4) respectively. Thus GR32191 is at least 600 times more potent as a TxRB drug than either R68070 or CV-4151. Consistent with these data, the threshold concentration (0.1μM) of GR32191 for antagonising collagen-induced aggregation was at least 100 fold less than that for R68070 or CV-4151. In contrast to their weak TxRB activity R68070 and CV-4151 exhibited potent TxSI activity with mean (n = 8) pIC_{50} values for inhibition of serum TxB_2 of 7.4 (7.1-7.6) and 6.9 (6.7-7.1) respectively. GR32191, however possessed negligible TxSI activity (<3.54 (3.51-3.56) n = 5).

In the conscious dog GR32191 ($1 mgkg^{-1}$ p.o.; n = 19) produced a marked rightward displacement of the collagen-induced platelet aggregation curve which was maximum at 1 hour post-dose (Concentration Ratio (CR) = 13.3 (9.3-19.2)) with inhibition still evident at 6 hours (5.6 (4.2-7.3)) and 12 hours (3.3 (2.9-3.9)) post-dose. In contrast, doses of $10 mgkg^{-1}$ p.o. of R68070 and CV-4151 produced maximum mean (n = 4) collagen CR values at 1-2 hours post-dose of only 3.3 (2.9-3.8) and 2.9 (0.9-9.3) respectively, again confirming the relatively weak TxRB activity of these compounds. However, at doses an order of magnitude lower, both R68070 and CV-4151 ($1 mgkg^{-1}$) produced substantial (> 90%) inhibition of serum TxB_2 formation with mean (n = 4-5) $T_{½}$ values (the period over which 50% inhibition of TxB_2 was maintained) of 33 ± 1 and 7 ± 2 hours respectively. Serum TxB_2 formation in GR32191-treated dogs was, however, unaffected.

In conclusion both R68070 and CV-4151 are potent TxSI drugs in vitro and ex vivo. In contrast, both compounds exhibit only weak TxRB activity. On the basis of the test systems used, despite possessing both TxRB and TxSI activities, R68070 and CV-4151 were no more effective as inhibitors of platelet aggregation than GR32191.

De Clerck, F. et al. (1989) Thromb, Haemostas., 61, 35-42
Gresele, P. et al. (1984) Thromb. Haemostas., 52, 364
Imura, Y. et al. (1988) Eur. J. Pharmacol, 147, 359-365
Thomas, M. et al. (1987) Thromb. Haemostas. 58, 181

CHARACTERISTICS OF [^{3}H]-GR 32191 BINDING TO THE THROMBOXANE (TP) RECEPTOR OF HUMAN PLATELETS

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Repeated dosing of patients with the TP-receptor antagonist GR 32191 leads to a profound and long lasting block of TP-receptor activation (Thomas et al, 1987). To investigate the long duration of action, the binding of [^{3}H]-GR 32191 to the platelet TP-receptor has been characterised. Methods used were similar to those previously reported (Armstrong et al, 1986). Briefly, bound and free radioligand were separated by filtration using a Brandell cell harvester, Whatman GF/B filter strips and an assay buffer of 50 mM Tris, pH 7.5 containing 100 mM NaCl, 5mM glucose and 1μM indomethacin.

Human platelet membranes: Scatchard analysis of [^{3}H]-GR 32191 binding was determined by either increasing ligand concentration or increasing unlabelled GR 32191 in the presence of 2 nM [^{3}H] GR 32191. Both methods indicated one class of binding site with K_d values of 1.7 ± 0.1 (n=4) and 2.4± 0.1 (n=8) nM, respectively. Onset of binding of 2nM [^{3}H]-GR 32191 at 37°C was complete within 5 min, giving K_{obs} of 0.69 ± 0.16 and K_{+1} of 3.2 x 10^8 $M^{-1}min^{-1}$ (n = 4). Dissociation of [^{3}H]-GR 32191 (2 nM, 37°C, 30 min preincubation) with 5μM unlabelled GR 32191 was slow, with a $t_{1/2}$ of 17.7 ± 2.8 min and K_{-1} of 0.044 ± 0.004 min^{-1} (n = 8). The kinetically determined K_d was 0.13 nM.

Intact human platelets: Binding of 2 nM [^{3}H]-GR 32191 was slower and more complex. Although 80% of total binding was complete within 10 min, equilibration of a slower component required 30-60 min. Similarly when dissociation was measured as above, displacement from 2 sites was detected: one with a $t_{1/2}$ of 8.1 ± 1.5 min, K_{-1} of 0.093 ± 0.015 min^{-1}, and one with a $t_{1/2}$ of 65.6 ± 8.8 min, K_{-1} of 0.011 ± 0.001min^{-1} (n = 4). The K_d for the fast offset site was 0.48 nM and for the slow site 0.27 nM.

In intact platelets, the ability of three TP -receptor agonists to displace either [^{3}H]-GR 32191 or [^{3}H]-SQ 29548 (Hedberg et al, 1988) was examined. IC_{50}values for inhibition of [^{3}H]-GR 32191 binding were higher than those obtained with other TP-receptor radioligands such as [^{3}H]-SQ 29548.(Table 1) and [^{125}I]-PTA-OH (Jones et al, 1989).

Table 1: IC_{50} values (nM) for inhibition of [^{3}H]-GR 32191 and [^{3}H]-SQ 29548 binding

Prostanoid	[^{3}H]-GR 32191 (2 nM)	[^{3}H]-SQ 29548 (5 nM)	
U-46619	947 ± 134	220 ± 21	
STA_2	205 ± 39	87 ± 19	(all n = 4)
EP 171	23.2 ± 2.0	5.4 ± 0.8	
GR 32191	2.3 ± 0.12	1.2 ± 0.12	

Whilst the initial binding of GR 32191 to TP -receptors is rapid, this is not matched by a rapid dissociation. The K_d determined kinetically is 10-fold higher than those obtained under equilibrium conditions. These results suggest that the nature of the binding of GR 32191 to the platelet TP-receptor is complex and associated with a slow dissociation which could account for the long duration of action in man.

Armstrong, R. A. et al (1986) Br. J. Pharmacol. 87,543-964.
Hedberg, A. et al (1988) J. Pharmacol. Exp. Ther. 245, 786-792.
Jones, R.L. et al (1989) Br.J.Pharmacol. 96, 875-887.
Thomas, M. et al (1987) Thromb. Haem. 58, 181.

CHARACTERISATION OF PLATELET-ACTIVATING FACTOR-INDUCED DIACYLGLYCEROL GENERATION IN RABBIT PLATELETS

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We have previously demonstrated that ionomycin-induced 5-HT release and thromboxane B_2 (TxB_2) generation are readily modulated by activation or inhibition of protein kinase C (PKC). However, sub-optimal platelet-activating factor (PAF)-induced elevation of cytosolic calcium concentration ($[Ca^{++}]_i$), 5-HT release and TxB_2 generation are not similarly modified (Murphy & Westwick, 1989). To analyse this paradox, we have determined PAF-induced formation of diacylglycerol (DAG), the endogenous activator of PKC. DAG was measured using the Amersham kit; $[Ca^{++}]_i$ and TxB_2 generation were measured as previously described (Murphy & Westwick, 1989; Poll *et al.*, 1986) in rabbit platelets.

Addition of 300nM PAF produced a rapid elevation of DAG concentration, which peaked at 10s; the values at 5, 10, 15, 20, 30 and 60s were 86.8±15, 280±52, 65±11, 87±31, 66±18 and 69±10 pmoles/10^9 platelets, above the basal value of 117±3 (n=3-9). This high concentration of PAF produced a peak elevation of $[Ca^{++}]_i$ of 1023 ± 72 nM, which occurred at 7.3±0.6s (n=13) and 62.7±12.9% of total TxB_2 was produced within 10s. At 10s after the addition of 0.3, 3, 30 and 300 nM PAF, the generation of 18±6, 8±6, 80±19 and 280±52 pmol DAG/10^9 platelets (n=3-9) above basal values was induced. Thus sub-optimal concentrations of PAF (0.3-3nM) do not increase DAG above basal values. Therefore, it is unlikely that PKC can exert a negative feedback on low-dose PAF-activated systems. For example, pre-treatment of platelets for 2 min with 1μM staurosporine (an inhibitor of PKC, Tamaoki *et al.*,1986), caused an approximately 3-fold enhancement of 300 nM PAF-induced DAG level, from 413±8.5 to 1147±227, but had no significant effect on 3nM PAF-induced DAG level, from 112.5±7 to 146±35 pmol/10^9 platelets. These results are paralleled by the staurosporine (1μM)-induced tripling of duration of 300nM (but not 3nM) PAF-elevated $[Ca^{++}]_i$ from t½ 34±4 to 105±4 s. Similarly, the 300nM PAF-induced TxB_2 generation is doubled by pretreatment with 1μM staurosporine from 170±19 to 307±17 pmol TxB_2/10^8 platelets.

Pretreatment with exogenous PKC activator 12-0-tetradecanoyl phorbol 13-acetate (100nM) reduced 3nM and 300nM PAF-induced DAG formation from 112±5 to 45±32 and from 413±8.5 to 140±37.4 pmol/10^9 platelets respectively.

Thus, with high concentrations of PAF, the diacylglyerol levels, duration of elevation of $[Ca^{++}]_i$ and generation of TxB_2 are significantly enhanced by inhibition of PKC.

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Murphy, C.T. & Westwick, J. (1989) Br. J. Pharmacol. **96**, 70P.
Poll, C. et al. (1986) Biochem. Biophys. Res. Commun. **136**, 381-389.
Tamaoki, T. et al. (1986) Biochem. Biophys. Res. Commun. **135**, 397-402.

TRANSLOCATION OF PROTEIN KINASE C (PKC) IN STIMULATED PLATELETS: A ROLE FOR AGGREGATION IN PKC DEGRADATION

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Tumour-promoting phorbol esters such as phorbol 12-myristate 13-acetate (PMA) can substitute for diacylglycerol as activators of the Ca^{2+}/phospholipid-dependent kinase (PKC). Evidence from several tissues suggests that phorbol esters promote translocation of PKC to the plasma membrane and that rapid enzyme degradation then occurs via the action of a membrane-associated Ca^{2+}-dependent protease (calpain) [Nishizuka, 1988]. However, the relevance of translocation to receptor-operated functions in platelets is unclear. In the present study we have assessed the distribution of PKC activity in agonist-stimulated platelets and have also addressed the role of aggregation in subsequent PKC degradation by measuring the time-course of PMA-induced translocation under aggregatory (stirred) and non-aggregatory (unstirred) conditions.

Washed, indomethacin (10μM)-treated platelets ($0.8\text{-}10 \times 10^9$/300μl) were incubated (± stirring) with either PMA (160nM), thrombin (2U/ml), collagen (40μg/ml) or ADP (100μM). PKC activity (cytosolic and membrane fractions) was assessed by measuring the incorporation of phosphate from [γ-^{32}P]-ATP into histone IIIs following partial enzyme purification on DEAE cellulose. PMA promoted aggregation in stirred platelets (18±4%, 1 min, n=9), caused a rapid loss of cytosolic PKC which was not paralleled by increases in membrane PKC, and induced a 52±12% (n=8) loss in total (membrane and cytosol) activity. In unstirred platelets, cytosolic activity was reduced by 21±2% (n=4), maintained elevations (1-15 min PMA) in membrane PKC occurred, and the decline in total activity was slower (66±7% after 15 min). In stirred platelets, total inhibition of PMA-induced aggregation by a fibrinogen-derived peptide (RGDS) [Gartner & Bennett, 1985] caused increases in membrane activity (240±9%, increase, n=3), and completely prevented the loss in total PKC. Addition of thrombin or collagen to stirred platelets (86 and 27% aggregation at 5 min respectively) resulted in a reduction in cytosolic PKC (39±11 and 60±5% reduction, respectively, n=3) and a loss in total activity, but a rise in membrane activity was only seen with collagen. ADP had no detectable effect on either the distribution or total cellular PKC activity.

These results demonstrate an agonist-induced redistribution of PKC and indicate that enzyme translocation is associated with the subsequent physiological responses. The degradation of PKC following PMA exposure under aggregating conditions, and our inability to detect increased membrane activity with a strongly aggregating agent such as thrombin, suggest that platelet aggregation may play an important role in the proteolysis and hence termination of PKC actions. Thus, an inhibitor of platelet aggregation, by indirectly inhibiting calpain, may be a valuable tool for assessing the role of this enzyme in platelet activation. The aggregation-induced loss in total activity could represent an intrinsic "switching-off" mechanism allowing termination of the effects of agonist or phorbol ester-mediated PKC activation.

Nishizuka, Y. (1988) Nature 334, 661-665.
Gartner, T.K. & Bennett, J.S. (1985) J. Biol. Chem. 260, 11891-11894.

EFFECTS OF THE CALPAIN INHIBITOR E64-D ON PLATELET ACTIVATION RESPONSES

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The Ca^{2+}, and phospholipid-dependent enzyme, protein kinase C (PKC), translocates from its primarily cytosolic location to the plasma membrane upon activation. At the plasma membrane, the enzyme is proteolytically degraded by a membrane-associated Ca^{2+}-dependent protease, calpain. This results in the release of the catalytic fragment of PKC into the cytosol, the significance of which is unknown. The principal aim of this study was to examine the role of calpain-induced proteolytic modification of protein substrates, especially that of PKC. In order to do this, we examined the effects of the recently described membrane-permeant inhibitor of calpain, E64-d (McGowan et al, 1989).

Washed platelets (10μM indomethacin and 250μM phenidone-treated) were resuspended in a modified Hepes-Tyrode (pH7.4) buffer (1mM Ca^{2+}) and pre-loaded with [^{14}C]-5HT (dense-granule marker), [^{3}H]-arachidonic acid (AA), quin 2 (fluorescent Ca^{2+} probe) or [^{32}P] inorganic phosphate. Pre-treatment with E64-d at concentrations reported to inhibit calpain in intact platelets (50-100μg/ml), inhibited platelet aggregation (shown first) and [^{14}C]-5HT secretion (shown second) induced by 20μg/ml collagen (40±6% and 71±8%, n=6), 16nM, phorbol 12-myristate 13-acetate (32±11% and 40±6% n=6) and 1μM U46619 (25±4% and 48±6%, n=5). Thrombin (0.04U/ml)-induced aggregation was unaffected although 5HT secretion was increased (collagen-induced 5HT secretion reduced from 9.6% to 2.7%, thrombin-induced secretion increased from 24.5% to 41%, $p<0.01$ in both cases). Thrombin (0.2U/ml) and collagen (20μg/ml)-induced [^{3}H]-AA release was significantly reduced (21±0.5 reduced to 17.8±0.5% and 9.8±0.7% reduced to 6.5±0.6%, respectively, $p<0.01$ in both cases), although [^{32}P]-phosphatidate formation in response to both agents was unaffected by drug pre-treatment.

The effect of E64-d on PKC activation/proteolysis was also assessed by monitoring PKC translocation following PMA or thrombin addition. In stirred aggregating platelets, both agents caused a rapid loss in cytosolic PKC (42.7 reduced to 25.9 fmoles [^{32}P-Pi incorporated/μg platelet protein with PMA) without any change in membrane-bound PKC. The total cellular PKC activity recovered was only 60-65% of the activity in resting platelets due to proteolytic degradation of the enzyme. Pre-treatment with E64-d (50μg/ml) prevented the loss in total PKC activity and caused sustained increases in membrane-bound PKC following thrombin or PMA addition. In summary, i) inhibition of PKC proteolysis may be responsible for the reduced secretion seen in the presence of E64-d; proteolytic degradation of PKC may thus favour secretion with most agonists (except thrombin) and ii) calpain-mediated modification of cytoskeletal/membrane proteins may play an important role in thrombin and collagen-induced arachidonate release but not in phosphatidate formation.

McGowan, E.B., Becker, E. & Detwiler, T.C. (1989) Biochem. Biophys. Res. Commun. 158, 432-435.

EFFECT OF VERAPAMIL ON THE VASCULAR RESPONSES OF THE HUMAN ISOLATED SAPHENOUS VEIN

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Verapamil, an organic calcium antagonist, or entry blocker, is known to block the slow calcium channels in cardiac, smooth and skeletal muscle fibres (Fleckenstein, 1977; Sato & Ono, 1982).
In the present investigation, the effect of verapamil on contractility of the human isolated saphenous vein, and its response to neurotransmitters and to high potassium , was studied, to see if verapamil modified contractility of the blood vessel and its responses to neurotransmitters, such as acetylcholine (ACh) ,noradrenaline (NA),and 5-hydroxytryptamine (5-HT).

The saphenous vein was obtained from male patients, 45-65 years of age, undergoing coronary bypass operation (vein graft). Informed consent was obtained from each patient before the start of the operation.

Tissues were cut spirally,into strips 2-3 cm long and 2 mm wide (Campbell, Marshall and Winslow,1983; Docherty & Hyland,1985). The endothelium was lost during or after the operation. The preparation was set up in an organ bath containing 20 ml of Krebs-Henseleit solution maintained at 38± 2°C and bubbled with 5% CO_2 in O_2. The mechanical responses produced by drug action were recorded isometrically,in the absence and presence of verapamil. Means±s.e.,were calculated and Student's t test was employed for paired sets of observations. A probability $P<0.05$ was considered significant.

Verapamil (0.1-10 μM) produced concentration-dependent relaxation in the saphenous vein. The mean maximum relaxation was 1.2±0.1 g (n=6), and the mean concentration of verapamil which produced 50% of maximum response (EC_{50} value) was 1.0±0.1 uM. In addition, verapamil (1 μM) reduced the contractions produced by ACh (0.5-550 μM), NA (0.1-100 μM), 5-HT (0.25-250 μM) and KCL (10-100 mM). The results are shown in Table 1.

Table 1. Effect of verapamil on the responses produced by neurotransmitters and by KCL in the human isolated saphenous vein.

	Maximum Response (g)	Control (C) EC_{50} value (μM)/(mM*)	Verapamil (V) EC_{50} value (μM)/(mM*)	EC_{50} ratio (V/C)
ACh (uM)	0.9±0.1	115.5± 12.1	216.0±11.0	1.9:1.0
NA (uM)	2.8±0.3	4.3±0.5	29.5±3.2	6.9:1.0
5-HT(uM)	3.4±0.5	9.1±0.5	59.0±9.6	6.5:1.0
KCL (mM)	1.6±0.1	25.0±3.1*	180.0±12.5*	7.2:10

The results are means±s.e.,n=6. The difference between control (C) and verapamil (V),EC_{50} values, was significant ($P<0.001$). It was concluded that verapamil relaxed the human saphenous vein and modified its responses to KCL and neurotransmitters,suggesting that it may act on both voltage and receptor-operated slow calcium channels.

Campbell,J.K., Marshall,R.J.& Winslow,E.(1983). Eur.J.Pharmacol.,86,217-228
Docherty,J.R.& Hyland,L.(1985). Br.J.Pharmacol., 84 , 573-576
Fleckenstein,A.(1977). Ann.Rev.Pharmacol. Toxicol., 17 , 149-166
Sato,T. & Ono,H.(1982). Eur.J.Pharmacol., 83 , 177-181

INVESTIGATION OF THE INVOLVEMENT OF PROSTAGLANDINS IN BRADYKININ-INDUCED RAT PAW INFLAMMATION

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We have shown that subplantar injection of bradykinin (BK) into rat paws causes hyperalgesia, increased blood flow (measured as paw temperature) and oedema (Carey et al, 1988a). BK has been reported to stimulate prostaglandin synthesis (Ferreira et al, 1973) and the possibility that the pro-inflammatory effects of BK may be secondary to prostaglandin production has been investigated.

Inflammatory responses were induced by subplantar injection of BK (100nmol) into the right hind paw of groups of six female AP strain rats (60-100g). Nociceptive pressure threshold, paw temperature and paw volume were measured as described previously (Carey et al, 1988a) and compared with responses in the left, vehicle-injected (0.1ml) paw at each time interval. Paw exudates were harvested by lavage with saline (Haworth and Carey, 1985) and PGE_2 measured using a specific radioimmunoassay. PGE_2 levels in lavages from non-injected paws were $0.62\pm0.19 ngml^{-1}$ (mean±s.e.m.), close to the detection limit of the assay ($0.5ngml^{-1}$), and increased to $1.18\pm0.28ngml^{-1}$ at 5min post-BK injection, although this was not significantly different from vehicle-injected paws at that time ($0.88\pm0.11ngml^{-1}$). PGE_2 levels were close to basal at 10min and 20min ($0.75\pm0.07ngml^{-1}$ and $0.70\pm0.08ngml^{-1}$ respectively). Dosing rats with the cyclo-oxygenase inhibitor flurbiprofen ($2mgkg^{-1}$ p.o.) 1h before BK injection had no effect on hyperalgesia (41±4% compared with 40±4% in controls) or oedema (49±4% compared with 45±2% in controls) but the increse of paw temperature was reduced from 16±2% to 10±2%. There was a synergistic interaction between BK (100nmol) and PGE_2 (3nmol) to enhance oedema (BK, 38±4% oedema, PGE_2, 5±3% and BK + PGE_2, 54±5%) but in contrast, there was no difference between the hyperalgesic responses to BK and PGE_2 alone (37±9% and 45±9% respectively) and in combination (41±15%) despite being submaximal (yeast produced 83±3% hyperalgesia).

Thus, while PGE_2 was slightly elevated in BK-inflamed paws and a proportion of the increased blood flow may have been the result of prostaglandin-induced vasodilatation, BK-induced oedema and hyperalgesia appeared to be independent of prostaglandin synthesis. The hyperalgesic action of BK may be a consequence of activation of protein kinase C in peripheral nociceptors subsequent to receptor-mediated phophoinositide hydrolysis by phospholipase C (Carey et al, 1988b; Bettaney et al, 1988) contrasting with the proposed mechanism of prostaglandin hyperalgesia via activation of adenylate cyclase (Ferreira and Nakamura, 1979). The results suggest that these putative mechanisms of hyperalgesia may not be additive.

Bettaney, J. et al (1988) Br.J.Pharmacol. 93, 276P.
Carey, F. et al (1988a) Eur.J.Pharmacol. 156, 161.
Carey, F. et al (1988b) Biochem.Soc.Trans. 16, 818.
Ferreira, S.H. and Nakamura, M. (1979) Prostaglandins 18, 179.
Ferreira, S.H. et al (1973) Br.J.Pharmacol. 49, 86.
Haworth, D. and Carey, F. (1985) In Inflammatory Mediators, Eds. G.A.Higgs and T.J.Williams, MacMillan, Basingstoke, p37.

IS ANTIFLAMMIN P1 A PHOSPHOLIPASE A_2 INHIBITOR?

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Research into the actions of phospholipase A_2 (PLA_2) and its relevance to inflammatory processes is complicated by the lack of selective inhibitors which are active in-vivo. Recently, antiflammin P1 (P1), a lipocortin and uteroglobin fragment, was reported to have in-vitro inhibitory activity against porcine pancreatic PLA_2 and in-vivo anti-inflammatory activity in the rat carrageenin paw oedema model (Miele et al, 1988). We have synthesised this nonapeptide and evaluated its effects both in-vitro and in-vivo.

Extracellular PLA_2 activity was assessed by incubating human polymorphonuclear leukocytes (PMN), prelabelled with ^3H-arachidonic acid (AA), with Naja Naja PLA_2; in these experiments the pre-labelled PMN provided the substrate for the exogenous enzyme. Assessment of intracellular PLA_2 activity was performed by incubating prelabelled PMN with melittin or FMLP. BSA (0.1%) was included in FMLP and Naja Naja PLA_2 incubates. In all experiments PMN ($2x10^7$/ml in PBS) were incubated with stimuli for 20 minutes at 37°C after a 10 minute preincubation with inhibitors. Anti-inflammatory activity was investigated using the rat carrageenin paw oedema model. Male rats (200g) were dosed po with indomethacin, dexamethasone or BW755C in 1ml 0.5% w/v hydroxy-methyl cellulose, 0.1% w/v polysorbate 80, as standards, one hour before the administration of carrageenin (0.1ml 10mg/ml in saline sub-plantar). P1 was dosed in 0.1ml PBS subplantar 2 minutes after the carrageenin. The rats were killed after 4 hours and the degree of oedema was assessed by weighing the injected and non-injected feet.

Naja Naja PLA_2 (50U/ml) and FMLP (5µg/ml) released 5.2%±1.9 (n=3) and 3.7%±1.9 (n=4) of the incorporated AA from PMN while melittin (20µg/ml) released 28.4%±12.4 (n=3). Pretreatment of the Naja Naja enzyme with 100µM p-bromophenacyl bromide inhibited the release of AA by 86% while pretreatment of the cells was without any effect. Naja Naja PLA_2 induced AA release was significantly inhibited by pretreatment of the PMN with 100µM mepacrine and 20µM aristolochic acid. AA release from PMN stimulated with either melittin or FMLP was significantly inhibited by mepacrin (100µM) and p-bromophenacyl bromide (100µM). P1 was without effect on Naja Naja PLA_2, FMLP or melittin induced AA release at concentrations up to 28µM. P1 was also without effect on LTB_4 release induced by melittin. CFHB rats were routinely used to study carrageenin paw oedema. Significant anti-inflammatory effects were seen with dexamethasone (1mg/Kg) (-59% $p<0.001$), an agent reported to induce lipocortin, indomethacin (5mg/Kg) (-25% $p<0.01$) and BW755C (100mg/Kg)(-25% $p<0.01$) but P1 showed no significant activity at doses up to 30mg/Kg. Similar results were obtained using Alderley Park and Sprague Dawley rats.

In conclusion P1 did not inhibit snake venom PLA_2 activity nor did it inhibit intracellular PLA_2 at concentrations 500 times those previously reported to inhibit pancreatic PLA_2 (Miele et al, 1988). In contrast to a previous report (Miele et al, 1988), P1 was not anti-inflammatory against carrageenin induced oedema. Studies are in progress to investigate the reasons for these divergent results but we conclude that P1 is not a useful pharmacological agent for evaluating the biological importance of PLA_2.

Miele, L., Cordella-Miele, E., Facchiano, A., and Mukherjee, A. (1988). Nature, 335, 726.

EFFECT OF INTERLEUKIN 1_A ON RESPONSES OF THE RABBIT AORTA *IN VITRO* TO DES-ARG^9-BK, 5-HT AND NORADRENALINE

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Two main types of bradykinin receptor have been described being designated B_1 and B_2 (Regoli & Barabé, 1980). The B_1 receptor can be characterised by the selective agonist des-Arg^9-BK and the selective competitive antagonist des-Arg^9-Leu^8-BK (Regoli & Barabé, 1980). The B_1-receptor is not normally present in vascular tissue but appears to be generated de-novo in vitro and in vivo being dependent on protein synthesis (Regoli et al, 1978; Whalley et al, 1983). A variety of substances including bacterial lipopolysaccharide, muramyl dipeptide, epidermal growth factor, endothelial growth factor and interleukins (1L) 1+2, but not tumour necrosis factor-α appear to enhance this protein-synthesis dependent up-regulation process in vitro (Bouthillier et al, 1987; Deblois et al, 1988). This study evaluates the action of $1L_{1\alpha}$ on this process in the rabbit aorta in vitro.

Male New Zealand White rabbits were killed with an overdose of pentobarbitone (60mgkg i.v.). Intact and de-endothelialised vascular rings of aorta were prepared as described previously (Nwator & Whalley, 1989) being placed in Krebs Henseleit solution at 37°C bubbled with 95% O_2 and 5% CO_2 under an isometric tension of 2g. At exactly time 1h and 4h after setting up the tissues concentration-effect curves were constructed to either des-Arg^9-BK, 5HT, NAd or KCl in the presence and absence of human recombinant $1L_{1\alpha}$ ($100ngml^{-1}$). The effect of cycloheximide ($7.2x10^{-5}M$) added at t=0h or at later times on responses to the above agents in the absence and presence of $1L_{1\alpha}$ was also studied.

On intact RbA responsiveness and sensitivity to des-Arg^9-BK but not NAd or 5-HT increased with time. Cycloheximide given at t=0h selectively blocked des-Arg^9-BK responses at t=1h and 4h. Incubation of the tissues with $1L_{1\alpha}$ from t=0h significantly enhanced responses to des-Arg^9-BK, significantly inhibited responses to 5HT and NAd and had no effect on response curves to KCl at t=1h and t=4h. When intact tissues were incubated with cycloheximide from t=1½h $1L_{1\alpha}$ significantly enhanced responses to des-Arg^9-BK and significantly inhibited responses to 5-HT and NAd at t=4h. When these experiments were repeated using de-endothelialised preparations sensitivity and responsiveness was seen to increase with time with des-Arg^9-BK but not 5-HT or NAd. Incubation of de-endothelialised tissues with $1L_{1\alpha}$ ($100ngml^{-1}$) had no effect on response curves to des-Arg^9-BK, or KCl at t=1h or t=4h but produced significant inhibition of responses to 5-HT and NAd.

These results demonstrate that $1L_{1\alpha}$ appears to produce enhancement of responses to the bradykinin B_1 agonist, des-Arg^9-BK on the rabbit aorta in vitro via a mechanism which does not involve protein synthesis in contrast to the conclusions of Deblois et al (1988). The effect appears to involve a sensitisation phenomenon which is dependent on the presence of an intact endothelium. In addition in contrast to the observations of Deblois et al (1988) $1L_{1\alpha}$ produced significant inhibition of responses to NAd in addition to 5HT an effect which is not dependent on the endothelium. The relevance of these results to local vascular control mechanisms requires further study.

Bouthillier, J., Deblois, D. & Marceau, F. (1987) Br. J. Pharmacol. 92, 257-264.
Deblois, D., Bouthillier, J. & Marceau, F. (1988) Br. J. Pharmacol. 93, 969-977.
Regoli, D. & Barabé, J. (1980) Pharmacol. Rev. 32, 1-46.
Whalley, E.T., Fritz, H. & Geiger, R. (1983) N-Schm.Arch.Pharmacol.324, 296-301.
Nwator, A.A. & Whalley, E.T. (1989) Eur. J. Pharmacol. 160, 125-132.
Regoli, D., Marceau, F. & Barabé, J.(1978) Can.J.Physiol.Pharmacol. 56, 674-677.

INTERACTIONS BETWEEN PALMITOYL CARNITINE AND VASOCONSTRICTORS IN THE MESENTERIC VASCULAR BED AND AORTA OF THE RAT

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Acyl carnitines accumulate in the ischaemic myocardium and have been reported to increase myocardial alpha adrenoceptor numbers (Heathers et al 1987). but their actions on vascular tissues have received little attention. For this reason we have studied the interactions of palmitoyl carnitine(PC) and the vasoconstrictor alpha agonist phenylephrine in the isolated perfused mesenteric vascular bed and thoracic aortic ring preparation. For comparison, the effects of two other vasoconstrictors endothelin and high potassium have also been studied. Mesenteric arteries were perfused at $4ml.min^{-1}$ and perfusion pressure recorded; aortas were set up for isometric recording under a resting tension of 2g. The physiological medium was Krebs-Henseleit solution at 37° with a potassium concentration of 5.9mM. When the potassium level was increased NaCl was reduced to maintain osmolarity.

In the mesenteric artery, bolus injections of PC(10-300nmoles) elicited a dose dependent vasoconstriction. If the tone of the preparation was raised from 31.0+2.5mmHg to 143+4.5mmHg (n=9) with phenylephrine(10UM) this caused a large potentiation (up to 400%) of the vasoconstrictor response to PC which was followed by a small dilator component. In order to see if this potentiation of the vasoconstrictor response to PC was simply the result of the raised basal tone, experiments were performed with basal tone raised by high K^+. At 65mM K^+ tone was increased to 53.8+2.6mmHg(n=4) and the response to PC was not significantly altered. With 110mM K^+ tone was increased to 154.9+10.0mmHg (n=8), under these conditions the response to low doses of PC(10-30nmoles) varied, half of the preparations showed dilation while the others showed constriction. The vasoconstrictor responses to the higher doses of PC(100-300nmoles) were potentiated under these conditions. In contrast to the interaction seen with phenylephrine, when tone was increased to 150.4+12.0mmHg with endothelin(1nM), the vasoconstrictor action of PC was converted to a marked dilator response with falls in perfusion pressure of 100mmHg being recorded at the highest doses used(n=5).

In aortic ring preparations PC had no effect on basal tension upto concentrations of 64uM. In preparations where basal tone had been raised by 110mM K^+ or phenylephrine (100nM), PC produced a gradual relaxation reaching a maximum of ~30% at the highest concentrations used. In endothelin(1nM) contracted aortae PC had no significant effect on the raised tone(n=4)

In conclusion PC contracts small resistance vessels more than large vessels and there are complex interactions between PC and agonist induced vascular responses which could be important in those situations where PC levels are elevated pathologically.

D.N.C. is an SERC/CASE student.

Heathers G.P.,Yamada K.A.,Kanter E.M. & Corr P.B.(1987) Circ.Res.61 735-746.

LIGUSTRAZINE, A CHINESE HERBAL EXTRACT, DILATES HUMAN PULMONARY RESISTANCE VESSELS

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Ligustrazine is a chemically identified and semisynthesized compound composed mainly of an extract from a traditional Chinese herb. Ligustrazine inhibits hypoxic pulmonary vasoconstriction in isolated perfused rat lungs(Cai & Suggett, 1988) and the right lower lobe of ferrets (Cai et al, 1988) and when given chronically, inhibits the development of pulmonary hypertension in rats exposed to 10% O_2 for two weeks (Cai & Barer, 1989). Ligustrazine also causes endothelium-independent relaxation of precontracted human small pulmonary arteries (Liu et al, 1989). The aim of present study was to compare the vasodilator effects of ligustrazine on human large and small pulmonary arteries and to investigate its mechanism of action.

Human pulmonary arteries were obtained from patients undergoing thoractomy. Rat pulmonary arteries and aorta were dissected from Wistar rats (250 g). The vessels were cut into rings of 3-4 mm in length and mounted over a pair of rigid wires connected to a force transducer and suspended in organ bath containing Krebs-Hesenleit solution (37^{O} C). In relaxation studies human and rat pulmonary arteries were precontracted with prostaglandin $F_{2\alpha}$ (1 uM), serotonin (1uM) and phenylephrine (0.2 uM) and dose-response curves to ligustrazine were obtained. In the Ca^{2+}-free solution studies, dose-response curves to calcium were constructed before and after incubating the rat aorta with different concentrations of ligustrazine for 30 min in calcium-free K^{+}-depolarizing medium.

The -log IC_{50} values for ligustrazine causing relaxation in human small (300-500 uM i. d.), and lobar pulmonary arteries (5-7 mm i. d.) were 4.36 $\pm$ 0.11, and 3.33 $\pm$ 0.14 respectively, with a significantly ($P < 0.05$) greater effects on small than on large vessels. Ligustrazine was equally potent in relaxing $PGF_{2\alpha}$- or 5-HT-precontracted arteries and pretreatment of the vessels with propranol (1 uM) or indomethacin (10 uM) had no significant effect on its vasodilator effects. Thus ligustrazine is not a $PGF_{2\alpha}$ or 5-HT antagonist and neither β-adrenoceptors nor cyclooxygenase products are involved in its vasodilator action. Ligustrazine caused dose-dependent inhibition of calcium induced contraction in calcium-free K^{+}-depolarizing medium and a Schild plot gave a PA_2 value of 3.42.

Our results demonstrate that ligustrazine is a vasodilator of human pulmonary artery in vitro with a preferential effect on small resistance vessels rather than large conduit pulmonary arteries. Ligustrazine is probably a naturally occuring calcium antagonist.

Cai, Y. & Suggett, A.J. (1988): J. Physiol., 399, 45P.
Cai, Y. Bee, D. & Barer, G.R. (1988): Clin. Sci. 75 (suppl 19), 55P.
Cai, Y. & Barer, G. R. (1989): Clin. Sci. in press.
Liu, S.F. et al. (1989): Clin. Sci. 76 (suppl), 27P.

LOW REFLOW AND REPERFUSION INJURY IN A RAT MODEL OF ACUTE LOWER LIMB ISCHAEMIA

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Much interest currently centres on the changes in blood flow during reperfusion following acute ischaemia (Feng et al. 1988). Drugs influencing free radical production, eicosanoid synthesis, leucocyte activity, or peptide factors might theoretically be able to modify these changes and could be of considerable benefit if given following arterial surgery upon the acutely ischaemic lower limb. To evaluate their effect we have developed a simple model in the rat demonstrating the changes in muscle perfusion following acute lower limb ischaemia.

Male Sprague Dawley rats (400-500g) were anaesthetised with phenobarbitone 40 mg/kg i.p. A rubber band tourniquet was placed around the limb to occlude the circulation for up to 6 hours. The left internal jugular was cannulated and also the left ventricle via the right carotid artery. The tourniquet was removed to allow reperfusion and 15 μm radioactive microspheres (Sc^{46}, St^{85}, and Ce^{141}) were injected intraventricularly after 0, 10, 60, 120 and 240 min of reperfusion. The ratio of radioactivity in the gastrocnemius muscle of the ischaemic perfused limb to the other limb was subsequently calculated to give an index of muscle microcirculatory blood flow.

Table 1. Changes in the reperfusion index of lower limb microcirculatory blood flow following cessation and restoration of blood flow.

Treatment	N	Time following tourniquet removal (mins) 0	10	60	120	240
		Reperfusion Index (median and i.q. range)				
Controls	10	1.05 0.68-1.18		0.97 0.79-1.13		
6 hrs Ischaemia	10	0.15** 0.04-0.23	0.08* 0.01-0.51	0.18* 0.03-0.49	0.48* 0.11-0.75	0.05** 0.01-0.28

*P<0.05, **P<0.01 vs controls Mann Whitney

Muscle blood flow during reperfusion was shown to demonstrate 3 phases. There was an initial low reflow with blood flow only one tenth that of normal values. This was followed by a relative hyperaemia during which the flow increased three to four fold and the limb became red and oedematous. Finally, there was a return to low flow values (reperfusion injury). Although during this latter period the limbs were slightly swollen, their colour was normal, with nothing to suggest that the muscle microcirculation had such a poor blood supply.

The aetiology of low reflow, as well as the increased capillary permeability and reperfusion injury are all poorly understood. A number of patho-physiological mechanisms would seem to be operating during the different stages (Feng et al. 1988; Koithuis et al. 1985) and the scope for pharmacological manipulation may be large. However, using this model, compounds capable of influencing many aspects of the ischaemic process can be assessed for activity during reperfusion following lower limb ischaemia.

Feng,L-J. et al. (1988) Plast. Reconstr. Surg. 81, 755-764
Korthuis, R.J. et al. (1985) Circ. Res. 57, 599-609

SELECTIVE INFLUENCE OF ENDOTOXIN ON CATECHOLAMINE CONTENT OF RAT TISSUES

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Bacterial infection in man and experimental animals is generally associated with raised plasma levels of catecholamines (Benedict & Grahame-Smith, 1978; Smith et al., 1977). However, measurement of circulating catecholamines permits no conclusion about the sympathetic nervous system (SNS) activity in individual tissues. Measurement of dopamine [D] accumulation (and the decline in noradrenaline [NA]) following pharmacological blockade of dopamine ß-hydroxylase (DßH)(EC 1.14.2.1), the enzyme which catalyzes the hydroxylation of D to NA, is an effective means of assessing SNS activity in specific tissues (Fuller et al., 1982). The present work used DßH inhibition to assess SNS activity in various tissues of rats treated 24 h previously with bacterial endotoxin.

Twenty-four male Sprague-Dawley rats (150-175 g) were divided into three weight-matched groups: the endotoxin group was given a s.c. injection of E. Coli endotoxin (1 mg/kg) while the two other groups received saline. One group receiving saline was pair-fed the same quantity of food consumed by the endotoxin group. Body temperature and weight and food intake were then monitored over the ensuing 24 h period whereupon all the rats were injected with disulfiram (400 mg/kg, i.p.), a DßH inhibitor. Two hours thereafter rats were killed and tissues were rapidly removed and frozen in liquid nitrogen. Catecholamines were extracted from acid homogenates of the tissues by standard alumina techniques. [D] and [NA] were determined by high performance liquid chromatography.

Body temperature was elevated nearly 0.5°C 24 h after endotoxin injection while food intake and final body weight were reduced nearly 25% and 5%, respectively ($p<0.05$) compared to the ad libitum-fed controls. Spleen, heart, liver and interscapular brown adipose tissue (IBAT) weights were greater in endotoxin injected rats compared to pair-fed animals, while the weight of gastrocnemius muscle (GM) was reduced. The [NA] in each tissue studied, except GM and IBAT, was similar in the control and pair-fed rats. [NA] was reduced by 25-30% in heart, liver, and IBAT of endotoxin treated rats ($p<0.05$) with no change in GM and spleen. Endotoxin caused a near doubling in [D] of heart, GM, IBAT and liver ($p<0.01$) compared to controls while it seemed to have no effect on the spleen. The results suggest that endotoxin causes increased SNS drive to heart, liver, IBAT and possibly GM of rats.

Benedict, C.R. & Grahame-Smith, D.G. (1978) Q. J. Med. 185, 1-20.
Fuller, R.W., Snoddy, H.D. & Perry, K.W. (1982) Life Sci. 31, 563-570.
Smith, I.M., Kennedy, L.R., Regne-Karlsson, M.H., Johnson, V.L. & Burmeister, L.F. (1977) Am. J. Clin. Nutr. 30, 1285-1288.

PARALLEL FORMATION OF IMP AND ADENOSINE FROM ADENINE NUCLEOTIDES AT THE INNERVATED FROG SARTORIUS MUSCLE

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It has been suggested that ectonucleotidases are involved in the formation of adenosine from released adenine nucleotides at the frog neuromuscular junction (Ribeiro & Sebastiao,1987). In the present work we investigated the degradation kinetics of exogenously applied adenine nucleotides at the innervated frog sartorius muscle preparation.

The preparations were mounted in a 2 ml bath at room temperature (22-25°C). The bathing solution (pH=6.8) contained (mM): NaCl 117, KCl 2.5, $CaCl_2$ 1.8, $MgCl_2$ 1.2, NaH_2PO_4 1, Na_2HPO_4 1. Unless otherwise stated, solutions contained dipyridamole (0.5 µM). 10 µM of ATP or of its metabolites were incubated with the preparations. Throughout the assay air was bubbled into the bath to facilitate diffusion. Samples of 75 µl were collected from the bath at different times up to 120 min, and analysed by reverse-phase HPLC.

ATP was degraded into ADP, AMP, IMP, adenosine (Ado) and inosine (Ino). The first substance to appear was ADP, which reached a peak concentration (3.3±0.1 µM) at 18±1 min (n=8). Neither ADP (50µM, n=3) nor p-nitrophenylphosphate (1 mM, n=3), a substrate for phosphatases, modified the kinetics of degradation of ATP. ADP was degraded into AMP, IMP, Ado and Ino. The first substance to appear was AMP, which reached a peak concentration (4.1±0.2 µM) at 13±1 min (n=3). The kinetics of degradation of ADP was not modified by AMP (50 µM, n=1). AMP was degraded into IMP, Ado and Ino. Peak concentrations of IMP and Ado were 4.0±0.1 µM and 2.0±0.1 µM, respectively; both attained peak concentrations at 62±1 min (n=19). IMP (n=7) and Ado (n=9) were both degraded into inosine. Half degradation times of ATP, ADP, AMP, IMP and Ado occurred at 10±0.7 min (n=8), 10±1 min (n=3), 19±0.7 min (n=19), 50±7 min (n=7) and 63±2 min (n=9) respectively. In the absence of dipyridamole, the disappearance of Ado was faster (half degradation time: 52±3 min, n=3) and the formation of Ino was enhanced (46±9% increase at 90 min). The adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA, 5-25 µM), applied in the presence of dipyridamole, did not modify the adenosine degradation kinetics (n=4). The 5'-nucleotidase inhibitor, α,β-methylene ADP (AOPCP, 200 µM) inhibited by 100% adenosine formation from AMP and inosine formation from IMP. In the absence of exogenously applied nucleotides or nucleosides, only trace amounts of inosine (0.19±0.01 µM at 120 min, n=25) were formed. All the other compounds could not be detected.

The results indicate that ATP can be sequentially degraded into AMP at the frog sartorius muscle. Degradation of AMP might occur through deamination yielding IMP, as well as through 5'-nucleotidase, yielding Ado. Formation of Ino from IMP might occur extracellularly through the action of 5'-nucleotidase, whereas formation of Ino from Ado probably occurs intracellularly.

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Ribeiro, J.A. & Sebastião A.M. (1987) J.Physiol. 384,571-585

THE ANABOLIC ACTION OF A B_2-ADRENOCEPTOR AGONIST CLENBUTEROL, ON MUSCLE, IS MEDIATED THROUGH B_2-ADRENOCEPTOR STIMULATION

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It has been demonstrated that some β_2-adrenoceptor agonists such as clenbuterol and cimaterol increase skeletal muscle protein content in experimental animals (Stock and Rothwell, 1986). However, this anabolic action has not yet been shown to be due to β_2-adrenoceptor stimulation and Reeds et al. (1988) have reported that a nonselective β-antagonist propranolol fails to inhibit the increase in muscle protein content by clenbuterol. We have examined whether a selective β_2-antagonist ICI 118551 is able to modify the action of clenbuterol on muscle protein. In addition, the effects of propranolol were also retested.

Male, Wistar rats (170g) were divided into four groups (n=7). One group was fed a normal powdered diet whereas the other three groups received diets containing either clenbuterol (4mg/kg), clenbuterol (4mg/kg) plus propranolol (200mg/kg), or clenbuterol (4mg/kg) plus ICI 118551 (200mg/kg). All diets were fed ad libitum. After 4 days animals were killed by cervical dislocation, and gastrocnemius muscles were immediately dissected for the determination of protein and RNA content.

Administration of clenbuterol significantly increased the mass, protein and RNA content, and ratio of RNA to protein of muscle by 15, 16, 31 and 13%, respectively. The latter effect suggests that clenbuterol stimulated muscle protein synthesis. In agreement with previous observations (Reed et al., 1988), none of these effects of clenbuterol was inhibited by propranolol (e.g. muscle protein content: control, 140±4; clenbuterol, 163±6; clenbuterol plus propranolol, 157±5mg). However, ICI 118551 completely prevented all of these effects of clenbuterol. There was no difference in any of the parameters compared in control and clenbuterol plus ICI 118551 rats (e.g. muscle protein content: clenbuterol plus ICI 118551, 137±4mg; $P<0.001$ vs clenbuterol).

These results indicate that the anabolic action of clenbuterol on muscle protein is mediated by β_2-adrenoceptor stimulation. The failure of propranolol to inhibit clenbuterol action might be due to its low affinity for muscle adrenoceptors.

Reeds, P.J., Hay, S.M., Dorward, P.M. & Palmer, R.M. (1988) Comp. Biochem. Physiol. 89C(2), 337-341.

Stock, M.J. & Rothwell, N.J. (1986) in 'Control and manipulation of animal growth': Buttery, P.J., Hayes, N.B. & Lindsay, D.B. (ed.), pp249-257. London, Butterworths.

VASCULAR AND RENAL CLEARANCE RESPONSES TO ATRIAL NATRIURETIC FACTOR ADMINISTRATION IN THE RAT

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The mechanisms responsible for atrial natriuretic factor (ANF) induced diuresis and natriuresis remain unclear, although both glomerular and tubular actions have been implicated (Baxter et al., 1988). Accordingly, the renal actions of ANF have been examined in rats infused with para-amino-hippuric acid (PAH) ^{3}H inulin and lithium chloride as markers of effective renal plasma flow, glomerular filtration rate (gfr) and proximal tubule reabsorption (Thomsen, 1984), respectively.

Inactin (5-ethyl - 5- (1' - methyl propyl 1) - 2 thiobarbiturate) anaesthethised (0.11g/kg) male Sprague-Dawley rats underwent jugular vein, urinary bladder and femoral artery cannulation and a tracheostomy was performed. Animals were placed on continuous i.v. hypotonic saline (0.067M NaCl, 2mmol L_iCl, 9.5 mmol PAH, 0.6 μC_i/^{3}Hinulin) infusion at 150 µl/min. After a 3h equilibration period and 1h of control collections, in half of the rats the infusate was switched to one of identical composition but containing rat-ANF (rANF, 28aa, Penninsula) at 75ng/min. Mean arterial blood pressure (MAP) was monitored continuously and urine collected every 20 min. Blood samples (600 µl) were taken from the femoral artery at 3h, 4.3h, 5.6h and at 7h. Values are presented as mean ± s.e. mean and comparisons are by paired or unpaired t-test as appropriate.

Table 1. Fluid and Electrolyte Excretion and PAH, Inulin and Lithium Clearances during ANF Administration

	Control n = 6	rANF (75ng/min) n = 5
MAP mm Hg	113.7±2.2	110.6±3.6
Inulin clearance µl/min	2,370±363	3,031±1234
PAH clearance µl/min	11,832±1632	10,052±854
L_i clearance µl/min	1,483±118	2,438±275*
Urine flow µl/min	128.3±8.2	296.8±18.2 ***
Na^+ excretion µmol/min	9.2±1.5	32.0±2.0 ***

Comparisons between Control (vehicle) and rANF treated groups are by t-test *$p<0.05$, ***$p<0.001$.

The addition of rANF to the infusate was associated with a rapid diuretic and natriuretic response which was maximal during the initial 20 min clearance period. Fractional Na^+ reabsorption fell from 91.8±2.0% to 85.3±3.3% ($p<0.02$). Table 1 indicates the contrasting measures in control and ANF treated groups during this 20 min period. There were no significant changes in renal plasma flow (PAH), or mean blood pressure, but gfr's were clearly very variable. The increase in urine flow and Na^+ excretion was, however, associated with an increased rate of L_i clearance. Clearance of lithium has been reported (Thomsen, 1984) to provide a reliable indication of proximal tubule handling of sodium.

Thus the current work provides no indication of altered renal vascular activity, but supports a possible proximal tubule site of ANF action. Reduced Na^+ reabsorption proximally would not only lead to increased Na^+ excretion, but also to elevated tubular fluid flow which may contribute to the associated diuresis.

P.A. Sainsbury is an SERC scholar.

Baxter, J.D., Lewichi, J.A. & Gardner, G. (1988) Biotechnology 6, 529-546.
Thomsen, K. (1984) Nehpron 37, 217-223.

ATRIAL NATRIURETIC FACTOR ANTAGONISES CENTRAL VASOPRESSIN AND ANGIOTENSIN II NATRIURETIC ACTION IN THE ANAESTHETISED RAT

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Vasopressin (AVP), angiotensin II (ANG II) and atrial natriuretic factor (ANF) play an important role in peripheral mechanisms of water and sodium management. Whilst ANF antagonises the peripheral actions of AVP, (Lynch et al, 1986) and ANG II (McMurray & Struthers, 1988) on renal water and sodium handling, the interaction of these peptides centrally is still unclear.

Under Inactin (5-ethyl-5- (1'- methyl propyl)-2-thiobarbiturate) anaesthesia (O.1 g/kg, i.p.) male Sprague-Dawley (3OO-35O g) rats were placed on continuous i.v. hypotonic saline infusion (O.O77M NaCl, 15O μl/min) following jugular vein, femoral artery and urinary bladder cannulation. A further cannula was located stereotaxically in the lateral ventricle. Following a 3h equilibration period, three 2O min control urine collections were made while all animals received intracerebroventricular(icv) vehicle (O.154M NaCl, O.8μl/min). Then for 8Omin animals received i.c.v. either ANG II (O.O9ng/min Val^5 - All, Sigma), or ANG II + ANF (O.4ng/min rANF, 28aa, Penninsula), or AVP (3.Opg/min Grade V, Sigma) or AVP + ANF. Values are mean $\pm$ SE and comparisons between groups are by t-test, paired and unpaired as appropriate.

By comparison with the control period, the 8O min of central ANG II infusion was associated with decreased urine flow (μl/min) (128.4+ 1O.6 vs 9O.9+ 7.7, n=9, p<O.O5), and increased sodium excretion (5.8+O.7 vs 17.4+1.7 μmol/min, n=9, p<O.OO1). Central AVP infusion was associated with increased urine flow (149 $\pm$ 11.4 vs 186.6 $\pm$ 5.7, n=7, p<O.O2) and elevated sodium excretion (12.1+1.7 vs 18.5+1.1, n=7, p<O.O1). Co-administration of ANF did not alter the antidiuretic response to ANG II (ANG II + ANF 1O1.3+ 15.3 μl/min, n = 7) nor the diuretic response to central AVP (AVP +ANF 174.3+5.8 μl/min, n = 9). However, co-administration of ANF abolished natriuretic responses to both ANG II (ANG II + ANF 9.6$\pm$1.6 μmol/min, n = 7, p<O.O1) and AVP (14.8$\pm$O.9 μmol/min, n = 9, p<O.O5).

It appears that the central actions of ANG II and AVP to influence renal water handling are effected through different brain sites from those which mediate the associated natriuretic responses. Only the latter sites are antagonised by the i.c.v. administration of ANF.

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Lynch, D.R., Brass, K.M. & Snyder, S.H. (1986) Proc. Nat. Acad Sci. (USA) 83 3357-3361.
McMurray, J.K. & Struthers, A.D. (1988) Clin. Sci. 74, 419-425

AFFINITIES OF 5-HT UPTAKE INHIBITORS FOR 5-HT$_3$ RECEPTORS IN BOTH BINDING AND FUNCTIONAL STUDIES

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5-Hydroxytryptamine (5-HT) receptors of the 5-HT$_3$ type have been identified in brain tissue using ligands such as ^{3}H-GR67330, ^{3}H-zacopride and ^{3}H-quipazine. 5-HT$_3$ receptor affinity (K_i) values derived from these binding assays correlate well with those obtained in functional 5-HT$_3$ receptor assays (Kilpatrick et al., 1987). Schmidt and Peroutka (1989) have recently reported that antidepressants, 5-HT uptake inhibitors and mianserin, have high affinities for 5-HT$_3$ sites labelled with ^{3}H-quipazine. This contrasts with a functional model of 5-HT$_3$ receptors, 5-HT-induced depolarization of the rat vagus nerve, in which paroxetine has only low affinity (Ireland et al., 1987). We have examined four 5-HT uptake inhibitors and mianserin for their abilities to inhibit ^{3}H-GR67330 binding to 5-HT$_3$ receptors and to antagonise 5-HT-induced depolarizations of the rat isolated vagus nerve (RVN).

^{3}H-GR67330 (0.1nM) binding to 5-HT$_3$ sites in the rat entorhinal cortex and 5-HT-induced depolarizations of the RVN were assayed as described previously (Kilpatrick et al., 1989; Ireland et al., 1987). Affinity (pK_B) values in the latter preparation were calculated from single antagonist concentrations using the equation: pK_B=log(dose-ratio-1)-log[antagonist]. Affinity (pK_i) values in the receptor binding assays were calculated using the computer program LIGAND. The results obtained are given in Table 1.

Table 1 The affinity of 5-HT uptake inhibitors and mianserin for 5-HT$_3$ receptors

	Affinity values			
	^{3}H-GR67330	RVN	^{3}H-quipazine*	^{3}H-paroxetine*
	pK_i	pK_B	pK_i	pK_i
Paroxetine	6.4	<6	8.3	8.9
Fluoxetine	5.2	<6	8.0	7.8
Imipramine	5.7	<6	7.0	7.4
Des-methylimipramine	5.5	<6	6.4	6.3
Mianserin	7.6	6.9	7.2	4.6

* Schmidt & Peroutka, 1989

Mianserin has affinity for both 5-HT$_3$ binding sites and functional receptors. None of the remaining compounds in Table 1 showed agonist or antagonist activity on the RVN at 10^{-6}M. Furthermore, affinity values obtained against ^{3}H-GR67330 binding, were uniformly low. Thus, there is a clear disparity when comparing these models with the high affinity values obtained for ^{3}H-quipazine binding by Schmidt and Peroutka. The relatively high affinity of mianserin for ^{3}H-quipazine binding sites is consistent with its affinity for 5-HT$_3$ receptors reported previously (Hoyer and Neijt, 1987). There are two likely explanations for the discrepancies; either ^{3}H-quipazine labels a subtype of the 5-HT$_3$ receptor with a high affinity for 5-HT uptake inhibitors or, more likely, ^{3}H-quipazine, or a metabolite, labels the 5-HT uptake site as well as the 5-HT$_3$ receptor.

Hoyer, D & Neijt, H.C. (1988) Mol. Pharmac. 33, 303-309
Ireland, S.J. et al. (1987) Br. J. Pharmac. 90, 151-160.
Kilpatrick, G.J. et al. (1987) Nature 330, 746-748.
Kilpatrick, G.J. et al. (1989) Br. J. Pharmac., 96, 8P.
Schmidt & Peroutka (1989) Eur. J. Pharmac. 163, 397-398.

PRELIMINARY STUDIES ON THE ACTION OF 8-OH-DPAT ON THE HUMAN BASILAR ARTERY *IN VITRO*

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Previous studies have demonstrated that 5-hydroxytryptamine (5-HT) is a potent contractile agent in human basilar artery (hba) in vitro (Forster & Whalley, 1982). This effect is thought to be mediated via activation of a $5HT_{1-like}$ receptor, since GR43175, a selective agonist at this receptor (Humphrey et al, 1988) contracts the hba, an effect which is blocked by the $5HT_{-1-like}$ antagonist methiothepin (Parsons et al, 1989). We have also demonstrated that the $5HT_{1A}$ ligand 8-OH-DPAT (Middlesmiss & Fozard, 1983) also contracts the hba in vitro (Parsons et al, 1989). The effect of 8-OH-DPAT on hba was studied further and compared with a second $5HT_{1A}$ ligand flesinoxan (Wouters et al, 1988).

Human basilar arteries were obtained 6-18h post mortem and set up for isometric recording of tension as described previously (Parsons et al, 1989). Following a 2h equilibration period in Krebs Henseleit solution at 37°C bubbled with 95%O_2 and 5%CO_2 a cumulative concentration-effect (C-E) curve to 5-HT was constructed and repeated 1h later on all tissues. After a further 1h C-E curves were constructed to either 5-HT, 8-OH-DPAT or flesinoxan. The effect of phentolamine was studied against nor-adrenaline (NAd), 5-HT, 8-OH-DPAT and phenylephrine (PhE). Methiothepin, labetalol and flesinoxan were studied against 5-HT, 8-OH-DPAT and U-46619.

5-HT, 8-OH-DPAT and flesinoxan produced concentration-related contractions of hba the pD_2 values (± s.e.m.) for each being 6.99±0.17 (n=17) 5.73±0.05 (n=13) and 5.49 ± 0.01 (n=4) respectively. The maximum responses obtained for 8-OH-DPAT and flesinoxan relative to 5-HT were 43.5±10.3% and 7.0±2.3% respectively. Analysis of the effect of phentolamine against NAd was made difficult due to the biphasic (contraction at low and relaxation at high concentrations) nature of the response curve. A pA_2 of 7.95 (7.52 - 8.37, 95% C.l n=5) and slope of 1.12 (0.9 -1.3, 95% C.l) was obtained for phentolamine against PhE. Phentolamine (1 and 10μM) produced significant inhibition of 8-OH-DPAT responses. Methiothepin (100nM) produced significant inhibition of both 5-HT and 8-OH-DPAT but not U-46619 responses. In the presence of flesinoxan (10μM) responses to 8-OH-DPAT were almost totally blocked and those to 5HT but not U-46619 significantly attenuated.

These preliminary results suggest that 8-OH-DPAT and flesinoxan produce contraction of the hba in vitro via activation of the same receptor. Whether this receptor is of the same $5HT_{1-like}$ type via which GR43175 produces contraction requires further study.

Forster, C. & Whalley, E.T. (1982) N-Sch. Arch. Pharamcol. 319, 12-17.
Humphrey, P.P.A., Feniuk, W., Perren, M.J., Connor, H., Oxford, A.W., Coates, I.H. & Butina, D. (1988) Br. J. Pharmacol. 94, 1123-1132.
Middlemiss, D.N. & Fozard, J.R. (1983) Eur. J. Pharmacol. 90, 151-153.
Parsons, A.A., Whalley, E.T., Feniuk, W., Connor, H.E. & Humphrey, P.P.A. (1989) Br. J. Pharmacol. 96, 434-449.
Wouters, W., Tulp, M.Th.M. & Bevan, P. (1988) Eur. J. Pharmacol. 149, 213-223.

AUGMENTATION OF 5-HT CONSTRICTOR RESPONSES IN SHEEP MIDDLE CEREBRAL ARTERIES BY HAEMOLYSATE

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Following cerebral haemorrhage, lysed blood surrounding cerebral arteries may release products that cause vasoconstriction. We have investigated the interaction of two blood products, 5-HT and haemolysate, applied to intraluminal and extraluminal surfaces of the middle cerebral artery (MCA) of the sheep.

Sheep heads were obtained from an abattoir and the MCA dissected free from the brain. The MCA was either cut into rings 3-4 mm long that were suspended in Krebs solution and contraction recorded isometrically or was perfused after ligation of all major side branches. Some tissues were perfused intraluminally with triton X-100 to remove the endothelium. In perfusion experiments, the cannulated artery was connected to a perfusion system which allowed changes in the intraluminal and extraluminal bathing solution (Krebs solution, 37°) to be carried out independently. The flow rate was set at 1.0ml/min for 30 minutes and then increased to 3.0ml/min for the duration of the experiment. The tissues were then left for a 30 minute equilibration period before treatment. Bolus doses of 5-HT (1μM, 10μM and 100μM) were infused intraluminally every 10 minutes until reproducible concentration-effect (C-E) curves were obtained. Haemolysate (10μl/ml) was added extraluminally and 5-HT infusions were repeated to obtain reproducible responses. Haemolysate (10μl/ml) was then added intraluminally and 5-HT infusions were repeated until reproducible responses were obtained. All responses were expressed as a percentage of the responses obtained with 100μM 5-HT in the absence of haemolysate.

In ring preparations, haemolysate increased the maximal contraction caused by 5-HT (from 0.80±0.17 to 1.3±0.19g) but had no effect on the pD_2 (control 6.87±0.08 haemolysate 6.96±0.06). Haemolysate had no effect on endothelium-dunuded rings. In perfused MCA (both endothelium intact and denuded) extraluminal haemolysate had no effect on resting pressure. Intraluminal haemolysate increased resting pressure only in the endothelium intact preprations (11.0±2.2mmHg, n=6). In endothelium intact perfused MCA, extraluminal haemolysate significantly increased the changes in perfusion pressure observed with 1μM, 10μM and 100μM 5-HT from 11±2%, 36±6% and 100% to 23±6%, 47±6% and 120±7% respectively. Intraluminal infusion of haemolysate caused a further increase in the responses elicited by 5-HT to 40±7% (1μM), 102±9% (10μM) and 151±8% (100μM). In endothelium-denuded preparations haemolysate did not cause any augmentation of constrictor responses.

These results indicate that, in the sheep MCA, haemolysate can cause vasoconstriction and augmentation of the vasoconstrictor responses of 5-HT but only in the presence of an intact endothelium. Haemolysate causes substantial augmentation when applied to the external surface, suggesting an interaction that may contribute to vasospasm following cerebral haemorrhage.

BMY 7378: ANTAGONIST EFFECTS AT FUNCTIONAL 5-HT_{1A} RECEPTORS

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BMY 7378 has a high affinity and low efficacy at 5-HT_{1A} receptors in rat and guinea-pig hippocampal membranes (Yocca et al., 1987). We have investigated the antagonist effects of BMY 7378 in two functional models of 5-HT_{1A} receptor activation. Proximal ileum was removed from male guinea-pigs (250-350g) and segments were set up in modified Krebs solution (mM: Na^+ 143.4, K^+ 5.9, Mg^{2+} 0.6, Ca^{2+} 0.65,Cl^- 123.2, $H_2PO_4^-$ 1.2, SO_4^{2-} 0.6, HCO_3^- 25.0, glucose 11.1). Electrical field stimulation was applied (0.5msec, 0.1Hz, supramaximal voltage) and changes in isometric tension were recorded. Male DOCA/salt hypertensive rats (Lister Hooded, 250-350g) were anaesthetised (thiobutobarbitone 120mg/kg i.p.). Blood pressure (BP) and heart rate (HR) were recorded via a cannula in the carotid artery; agonists were injected via the jugular vein. BMY 7378 or vehicle (distilled water, 1ml/kg) were administered s.c. 1 hour previously.

8-OH-DPAT and flesinoxan caused a concentration-related inhibition of twitch height in the field stimulated guinea-pig ileum; the EC_{20} values (concentrations producing 20% inhibition) were 37 (22-64)nM and 19(10-36)nM respectively (geometric mean (range) n = 4). BMY 7378 (0.1μM) antagonised the effects of 8-OH-DPAT and flesinoxan; apparent pA_2 values were 7.7 ± 0.1 and 7.9 ± 0.1 respectively, n = 4. This concentration of BMY 7378 alone had little effect on the twitch height and also did not modify clonidine-induced inhibition of the twitch contraction (concentration ratio = 1.3 (0.5-2.3) geometric mean (range), n = 4).

In anaesthetised vehicle (V)-pretreated rats, 8-OH-DPAT (10μg/kg i.v.) decreased BP and HR; maximum changes are shown in Table 1. In contrast, in animals pretreated with BMY 7378, 0.5mg/kg s.c., (BMY), the effects of 8-OH-DPAT were reduced. In separate experiments, although this dose of BMY 7378 reduced resting BP and HR in anaesthetised DOCA hypertensive rats, the vasodepressor responses to the 5-HT_1-like receptor agonist, 5-carboxamidotryptamine (10-100ng/kg i.v.), were not attenuated by BMY 7378 and indeed, the pressor responses to phenylephrine (3-30μg/kg i.v.) were enhanced.

Table 1 Effect of BMY on 8-OH-DPAT-induced hypotension and bradycardia

Treatment μg/kg i.v.			Basal BP (mmHg)	ΔBP	Basal HR (bpm)	ΔHR
V	8-OH-DPAT	10	138 ± 10	-40 ± 3	396 ± 21	-30 ± 5
BMY	8-OH-DPAT	10	141 ± 11	-10 ± 5**	298 ± 15**	-8 ± 5*

Resting mean BP and HR in V or BMY pretreated rats and maximum changes produced by 8-OH-DPAT 10μg/kg i.v. Values are means ± S.E.M., n = 4-5; *P<0.05, **P<0.01, Student's t-test compared to corresponding V control.

In conclusion, these results demonstrate that BMY 7378 acts as a relatively potent and selective 5-HT_{1A} receptor antagonist in two functional models of 5-HT_{1A} receptor activity. BMY 7378 is therefore a useful tool to evaluate activity at this receptor type.

Yocca, F.D. et al. (1987) Eur. J. Pharmacol., 137, 293-294.

EFFECTS OF ADRENAL DEMEDULLATION ON THE RENAL ACTIONS OF ADRENALINE AND NORADRENALINE IN THE RAT

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Our recent studies have drawn attention to the possible role of the adrenal medulla in mediating the renal responses to Kappa opioid agonists (Ashton et al, 1989). The current work examines the renal actions of catecholamines in the presence and absence of the adrenal medulla.

Groups of Long Evans rats were left intact (n=22) or were demedullated (n=18) one week prior to cannulation of jugular vein, femoral artery and urinary bladder under Inactin (5 - ethyl - 5 -(1' - methyl propyl) - 2 thiobarbiturate) anaesthesia (0.11g/kg). Animals were placed on continuous i.v. hypotonic saline (0.077M NaCl) infusion (150µl/min) and after 3h equilibration period and 1h control collections animals received infusate supplemented (1.05 ng/min) with noradrenaline, or adrenaline with 10-6 M ascorbic acid to prevent oxidation.

Table 1. Mean Arterial Blood Pressure and Renal Excretion During Initial 20 min of Catecholamine Infusion.

	Blood Pressure (mmHg)	Urine Flow (µl/min)	Na+excretion (µmol/min)	K+excretion (µmol/min)
Intact				
Vehicle n=6	118.3±2.1	131±10	10.2±1.1	5.4±0.2
Noradrenaline n=8	129.6±7.3	230±10***	21.3±3.6***	5.3±0.4
Adrenaline n=8	108±1.3***	100±8*	7.5±1.5	3.6±0.4**
Demedullated				
Vehicle n=6	98.7±4.7***	78±8***	1.4±0.3***	2.9±0.6***
Noradrenaline n=6	106.8±1.7	152±10+++	1.6±0.3	4.1±0.5
Adrenaline n=6	101.3±1.6	107±6++	3.2±1.0	4.6±0.3++

Values are mean ± S.R. Mean. Differences from intact control -* $p<0.05$, ** $p<0.01$, *** $p<0.001$ and differences from demedullated control ++ $p<0.01$, +++ $p<0.001$ were assessed by t-test.

In intact animals noradrenaline administration resulted in significant diuresis and natriuresis (Table 1). By contrast, adrenaline produced a fall in blood pressure, urine flow and potassium excretion. Adrenal demedullated rats exhibited reduced blood pressure, urine flow, sodium and potassium excretion by comparison with intact animals. Although the diuretic response to noradrenaline was still evident its natriuretic action was absent in demedullated animals. In contrast to its action in intact rats adrenaline did not alter blood pressure but raised urine flow and potassium excretion in demedullated animals.

The overall pattern of responses to catecholomines in intact animals is consistent with that reported by Brownlee et al (1988). The intact adrenal medulla would, however, appear to be essential in determining the character and the extent of the renal actions of administered catecholamines.

Ashton, N., Balment, R.J. & Blackburn, T.P. (1989) Brit. J. Pharmacol (In Press).
Brownlee, A.A., Lee, G. & Mills, I.H. (1988) J. Physiol. 403, 9P.

THE EFFECT OF MORPHINE AND SODIUM CROMOGLYCATE ON THE RELEASE OF SUBSTANCE P FROM CAPSAICIN-SENSITIVE NEURONS IN RAT TRACHEA

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In rat trachea, changes in vascular permeability take place upon antidromic activation of capsaicin-sensitive sensory neurons in vivo (Lundberg & Saria, 1982). Substance P has been proposed as a candidate mediator for these vascular responses and it may play a role in respiratory disease, notably neurogenic plasma extravasation in vivo (Belvisi et al., 1989). In addition, sodium cromoglycate (SCG) has been reported to suppress C-fibre excitability in dog airways in vivo (Dixon et al., 1980). We have utilised a multi-superfusion system to investigate the effect of morphine and SCG on potassium (K^+) stimulated release of substance P-like immunoreactivity (SP-LI) from rat trachea in vitro.

Ten spirally cut trachea from male rats (Wistar 350-400g) were mounted in parallel oxygenated glass chambers and superfused with Krebs' solution (pH 7.4, 37°C gassed with 95% O_2/5% CO_2) at 5 ml/min to allow equilibration. After 60 min. phosphoramidon (1 μM), captopril (100 μM), and bacitracin (20 mg/l) were added to the Krebs' solution and the flow rate adjusted to 1 ml/min. After a further 15 min. four fractions of 5 min. from each trachea were simultaneously collected in vials containing a final concentration of 0.1% trifluoracetic acid. Fractions were then concentrated on Sep-Pak C cartridges, lyophilised and reconstituted in barbitone buffer (pH 8.6) for radioimmunoassay (RIA) of SP-LI. The K^+ concentration of the superfusion fluid was isosmotically increased to 60 mM for 4 min. during the third fraction. Changes in SP-LI release were calculated as the difference in fractional release constants between the mean of fractions 1 and 2 and the mean of fractions 3 and 4 based on the recovery of exogenous SP (40 pg/ml) determined as 62.2 ± 3.2% (n=12). None of the agents interfered with the RIA at the concentrations used.

We have previously reported that capsaicin (50 mg/kg s.c.), administered neonatally, caused a 93.2 ± 6.4% reduction in tracheal SP-LI content and that high K^+ concentrations cause dose-related, Ca^{2+}-dependent release of SP-LI (Ray et al., 1989).

Morphine (0.1-100 μM), when added 2 min. prior to and during exposure to 60 mM K^+, produced dose-dependent inhibition of SP-LI release with a maximum decrease of 60.4 ± 13.7% ($P < 0.001$, n=10). This inhibition was completely abolished by 100 nM naloxone ($P < 0.001$). The κ-receptor agonist U50,488H (10 μM), did not affect K^+ stimulated SP-LI release ($P > 0.05$, n=14). Spontaneous SP-LI release was not significantly altered by either morphine or U50,488H.

The effect of SCG (10 μM) was also investigated. SCG, when present throughout the equilibration and sample collection periods, had no apparent effect on either spontaneous or 60 mM K^+ stimulated SP-LI release ($P > 0.05$, n=15).

We conclude that μ-receptor activation can inhibit SP-LI release from capsaicin-sensitive neurons and that centrally inactive μ-receptor agonists may have therapeutic potential in the treatment of asthma.

Barnes, P.J. (1986) Lancet, i, 242-245.
Belvisi, M.G. et al. (1989) J. Appl. Physiol., 66, 268-272.
Dixon, M. et al. (1980) Br. J. Pharmac., 70, 11-13.
Lundberg, J.M. & Saria, A. (1982) Acta Physiol. Scand., 115, 521-523.
Ray, N.J. et al. (1989) Br. J. Pharmac. (in press).

MODULATION OF CHOLINERGIC NEUROTRANSMISSION IN GUINEA-PIG AIRWAYS BY OPIOIDS

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Opioid receptors are present on capsaicin-sensitive sensory nerves (Laduron et al., 1984) which contain neuropeptides including substance P (SP) and neurokinin A. Opioids have been shown to inhibit non-adrenergic non-cholinergic (NANC) neurotransmission in guinea-pig bronchi in vitro (Bartho et al, 1987; Frossard and Barnes,1987) and in vivo (Belvisi et al, 1988). We have now investigated whether an inhibitory effect could be demonstrated on cholinergic neurotransmission in guinea-pig airways in vitro.

Guinea-pig trachea and main bronchi were dissected and mounted in 10ml organ baths containing Krebs-Henseleit solution at 37^0C, bubbled with 95% O_2/5% CO_2 at pH 7.4. Electrical field stimulation (EFS)(8Hz, 0.5ms, 40V for 20s) produced a rapid cholinergic response in upper trachea but in lower trachea and main bronchi a longer lasting NANC contraction was also demonstrated.

DAGOL, a selective u-opioid agonist, inhibited the cholinergic response to EFS in a dose-dependent manner in main bronchi (EC_{50} = 113nM with a maximal inhibition of 35.7 $\pm$ 5.6% at 10uM). In lower trachea DAGOL inhibited the cholinergic response to a similar extent with an inhibition of 35.8$\pm$ 3.5% at 10uM. However DAGOL had no inhibitory effect on the contractile response to exogenously applied acetylcholine (ACh). By contrast opioids had no inhibitory effect on cholinergic neurotransmission in upper trachea. The d-agonist, DPDPE, significantly inhibited the cholinergic component of the constrictor response to EFS in the bronchi but only at the highest dose used (10uM). U-50,488H, a k-receptor agonist, had no inhibitory effect on the cholinergic constrictor component in the main bronchi (10uM). DAGOL inhibited the NANC responses to EFS in main bronchi in a dose-dependent manner (with an EC_{50} = 36nM and a maximal inhibition of 63.4 $\pm$ 8.3% at 1uM). DAGOL had no effect on contractile responses to exogeously applied SP. DPDPE also inhibited the NANC constrictor component, but was less potent with a maximal inhibition of only 29.2 $\pm$ 4.2%, whereas U-50,488H (10uM) had no inhibitory effect. In each case the inhibitory effects of opioids were reversed by the opioid receptor antagonist naloxone (10uM). After capsaicin pretreatment, which depletes sensory nerves of neuropeptides, the inhibitory effect of DAGOL (1uM) on cholinergic constriction in main bronchi was only 13.4 $\pm$ 1.9% compared with 32.9 $\pm$ 4.0% inhibition in vehicle treated controls ($P<0.001$).

In conclusion opioids may reduce the cholinergic neural responses in airways largely via an action on excitatory NANC nerves. The opioid receptor involved is probably of the u-receptor subtype.

Bartho, L. et al. (1987) Naunyn-Schmied. Arch. Pharm.,**336**, 316-320.
Belvisi, M.G. et al. (1988). Br. J. Pharmacol., **95**, 413-418.
Frossard, N & Barnes, P.J. (1987). Eur. J. Pharm., **141**, 519-522.
Laduron, P.M. (1984). Brain Res., **294**, 157-160.

LACK OF INVOLVEMENT OF PRESYNAPTIC SIGMA RECEPTORS IN THE PCP-STIMULATED ENHANCEMENT OF TWITCHES IN RAT VAS DEFERENS

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The evaluation of pharmacological activity at the sigma binding site has been hampered by the lack of a functional bioassay in which agonist or antagonist activity at this putative receptor can be evaluated. Recently Vaupel et al., (1987) and Campbell et al., (1987) have proposed that phencyclidine (PCP), and other sigma ligands, potentiate electrically-induced twitches of guinea-pig and mouse vas deferens by sigma receptor mediated enhancement of noradrenaline release. Other workers have suggested that the twitch potentiation may be due to M current inhibition (Kennedy et al., 1989). To further examine the potential role of sigma receptors in this phenomenon, we have examined the effects of PCP in rat vas deferens. All results presented are means±s.e.mean ($n \geq 4$).

In an initial series of experiments the effect of PCP on twitch developed tension in vas deferens, prepared and electrically stimulated according to McGrath, 1984), was examined. The compound produced a concentration-dependent (0.1μmol/l - 30μmol/l) increase in twitch tension (increase over control: 54±4% at 30μmol/l); higher concentrations induced spontaneous activity in the preparation. In the presence of haloperidol (3μmol/l), which has been postulated to inhibit the effect of PCP at sigma receptors, PCP produced a less marked twitch potentiation (31±11% at 30μmol/l PCP). These data reproduce effects reported by Vaupel et al., (1987) and Campbell et al., (1987) in guinea-pig and mouse vas deferens, respectively. To further examine the phenomenon the effect of PCP on exogenous noradrenaline, oxymetazoline and tyramine-induced contractions in rat vas deferens were examined. PCP (10μmol/l) induced a leftward shift of the noradrenaline concentration-response curve and increased the maximum response attainable to noradrenaline (maximum developed tension increased to 182±14% of the pre-PCP maximum). The oxymetazoline concentration-response curve was not shifted to the left but the maximum response attainable, expressed as a percent of the pre-PCP noradrenaline maximum, was increased, by PCP, from 42±9 to 66±7%. The tyramine response was not inhibited by PCP. These effects of PCP cannot be mediated by a presynaptic enhancement of noradrenaline release and, thus, strongly suggest that the effects of PCP on electrically-induced twitch have little to do with a presynaptic sigma receptor mediated effect on noradrenaline release. The PCP-induced effects on the noradrenaline and the twitch response may be, at least partially, due to inhibition of noradrenaline uptake, as PCP is a potent inhibitor of noradrenaline neuronal re-uptake (IC_{50}: 0.52μM; Smith et al., 1977). However, as oxymetazoline, which is not subjected to uptake, is potentiated by PCP, and because responses to tyramine are not inhibited by this compound, a postsynaptic potentiation of agonist activity must also be postulated as being involved in the PCP-induced potentiation of twitch responses. Whilst these investigations do not clarify the mechanism of action of PCP, they do cast severe doubt on the utility of the vas deferens test as a functional bioassay for sigma receptors.

Campbell, B.G. et al., (1987) Eur. J. Pharmac. 138, 447-449.
Kennedy, C. & Henderson, G. (1989) Br. J. Pharmac. 96, 125P.
McGrath, J.C. (1984) Br. J. Pharmac. 82, 769-781.
Smith , R.C. et al., (1977) Biochem. Pharmac. 26, 1435-1439.
Vaupel, D.B. & Su, T.P. (1987) Eur. J. Pharmac. 139, 125-128.

THE SLOW NON-CHOLINERGIC CONTRACTION OF ELECTRICALLY STIMULATED BOVINE IRIS SPHINCTER MUSCLE

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When isolated strips of bovine iris sphincter muscle are subjected to a train of field stimulation, a compound response occurs. This has been variously reported to consist of a fast contraction followed by slow relaxation (Kalsner, 1983) or by another slow contraction (Butler & Bacon, 1988). We have demonstrated a triphasic response to stimulation (1 to 10 s train, 0.5 ms pulse width, 1 to 50 Hz) confirming that all these phenomena occur simultaneously. All three components are TTX-sensitive. The initial phase of the response is brief and rapidly declining, even before cessation of a 10 s train; it is atropine-sensitive. The relaxation which follows is maximal at about 23 s after the start of stimulation, is mimicked by isoprenaline and is at least partially inhibited by propranolol or timolol (10^{-5}M). The third phase peaks at approx. 60 s, is frequency-dependent, resists atropine and has been suggested as mediated by a neurokinin such as substance P (SP), released from primary afferent neurones; a similar slow contraction in the rabbit iris is abolished by the SP antagonist, Spantide (Leander et al., 1981). We now present evidence that in the bovine iris this last phase of contraction is not mediated by SP.

A 2-3 mm strip of sphincter muscle was suspended under 150 mg tension (isotonic) in Krebs solution containing indomethacin (10^{-6}M) and, for most experiments, timolol ($4x10^{-6}$M). Using Ag-AgCl electrodes, field stimulation was applied for 10 s at 30 Hz, 2 min after addition of drugs. Tachyphylaxis to SP was induced by adding cumulative doses to bring the concentration from $1.4x10^{-8}$ up to $4.2x10^{-7}$M over 6 min. This reduced the response to a submaximal dose ($5.6x10^{-8}$M) applied 10 min later, to 39% of that to the same concentration applied before the cumulative doses. The response to a train of stimuli was unaltered. The response to SP ($5.6x10^{-8}$M) was reduced by >50% using Spantide ($2x10^{-5}$M), but this had no effect on the slow electrically-evoked contraction.

Noradrenaline (NA) in the presence of timolol caused the sphincter muscle to contract. The threshold concentration for this effect was about 10^{-6}M. Exposure of the muscle to cumulative concentrations of NA from $4x10^{-6}$M up to $6.4x10^{-5}$M over a period of 5 min abolished the slow contractile response to electrical stimulation. Phentolamine (10^{-6}M) produced marked inhibition of the slow contraction to field stimulation, while it had no effect on the relaxation component of the triphasic response. Dopamine (10^{-6}M) reliably abolishes the slow electrically-evoked contraction (Butler & Bacon, 1988) yet this concentration of dopamine only inhibited the response to SP ($5.6x10^{-8}$M) by approx. 40%. This action of dopamine on the slow response to stimulation is mimicked by the selective D_1 agonist SKF 38393 (at $2x10^{-6}$M) and blocked by the D_1 antagonist SCH 23390 (10^{-6}M).

The evidence suggests that in the bovine iris sphincter muscle the slow electrically-evoked contraction is not due to SP release by sensory nerves, but that it involves an α-adrenergic mechanism.

Butler, J.M. & Bacon, A. (1988) Br.J.Pharmac. 94, 459P
Kalsner, S. (1983) Br.J.Pharmac. 78, 247-253
Leander, S. et al. (1981) Nature 294, 467-469

LOCAL APPLICATION OF CLONIDINE, YOHIMBINE AND TYRAMINE TO SYMPATHETIC NERVE TERMINALS: ROLE OF PREJUNCTIONAL α–ADRENOCEPTORS

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It is generally accepted that transmitter release from postganglionic sympathetic nerve terminals is regulated locally through prejunctional α-adrenoceptors (Starke, 1987). However, previous studies have questioned whether 'local regulation' of transmitter release occurs (Blakeley et al. 1982). Briefly, when the activity of only a few nerve fibres are studied, pulse-by-pulse modulation cannot be demonstrated in the absence of drugs. Paradoxically α-adrenoceptor antagonists potentiate the amplitude of excitatory junction potentials in many tissues suggesting that modulation of transmitter release occurs when many nerve fibres are activated. Recently, we reported a method using focal extracellular electrodes which allows transmitter release from a small population of varicosities located on a single nerve fibre to be investigated (Brock & Cunnane, 1988). The aim of the present study was to determine using this new approach whether there is any evidence for local regulation of transmitter release.

The method used was essentially the same as previously described and involved the placing of a small suction electrode (diameter about 50 μm) onto the surface of the guinea-pig vas deferens to record simultaneously the nerve terminal impulse and the associated excitatory junction current (Brock & Cunnane, 1988), which was taken as a measure of evoked transmitter release. Drugs were applied locally by internally perfusing the recording electrode.

The α-adrenoceptor agonist clonidine (0.1 μM) powerfully inhibited transmitter release and this effect was reversed by the α-adrenoceptor antagonist yohimbine (0.1 - 1 μM), suggesting that α_2 adrenoceptors are present on these nerve terminals. However, locally applied yohimbine (0.1 μM) did not cause a consistent increase in transmitter release. Thus NA released from sympathetic nerve terminals by action potentials does not appear to inhibit transmitter release locally. When tyramine (10 - 100 μM) was used to release endogenous NA, electrically evoked transmitter release was powerfully inhibited. The inhibitory action of tyramine was reversed by yohimbine (1 μM) and phentolamine (1 μM). These effects of tyramine were not mediated directly by activation of prejunctional α-adrenoceptors since the inhibitory actions of tyramine (100 μM) were absent or markedly reduced in reserpinized tissues; locally applied clonidine (0.1 - 0.3 μM) still inhibited transmitter release showing that prejunctional α-adrenoceptors were functional. The reason why action potential evoked NA release has little or no effect locally on subsequent transmitter release remains to be established.

Blakeley, A.G.H., Cunnane, T.C. & Petersen, S.A. (1982) *J.Physiol.* **325**: 93-109
Brock, J.A. & Cunnane, T.C. (1988) J.Physiol. **339**: 607-632
Starke, K. (1987) *Rev. Physiol. Biochem. Pharmacol.* **107**: 73-146

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EFFECTS OF CAFFEINE AND RYANODINE ON $INSP_3$ AND GTPγS-INDUCED INTRACELLULAR CA^{2+} RELEASE IN CULTURED RABBIT AIRWAY SMOOTH MUSCLE

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Guanosine triphosphate (GTP) and its non-hydrolysable analogue GTPγS can, like inositol (1,4,5) trisphosphate ($InsP_3$), release Ca^{2+} from intracellular stores in smooth muscle (Cheuh et al, 1987; Somylo et al, 1988). The mechanisms of this release is unclear and it is possible that $InsP_3$ and GTP may act at different sites. In order to clarify the relationship between $InsP_3$ and GTP regulated mechanisms of Ca^{2+} release, we have investigated the effect of two pharmacological probes of intracellular Ca^{2+} stores, ryanodine and caffeine, on the response to $InsP_3$ and GTPγS in ^{45}Ca-labelled monolayers of airway smooth muscle cultured from rabbit trachealis.

The release of Ca^{2+} from the intracellular store was estimated by measurement of ^{45}Ca efflux in digitonin-permeabilised monolayers of cells, bathed in a mock intracellular medium containing ^{45}Ca-labelled 1μM [Ca^{2+}] at pH 6.8. Addition of $InsP_3$ and GTPγS caused a dose-dependent reduction in Ca^{2+} content. Maximally effective 10μM $InsP_3$ released 0.5 ± 0.12 nmol Ca^{2+}/mg cell protein (mean ± s.e.mean, n = 5), while 100μM GTPγS released 0.12 ± 0.03 nmol Ca^{2+}/mg. Pretreatment with 100μM ryanodine, which released 0.42 ± 0.06 nmol Ca^{2+}/mg, reduced the subsequent $InsP_3$ induced Ca^{2+} release to 0.12 ± 0.05 nmol Ca^{2+}/mg and abolished the response to GTPγS. Pretreatment with 50mM caffeine, which released 0.11 ± 0.01 nmol Ca^{2+}/mg, also reduced subsequent $InsP_3$ induced release to 0.21 ± 0.01 nmol Ca^{2+}/mg, but did not affect GTPγS induced release.

Ryanodine therefore suppresses GTPγS induced Ca^{2+} release, possibly by pre-releasing the GTPγS sensitive pool, whereas caffeine has no effect. Both caffeine and ryanodine reduce $InsP_3$ induced Ca^{2+} release but to differing extents.

These results can be interpreted as evidence for heterogeneity of intracellular Ca^{2+} pools in airway smooth muscle. They suggest that the GTPγS releasable fraction may be part of a larger $InsP_3$ releasable pool, and that it is sensitive to ryanodine, but not caffeine.

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Cheuh, S-H., Mullaney, J.M., Ghosh, T.K., Zachary, A.L. & Gill, D.L. (1987) J. Biol. Chem. 262, 13859-13864.
Somlyo, A.P., Walker, J.W., Goldman, Y.E., Trentham, D.R., Kobayashi, S., Kitazawa, T. & Somlyo, A.V. (1988) Phil. Trans. R. Soc. Lond. B 320, 399-414.

SODIUM AND CALCIUM DEPENDENCE OF PHORBOL ESTER-INDUCED CONTRACTION OF BOVINE AIRWAY SMOOTH MUSCLE

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Phorbol esters have been shown to contract bovine trachea (Park & Rasmussen 1985) suggesting a role for protein kinase C in the sustained phase of the contractile response. The precise mechanisms involved in this contraction are as yet unknown.

We have studied the calcium dependence and the role of different sodium channels on the contraction produced by phorbol 12,13 dibutyrate (PDB) in bovine bronchial rings. Fresh bovine trachea was obtained immediately after death and bathed in Krebs-Henseleit solution. Bronchial rings were suspended under 2g tension in organ baths containing Krebs at 37^{o}C, continuously aerated with 95% O_2 5% CO_2. Changes in tension were measured using Grass FTO3 force displacement transducers and displayed on 2 CR600 2 channel flat bed recorders. PDB (10^{-6}M) produced a slowly developing contraction of bronchial rings reaching a maximum at 90 minutes.

Calcium free solutions and nifedipine (10^{-5}M) markedly inhibited the response. Amiloride (10^{-6}M) an inhibitor of Na entry channels, 5-(N,N-hexamethylene) amiloride (10^{-5}M) a specific inhibitor of Na/H exchange, and frusemide (10^{-5}M) an inhibitor of Na-K-Cl cotransport did not affect the response.

Our results suggest that phorbol ester induced contraction of bovine airway smooth muscle is dependent on calcium influx via voltage dependent calcium channels, but is independent of Na entry, Na/H and Na-K-Cl transport. This could be explained by protein kinase C activation being calcium dependent or by protein kinase C causing contraction by opening voltage dependent calcium channels.

Park S, Rasmussen H (1985). Proc Natl Acad Sci USA 1985 82 8835-8839.

AH 21-132 AND ITS ENANTIOMERS: THEIR TRACHEAL RELAXANT AND PHOSPHODIESTERASE INHIBITORY ACTIVITIES

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AH 21-132 ((±) cis-6-(p-acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methylbenzo-[c][1,6]naphthyridine) evokes concentration-dependent relaxation of both guinea-pig (Bewley & Chapman, 1988; Small et al., 1989) and bovine (Giembycz et al., 1989) isolated trachealis. In guinea-pig trachea AH 21-132 more potently inhibits cAMP-dependent phosphodiesterase (PDE) than cGMP-dependent-PDE, an observation consistent with its reported (Berry et al., 1989; Giembycz et al., 1989) selective inhibition of PDE isoenzyme types III and IV. The present experiments were designed to clarify whether the tracheal relaxant activity of AH 21-132 depends on its ability to inhibit cyclic nucleotide- dependent PDE.

Tested against the spontaneous tone of guinea-pig isolated trachea, AH 21-132 and its (-)-enantiomer proved more potent relaxants than the (+)-enantiomer, with -log EC50 values (mean ± s.e. mean) of 5.79 ± 0.04, 5.75 ± 0.05 and 4.56 ± 0.04 respectively. Tested against total cAMP- or cGMP-dependent phosphodiesterase (PDE) activity of guinea-pig trachea and PDE isoenzymes I and IV from bovine trachea or PDE isoenzyme III from guinea-pig cardiac ventricle, AH 21-132 and its (-) enantiomer proved more potent enzyme inhibitors than the (+)- enantiomer (Table 1).

	AH 21-132	(-)-enantiomer	(+)-enantiomer
Total cAMP-PDE (guinea-pig trachea)	5.4 ± 0.20*	6.19 ± 0.07	4.30 ± 0.03
Total cGMP-PDE (guinea-pig trachea)	4.16 ± 0.11*	4.45 ± 0.06	>>4
PDE I (bovine trachea)	4.02 ± 0.09	4.04 ± 0.08	>>3
PDE III (guinea-pig ventricle)	5.81 ± 0.06	6.16 ± 0.04	4.23 ± 0.09
PDE IV (bovine trachea)	5.85 ± 0.09	6.07 ± 0.10	4.45 ± 0.10

Table 1 Mean (± s.e. mean) values of -log IC50 for AH 21-132 and its enantiomers tested against cyclic-nucleotide dependent PDE. Substrate = 1 μM cAMP for all enzymes except total cGMP-PDE (1 μM cGMP). * data from Small et al. (1989).

Our data suggest that the tracheal relaxant and PDE inhibitory activity of AH 21-132 reside principally in the (-)-enantiomer. Like the racemate, the (-) enantiomer provides selective inhibition of (a) cAMP-dependent PDE as opposed to cGMP-dependent PDE and (b) the type III and IV isoenzymes as opposed to type I. The (-) enantiomer of AH 21-132 was more potent than the (+) enantiomer both as regards tracheal relaxation and PDE inhibition, a finding consistent with the suggestion that PDE inhibition underlies the tracheal relaxant action.

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Berry, J.L., Boyle, J.P., Elliott, K.R.F., Foster, R.W., Small, R.C. & Watt, A.J. (1989). Br. J. Pharmacol. (In press).
Bewley, J.S. & Chapman, E.D. (1988). Br. J. Pharmacol., 93, 52P.
Giembycz, M.A., Mak, J.C.W., Ukena, D., Chilvers, E.R. & Barnes, P.J. (1989). Br. J. Pharmacol. (In press).
Small, R.C., Boyle, J.P., Duty, S., Elliott, K.R.F., Foster, R.W. & Watt, A.J. (1989). Br. J. Pharmacol. (In press).

EFFECTS OF AQUEOUS ONION AND GARLIC EXTRACTS ON CONTRACTIONS OF GUINEA-PIG AIRWAYS *IN VITRO*

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Ingestion of ethanolic extracts from onion (Allium cepa) bulbs have been reported to inhibit allergic bronchoconstriction (Dorsch & Weber, 1984). The active products appear to be alk(en)ylsulfinothioic acid alk(en)yl esters which inhibit arachidonic acid (AA) metabolism in a number of tissues (Dorsch et al., 1988). Aqueous extracts of garlic (Allium sativum) also inhibit thromboxane synthesis in rabbit platelets (Ali & Mohammed, 1986).

We had developed an in vitro method which differentiated whether putative anti-allergic compounds inhibited the cyclooxygenase or lipoxygenase pathways of AA metabolism in guinea-pig airways (Burka et al., 1989) and wished to examine whether the aqueous extracts from onion or garlic affected antigenic and AA-induced contractions. Tracheal spirals, in the presence of indomethacin (8.4 uM), and lung parenchymal strips, in the absence of indomethacin, from ovalbumin (OA)-sensitized guinea pigs were set up under isometric conditions in 10 ml organ baths in Krebs-Henseleit solution at 37°C. Aqueous extracts of fresh garlic cloves and onion bulbs were prepared by the method of Ali & Mohammed (1986). Following a control contraction with histamine (10^{-4}M) and a return to baseline, onion or garlic extract (10, 100, or 300 ul) was added to the baths 30 min prior to the addition of AA (66 uM) or ovalbumin (1 uM). The resulting contraction was measured over the next 60 min as a percentage of the control histamine contraction. The area under the contractile curve in the presence of onion or garlic extract was measured and compared to that of a paired tissue in the absence of extract. In addition, concentration-response curves to histamine and KCl were carried out in the presence and absence of onion or garlic extract.

Garlic extract caused a concentration-dependent inhibition of AA- and OA-induced contractions of both tracheal spirals and parenchymal strips. Complete inhibition was achieved with 300 ul garlic extract/10 ml. Histamine and KCl contractions were similarly reduced. Garlic extract also discoloured the parenchymal strip. If the garlic extract was boiled for 10 min prior to use, the inhibitory activity was either reduced (trachea) or eliminated (parenchyma) and no discolouration of the parenchyma was observed. Extracts from onions did not affect any of the parameters studied.

The results suggest that a heat-labile substance(s) in aqueous garlic extract causes a non-specific depression of guinea-pig airways if applied in vitro. Extracts from onions had no effects, suggesting that the concentration of the 'toxic' substance was not as high, or absent, in onions. The results also indicate that the anti-asthmatic factor found in the ethanolic extract of onions (Dorsch et al., 1988) is not found in an aqueous extract.

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Ali, M. & Mohammed, S.Y. (1986) Prostaglandins Leukotrienes Med. 25, 139-146
Burka, J.F. et al. (1989) Can. J. Physiol. Pharmacol. 67, in press.
Dorsch, W. & Weber, J. (1984) Agents Actions 14, 626-629
Dorsch, W. et al. (1988) Biochem. Pharmacol. 37, 4479-4486

IONOPHORE A23187 CAUSES PAF RELEASE IN THE ISOLATED PERFUSED LUNGS OF OXYGEN-TREATED RATS

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In a previous study we detected Platelet-activating Factor (PAF) in the bronchoalveolar lavage (BAL) fluid from rats which had been exposed to 100% oxygen (O_2) for 12 h. Exposure to O_2 for 24 h and 48 h produced only changes in lyso PAF levels (Court & Kingston, 1987). These results could not be accounted for by changes in the uptake or metabolism of PAF (Court & Kingston, 1989). In the present study we have examined the ability of ionophore A23187 to release PAF from the isolated perfused lungs of O_2 treated rats.

Male Wistar rats (250-300g) were exposed to O_2 as described by Court & Kingston (1987) for periods of either 12 h, 24 h or 48 h. Control rats (0 h) were not exposed to 100% O_2. The lungs were excised and perfused through either the vasculature or through the airways as previously described (Court & Kingston, 1988). The perfusing fluid was Greenberg - Bohr Buffer containing 0.25% w/v bovine serum albumin (GBB-BSA). After perfusing the lungs with GBB-BSA for 25 min the perfusate was collected for 15 min. Ionophore was then infused. The rate of ionophore infusion was 0.1 ml/min and throughout the 5 min infusion the ionophore concentration entering the lungs was 480 nM. On completion of the ionophore infusion the perfusate was collected over 15 min intervals for a total of 1 h. PAF was extracted from the perfusate and assayed as described by Court et al (1987). PAF was not detected prior to, or 45 min after ionophore infusion in any group of rats. The ionophore did not cause the lungs of 0 h and 48 h O_2 treated rats to release PAF. PAF release was observed in 12 h and 24 h O_2 treated rats following ionophore infusion (Table 1).

Table 1. The PAF content (pmol) in the perfusate following ionophore A23187 infusion into the isolated lungs of oxygen treated rats perfused via the vasculature (Vasc.) or through the airways (Air.). Results are expressed as the mean + s.e. mean (n=4) (ND = not detected).

Time after ionophore infusion (min)	period of oxygen exposure 12 h		24 h	
	Air.	Vasc.	Air.	Vasc.
0-15	132±5	387±21	ND	ND
15-30	112±6	314±16	118±7	310±17
30-45	ND	ND	98±4	242±8

These results indicate that lungs from 12 h and 24 h O_2 treated rats release more PAF than those from 0 h and 48 h O_2 treated rats. The PAF detected in the BAL fluid of 12 h O_2 treated rats (Court & Kingston, 1987) may thus be due to the increased ability of the lungs to synthesize and release PAF.

Court, E.N. & Kingston, W.P. (1987). Br. J. Pharmac., 92, 630P.
Court, E.N. et al (1987) Br. J. Clin. Pharmac., 24, 258P.
Court, E.N. & Kingston, W.P. (1988). Br. J. Pharmac., 95, 807P.
Court, E.N. & Kingston, W.P. (1989). Br. J. Pharmac., (In press).

BRADYKININ B_1 AND B_2 RECEPTOR ACTIVATION STIMULATES CHLORIDE TRANSPORT IN ISOLATED RAT COLON

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Bradykinin (Bk) has been shown to stimulate a net secretion of chloride ions when administered to the serosal side of the colonic epithelium (Cuthbert & Margolius, 1982). This effect is likely mediated via Bk receptors present in colonic crypts and villi (Manning et al 1982). In the present study we have used a number of Bk-analogues to characterize the receptors involved.

Segments of proximal decending colon were stripped of muscle coat and submucosal layers. Two adjacent section of tissue, one acting as control, were mounted in Ussing-type chambers and bathed on both sides with oxygenated physiological solution at 37°C (Perkins et al 1988). The trans-epithelial potential was held at zero and the effect of drugs, added to the serosal side, was monitored by measuring changes in the short circuit current (scc).

As in our previous study (Perkins et al, 1988), Bk induced a chloride-dependent, dose-related increase in scc (EC_{50} = 5nM: maximum amplitude 24.1 ± 1.0μA 0.6cm^{-2}, n=10). The B_1-receptor ligand des-Arg^9 Bk (Regoli & Barabe, 1980) produced little or no response on the first application but tissue responsiveness gradually increased and stabilized after 2-3 hours. At this time the EC_{50} was 0.2 ± 0.04 μM, n=12. The changes in responsiveness were independent of concentration or dosing frequency. The response to des-Arg^9 Bk was severely attenuated when chloride ions were substituted with gluconate in the bathing solution. When tested at the EC_{50} dose, responses produced by des-Arg^9 Bk, but not those produced by Bk, were reduced by the B_1-receptor antagonist des-Arg^9,Leu^8 Bk (IC_{50}= 0.5μM). This compound showed initial agonistic activity (EC_{50} = 0.7 ± 0.1 μM, n=10). The Bk antagonist D-Arg [Hyp^3,Thi^{5,8},D-Phe^7] Bk (Vavrek & Stewart, 1985), was equieffective against both des-Arg^9 Bk and Bk (IC_{50} = 0.3-0.4μM).

These data suggest that chloride secretion in the colonic epithelium is mediated by both B_1- and B_2-receptor subtypes and that des-Arg^9,Leu^8 Bk acts as a partial agonist at B_1-receptors in this tissue. The antagonist D-Arg [Hyp^3,Thi^{5,8},D-Phe^7] Bk acts non-selectively on both receptors.

Cuthbert, A.W. & Margolius, M.S. (1982) Br. J. Pharmacol. 75, 587-598.
Manning, D.C., Snyder,S.H., Kachur, J.F. & Field, M. (1982) Nature, 229, 256-259.
Perkins M.N., Forster, P.L. & Dray, A. (1988) Br. J. Pharmacol. 94, 47-54.
Regoli, D. & Barabe, J. (1980) Pharmacol. Rev. 32, 1-46.
Vavrek, R.J. & Stewart, J.M. (1985) Peptides, 6, 161-164.

COMPARISON OF EFFECTS OF LOPERAMIDE AND VERAPAMIL ON COLONIC PROPULSION AND VASCULAR MUSCLE CONTRACTION

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Loperamide, a widely used antidiarrhoeal drug, was shown previously to possess activity similar to that of the calcium channel blocker verapamil in inhibiting agonist-induced contractions of guinea-pig ileum, although it was consistently less potent (Ali et al., 1988). In the present work we compare the actions of loperamide (lop) with verapamil (verap) in vascular smooth muscle and in a model of integrated gut motor activity, faecal pellet expulsion from the isolated guinea-pig colon.

Colons containing formed faecal pellets were bisected to provide proximal and distal preparations which were individually incubated in oxygenated Krebs' solution at 25°C. Each half colon contained approximately 14 pellets and the percentage of these extruded per 15 min was recorded. Control preparations emptied within 120 min. One segment from each animal was randomly assigned as a control and the other was incubated throughout with one concentration of lop or verap ($n \geqslant 5$ for each concentration). Lop (0.1-10μM) and verap (0.3-3μM) caused concentration-related reduction in the total number of pellets extruded, loperamide being more potent than verapamil. The maximum inhibition observed was 81±2%, with loperamide (10μM). The effect of naloxone (0.1μM) was studied on the actions of lop (0.1μM) and verap (3μM) (concentrations causing approximately 50% reductions in pellet extrusion). Naloxone completely prevented the effect of lop while the effect of verap was not significantly reduced. Naloxone alone had no effect. The effect of lop but not verap at these concentrations in intact colon thus appears to be due to opiate activity.

Spedding and Cavero (1984) suggested a simple screen to assess calcium antagonist properties in a compound, involving comparisons of the inhibition of potassium-(K) and noradrenaline-(NA) evoked contractions in aorta and portal vein (PV). In the present experiments,cumulative concentration-response curves to K (10-80mM) and NA (1nM-30μM) were investigated in rat PV and aortic rings before and after exposure to lop (1-3μM) and verap (10nM-1μM). Tissues were maintained in oxygenated Krebs' solution at 37°C under a resting tension of 1g. Concentrations of lop (3μM) and verap (0.1μM) which produced comparable reductions in maximal contractions to K (K_{max}) and NA (NA_{max}) in PV caused differential effects in aorta. In PV, lop and verap caused 77±6% and 80±4% reduction in K_{max} and 59±10% and 60±3% reductions in NA_{max} respectively. The height, but not the rate, of spontaneous contraction was decreased by both lop and verap, by 47±8% and 50±3% respectively. In aorta, verap reduced K_{max} by 37±8%, with no effect on NA_{max}, whereas lop reduced K_{max} by 61±4% and NA_{max} by 44±6%.

Thus, in vascular smooth muscle, lop did not show the same profile of activity as verapamil, suggesting that it may possess intracellular actions in addition to calcium channel blockade (Spedding & Cavero, 1984). However, in the intact colon, and at a lower concentration, opiate effects appear to dominate.

Ali, I.M. et al. (1988) Br. J. Pharmac. 95: 605P
Spedding, M. & Cavero, I. (1984) Life Sci. 35: 575-587

CONTRACTILE MECHANISMS OF RAINBOW TROUT (*SALMO GAIRDNERI*) INTESTINE: EFFECT OF TEMPERATURE

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Carbachol, 5-hydroxytryptamine (5-HT), and potassium chloride (KCl) contract rainbow trout intestine in a concentration-dependent manner. The effects of carbachol and 5-HT appear to be mediated through M-2 and 5-HT_2 receptors, respectively (Burka et al., 1989). Receptor properties of mammalian smooth muscle change with temperature (Cook et al., 1977; Ishii and Shimo, 1986). Whether this occurs in poikilothermic animals is not known. We therefore wished to examine whether the contractility of trout smooth muscle varied with temperature and to obtain an indication of whether changes occurred at the receptor or intracellular levels. The role of calcium at various temperatures was also investigated.

Isolated trout intestinal segments, approximately 2 cm in length, were suspended isometrically under 2 g tension in 10 ml organ baths containing trout Ringer's solution aerated with O_2 and CO_2 (95:5).

Contractions of trout intestine were identical at 10°C and 20°C for the three agonists studied (Table 1). However, the efficacy, but not the potency, of each agonist was decreased at 2°C. Receptor-induced contractions were reduced to a greater extent at 2°C than those induced by depolarization. The results also indicated that the calcium source for contractility was dependent on temperature (Table 2). As temperatures increased, utilization of intracellular calcium increased, as indicated by increased contractility in the absence of extracellular calcium.

Table 1. Maximal tension developed (mg/mm^2); values are means ± SEM, n = 6.

Agonist	Temperature		
	2°C	10°C	20°C
KCl	159.1 ± 26.7	262.2 ± 17.1	327.0 ± 44.9
5-HT	37.3 ± 7.9	242.1 ± 22.2	243.7 ± 38.4
Carbachol	37.4 ± 6.0	209.9 ± 16.4	251.8 ± 55.5

Table 2. Responses of tissues in zero calcium as a percentage of control; values are means ± SEM, n = 6.

Agonist	Temperature		
	2°C	10°C	20°C
KCl (70 mM)	0	4.3 ± 2.4	15.2 ± 5.3
5-HT (10^{-4}M)	21.0 ± 12.7	23.5 ± 5.3	60.0 ± 23.5
Carb (10^{-4}M)	9.1 ± 2.2	14.7 ± 4.8	69.0 ± 22.6

Thus, low temperatures decrease smooth muscle contractility by affecting receptor mediated events rather than the intracellular contractile mechanisms. Receptor-operated agonists appear to have a higher capability of using intracellular calcium than depolarizing agents.

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Burka, J.F., Blair, R.M.J. & Hogan, J.E. (1989) Can. J. Physiol. Pharmacol. 67, in press
Cook, D.A., Kenakin, T.P. & Krueger, C.A. (1977) Fed. Proc. 36, 2584-2589
Ishii, T. & Shimo, Y. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol. 332, 219-223

THE EFFECTS OF SIBUTRAMINE HCL AND ITS METABOLITES ON HISTAMINE-EVOKED RESPONSES IN THE GUINEA-PIG ILEUM

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Tricyclic antidepressants have anti-muscarinic (Golds et al, 1980; Mahony et al, 1988) and anti-histamine (H_1) (Richelson, 1983) actions both centrally and peripherally which are responsible for some of their clinical side effects, for example, sedation. The putative antidepressant sibutramine HCl (BTS 54 524) has recently been reported to rapidly down-regulate cortical B-adrenoceptors (Buckett et al, 1988), a response probably mediated by potent inhibition of monoamine uptake by its metabolites (Luscombe et al, 1989). We report on a comparison of the effects of sibutramine HCl and its metabolites with the antidepressants imipramine, dothiepin, iprindole and mianserin on histamine-induced responses in the guinea-pig ileum.

Histamine dose-response curves were obtained with an *in vitro* guinea-pig ileal preparation using a 2 min cycle in the absence and presence of mepyramine or the antidepressants. Affinity constants and pA_2 values were computed and compared with those found with mepyramine (pA_2 = 9.4). Dothiepin (pA_2 = 8.4) and imipramine (pA_2 = 7.4) were found to exhibit competitive antagonism, while iprindole and mianserin exhibited non-competitive antagonism to histamine. Sibutramine HCl (BTS 54 524) and its secondary (BTS 54 354) and primary (BTS 54 505) amine metabolites (at concentrations $<10^{-6}$M) produced no changes in the histamine dose-response curve. At concentrations exceeding $5x10^{-6}$M sibutramine HCl and its metabolites exhibited non-competitive antagonism, as revealed by an immediate reduction in the maximum response and slope of the dose-response curves, which was readily reversible after washing. At concentrations which induced non-competitive antagonism it was observed, particularly with sibutramine HCl and its metabolites, that the individual contractions showed a rapid decay (within 5-10 seconds) during the period of agonist application.

The sedative side-effects of antidepressants reflect their ability to block histamine responses at H_1 receptors (Richelson, 1983), thus clinically dothiepin is found to be more sedative than imipramine, an observation supported by the present *in vitro* studies. The present evidence also suggests that sibutramine HCl and its metabolites would not be expected to produce sedation in clinical use.

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Buckett, W.R. et al (1988) Prog. Neuro-psychopharmacol. Biol. Pschiat. 12, 575-584.
Golds, P.R. et al (1980) Br. J. Pharmacol. 68, 541-549.
Luscombe, G.P. et al (1989) Neuropharmacology 28, 129-134.
Mahony, M.T. et al (1988) Brit. J. Pharmacol. 85, 885P.
Richelson, E. (1983) Mayo Clin. Proc. 58, 40-46.

SEPARATION OF THE ANTIMALARIAL AND TOXIC EFFECTS IN MICE OF EXTRACTS FROM *BRUCEA JAVANICA* FRUITS

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Extracts of Brucea javanica fruits are antimalarial in mice, but death occurs with high doses (Anderson et al, 1988; O'Neill et al, 1987). We have now studied the toxicology of these extracts and the possibility of separating the antimalarial activity from the adverse effects by chemical treatment of an aqueous extract.

An aqueous extract of B. javanica fruits and its chloroform soluble product after acid treatment were prepared as described previously (Anderson et al, 1988). Antimalarial activity was measured by the reduction in the percentage of Leishman-positive erythrocytes in male mice (18-30g) inoculated with Plasmodium berghei ($2x10^7$ Leishman-positive murine erythrocytes/mouse i.v.). The acute behavioural and physiological effects of extracts were assessed in normal mice using observational tests (Irwin 1968), Animex activity meter and rectal thermistor probe 60-75 min after one injection of doses (aqueous 100mg/kg, chloroformic 25mg/kg i.p.) which produced similar antimalarial effects in mice on repeated administration.

The aqueous extract (100mg/kg i.p. twice daily for 3 days starting within 1h of inoculation) reduced parasitaemia (6± s.e.mean 2%, $P<0.001$ compared with the control value of 58±4%, n=6) measured 72h after inoculation. A 25mg/kg dose had no effect (parasitaemia 59±4%). A similar regimen with the chloroformic extract (12.5 and 25 mg/kg) caused a dose-related decrease in parasitaemia (25±4% and 3±1% respectively at 72h). This treatment with the chloroformic extract (12.5 and 25mg/kg) caused some deaths in normal mice (2/6 and 3/6 mice respectively), but none in infected animals. The aqueous extract did not cause any deaths. B. javanica treatment started 24 or 48h after inoculation was ineffective except for chloroformic extract 25mg/kg given twice daily for 3 days from 24h (parasitaemia 10±2% at 96h compared with 51±2% in vehicle-treated malarial mice). Doses (aqueous 100mg/kg, chloroformic 25mg/kg), which did not cause death in infected mice when treatment began within 1h of inoculation, did when treatment started at 48h and when concurrent controls were alive with a parasitaemia of 63±1%. Motor activity measured by Animex meter was reduced by a single dose of the aqueous (100mg/kg i.p., 15±6 counts/5min, $P<0.001$) and the chloroformic extract (25mg/kg i.p., 35±12 counts/5min, $P<0.05$) when compared with activity in control mice (84±13 counts/5min, n=6). In observational tests, both extracts decreased defecation and urination, but only the aqueous extract caused palpebral closure and decreased abdominal tone, spontaneous locomotor activity and spatial locomotion on removal to a new environment ($P<0.05$, 2-tailed Mann-Whitney U). Arousal and the righting reflex were not affected. The aqueous, but not the chloroformic extract caused a fall in colonic temperature from pre-injection values (-1.9±0.3°C, $P<0.05$) when compared with the response to vehicle (-0.8±0.2°C, n=6).

These data indicate that acid treatment of an aqueous extract of B. javanica fruits followed by chloroform extraction produces a preparation with less acute toxicity (hypothermia, decreased motor activity) in mice than the aqueous extract in doses that are antimalarial on repeated injection. The increased toxicity of B. javanica extracts in the presence of an established infection may be due to malaria affecting the disposition and/or the metabolism of these extracts.

Anderson, M.M. et al (1988) J. Pharm. Pharmacol. 40(Suppl.), 52P
Irwin, S. (1968) Psychopharmacol. 13, 222-257
O'Neill, M.J. et al (1987) J. Nat. Prod 50(1), 41-48

INHIBITION BY AMINOQUINOLINE AND TRIAZINE, ANTIMALARIAL AGENTS, OF THE STIMULATION OF ^{86}-RUBIDIUM EFFLUX BY CROMAKALIM

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Cromakalim has been shown to activate K^+ channels in a variety of smooth muscles (Hamilton & Weston, 1989) although the precise site of action is as yet unknown. Attempts at identifying interactions of cromakalim with a number of neurotransmitter receptor binding sites and second messenger systems have been unsuccessful (Coldwell & Howlett, 1987), although recently it has been suggested that the activation of protein kinase C may be involved in determining the open state probability of the cromakalim activated channel (Coldwell, 1988). As part of our continuing attempts to throw further light on the mechanism involved in K^+ channel activation, this communication describes the effects of phospholipase A_2 inhibitors and a number of antimalarial agents on cromakalim stimulated 86-rubidium efflux.

86-Rubidium efflux was measured in rabbit isolated mesenteric artery (RIMA) as described previously (Coldwell & Howlett, 1987). Examination of a number of 4- and 8-aminoquinolines showed an ability of the compounds to inhibit cromakalim stimulated 86-rubidium efflux rate, without affecting the basal efflux rate. Thus, cromakalim (10 μM) produced a 70.4 ± 20.7% stimulation of the basal efflux rate. This was significantly inhibited by mepacrine (30 μM), 1.5 ± 6.4% stimulation; $p<0.001$; $n=6$), primaquine (30 μM, 31.4 ± 4.4%; $p<0.02$; $n=7$), pamaquine (30 μM, 34.1 ± 10.0%; $p<0.05$; $n=10$) and chloroquine (100 μM, 32.1 ± 8.5%; $p<0.05$; $n=6$). The order of potency of these compounds for inhibiting 86-rubidium efflux parallels their phospholipase A_2 inhibitory activity (R. Melarange, personal communication). Other putative phospholipase A_2 inhibitors, such as papaverine, nordihydroguaretic acid and bromophenacyl bromide, however, had no effect on cromakalim stimulation of 86-rubidium efflux. In separate experiments, rabbits were pretreated (90 min) with dexamethasone (5 mg/kg s.c.). The mesenteric artery was subsequently removed and loaded with 86-rubidium, in the presence of 10 μM dexamethasone. This pretreatment had no effect on the ability of cromakalim to stimulate 86-rubidium efflux. Thus, under conditions where phospholipase A_2 would be largely inhibited, cromakalim stimulated efflux was unaffected.

We have previously reported that cromakalim stimulated rubidium efflux is inhibited by methylene blue through a non-cyclic GMP dependent mechanism (Coldwell & Howlett, 1986; Coldwell & Howlett, 1987). Like the aminoquinolines, methylene blue has been reported to display antimalarial activity (see Carson, 1985). Thus, it appeared appropriate to examine other antimalarial agents, not known to possess phospholipase A_2 inhibitory properties, as inhibitors of cromakalim stimulated efflux. Of these, the triazine, clociguanil (30 μM) inhibited the response from a control stimulation with 10 μM cromakalim of 54.3 ± 11.4% to 18.2 ± 7.1% ($p<0.5$; $n=4$). Similarly, although slightly less potent, cycloguanil (100 μM) decreased efflux from a control response of 119.5 ± 26.7% to 43.9 ± 13.6% ($p<0.05$; $n=5$). These results would not support the implication of phospholipase A_2 activation in the mode of action of cromakalim. However, the apparent activity of the antimalarial agents as potassium channel blockers would suggest some common pathway in their mode of action and that of cromakalim.

Carson, P.E. (1985) in Handbook of Experimental Pharmacol., 68.
Coldwell, M.C. (1988) Brit. J. Pharm. 93, 207P.
Coldwell, M.C. & Howlett, D.R. (1986) Brit. J. Pharm. 88, 443P.
Coldwell, M.C. & Howlett, D.R. (1987) Biochem. Pharm. 21, 3663-6.
Hamilton, T.C. & Weston, A.H. (1989) Gen. Pharmacol. 1, 1-9.

EFFECTS OF PROPRANOLOL AND DIMETHYLQUATERNARY PROPRANOLOL ON K^+ INFLUX IN GUINEA-PIG HEART VENTRICLE SLICES

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It was previously reported (Nowell *et al.* 1987), that dimethylquaternary propranolol (DMP) selectively inhibited K^+ stimulation of purified cardiac Na, K-ATPase by being competitive against K^+, with a similar competitive inhibition of ouabain-sensitive ^{86}Rb influx in trout erythrocytes, although in this case the drug markedly inhibited frusemide-sensitive ^{86}Rb influx as well. In other work, propranolol (Prop) exhibited mixed-type inhibition against K^+ on the enzyme (Abdul-Cader *et al.*, 1984) under normal pH conditions (pH 7.5) and more complicated effects than DMP on trout erythrocytes. The effects of Prop and DMP were further investigated on ^{86}Rb influx in guinea-pig heart left ventricle slices using the method of Ku *et al.* (1974) with minor modifications. At normal K^+ concentration (6 mmol l^{-1}), Prop concentrations of 10, 100 and 300 μmol l^{-1} increased influx by 37, 17 and 18% respectively, with higher concentrations of 1 - 9 mmol l^{-1} giving 11-90% inhibition and DMP being less effective in both respects. When the K^+ concentration was reduced to 1.2 mmol l^{-1}, the stimulatory potency of Prop was increased approximately 10-fold by increases of 22-38% being obtained with 0.3 - 3 μmol l^{-1} concentrations and similar degrees of stimulation up to 300 μmol l^{-1} Prop , with no increase in inhibitory potency at higher concentrations. In contrast with DMP, K^+ sensitivity was only evident in the inhibition phase by 3-10 mmol l^{-1} DMP giving 12-45% inhibition compared with 0-24% at 6 mmol l^{-1} K^+, ie in keeping with the DMP/K^+ competition observed with similar DMP concentrations in trout erythrocytes. The effects of some stimulatory Prop and DMP concentrations are shown in Table 1, using ouabain (1 mmol l^{-1}) or frusemide (1 mmol l^{-1}) to indicate the component of K^+ influx (Na(K) pump or NaKCl cotransporter) which was being affected.

TABLE 1 Effects of Prop. and DMP on K^+ Influx in Guinea-Pig Heart Ventricle Slices

Drug/ Concn. (μmol l^{-1})	K^+ Concn. (mmol l^{-1})	Mean % Total K^+ Influx (± SEM) Control	Ouabain Insensitive	Frusemide Insensitive
-	6	100 ± 0	27.6 ± 1.1	88.0 ± 6.0
Prop/10	6	137.1 ± 12.7*	22.8 ± 2.6	90.0 ± 7.2
DMP/30	6	112.2 ± 3.7*	22.8 ± 3.3	94.7 ± 8.9
-	1.2	100 ± 0	21.6 ± 1.7	88.8 ± 4
Prop/3	1.2	138.2 ± 8.6*	21.0 ± 1.8	122.3 ± 7*
DMP/10	1.2	110.6 ± 9.9	19.8 ± 2.3	ND
DMP/30	1.2	113.4 ± 12.1	ND	134.1 ± 18.1

*$p < 0.05$ (unpaired Student's t test and Wilcoxon rank sum test, N = 4 or 5). ND = Not determined.

Since with the lowest concentration of Prop (3 μmol l^{-1}) tested at 1.2 mmol l^{-1} K^+, the stimulation was abolished by ouabain but not by frusemide, it appeared that the drug was primarily stimulating the Na(K) pump component of K^+ influx under these conditions. In other cases, neither the frusemide - insensitive nor the ouabain-insensitive components of the influx were increased, suggesting that both the pump and cotransport components may be involved in the effect. Previous reports implicating the effects of Prop on K^+ transport include stimulation of the ouabain-insensitive K^+ influx in rat thymocytes (Murphy & Ryan, 1983) and it adversely affecting the ability of human subjects to handle an acute K^+ load (Rosa *et al.*, 1980). It is felt that the stimulatory effects of low concentrations of Prop and DMP on K^+ influx merit further investigation as they may contribute to the cardiac actions of these drugs in clinical situations.

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Abdul-Cader, Z. *et al.* (1984), 9th Int. Cong. Pharmacol., London, 915P
Ku D.D. *et al.* (1974), Naunyn-Schmied Arch. Pharmacol. 285, 185-200
Murphy J.J. & Ryan M.P. (1983), Br. J. Pharmacol. 80, 609P
Nowell P.T. *et al.* (1987), 10th Int. Cong. Pharmacol., Sydney, P1079
Rosa R.M. *et al.* (1980), New Engl. J. Med. 302, 431-434

BRL 31660 INHIBITS THE EFFECTS OF CROMAKALIM AND ITS ENANTIOMER BRL 38277 ON TENSION AND $^{43/42}K^+$ EFFLUX IN GUINEA-PIG TRACHEA

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The relaxant effect of cromakalim (BRL34915) in vascular (Wilson et al., 1988) and bronchial (Allen et al., 1986) tissue is inhibited by standard potassium channel blockers (eg 4-aminopyridine and procaine), which supports other evidence that cromakalim activates potassium channels (Hamilton et al., 1986). Cromakalim inhibits tone in tracheal spirals (Taylor et al., 1989) and has potential as a bronchodilator for the treatment of asthma (Arch et al., 1988). However, the use of known potassium channel blockers in tracheal spirals presents practical problems in that they induce marked phasic activity in the tissues, making interpretation of the data difficult.

In the present study we have investigated the action of BRL31660, a novel antiarrhythmic agent which has been reported to have both class I (membrane stabilising) and class IV (calcium antagonist) properties (Bruckner et al ., 1985), on the effects of cromakalim and its active enantiomer BRL 38227 (Buckingham et al., 1986) in both tension and $^{43/42}K^+$ efflux experiments.

Guinea-pig tracheal spirals (2 per animal) were suspended for isometric recording under 2g tension in Krebs solution bubbled with 95%O_2/5%CO_2 at 37°C. Tissues were allowed to equilibrate over a one hour time period and as spontaneous tone developed, tension was readjusted to 2g.

BRL31660 (10^{-6} - 10^{-4}M), equilibrated with the tissues for 30 mins at each concentration, reduced the effects of cromakalim and BRL 38227 (10^{-8} - 10^{-4}M) in a non-competitive manner. A concentration of BRL31660 (10^{-5}M), which reduced the maximal inhibitory effect of cromakalim by approximately 70%, did not block the effects of isoprenaline (10^{-9}-10^{-6}M), salbutamol (10^{-9} - 10^{-6}M), sodium nitroprusside (10^{-8}-10^{-5}M) or indomethacin (10^{-8} - 10^{-5}M). Furthermore, BRL31660 alone had no spasmogenic or inhibitory effect.

For the efflux experiments, the trachealis muscle was dissected away from the cartilage and equilibrated for 60 min in Krebs containing $^{43/42}K^+$ (2μCiml^{-1}). The tissues were then allowed to efflux into ordinary Krebs for eleven 3 min collection periods before challenge with cromakalim (10^{-5}M) or BRL 38227 ($5x10^{-6}$M), cromakalim or BRL 38227 and BRL31660 (10^{-5}M) or vehicle control for four 3 min periods. BRL31660 (10^{-5}M) had no effect on the basal efflux of $^{43/42}K^+$ but reduced (by 75 to 100%) the cromakalim-or BRL 38227-stimulated increase in $^{43/42}K^+$ efflux from the trachealis muscle.

In conclusion, BRL31660 may be useful as a pharmacological tool to help to elucidate the role of potassium channel activation in the mechanisms of action of novel compounds.

Allen, S.L et al. (1986) Br.J. Pharmac. 89, 395-405
Arch, J.R.S. et al. (1987) Br.J. Pharmac. 95, 763-770
Bruckner, R et al. (1985) Naunyn-Schmiedebergs Arch Pharmacol. 329, 86-93
Buckingham et al. (1986) Br. J. Pharmac. 87, Proc. Suppl., 78P.
Hamilton, T.C et al. (1986) Br.J Pharmac. 88, 103-111
Taylor, S.G et al. (1989) Br. J. Pharmac. In Press 95, Proc. Suppl., 795P.
Wilson, C et al. (1988) Eur. J. Pharmac. 152, 331-339.

EFFECT OF POTASSIUM CHANNEL OPENERS ON CA $^{2+}$INFLUX, NA^+/CA^{2+} EXCHANGE AND NA^+/K^+ PUMPING IN GUINEA-PIG TRACHEA *IN VITRO*

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Potassium channel openers (KCOs) are proposed to relax smooth muscle by increasing K^+ conductance across the sarcolemma. The resultant hyperpolarization decreases the membrane potential below the threshold required for the opening of voltage operated Ca^{2+} channels (VOCs) and reduces the influx of Ca^{2+} thereby uncoupling the excitation-contraction sequence (Hamilton et al., 1986). The present study compares the actions of two KCOs, RP49356 ((±)-N-methyl(pyridyl-3)-2-tetrahydrothiopyrancarbothioamide-2-oxide-1) and cromakalim, with the Ca^{2+} entry blockers (CEBs) verapamil and nifedipine on other ionic processes affecting guinea-pig trachealis contractility in vitro.

Tracheas were removed from male Dunkin-Hartley guinea pigs (400 - 600 g.), cut into transverse strips and suspended under a 2g load in Krebs Henseleit solution maintained at 37°C and gassed with 5% CO_2 / 95% O_2. Changes in the force of isometric contraction were recorded by conventional means. Ca^{2+} induced contractions through VOCs were measured by adding back Ca^{2+} (0.1 or 2.5 mM) to tissues depolarized with 17.5 or 40 mM K^+ in the absence of extracellular Ca^{2+}. In separate experiments Na^+/Ca^{2+} exchange processes were studied by transferring tissues to a low Na^+ bathing solution; Na^+/K^+ ATPase activity was assessed using ouabain (1 uM) as an inhibitor. The concentrations of RP49356 and cromakalim used are equieffective at opening potassium channels in this tissue (Brown et al., 1989).

Nifedipine (1 uM) inhibited responses to Ca^{2+} influx in low and high K^+ depolarized tissues by more than 90% (n = 4) but RP49356 and cromakalim were effective only against Ca^{2+} (0.1 mM) in tissues depolarized with low K^+. No compound significantly affected Na^+/Ca^{2+} exchange in terms of the time course or magnitude of the contraction but all affected to some degree the contraction following ouabain treatment (see Table). The CEBs markedly inhibited the contractile response and tended to reduce the time to half maximum; both KCOs increased the time to half maximum response but only RP49356 reduced the magnitude of the response.

COMPOUND	% CONTROL MAX. CONTRACTION	TIME (min) TO HALF MAX. CONTRACTION
vehicle (n = 42)	100	4.75 ± 0.2
RP49356 (10 uM, n = 9)	56 ± 6*	6.19 ± 1.1*
cromakalim (1 uM, n = 9)	92 ± 8	5.85 ± 0.9*
verapamil (1 uM, n = 6)	44 ± 11*	4.09 ± 0.5
nifedipine (1 uM, n = 3)	13 ± 3*	3.85 ± 0.1

* $p < 0.05$ compared with vehicle control

These results suggest: 1) that the contraction seen in low Na^+ buffer was indeed due to Na^+/Ca^{2+} exchange rather than Ca^{2+} entry through VOCs since the CEBs were without effect 2) that at the concentrations studied KCOs do not affect Na^+/Ca^{2+} exchange and 3) that RP49356 and the CEBs but not cromakalim inhibit the contraction following inhibition of Na^+/K^+ ATPase activity. RP49356 and the CEBs differ since the KCO affected only responses to low Ca^{2+} in low K^+ depolarized tissues, an action on Na^+/K^+ ATPase rather than inhibition of the subsequent Ca^{2+} influx. The delayed action of ouabain in the presence of cromakalim cannot be explained at present.

Brown, T.B., Sweetland, J. & Raeburn, D. (1989) Pflugers Arch. (in press)
Hamilton, T.C., Weir, S.W. & Weston, A.H. (1986) Br. J. Pharmac. 88, 103 - 111.

STUDIES ON THE BRONCHODILATOR EFFECTS OF B_2-AGONISTS ADMINISTERED BY DRY POWDER OR BY AEROSOL TO THE ANESTHETISED GUINEA-PIG

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In the routine treatment of stable asthma, the most effective route of administration of bronchodilators is by inhalation. Difficulties experienced by some patients in using a metered dose pressurised aerosol, and the side effects attributed to aerosol propellants, have resulted in a recent trend towards administering drugs by inhalation of "dry powder" formulations. Because there are few animal models which permit the evaluation of such formulations, we have developed a procedure which allows compounds diluted with a lactose carrier to be administered to positively ventilated, anaesthetised guinea pigs. This method has been used to compare the bronchodilator efficacy of the B_2 agonists salbutamol and RP58802 (([benzimidazolyl-1]-H[(hydroxy-4-methoxy-3 phenyl)-2 hydroxy-2 ethyl] amino-3 butane dichlorhydrate, dihydrate) administered as dry powders or as nebulised aerosols.

Dry powder formulations of salbutamol and RP58802 were prepared by mixing micronised drug substance (median particle size 2-5 u) in a mortar with lactose (median particle size 60 u). The homogeneity of the mixture was assessed by U.V. spectroscopy on random samples of each powder mix. A bolus of dry powder was blown into the tracheal cannula by a rubber bulb from a tube fitted as a side arm to the air inlet from the respiration pump. The direction of flow was controlled with a three-way valve. Solutions for administration by aerosol were prepared in normal saline and nebulized for 1 min. using a deVilbiss "Pulmosonic" nebuliser. The amount of drug received by the animal was estimated from the residue remaining in the dry powder device or the nebulizer.

Male, Dunkin-Hartley guinea pigs (400-600 g) were anaesthetised with sodium pentobarbitone (60 mg/kg, i.p.) to measure histamine-induced bronchospasm using a modified Dixon-Brodie technique. Histamine (5-15 ug/kg, i.v. which produces approx. 60-70 % max. bronchospasm) was administered at 8 min intervals until responses were stable. Bronchodilator was given and the responses to histamine recorded until they returned to their pretreatment levels. The table shows the results for an inhaled dose of 5 ug of each drug powder (in a total of 5 mg lactose) or an equivalent dose of nebulized solution. Up to 40 mg of lactose alone or nebulizer drug vehicle had no effect on airway responses.

TREATMENT	EFFECT ON BRONCHOSPASM % INHIBITION	DURATION OF ACTION (min)
SALBUTAMOL Dry powder (n = 5)	63 ± 12	47 ± 10
SALBUTAMOL Aerosol (n = 6)	73 ± 4	35 ± 5
RP58802 Dry powder (n = 5)	65 ± 7	>90
RP58802 Aerosol (n = 5)	71 ± 6	>90

These results demonstrate that: 1) For each drug substance, there was no significant difference between the inhibition of histamine-induced bronchospasm achieved with dry powder formulation or aerosolised solution. From this we conclude that the dry powder technique is a valuable new method for evaluating bronchodilator activity in anaesthetised animals. 2) At the dose examined, RP58802 was equipotent with salbutamol but had a significantly longer duration of action when administered by either method.

EFFECTS OF CROMAKALIM, GALANIN AND GLIBENCLAMIDE IN VASCULAR SMOOTH MUSCLE

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Potassium (K) channels which are sensitive to changes in intracellular concentrations of ATP ([ATP]i) have been described in pancreatic ß-cells (Stanfield, 1987). These channels can be blocked by glibenclamide and are opened by galanin (de Weille et al., 1988). Recently it has been suggested that an ATP-sensitive K channel also exists in vascular smooth muscle (Davies et al., 1989) but it is not clear whether this K channel is involved in the vasorelaxant actions of cromakalim (CK). Therefore we have examined the effects of glibenclamide and lowering [ATP]i on the action of CK and also the effects of galanin in vascular smooth muscle.

Endothelium-free strips of aorta or whole portal veins were isolated from male Sprague Dawley (300-500g) rats and mounted in a tissue bath for either isometric tension recording or for [ATP]i assessment using a bioluminescence assay, or on a manifold for ^{86}Rb efflux studies (37°C, pH 7.4).

In rat aorta CK inhibited KCl (20mM)-induced contractions. This inhibition was antagonized by glibenclamide which altered the IC50 concentrations for CK from 0.07µM (control) to 0.20, 0.84 and 2.25µM (glibenclamide 0.1, 0.3, 1.0µM respectively; n = 6). Galanin acted as a mechanical stimulant. Efflux studies showed that galanin (100nM) or glibenclamide (1µM) had no effect on ^{86}Rb efflux, whereas CK (10µM) increased ^{86}Rb exchange. This stimulatory effect of CK was reduced in the presence of glibenclamide (1µM; see Table 1).

Table 1. Mean ^{86}Rb efflux rate coefficient (relative to control = 1)

	Aorta		Portal vein	
	CK	Galanin	CK	Galanin
Control	3.03	1.00	1.95	1.70
1µM Glibenclamide	1.10	not tested	1.48	not tested

In rat portal vein, CK inhibited spontaneous mechanical activity. This effect was antagonized by glibenclamide which shifted the IC50 values for CK from 0.09µM (control) to 0.20, 0.23 and 1.51µM (0.1, 0.3 and 1.0µM glibenclamide respectively; n = 4-6). Galanin caused an increase in the spontaneous mechanical activity. Following a 2-3h incubation period in DG buffer (ie MOPS physiological salt solution (PSS) containing 0mM glucose, 1mM 2-deoxyglucose and 0.24µg/ml oligomycin) [ATP]i was reduced from 0.05 ± 0.01µg/mg protein (control) to 0.02 ± 0.004µg/mg protein (n = 4) and also spontaneous mechanical activity of the portal veins was diminished. The activity was restored partially by incubation with glibenclamide (1µM), and it could subsequently be abolished by addition of CK (IC50 values for CK in normal MOPS and DG MOPS were 1.0 ± 0.10µM and 0.53 ± 0.07µM respectively; n = 4). Efflux studies showed that glibenclamide (1µM) had no effect on ^{86}Rb efflux, whereas galanin (10nM) and CK (10µM) increased ^{86}Rb exchange. CK (10µM)-induced ^{86}Rb efflux was inhibited by glibenclamide (see Table 1).

The loss of spontaneous mechanical activity in portal vein observed under conditions of lowered [ATP]i may reflect the opening of K channels which are sensitive to the effects of glibenclamide and CK. However, the results show that the concentrations of glibenclamide required to inhibit the action of CK in vascular smooth muscle are much higher than those required to exert effects in pancreatic ß-cells (de Weille et al., 1988). Furthermore, galanin produced excitatory effects on both types of smooth muscle examined. These observations suggest that the K-channel involved in the action of CK in smooth muscle is not identical to the ATP-sensitive K-channel found on pancreatic ß-cells.

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Davies, N.W., Nelson, M.T., Quayle, J.M., Standen, N.B. & Huang Yu (1989) J. Physiol., in press.
Stanfield, P.R. (1987) Tr. Neurosci., 10, 335-339.
de Weille, J., Schmid-Antomarchi, H., Fossett, M. & Lazdunski, M. (1988) Proc. Natl. Acad Sci. (USA), 85, 1312-1316.

DIFFERENTIAL CONCENTRATION-DEPENDENT EFFECTS OF K CHANNEL OPENERS ON ^{42}K AND ^{86}RB EFFLUX IN RABBIT ISOLATED AORTA

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$^{86}Rb^+$ is frequently used as a marker for K^+, although the basal efflux of $^{86}Rb^+$ is lower than that of $^{42}K^+$ and the use of $^{86}Rb^+$ is thought to underestimate stimulated $^{42}K^+$ efflux (Smith, Sanchez & Jones, 1986; Quast & Baumlin, 1988). In the present study the concentration - dependent effects of the K^+ - channel openers cromakalim and pinacidil on $^{42}K^+$ - and $^{86}Rb^+$ - efflux, have been examined.

Endothelium - free strips of rabbit aorta were prepared from male Half - lop rabbits (2 -3Kg). Each strip was impaled on to a syringe needle and attached to a perspex gassing manifold. After an initial equilibration period in MOPS - buffered physiological salt solution (PSS), tissues were transferred to PSS containing $^{42}K^+$ (1.57μCi. ml-1) or $^{86}Rb^+$ (5μCi.ml-1) for 180 and 90min, respectively. Exposure to cromakalim (CK) or pinacidil was made between the 16th and 26th minute of efflux into PSS.

In the 16min prior to drug exposure the mean efflux of $^{42}K^+$ and $^{86}Rb^+$ from aortic strips was 0.34 ± 0.02 %.min^{-1} and 0.27 ± 0.02%.min^{-1}, respectively. Thus there was a small but significantly greater basal efflux of $^{42}K^+$ from these tissues. Mean increases in efflux (Δ%) averaged over the period of exposure to cromakalim or pinacidil were calculated and are shown in Table 1.

Table 1

	Δ efflux %		
	$^{42}K^+$	$^{86}Rb^+$	$^{42}K^+/^{86}Rb^+$
cromakalim			
0.3μM	0.116 ± 0.004	0.025 ± 0.001	4.64±0.42
1μM	0.926 ± 0.010	0.100 ± 0.004	9.26±0.61
10μM	1.560 ± 0.100	0.680 ± 0.020	2.29±0.08
pinacidil			
1μM	0.116 ± 0.005	0.020 ± 0.004	5.80±1.28
10μM	0.794 ± 0.090	0.330 ± 0.060	2.40±0.34

values are mean ± s. e. mean, n = 6.

In a separate series of experiments the mean control (ethanol) efflux of $^{42}K^+$ in MOPS - PSS and Ca^{2+} - free - PSS containing EGTA (2mM) and $MgCl_2$ (10mM) was 0.41 ± 0.01%.min^{-1} and 0.62 ± 0.08%.min-1, respectively. Thus, there was a significant increase in the permeability of tissues to $^{42}K^+$ under Ca^{2+} - free conditions. In the presence of CK (1μM) the mean efflux of of $^{42}K^+$ in MOPS - PSS and Ca^{2+} - free PSS increased 3.3 - and 3.9 - fold, respectively.

Exposure of tissues to CK (0.3 and 1μM) and pinacidil (1μM) resulted in increased $^{42}K^+$ permeability relative to changes in $^{86}Rb^+$ permeability. However, when the concentration of these relaxants was increased to 10μM the ratio of $^{42}K^+$ / $^{86}Rb^+$ efflux fell due to a large increase in $^{86}Rb^+$ permeability. Thus, cromakalim and pinacidil may exert their relaxant effects via the concentration - dependent opening of more than one type of K^+ - channel; one permeable to K^+ and Rb^+ and one relatively impermeable to Rb^+ . Furthermore, the opening of these channels is independent of extracellular Ca^{2+} as suggested by Howlett & Coldwell (1987).

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Coldwell, M. C. & Howlett, D. R. (1988). Biochem. Pharmacol., 37, 4105 - 4110.
Quast, U. & Baumlin, Y. (1988). Naunyn - Schmiedeberg's Arch. Pharmacol., 338, 319 - 326.
Smith, J. M., Sanchez, A. A. & Jones, A. W. (1986). Blood Vessels, 23, 297 - 309.

CARDIOVASCULAR TOLERANCE TO CROMAKALIM IN THE RAT *IN VIVO*

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Cromakalim is a potent relaxant of smooth muscles including those of vascular and uterine tissues. A single dose of cromakalim produced sustained reduction in blood pressure (BP) and prolonged inhibition of uterine contractions in vivo in the rat (Buckingham et al., 1986, Downing et al., 1989). Inhibition of uterine contractions was not maintained with repeated doses of cromakalim. After 20h of cromakalim treatment, uterine sensitivity to the drug was 25-fold lower than that seen in vehicle treated rats. The following study was performed to determine whether tolerance to cromakalim in vivo extended to the cardiovascular system.

Female rats (200-250g) were anaesthetized with tribromoethanol (240 mg kg^{-1}, i.p.), bilaterally ovariectomized and the left carotid artery and right jugular vein cannulated. One day following surgery, BP and heart rate were recorded before and after administration of a single iv sensitivity test bolus dose of cromakalim (0.01, 0.05, 0.1, 0.5 or 2 $mgkg^{-1}$, 10 rats per dose). Effects on the cardiovascular system were determined at 30 min after cromakalim and expressed as percent change in BP and heart rate compared with means of the parameters recorded prior to drug administration. Four hours later, the rats were given the first of 3 bolus iv doses of either cromakalim (1 mg kg^{-1}) or vehicle (15% ethanol/saline, 1 ml kg^{-1}), 8 h apart. Mean BP was determined at 1 h intervals after each of the 3 doses of cromakalim or vehicle. Four hours after the third cromakalim or vehicle injection, the rats received the same sensitivity bolus dose of cromakalim and the cardiovascular response determined as before.

The first dose of cromakalim treatment (1 mg kg^{-1}) produced a mean maximum fall in BP of 35.9 ± 5.4%. BP remained significantly lower than in vehicle treated rats for 6h. The second and third doses of cromakalim (1 mg kg^{-1}) produced significantly smaller mean maximum reductions in BP (26.4 ± 4.6%, $p<0.02$ and 18.2 ± 6.3%, $p<0.001$, respectively) compared with that produced by the first dose, and their effects were of shorter duration.

Sensitivity of the cardiovascular system to cromakalim was similar pre- and post-vehicle treatment for both BP (pre-vehicle ID_{20}, 0.07 mg kg^{-1}; post-vehicle ID_{20}, 0.06 mg kg^{-1}) and heart rate (pre-vehicle ED_{20}, 0.06 mg kg^{-1}; post-vehicle ED_{20}, 0.06 mg kg^{-1}). Falls in BP in response to bolus doses of cromakalim were significantly less after prolonged treatment with the drug ($p < 0.001$) and an increase in ID_{20} was observed in cromakalim-treated rats (0.48 mg kg^{-1}) compared with pre-cromakalim treatment (0.095 mg kg^{-1}). Changes in heart rate in response to sensitivity bolus doses of cromakalim were inconsistent in cromakalim treated rats and it was not possible to determine the ED_{20}.

Tolerance to cromakalim was observed in the cardiovascular system in vivo, with a 5-fold reduction in sensitivity for mean BP after repeated cromakalim treatment. This phenomenon occurred over a similar time period, although to a lesser extent, to that observed previously in uterus (Downing, et al., 1989).

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Buckingham, R.E., Clapham, J.C., Hamilton, T.C., Longman, S.D., Norton, J. & Poyser, R.H. J. Cardiovasc. Pharmacol., 8, 798-804 (1986).
Downing, S.J., Miller, M. & Hollingsworth, M. Br. J. Pharmacol., 96, 732-738 (1989).

CROMAKALIM, RP49356 AND RAT UTERUS *IN VIVO* - ANTAGONISM BY GLIBENCLAMIDE

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Cromakalim inhibits uterine contractions in the rat in vivo at doses which lower blood pressure (Downing et al., 1989). RP 49356, another putative K+ channel opener, is also a vasodilator (Mondot et al., 1988). Cromakalim and RP 49356 are relaxants of the isolated rat uterus and are antagonised by glibenclamide (Piper & Hollingsworth, 1989). We aimed to determine if the uterine relaxant effects of RP 49356 extended to the rat in vivo and whether cromakalim and RP 49356 were antagonised by glibenclamide in this situation.

Female rats (200-250g) were anaesthetized with tribromoethanol (240 mg kg-1, i.p.) bilaterally ovariectomized, the right jugular vein cannulated and an intrauterine microballoon inserted. One day following surgery, uterine contractions were recorded for 30 min before and 2-3 h after an i.v. bolus dose of cromakalim (0.1 mg kg-1) or RP 49356 (0.1 mg kg-1). Glibenclamide (20 mg kg-1) or solvent (0.02 N sodium hydroxide in 4% w/v glucose; 5 ml kg-1) was then infused over 5 min. The same dose of cromakalim or RP 49356 was repeated 30 min later. The integral of contractions for the 30 min after each dose of cromakalim or RP 49356 was expressed as a % of the integral for 30 min prior to the injection. In a second group of rats, the left carotid artery was cannulated, instead of uterine balloon placement. The same experimental design was adopted to enable measurement of the effects of cromakalim and RP 49356 on blood pressure (BP) and heart rate before and after glibenclamide or vehicle infusion.

The pre-infusion dose of cromakalim and RP 49356 produced inhibition of uterine contractions of 77.3 ± 4.7% (n=9) and 51.1 ± 13.8% (n=5) respectively. There was a peak fall in BP of 41.6 ± 3.9% (n=8) and 26.6 ± 4.3% (n=6) and a tachycardia of 17.6 ± 2.8% (n=8) and 13.9 ± 2.5% (n=6) respectively. By 2-3 h the uterine inhibitory effects of cromakalim and RP 49356 had largely ceased. The durations of the actions of RP 49356 were less than those of cromakalim. The post-infusion dose of these compounds in vehicle-treated controls produced inhibitions of uterine contractions of 64.5 ± 20.3% (n=4) and 50.8 ± 4.8% (n=2) respectively, similar to pre-infusion effects and produced similar falls in BP and tachycardia as for the first dose. Cromakalim and RP 49356 inhibited uterine contractions after glibenclamide infusion by only 23.3 ± 5.9% (n=5) and 22.8 ± 3.7% (n=3). After glibenclamide, cromakalim produced only a small fall in BP of 9.3 ± 3.3% (n=4) by 60 min and RP 49356 failed to lower BP (n=3).

RP 49356, like cromakalim, is a uterine relaxant in the rat in vivo at a dose which also lowers BP. Both cromakalim and RP 49356 can be antagonised by glibenclamide in the rat uterus in vivo suggesting that their mechanism(s) of action in this tissue is similar to that in the cardiovascular system (Buckingham et al., 1989).

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Buckingham, R.E., Hamilton, T.C., Howlett, D.R., Mootoo, S. & Wilson, C. (1989) Br. J. Pharmacol. 97, 57-64.
Downing, S.J., Miller, M. & Hollingsworth, M. (1989) Br. J. Pharmacol. 96, 732-738.
Mondot, S., Mestre, S., Gaillard, C.G. & Cavero, I. (1988) Br. J. Pharmacol. 95, 813P.
Piper, I. & Hollingsworth, M. This meeting.

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POTASSIUM CHANNEL ACTIVATION IN SMOOTH MUSCLE CELLS MAY BE ASSOCIATED WITH DECREASES IN INTRACELLULAR ATP

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Previous studies with the novel antihypertensive agent, cromakalim, have suggested that vasorelaxation, and the ability to hyperpolarise the smooth muscle cell membrane, may reflect activation of ATP-sensitive potassium channels (Howlett & Longman, 1989). More recently, potassium channels, whose open-state probability is dependent on intracellular ATP levels, have recently been demonstrated in vascular smooth muscle (Davies et al, 1989). Since cromakalim has been shown to act by a stereospecific mechanism with biological activity residing predominantly in the (-)-enantiomer (BRL 38227) (Buckingham et al, 1986), this study attempts to investigate the stereospecific nature of any involvement with intracellular ATP and whether this may be associated with potassium channel opening.

Smooth muscle cells were dispersed enzymatically from rabbit mesenteric arteries, cultured as previously described (Howlett & Longman, 1989), and were used between passages 3 to 12. During experiments, cells were incubated in vehicle/drug made up in HEPES buffer (KCl content 5 mM). Intracellular ATP was measured using luciferin-luciferase as described by Nguyen et al (1988). Exposure to BRL 38227 (20nM - 100nM) for 120 min significantly decreased ATP levels with a maximum effect at 100nM (120 min control 387±42ng ATP/well; BRL 38227 275±33; $P<0.001$; n=8). No significant changes in ATP levels occurred at higher (200nM) or lower (10nM) concentrations of BRL 38227 or with 100nM BRL 38226 ((+)-enantiomer of cromakalim) for 120 min. In separate experiments, the fall in ATP levels following 120 min exposure to BRL 38227 (100nM) was markedly reduced by the sulphonylurea, glibenclamide (GBC) (3μM prior to/during BRL 38227 exposure). Furthermore, the fall in ATP levels witnessed following 120 min exposure to BRL 38227 (100nM) diminished as the buffer KCl concentration increased. Thus, significant reductions in intracellular ATP levels were evident at 5, 15 and 30mMKCl but not at 60 and 90mMKCl. Interestingly, control ATP levels were also diminished with increasing KCl concentration (5mMKCl 390 ± 29 ng ATP/well (n=8); 60mMKCl 261±12ng ATP/well (n=7), $P<0.01$; 90mMKCl 224±20ng ATP/well (n=7), $P<0.001$).

The present study with BRL 38227 has confirmed previous observations with cromakalim demonstrating an ability to decrease intracellular ATP in vascular smooth muscle cells. This effect is stereospecific since BRL 38226 was impotent in this respect. The ability of BRL 38227 to evoke GBC-sensitive falls in intracellular ATP, coupled with earlier data showing enhanced 86-rubidium efflux with cromakalim (Howlett & Longman, 1989) supports the theory that both compounds may act by opening ATP-sensitive potassium channels. This may be substantiated by recent electrophysiological evidence of an interaction of cromakalim with ATP-sensitive potassium channels in vascular tissue (Standen et al, in press). The inability of BRL 38227 to lower intracellular levels of ATP at high (60 and 90mMKCl) concentrations of potassium, where the smooth muscle cell membrane would be depolarised, is consistent with previous studies where the vasorelaxant effects of cromakalim were only seen at low potassium concentrations (Hamilton et al, 1986). This latter effect, together with the reversal of the BRL 38227 response (in 5mMKCl) by GBC, suggests that the decreases in ATP with BRL 38227 are a function of the potassium channel opening.

Buckingham, R.E. et al (1986), Br. J. Pharmac; 87, 78P.
Davies, N.W. et al (1989), J. Physiol; (London meeting, in press).
Hamilton, T.C. et al (1986), Br. J. Pharmac; 88, 103.
Howlett, D.R. and Longman, S.D. (1989), Br. J. Pharmac; 96, 256P
Nguyen, V.T. et al (1988), Anal. Biochem; 171, 404.
Standen, N.B. et al (1989), Science; (in press).

COMPARATIVE EFFECT OF GLIBENCLAMIDE ON RESPONSES TO CROMAKALIM AND HYPOXIA *IN VITRO*

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The vasorelaxant effect of the K^+ channel activator, cromakalim (CRK), in noradrenaline-contracted rabbit isolated mesenteric artery (RIMA) was antagonised by the sulphonylurea, glibenclamide (GLIB), with a pA_2 value of 7.2 (Wilson, 1989). Since GLIB blocks ATP-sensitive K^+ channels in various tissues, this raises the possibility that CRK may activate such a channel in RIMA. Hypoxia relaxes RIMA (Marriot & Marshall, 1988) and also increases K^+ efflux from e.g. cardiac muscle via an ATP-sensitive K^+ channel (Noma, 1983). More recently, it has been suggested that CRK, like hypoxia, activates ATP sensitive K^+ channels in the heart (Sanguinetti et al, 1989). In order to investigate whether CRK and hypoxia share a common mechanism in RIMA, the comparative effect of GLIB on CRK- and hypoxia-induced vasorelaxation and ^{86}Rb (marker for K^+) efflux was studied.

Isometric tension was recorded from rings of RIMA superfused with HEPES buffer gassed with 5% CO_2 in O_2. Tissues were contracted by introducing phenylephrine (PHE; 0.1 mM) into the superfusing solution. CRK (3 μM) significantly reduced developed tension from 4992 ± 736 to 820 ± 576 mg (n=5; $p<0.001$). In tissues pretreated for 15 min with GLIB (3 μM), and in which GLIB itself did not affect tension (n=5), CRK (3 μM) only reduced tension from 4910 ± 465 to 4167 ± 619 mg (n.s. $p>0.05$). In separate experiments (n=5) where CRK (3 μM) reduced tension from 3471 ± 702 to 697 ± 163 mg, GLIB (3 μM) restored tension to 2983 ± 633 mg ($p<0.001$). In other tissues, hypoxia was induced by replacing glucose in the HEPES buffer with mannitol and gassing with 100% N_2. Hypoxia (bath pO_2 = 30 mmHg) relaxed PHE-contracted RIMA from 5425 ± 918 to 280 ± 129 mg (n=5; $p<0.001$). In tissues pretreated with GLIB (3 μM), hypoxia similarly reduced tension from 4884 ± 1079 to 520 ± 206 mg (n=5; $p<0.001$). GLIB (3 μM) also failed to restore tension in tissues already relaxed by hypoxia. In a further study in which the endothelium had been removed from RIMA, relaxation to hypoxia was attenuated. GLIB (3 μM), however, had no effect upon the remaining response.

^{86}Rb efflux was measured in RIMA incubated in HEPES buffer as described previously (Coldwell & Howlett, 1987). CRK (10 μM) increased the efflux rate by 92 ± 20% (basal rate = 2.17%/3 min; n=14). This response was reduced to 74 ± 15% (n=10; n.s.), 49 ± 8% (n=7; $p<0.05$) and 10 ± 4% (n=7; $p<0.01$) by GLIB, 0.1, 0.3 and 1.0 μM respectively. Hypoxia, induced as before, increased the ^{86}Rb efflux rate by 85% ± 23% (n=7). In the presence of GLIB (1 μM), this stimulation to hypoxia was reduced to 22 ± 16% (n=8; $p<0.05$).

The results demonstrate that GLIB inhibits both CRK- and hypoxia-induced Rb efflux, but only CRK-induced vasorelaxation. Hypoxia-induced relaxation was partly endothelium-dependent but not sensitive to GLIB. Thus, CRK and hypoxia appear to activate a GLIB-sensitive, ^{86}Rb-permeable K^+ channel (which may or may not be ATP-sensitive) in RIMA. This mechanism appears to underlie the relaxant action of CRK but not that of hypoxia.

Coldwell, M.C. & Howlett, D.R. (1987) Biochem. Pharmac., 36, 3663-3669.
Marriott, J.F. & Marshall, J.M. (1988) J. Physiol., 403, 120P.
Noma, A. (1983) Nature, 305, 147-148.
Sanguinetti, M.C., Scott, A.L., Zingaro, G.J. & Siegl, P.K.S. (1989) Proc. Natl. Acad. Sci. USA, 85, 8360-8364.
Wilson, C. (1989) J. Autonom. Pharmac., 9, 9-16.

TOLERANCE TO THE OESTROGEN-LOWERING EFFECT OF AMINOGLUTETHAMIDE IN THE RAT

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While the acute interaction of the aromatase inhibitor, aminoglutethimide (AG) with its target enzyme has been extensively examined experimentally in vitro and in vivo (Brodie et al. 1986), less is known of its activity on chronic dosing in animal models. Wing et al. (1985) suggested that, after 4 weeks of daily treatment with AG, tolerance to the effect of the drug occurs in female rats via a pituitary/hypothalamic feedback mechanism. The present work provides a more detailed study of the time-course of this phenomenon.

Female albino rats (250-300g; n=6) received daily oral doses ($2ml kg^{-1}$) of either AG (1, 10 or $25mg kg^{-1}$) or vehicle (1% w/v carboxymethylcellulose containing 0.1% v/v Tween-80) for 35 days. Blood samples were collected by cardiac puncture under ether anaesthesia for the determination of oestradiol (by RIA) on days 1, 9, 18, 25 and 35, 3h after AG administration. Four days prior to each blood sampling (viz. days -3, 5, 14, 21 and 31) the rats received pregnant mares' serum gonadotrophin (PMSG, 100 iu, sc).

Table 1: Effect of daily administration of AG ($25mg kg^{-1}$,po) on plasma oestradiol (pmol L^{-1})

	Day of AG treatment				
	1	9	18	25	35
Control	1798 (±382)	2912 (±92)	233 (±92)	198 (±99)	444 (±258)
AG	99* (±17)	177* (±40)	89 (±33)	148 (±26)	228 (±129)

Values are means ± SEM (n=6) *P<0.05

It may be observed (Table 1) that the response (elevation of plasma oestradiol levels) to PMSG appeared to diminish with repeated administration of the latter. Unpublished observations (Sadri) indicate that this is not due to a desensitisation but rather to a shift in the effect-time profile with peak oestradiol levels occurring earlier. At the $25mg kg^{-1}$ dose level, AG initially reduced oestradiol levels by 94%. By day 18, tolerance was evident (62% lowering) and this was complete by day 25. With the initial dose at the 1 and $10mg kg^{-1}$ levels, there were reductions of 27 and 84% respectively in the oestradiol levels. Tolerance to the effect at these doses was complete by day 25.

As autoinduction of AG metabolism in rats receiving the drug chronically is of a low order and is maximal within 10 days (Ahmad et al. 1988), the results indicate that other mechanisms must be involved in contributing to the tolerance observed in the present experiments.

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Ahmad, B. et al. (1988) J. Pharm. Pharmacol. 40, 99P
Brodie, A.H.M. et al. (1986) J. Steroid Biochem. 25, 859-865
Wing, L.Y. et al. (1985) Cancer Research 45, 2425-2428

COMPARISON OF AMINOGLUTETHIMIDE AND TWO NOVEL ANALOGUES AS INHIBITORS OF AROMATASE IN HUMAN BREAST TUMOURS

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Breast tumours convert partially-formed precursors into active steroids, some tumours aromatising androgens into oestrogen (Abul-Hajj 1982; Miller & Forrest 1976) and treatment of hormone-dependent breast cancer with aromatase inhibitors requires effective inhibition of oestrogen biosynthesis. This work investigates the ability of 4-pyridylglutethimide (PyG; Foster et al. 1985) and 3-(4 -aminophenyl)-pyrrolidine-2,5-dione (WSP-3; Pourgholami et al. 1985), both analogues of the aromatase inhbitor aminoglutethimide (AG), to inhibit in vitro aromatisation in human breast carcinoma.

Breast cancer tissue was obtained during mastectomy and stored at -20°C until assayed. The minced tumour (Hallam et al. 1988) was incubated with 7α-^{3}H-testosterone and a NADPH generating system for 2h at 37°C in the presence and absence of inhibitor (AG, PyG 50μM, WSP-3 100μM). The method detects conversions of >0.02% (Miller et al. 1974; Miller & Forrest 1976).

Evidence for oestrogen synthesis was obtained in 12 of the 14 tumours examined with conversion ranging from 0.02 to 1.86%. Conversion levels were reduced in 5 of the 7 tumours incubated with PyG, 4 of the 7 incubated with WSP-3 and 3 of the 5 incubated with AG. In the incubations where the novel analogues were compared to AG directly (Table 1) the latter appeared to be the most potent inhibitor.

Table 1. Percentage conversion of testosterone to oestradiol by breast carcinoma

Incubation	1	2	3	4	5
Control	0.31	<0.02	1.86	0.04	0.17
PyG (50μm)	0.07	<0.02	1.43	-	-
AG (50μm)	<0.02	<0.02	0.55	0.04	0.03
WSP-3 (100μM)	-	-	-	0.04	0.04

Values are the mean at 3 determinations

PyG and WSP-3 possess the advantage over AG of greater selectivity in not inhibiting the side chain cleavage of cholesterol (Foster et al. 1985; Pourgholami et al. 1987). Thus despite the lower potencies, the present results suggest that both new compounds are worth further investigation for their application in the management of advanced oestrogen-dependent breast cancer.

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Abul-Hajj, Y.R. (1982) Cancer Res. 42, 3373S-3375S
Foster, A.B. et al. (1985) J. Med. Chem. 28, 200-204
Hallam, A. et al. (1988) Br. J. clin. Pharmacol. 26, 633P
Miller, W.R. et al. (1974) Steroids 23, 379-385
Miller, W.R. and Forrest, A.P. (1976) Br. J. Cancer 33, 116-118
Pourgholami, M.H. et al. (1987) J. Steroid Biochem. 26, 309-312

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DEPENDENCE OF THE FIBRINOLYTIC RESPONSE ON PLASMA tPA AND PAI-1 LEVELS IN THE LABORATORY RAT

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A compromised fibrinolytic response has been assosciated with the development of thrombotic disorders contributing to cardiovascular disease. The main proteins responsible for the regulation of the fibrinolytic response are tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI).

It is well established that alterations in the tPA/PAI balance can lead to altered fibrinolytic activity and that the systemic levels of these proteins can be influenced by various pharmacological agents.

We have investigated changes in the fibrinolytic response of the rat following administration of agents reported to influence basal levels of either tPA or PAI.

tPA (IU/ml) and PAI activity (AU/ml, one AU quenching one IU of tPA activity) were measured in rat plasma using a functional chromogenic assay. Changes in the fibrinolytic capacity of the plasma were investigated using the euglobulin clot lysis time assay (ECLT). Values means ±S.E.M n=6.

Control rat plasma demonstrated basal tPA activity of 14.3 IU/ml ±1.5, PAI activity of 38.5 AU/ml ±1.7 and 50% ECLT of 124 mins ±11.7.

Acute administration of platelet activating factor (PAF) 0.3-3ug/kg I.V produced a dose related increase in functional tPA activity (57.5 IU/ml ±9.6) with no change in PAI activity (38.8 AU/ml ±0.5). Enhanced fibrinolytic activity was demonstrated by a reduction in 50% ECLT (12 mins ±1.4).

Acute administration of endotoxin (3-300ng/kg I.V, 3hr pretreatment) produced a marked increase in plasma PAI activity (50.9 AU/ml ±2.9) but no change in functional tPA activity (14.9 IU/ml ±3.6). Depression of the fibrinolytic response was demonstrated by prolongation of the 50% ECLT (>300 mins).

In conclusion, elevation of either tPA or PAI leads to dramatic changes in the fibrinolytic response and demonstrates the important role of these proteins in maintaining the systemic fibrinolytic balance of the laboratory rat.

PLASMINOGEN ACTIVATOR INHIBITOR LEVELS IN AN ARTHRITIC RABBIT MODEL - EFFECTS WITH THE ANABOLIC STEROID STANOZOLOL

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A compromised fibrinolytic response has been associated with the development of thrombotic disorders contributing to cardiovascular disease. The main proteins responsible for the regulation of the fibrinolytic response are tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI). Recent data suggests that PAI may behave like a typical acute phase reactant. The anabolic steroid Stanozolol has been shown to reduce elevated PAI levels in man leading to normalisation of a compromised fibrinolytic response. This agent has also been reported as demonstrating beneficial effects in rheumatoid arthritis.

The aim of this study was to investigate changes in circulating levels of PAI and fibrinogen (an acute phase reactant) during the development of chronic inflammation in the rabbit antigen-induced arthritis model, and to determine whether Stanozolol demonstrated any anti-inflammatory activity alongside changes in PAI and fibrinogen levels.

Monoarticular arthritis was induced in male Dutch rabbits (2-3kg) by immunisation to ovalbumin followed by an intra-articular (ia) injection of antigen into one knee. Stanozolol was dosed (10mg/kg/day im) for two days prior to the ia. challenge and up to 28 days post challenge. tPA (IU/ml) and PAI (AU/ml, one AU quenching one IU of tPA activity) were measured in rabbit plasma using a functional chromogenic assay. Fibrinogen levels were assayed according to the Clauss technique. Development of the arthritic response was monitored by Knee width measurement.

PAI and fibrinogen levels increased over the immunisation period. PAI activity increased from 9.9±0.5 AU/ml at the start of the study to 36.2±1.2 AU/ml prior to ia. challenge. Fibrinogen levels increased from 224±5.8 mg/dl to 336.9±9.2 mg/dl following immunisation. No change was seen in circulating tPA activity. Following the arthritic challenge a further dramatic and rapid increase in both PAI and fibrinogen levels was observed. PAI activity increased to 62.9±2 AU/ml and fibrinogen levels to 679.6±10.9 mg/dl. There was no change in the circulating tPA activity. After the acute inflammatory response had subsided (from day 8), PAI activity and fibrinogen levels recovered to post immunisation levels but did not normalise. Test rabbits given Stanozolol demonstrated no reduction in the elevated levels of PAI or fibrinogen, and no effect on the inflammatory response was seen (values means ±S.E.M; n=35).

In conclusion, this study demonstrates elevated levels of both PAI and fibrinogen in a model of antigen induced arthritis. These elevations are most pronounced in the acute phase immediately following arthritic challenge. Changes in PAI closely parallel changes in fibrinogen levels reflecting the acute phase reactant nature of the PAI response. We could not demonstrate a reduction in elevated PAI or any anti-inflammatory response following treatment with Stanozolol in this rabbit model.

A PHARMACOLOGICAL ACTION OF VITAMIN E RELATED TO ITS STRUCTURE

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Vitamin E (alpha-tocopherol) has previously been shown to protect the functioning of a number of isolated tissues from the deleterious effects of hypoxia (Kelly, 1988). In one of these tissues, guinea-pig colon, the vitamin stimulates mechanical activity in a dose-dependent manner during hypoxia but not during normoxia (Kelly, 1986). Although vitamin E is known to be an anti-oxidant (Century and Horwitt, 1965) this property does does not appear to be responsible for its pharmacological action in colonic tissue (Kelly, 1989). The possibility that the restorative action of the vitamin in hypoxia is related to structural features of the molecule was therfore investigated.

Small segments (2 cm long) of guinea-pig colon were suspended in tissue baths containing Krebs-Henseleit solution, maintained at 32°C ± 1°C and bubbled with a $95\%O_2$ /$5\%CO_2$ gas mixture. During this period spontaneous mechanical activity was observed in the colon and the effects of a range of drugs on this activity were measured. After 30 min. this 'normoxic' environment was converted into a 'hypoxic' one by bubbling with $95\%N_2/5\%CO_2$. After 15 min., when oxygen tension had fallen to less than 0.1% of its original level, spontaneous mechanical activity had ceased and drug effects on this reduced basal tension were recorded.

The stimulatory response to (+)-alpha-tocopherol in hypoxia was dose-dependent, with a mean EC_{50} value of 10.6±0.9μM (± s.e.mean) for 11 animals. Another lipid soluble vitamin, K_1, with a similar ring and phytyl side chain structure was also active, but less potent, in the colon while agents with the ring structure alone, like Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), were inactive.

The side chain element appeared to be an important entity in initiating this pharmacological response to vitamin E and therefore a number of individual chains, ranging from 3 carbon to 18 carbon lengths, were tested. Phytol was the most potent agent tested with an EC_{50} value of 1.0 +0.2μM (± s.e.mean) for 6 animals. Expressing the potency of phytol as 100%, the other agents tested had the following potencies: cetyl alcohol 40%, 1-tetradecanol 33%, stearyl alcohol 20%, lauryl alcohol 14%, (+) -alpha-tocopherol 10%, dodecane 1,12 diol 8%, vitamin K 5%, retinol 5%, geraniol 4%, farnesol 3%, undecan-1-ol 3%, 11 bromo undecan-1-ol 0%, 1-decanol 0%, 3-methyl-2 buten-1-ol 0%, allyl alcohol 0%.

Ring structures like Trolox and vitamin K_3 had no activity but appeared to be antagonists of both vitamin E and the alcohol chains. The results suggest that this pharmacological action of vitamin E is related to particular features of its structure.

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Century, B. & Horwitt, M.K. (1965) Fed. Proc. 24, 906-911.
Kelly, M.J. (1986) J. Pharmacol. 38, 66-68.
Kelly, M.J. (1988) Prog. Medicinal Chem. 25, 249-290.
Kelly, M.J. (1989) Leeds B.P.S. Meeting.

EFFECT OF DESIPRAMINE ON NORADRENALINE-INDUCED RESPONSES IN THE RAT ISOLATED SEMINAL VESICLE

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Desipramine is a tricyclic antidepressant which is the principal active metabolite of imipramine. Desipramine (DMI) has anticholinergic and anti-reserpine activities, which might contribute to its beneficial effect as a sedative. The antidepressant effect of DMI is thought to be due to inhibition of noradrenaline (NA) re-uptake by adrenergic neurones in the CNS, and thus increasing the availability of NA, resulting in a net enhancement of neurotransmission (Sulser, et.al., 1969; Carlsson, 1975; Bill, et.al., 1989).

The aim of the present investigation was to study the effects and interactions of DMI with NA and clonidine, to see if DMI inhibited the responses of exogenous and endogenous NA, in the presence of pharmacological antagonists, e.g., phentolamine, methysergide, α,β-Methylene ATP and atropine, and to characterise the NA response in the rat isolated seminal vesicle.

Seminal vesicles were obtained from male, adult, Sprague-Dawley rats, weighing 200-300 g. Tissues were cut spirally into strips 2-3 cm long and 2 mm wide (Campbell, et.al., 1983; Docherty & Hyland, 1985). The preparation was set up in an organ bath containing 20 ml Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% CO_2 in O_2. The contractile responses produced by drugs or field stimulation (FS), at 1-100 Hz, with 20 V (supramaximal) and 0.5 ms pulse duration, were recorded isometrically, and the results are shown in Table 1.

Table 1 Effect of desipramine on the responses produced by NA, clonidine and FS in the rat seminal vesicle.

	Maximum Response (g)	Control (C) $EC_{50}/Freq_{50}$* (μM) (Hz)*	Desipramine (D) $EC_{50}/Freq_{50}$* (μM) (Hz)*	Ratio of $EC_{50}/Freq_{50}$* (C/D)
NA (0.1-100 μM)	1.7±0.3	34.5±0.8	23.1±2.1	1.5:1.0
Clonidine (3.8-380 μM)	0.5±0.1	98.1±1.9	178.2±10.4	0.6:1.0
FS at 50 Hz	1.1±0.2	39.4±3.1 *	22.6±0.6*	1.8:1.0*

The results are means±s.e., n=6. NA (0.1-100 μM), clonidine (3.8-380 μM) and FS (1-100 Hz) produced concentration and frequency-dependent contractions in the rat seminal vesicle. The mean maximum contraction produced by NA, clonidine and FS were 1.7±0.3, 0.5±0.1 and 1.1±0.2 g respectively (means±s.e., n=6 rats, Table 1). Based on the EC_{50} values, NA was about 3 times as effective as clonidine in contracting the rat seminal vesicle. In addition, DMI, which on its own had little effect on the seminal vesicle, significantly ($P<0.001$) potentiated the NA responses, whereas it significantly reduced the clonidine response. DMI (3.3 μM) also significantly increased the FS-induced contractions in the presence of atropine (1 μM), methysergide (1 μM), α,β-Me-ATP (10 μM). Phentolamine (1 μM) reduced the FS contractions by 86±3.2% of the control value. Thus, DMI had a differential effect of the NA and clonidine-induced responses in the rat isolated seminal vesicle.

Bill, D.J., Hughes, I.E. & Stephens, R.J. (1989). Br.J.Pharmacol., 96, 144-152
Campbell, J.K., Marshall, R.J. & Winslow, E. (1983). Eur.J.Pharmacol., 86, 217-228
Carlsson, A. (1975). Pharmacol.Ther.Bull. 1, 393-400
Docherty, J.R. & Hyland, L. (1985). Br.J.Pharmacol., 84, 573-576
Sulser, F., Owns, M.L., Strada, S.J. & Dingell, J.V. (1969). J.Pharmacol.Exp.Ther., 168, 272-282

GLYCOSYLATION OF THROMBIN MODULATES BIOLOGICAL ACTIVITY

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Thrombin is a glycoprotein serine protease which plays a central role in haemostasis (1). Thrombin induces clot formation by a number of mechanisms including cleavage of fibrinogen to fibrin, activation of other factors in the coagulation cascade and by activating platelets (1). Although the glycoprotein nature of thrombin is well established the function of the carbohydrate moiety is uncertain. While the lectin Conconavalin A (Con A) has been shown to inhibit thrombin induced clot formation (2) removal of part of the carbohydrate chains by glycosidase treatment has been reported to have no effect on enzymatic activity (3). We have presently investigated the function of thrombin carbohydrate moieties in the hydrolysis of a model substrate, the conversion of fibrinogen to fibrin and the aggregation of platelets.

Deglycosylation of bovine thrombin (Sigma) was performed by incubation with either neuraminidase (NA) to remove sialic acid; endo β-galactosidase (BG) to cleave chains at galactose residues and glycopeptidase F (GF) to remove the entire carbohydrate chain. Efficiency of carbohydrate removal was assessed by lectin blotting on nitrocellulose and monosaccharide analysis. Thrombin activity before and after deglycosylation was determined :- (1) spectrophotometrically using the model substrate Chromozym TM; (2) Visually by assessing the hydrolysis of fibrinogen to fibrin, and (3) by monitoring the aggregation of washed rat platelets in an aggregometer.

Treatment of thrombin with NA, BG or GF resulted in the removal of 7%, 34% and 93% of carbohydrate content respectively as assesed by lectin binding. None of the deglycosylation procedures significantly affected the kinetic parameters of the hydrolysis of Cromozym TM. Treatment of thrombin with NA or BG had no effect on fibrin clot formation (Clot Time (secs) CON 14.6 $\pm$ 3.2; NA 12.6 $\pm$ 2.6; BG 13.6 $\pm$ 3.1). Deglycosylation of thrombin with GF however significantly increased clot time compared to control (CON 14.6 $\pm$ 3.2 secs; GF 24.8 $\pm$ 3.8 secs). Moreover, while treatment with NA had no effect on thrombin induced aggregation of platelets BG treatment significantly decreased thrombin ED 50 whereas GF treatment significantly increased the ED 50 of thrombin (ED 50, n=4:- CON 43 $\pm$ 5mU; NA 39 $\pm$ 3mU; BG 29 $\pm$ 6mU; GF 124 $\pm$ 16mU). In addition while preincubation of intact thrombin with Con A inhibited subsequent platelet aggregation similar treatment of GF treated thrombin had no effect on subsequent platelet aggregation.

The present results demonstrate that the oligosaccharide chains on thrombin are important in the regulation of its biological activity. As the effects of deglycosylation are only apparent with large physiological molecules and not with a small model substrate it is likely that the sugar residues influence the interaction of thrombin with its physiological substrates and receptors.

1. Mann KG. Methods in Enzymol. (1981). Vol. 80. p 286.
2. Karpatkin S. and Karpatkin M. (1974). Biochem. Biophys. Acta. 7 1111.
3. Skaug K. and Christensen TB. (1971). Biochem. Biophys. Acta. 30 627.

POTENTIATION OF ADRENALINE-INDUCED HUMAN PLATELET AGGREGATION BY ATP

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Adenosine 5'-triphosphate (ATP) is a competitive antagonist of adenosine 5'-diphosphate (ADP) - induced platelet aggregation (Cusack and Hourani, 1982). ATP has been shown to potentiate adrenaline (Adr) - induced aggregation (MacFarlane and Mills, 1975) and platelet fibrinogen receptor expression (Shattil et al 1989,) however this potentiation may be due to ADP derived from the added ATP.

Aggregation was quantified by single platelet counting (Thompson, et al 1986) using citrated (10mM) platelet-rich plasma (PRP). Aliquots of PRP were equilibrated at 37 °C and stirred at 1,100 rpm. Saline or Adr (0.1μM) was added, followed by the nucleotide at <10 sec. At two min. aggregation was terminated by the addition of 4 vols of 1% glutaraldehyde. Single platelets were counted by electronic particle counting. The results are number of single platelets nl^{-1} expressed as means ± SEM of 5 different donors compared with controls (saline) using a paired 't'-test (Table 1).

Table 1

	CONTROL	ATP (μM)		β-γ-methylene ATP (μM)		ADP (μM)	
		30	3	30	3	0.3	0.03
Adr (0.1μM)	169±16	90±9 p<0.01	113±17 p<0.01	167±18 NS	173±15 NS	50±9 p<0.01	141±20 p<0.05
Saline	207±4	147±12 p<0.01	172±10 p<0.01	206±7 NS	207±6 NS	97±24 p<0.01	181±10 p<0.05

Aggregation induced by a threshold concentration of Adr (0.1μM) was potentiated both by ATP and by ADP but not by β-γ-methylene ATP. However, ATP also caused primary platelet aggregation in the absence of Adr whereas β-γ-methylene ATP did not.

The failure of the ATP-ase resistant analogue β-γ-methylene ATP to potentiate Adr-induced aggregation suggests that the effect found with ATP may be due to conversion to ADP, this is supported by the finding that ATP caused significant aggregation in the absence of adrenaline. However, β-γ methylene ATP is a less potent antagonist of ADP (Cusack and Hourani, 1982) and may lack the specificity of ATP. Since the onset of action of ATP was as rapid as that with ADP it is possible that ATP is a partial agonist as suggested by Shattil, et al, (1989). These results suggest that ATP, which has been shown to accumulate at sites of vascular injury (Born adn Kratzer, 1984) could promote haemostasis both directly and through metabolism to ADP by platelet ecto-ATPase.

Born, G V R and Kratzer, M A A (1984) J Ohysiol, 354, 419-429.
Cusack, N J and Hourani, S M O (1982) Br J Pharmacol, 76, 221-227.
MacFarlane, D E and Mills, D C B (1975) Blood, 46, 309-320.
Shattil, S J, Budzynski, A and Scrutton, M C (1989) Blood, 73, 150-158.
Thompson, N T, Scrutton, M C and Wallis, R B (1986) Eur. J Biochem 161, 399-408.

POTENTIATION OF HUMAN PLATELET AGGREGATION BY DYNORPHIN

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Opioid receptors have been demonstrated on human platelets (Mehrishi et al 1983). Morphine has been shown to antagonize the PGE_1 and PGE_2- mediated inhibition of human platelet aggregation induced by ADP and collagen (Gryglewski et al 1975). In vitro, naloxone in millimolar concentrations inhibited gerbil platelet aggregation in a dose-dependent manner (Turcani et al 1986).

We have investigated the effects of the kappa opioid receptor agonist dynorphin on human platelet aggregation. Platelet-rich plasma was prepared from citrated venous blood obtained from drug-free healthy volunteers. Platelet count was adjusted to 300, 000 µl using autologous platelet poor plasma and aggregation recorded for 6 minutes at 37°C according to the method of Born. The effect on platelet aggregation of dynorphin alone in the concentration range 0.1-30 µM and of naloxone alone in the concentration of 0.1-50 µM was determined. The effect of dynorphin in this same concentration range on platelet aggregation in the presence of subthreshold concentrations of ADP (with and without naloxone 50 µM) and collagen was then determined. Platelets were pre-incubated for 2 minutes at 37°C with dynorphin 0.1-30 µM prior to addition of these agonists. Samples from fifteen subjects were studied in each group. Data were analysed by Friedman Two Way Analysis of Variance.
Neither dynorphin alone nor naloxone alone influenced platelet aggregation. Dynorphin potentiated in a dose dependent manner platelet aggregation induced by a subthreshold concentration of ADP and this effect was not blocked by 50 µM naloxone. Dynorphin had a similar but less marked effect on platelet aggregation induced by collagen.

	Mean Percentage Aggregation (SD)		
Conc of dynorphin	ADP	Naloxone+ADP	Collagen
Saline (Control)	20.20(12.35)	25.43(12.77)	17.00(9.06)
0.1µM	32.60(21.13)	44.36(22.77)	11.50(8.92)
0.3µM	34.30(20.93)	35.50(20.80)	12.60(8.25)
1.0µM	50.13(24.17)*	51.00(21.18)	15.40(10.80)
3.0µM	57.47(22.64)*	60.21(23.05)*	22.20(15.80)
10.0µM	64.13(20.68)*	71.14(13.29)*	31.90(21.40)
30.0µM	68.20(15.96)*	70.43(19.07)*	34.50(20.10)x

* $p<0.01$; x $p<0.05$ compared to control.

We conclude that kappa opioid peptides can synergize with other agonists in inducing platelet aggregation.

Mehrishi, J.N. and Mills, I.H. (1983) Clin.Immun & Immunopath. 27, 240-49.
Gryglewski, R.J. Szczeklik, A. and Bieron, K. (1975) Nature 256, 721-23.
Turcani, P., Gotoh, F., Ishihara, N., Tanaka, K., Gomi, S., Takashima, S. and Mihara, B. (1986) Thromb.Res. 4, 817-28.

EFFECT OF ADRENOCEPTOR-BLOCKING DRUGS ON PLATELET AGGREGATION INDUCED BY VARIOUS AGGREGATING AGENTS

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Anti-adrenergic drugs are widely used in the treatment of hypertension and ischaemic heart disease. However, effects of these drugs on platelet aggregation induced by various agents such as adrenaline (ADR), adenosine 5'-diphosphate (ADP), arachidonic acid (AA) and platelet-activating factor (PAF) have not been widely studied. In this investigation, effects of different anti-adrenergic agents on platelet aggregation are described.

Blood was obtained from normal adult volunteers who had not taken any aspirin-like drugs for 10 days. Platelet-rich plasma (PRP) was prepared by centrifugation at 260 x g and aggregation was monitored turbidometrically using a dual channel Chrono-Log aggregometer. All aggregation procedures were carried out at 37°C with PRP having platelet count of 2-2.5 x 10^8/ml plasma. Platelet aggregation was monitored with ADR (200 uM), ADP (2.2 uM), AA (1.7 mM) or PAF (0.8 uM). All drugs were tested at three to four concentrations in duplicate. The mean values ± SEM for inhibiting platelet aggregation by 50% (IC_{50}) for various drugs are given below.

Drugs	Aggregating agents			
	Adrenaline	ADP	Arachidonic acid	PAF
Alpha-blockers				
Tolazoline	55±3	NI	NI	NI
Phenoxybenzamine	86±5	NI	NI	NI
Phentolamine	7±2	NI	NI	29±7
Prazosin	52±5	30±3	54±3	NI
Beta-blockers				
Propranolol	67±7	42±2	NI	44±3
Pindolol	NI	NI	NI	NI
Atenolol	NI	NI	NI	NI
Metoprolol	NI	NI	NI	NI
Aspirin	NI	NI	140±7	NI

Values shown are mean±SEM of three to five determinations. NI: not inhibited up to 300 uM.

All alpha-blocking drugs inhibited adrenaline-induced platelet aggregation whereas aggregation induced by ADP or AA was inhibited by prazosin only. Similarly PAF-induced aggregation was inhibited by phentolamine and propranolol only. All beta-blockers except propranolol were found to be devoid of inhibitory action against all aggregating agents. Propranolol inhibited aggregation induced by all agents studied except AA. The inhibitory effects of tolazoline, phenoxybenzamine and phentolamine against adrenaline may be due to their alpha-blocking properties. However, prazosin and phentolamine appear to inhibit aggregation through mechanism(s) other than alpha-receptor blockade. Similarly our data suggest that propranolol may also exert its inhibitory activity that is independent of beta-receptor blockade.

900P

THE RESPONSE OF ISOLATED HUMAN RESISTANCE ARTERIES TO ENDOTHELIN-1: EFFECT OF CALCIUM CHANNEL BLOCKERS

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Endothelin-1 (ET-1) is a 21 amino acid vasoconstrictor peptide recently characterised from vascular endothelial cells and suggested to act by opening voltage-dependent Ca^{2+} channels (Yanagisawa et al, 1988). It has been shown to potently produce contractions of isolated human resistance arteries (Hughes et al, 1989). Dihydropyridine (DHP) Ca^{2+} channel blockers only partially antagonise, or have no affect on, its vasoconstrictive effects in rabbit, pig and rat arteries (Stasch & Kazda, 1989, D'Orleans-Juste et al, 1989). We have therefore investigated the effects of these and other Ca^{2+} channel blockers on the response to ET-1 in human arteries.

Arteries (I.D. 118-711 μm, median 279 μm) were dissected from omentum and subcutaneous fat, removed at surgery from 39 patients)aged 33-71 yr, median 58 yr), and mounted, in Krebs buffer, in a Mulvany myograph maintained at 37° C and aerated with 95% O_2/5%CO_2. Following 1 hr equilibration, the arteries were set to a normalised resting circumference equal to 0.9 x L100, where L100 is the internal circumference producing a passive wall tension equivalent to 100 mmHg, calculated by the Laplace relationship. Following a further 30 min equilibration viability was confirmed by demonstration of contractile responses to a depolarising K^+ (120 mM) in Krebs solution (KDS) and noradrenaline (NA) (10 μM). Arteries producing a tension equivalent to less than 90 mmHg were discarded. Each ET-1 dose response curve was prepared in a separate artery. Antagonist contact time was 30 min. DHP experiments were performed in a light-proof tissue chamber. Addition of ET-1 (100 pM-100 nM) produced a dose dependent contraction with an EC50 of 4 nM and a maximum tension (Emax) of 2.3 N/m (n = 16). This exceeded those produced by KDS (1.7 N/m) and NA (10 μM) (1.2 N/m). Expressed as a percentage of the KDS response the control ET-1 Emax was 135%. Incubation in Ca^{2+} free Krebs containing 0.1 mM EGTA reduced the Emax to 116% (n=5) and inclusion of the inorganic Ca^{2+} channel blocker, $CoCl_2$ (1 mM) reduced this to 78% (n=5) but neither treatment affected the EC50. Prior incubation with diltiazem (n=8), nimodipine (n=9), verapamil (n=5), flunarazine (n=8), and nisoldipine (n=6) (all 1 μM) reduced the ET-1 Emax to 131%, 104%, 101%, 90% and 43% of the KDS response, respectively, without altering the EC50. All these Ca^{2+} channel blockers completely reversed the contraction produced by K^+ (40 mM) in Krebs. Prior treatment with K^+ (40 mM) plus nimodipine (1 μM) (n=8) reduced the ET-1 Emax to 111%. These findings demonstrate that the contractile response to ET-1 in human resistence arteries is not entirely dependent upon extracellular Ca^{2+}. Of the Ca^{2+} channel blockers studied, only nisoldipine produced appreciable functional antagonism and this was non-competitive. This suggests that ET-1 acts through a mechanism other than direct opening of voltage-dependent Ca^{2+} channels and also a heterogeneity of action among the DHP Ca^{2+} channel blockers.

D'Orleans-Juste P et al (1989); J Cardiovasc Pharmacol 13(Suppl 5):S19-S22.

Hughes A D et al (1989); J Cardiovasc Pharmacol 13(Suppl 5): S225-S228.

Stash J P and Kazda S (1989); J Cardiovasc Pharmacol 13(Suppl 5): S63-S66.

Yanagisawa M et al (1988); Nature 332:411-415.

VASCULAR SMOOTH MUSCLE FACTOR CAUSES VASOCONSTRICTION FOLLOWED BY SLOW ONSET AND PROLONGED EDRF RELEASE

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We have assessed the ability of cultured vascular smooth muscle cells (SM) to synthesize vasoactive agents. Rabbit aorta SM were isolated using an explant method and cultured by non-enzymatic methods. Culture media (SMM) was removed after 3 days contact with confluent monolayers, filtered and stored. When given for 30s by superfusion to a de-endothelialized rat aortic ring under 1 gm resting tension SMM caused a 11±1% contraction (mean peak 1.3 min, mean duration 3 min) compared to a 12±2% contraction (mean peak 2.5 min, mean duration 11.5 min) caused by endothelial cell media (100% = contraction caused by 10^{-6}M phenylephrine).

SMM was also passed through a column of 10^7 endothelial cells grown on microcarrier beads and perfused with buffer at 2 ml/min. The endothelial cells were isolated from bovine pulmonary artery and propagated using non-enzymatic methods. The effluent from the column dripped directly onto a bioassay of de-endothelialized rat aorta pre-contracted with 10^{-6}M phenylephrine. After perfusion of the column for 30 s with SMM, the effluent from the column caused an initial contraction of 10±3%, (mean duration 1.9±0.2 min), followed by a 44±4% relaxation (mean peak 5.6 min, mean duration 17 min, n=6). Similar results were obtained if the bioassay was pre-contracted with 10^{-8}M U46619 (n=20) or 30 mM K^+ (n=6).

The effect of SMM given directly to rat aortic rings with intact endothelium was much longer lasting. When given for 30s to rings which had been contracted with 10-^{6}M phenylephrine there was a 22±2% further contraction (mean peak 1.6 min, mean duration 3.6 min) followed by a 45±3% relaxation (mean peak 25±8 min, duration >60 min, n=9). Results with endothelium intact rabbit aortic rings were similar (n=4). Results are mean ±s.e.mean.

The contraction caused by SMM was endothelium independent, unaltered by exposure of the endothelial cells or bioassay ring to indomethacin 10^{-5}M, phentolamine 10^{-6}M or chlorpheniramine 10^{-5}M, but completely blocked by verapamil 10^{-5}M. The relaxation was endothelium dependent, potentiated by superoxide dismutase 10 u/ml, unaltered by indomethacin but blocked by haemoglobin 10^{-6}M, methylene blue 5 x 10^{-6}M, L-methyl-N-arginine 10^{-4}M or by a 60s delay to the outflow of the column of endothelial cells, confirming the relaxation to be mediated by the release of endothelium-derived relaxing factor (EDRF). The activity of SMM was not affected by pre-heating it to 56°C for 30 min or storing at -4°C for 1 week.

The constrictor effect of SMM is endothelium independent and shorter acting than endothelium derived constricting factor. The slow onset and prolonged release of EDRF by SMM was not mimicked by known physiological stimuli to EDRF release. The factor(s) in SMM is an unknown product of vascular smooth muscle.

EFFECTS OF HYPOXIA ON SHEEP ISOLATED INTRAPULMONARY ARTERY

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The mechanism(s) underlying the pulmonary vasoconstriction that results from a fall in alveolar PO_2 has not yet been elucidated. The aims of this study were to investigate the effects of hypoxia on isometric tension development in sheep intrapulmonary artery rings and to determine the role, if any, of the endothelium.

Intrapulmonary arteries of large (2.2-4.1 mm) and small (.32-.64 mm) diameter were suspended under optimum resting tension of 5 and 0.5 g respectively. Isometric force generated per cross sectional area of the vessel was measured. The Krebs solution was aerated with 95% O_2 5% CO_2 or with 95% N_2 5% CO_2 to yield a PO_2 in the bath of 670 ± 5 and 4 ± 0.5 mmHg respectively. Removal of the endothelium in some arteries was confirmed by lack of relaxation to acetylcholine and by histological examination.

In endothelium containing large artery rings, precontracted with 5-HT at its EC_{85} ($7x10^{-6}$ M), hypoxia produced further contraction (182 ± 24 to 260 ± 14 g cm^{-2}, $P<0.05$). Removal of the endothelium did not significantly affect the 5-HT contraction but abolished the response to hypoxia. Hypoxia had no effect on large endothelium intact artery rings precontracted with 5-HT at its EC_{35} (10^{-6} M) or with KCl either at its EC_{35} (20 mM) or EC_{85} (50 mM). The contractions produced by KCl at its EC_{85}, alone and in the presence of hypoxia were 186 ± 42 and 191 ± 39 g cm^{-2}. In endothelium containing small artery rings, hypoxia caused an increase in force following precontraction with either the 5-HT EC_{35} ($7x10^{-8}$ M) or EC_{85} ($3x10^{-7}$ M). The effect of hypoxia was more marked in the small than the large arteries. The contraction in the presence of hypoxia was almost double (62 ± 10 g cm^{-2}) the contraction with 5-HT alone at its EC_{35} (34 ± 10 g cm^{-2}, $P<0.05$). Hypoxia either had no effect or caused a small contraction (35-110 mg) of artery rings under their optimum resting tension.

In 5-HT but not KCl precontracted large artery rings, acetylcholine (10^{-5} M) produced a relaxation of 20 ± 4 %. This relaxation was almost abolished (3 ± 2 %) by hypoxia.

The effects of hypoxia to augment the 5-HT contraction and abolish the acetylcholine relaxation in sheep intrapulmonary artery rings may be explained by inhibition of the release of a vasodilator from the endothelium under conditions of low PO_2. This effect may contribute to hypoxia-induced pulmonary vasoconstriction.

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EFFECTS OF DIMETHYLTHIOUREA AND DIZOCILPINE ON INFARCT SIZE FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT

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Both free radicals and excitatory amino acids have been implicated in tissue damage following ischaemia (McCord, 1985; Rothman & Olney, 1986). We have investigated the effect of the hydroxyl radical scavenger dimethylthiourea (DMTU) and the noncompetitive N-methyl-D-aspartate receptor antagonist dizocilpine on ischaemic brain damage, using nuclear magnetic resonance (NMR) imaging, which is a sensitive probe for oedema (Bryan *et al*, 1983).

Male Sprague-Dawley rats (200-250g) were anaesthetised (2l/min O_2, 1l/min N_2O, 1.5% halothane), and the left middle cerebral artery (MCA) was occluded by electro-coagulation. Treated animals were injected (i.p.) with either DMTU (750mg/kg) one hour before MCA occlusion, or dizocilpine (3mg/kg) 30 minutes after MCA occlusion. Control animals received saline only.

Twenty-four hours later, the animals were anaesthetised (pentobarbitone sodium, 70mg/kg i.p.) and subjected to NMR imaging using a 4.7 tesla Bruker Biospec imaging spectrometer. Eight contiguous coronal images with a slice thickness of 2mm and a resolution of 240μm×260μm were obtained from anterior to posterior of the brain, using a multislice SE(4500/65) pulse sequence. Oedema was visualised by regions of increased intensity in the images. The volume of oedema fluid was quantified by summing the number of voxels with increased intensity for the eight images.

The main areas affected, as assessed by NMR imaging, were the cortex and the caudate-putamen. Results are expressed in Table 1 as mean±s.e.mean[n] for the dizocilpine and DMTU treated animals and the control animals. With both drug treatments there was a significant reduction in oedema volume.

Table 1 Volume of Oedema Assessed by NMR Imaging 24 Hours after MCA Occlusion

Treatment	Oedema Volume (voxels)
Saline	1376±153[10]
Dizocilpine	756±59[6] **
DMTU	726±206[5] *

* P<0.05 ** P<0.01 (Student's t-test)

It would therefore appear that excitatory amino acids and free radicals contribute to the formation of oedema in this model.

D.J.L. is an SERC scholar.

Bryan, R.N., Willcott, M.R., Schneiders, N.J., Ford, J.F. & Derman, H.S. (1983) Radiology, 149, 189-192.
McCord, J.M. (1985) N. Engl. J. Med., 312, 159-163.
Rothman, S.M. & Olney, J.W. (1986) Ann. Neurol., 19, 105-111.

L-N^{G}-NITRO-ARGININE IS A POTENT, L-ARGININE REVERSIBLE, INHIBITOR OF NANC RELAXATIONS IN THE MOUSE ANOCOCCYGEUS

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L-N(G)-monomethyl-arginine (LNMMA) inhibits the synthesis of nitric oxide (NO) from L-arginine (Moncada et al., 1989). The observation that LNMMA reduces vasorelaxation mediated by release of endothelium-derived-relaxing-factor (Rees et al., 1989), and non-adrenergic, non-cholinergic (NANC) relaxation of the mouse anococcygeus muscle (Gibson & Mirzazadeh, 1989a) has been taken as evidence for the involvement of endogenous nitrovasodilator-like substances in these processes. We have been investigating other L-arginine derivatives for similar activity, and in this paper we report that one of these, L-N(G)-nitro-arginine (NOARG), is more potent than LNMMA as an inhibitor of NANC relaxations in the mouse anococcygeus.

Anococcygeus muscles from male mice were set up, in vitro, for the recording of isometric tension responses to drugs and field stimulation (Gibson & Mirzazadeh, 1989b). To observe relaxations to drugs and NANC stimulation muscle tone was raised with 50 μM carbachol. Results are expressed as mean ± standard error, with a minimum of 6 observations in each case.

Both LNMMA (10 - 200 μM) and NOARG (1 - 50 μM) produced concentration-related inhibitions of NANC relaxations (10 Hz ; 10 s). 10, 50, and 200 μM LNMMA reduced NANC responses by 5 ± 1%, 37 ± 7%, and 47 ± 7% respectively ; 1, 5, 10, and 50 μM NOARG reduced NANC relaxations by 25 ± 3%, 52 ± 6%, 75 ± 7%, and 97 ± 1% respectively. Thus, NOARG was some 20 - 30 times more potent than LNMMA. Inhibitions produced by both NOARG and LNMMA were reversed by L-arginine but not by D-arginine (100 - 400 μM in both cases) ; it was noticeable that the reversal was faster in the case of LNMMA.

Neither NOARG (10 μM) nor LNMMA (50 μM) affected relaxations induced by submaximal concentrations of sodium nitroprusside (100 nM), sodium azide (10 μM), hydroxylamine (10 μM), or NO (60 μM).

Both NOARG (10 μM) and LNMMA (50 μM) enhanced contractile responses of the mouse anococcygeus obtained by stimulating the atonic tissue (30 Hz ; 10 s) in the absence of sympatholytic drugs, presumably by negating the opposing influence of the simultaneously released NANC transmitter.

In conclusion, NOARG is a more potent, L-arginine reversible, inhibitor of NANC relaxation in the mouse anococcygeus than LNMMA. Its actions are selective against NANC transmission, and it appears to act presynaptically as expected, since relaxations to several nitrovasodilator drugs were unaffected ; thus, NOARG and LNMMA differ from N-methyl-hydroxylamine which inhibits relaxations to both NANC stimulation and nitrovasodilators (Gibson & Mirzazadeh, 1989b). The results suggest that NOARG, and the anococcygeus, may provide very useful and inexpensive tools with which to study the physiology and pharmacology of certain types of endogenous nitrate function.

Gibson, A. & Mirzazadeh, S. (1989a) BPS meeting, Leeds, July 1989.
Gibson, A. & Mirzazadeh, S. (1989b) Br. J. Pharmacol. 96, 637 - 644.
Moncada, S., Palmer, R.J. & Higgs, E.A. (1989) Biochem. Pharmacol. 38, 1709 - 1715.
Rees, D.D., Palmer, R.J., Hodson, H.F. & Moncada, S. (1989) Br. J. Pharmacol. 96, 418 - 424.

L-N^G-NITRO ARGININE (NOARG) INHIBITS ENDOTHELIUM-DEPENDENT VASODILATATION IN THE RABBIT AORTA AND PERFUSED RAT MESENTERY

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Endothelium derived relaxing factor (EDRF) mediates the relaxant effect of ACh and a number of other substances on isolated blood vessels and exhibits potent platelet anti-aggregatory activity. Evidence has been presented in the literature that EDRF is identical with nitric oxide (NO) and is formed from L-arginine (Palmer et al., 1988). Recently, L-N^G-monomethyl arginine (L-NMMA) was reported to inhibit NO biosynthesis in endothelial cells, macrophages and polymorphonuclear cells (reviewed in Moncada et al., 1989). We report here that a second guanidino-substituted L-arginine analogue, L-N^G-nitro arginine (NOARG), similarly inhibits the vasodilator effect of ACh in rabbit aortic rings and the perfused rat mesentery.

Rabbits (male, New Zealand White, 1.5-3.5 kg) were killed by an overdose of pentobarbitone (60 mg/kg, i.v.). Aortic rings (5 mm) were mounted in organ baths containing warmed (37°C), oxygenated (95% O_2:5% CO_2) Krebs solution and responses monitored using Grass FT03 force transducers. ACh (pD_2, 7.1 ± 0.2, n=22) produced dose-related relaxation of rings precontracted with a submaximal concentration of phenylephrine (750 nM). NOARG (1.5, 15 μM) or L-NMMA (3, 30 μM) reduced responses to ACh but not sodium nitroprusside. For example, the effect of an ED_{50} dose of ACh (0.1 μM) was decreased (P<0.05) by 24 ± 3 % (n=5) and 8 ± 1 % (n=4) at the lower concentration of each inhibitor and 44 ± 4% (n=11) and 32 ± 5% (n=5) at the higher concentration. L-arginine (100 μM) but not D-arginine (100 μM), L-citrulline (100 μM) or L-arginosuccinic acid (10 μM), completely reversed the inhibitory effect of NOARG (1.5 μM) and L-NMMA (3 μM).

In separate experiments, mesenteric vascular beds of rats (male, Sprague-Dawley, 180-280 g) were perfused (7 ml/min) with warmed (37°C), oxygenated (95% O_2:5% CO_2) Krebs solution containing noradrenaline (0.2-0.5 mM) as described elsewhere (Bhardwaj & Moore, 1988). Bolus injection of ACh (ED_{50}, 0.2 ± 0.1 nmol, n=21) or sodium nitroprusside (ED_{50}, 1.8 ± 0.4 nmol, n=5) elicited transient and dose related vasodilatation. NOARG (30 μM) or L-NMMA (30 μM) added to the perfusate inhibited ACh (but not sodium nitroprusside) induced mesenteric vasodilatation. NOARG was more potent (ED_{70} dose of ACh, 1.8 nmol, reduced by 58 ± 7%, n=8, P<0.05) than L-NMMA (ED_{70} dose of ACh reduced by 27 ± 5%, n=7, P<0.05). The inhibitory effect of both NOARG and L-NMMA was reversed by treatment (10 min) with L-arginine (100 μM) but not D-arginine (100 μM).

These results provide further evidence that an L-arginine-derived vasodilator, presumably NO, mediates the vasodilator effect of ACh in the rabbit aorta and perfused rat mesentery. NOARG is a more potent L-arginine-reversible inhibitor of endothelium-dependent vasodilatation in these preparations than L-NMMA. We propose that NOARG may prove to be a useful tool to investigate the biological significance of EDRF.

Bhardwaj, R. & Moore P.K. (1988) Br. J. Pharmacol., 95, 835-843
Moncada, S., Palmer, R.M.J. & Higgs, E.A. (1989) Biochem. Pharmacol., 38, 1709-1715.
Palmer, R.M.J., Ashton, D.S. & Moncada, S. (1988) Nature, 333, 664-666.

BIOLOGICAL ACTIVITIES OF THE EPOXIDES OF ARACHIDONIC ACID

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Arachidonic acid (AA) is metabolised by cytochrome P_{450} to produce four isomeric epoxy derivatives (EETs) [1]. Previous reports from this laboratory have indicated that the inhibitors of cytochrome P_{450} can modify responses of smooth muscle, implying that endogenous synthesis of EETs may have biological significance [2,3]. In this study we have now investigated the biological activity of the EETs in some smooth muscle preparations and also in platelets.

The EETs were synthesised as described by Oliw et al. [1] and separated by TLC using a solvent of ether/hexane/acetic acid (30:70:1). The EETs were extracted, methylated and their identity confirmed by GC-MS.

In de-endothelialised rat aortic strips pre-contracted with phenylephrine all four EETs induced-dose dependent relaxations, the 5(6)-EET being the most potent (EC_{50} $8.2\pm1.8\times10^{-8}$M, mean±SEM (n=4)). The 8(9)-, 11(12)- and 14(15)-EETs were far less potent (EC_{50} $4.7\pm2.7\times10^{-6}$M (n=3); $6.7\pm0.1\times10^{-6}$M (n=2) and $11.2\pm1.7\times10^{-6}$M (n=2) respectively). In strips with an intact endothelium, relaxant responses to 5(6)-EET were augmented (EC_{50} $4.9\pm0.3\times10^{-8}$M n=3), whereas responses to the remaining EETs were unchanged.

Similarly in guinea-pig tracheal strips precontracted with acetylcholine, all four EETs induced dose-dependent relaxations, the 5(6)-EET being most potent (EC_{50} $1.3\pm0.4\times10^{-6}$M (n=8)). The 8(9)-, 11(12)- and 14(15)-EETs (EC_{50} values of $1.2\pm0.1\times10^{-4}$M (n=5), $1.1\pm0.4\times10^{-4}$M (n=5) and $8.9\pm0.3\times10^{-5}$M (n=4) respectively). The relaxant effects of 5(6)-EET were attenuated by the presence of 3×10^{-6}M indomethacin (EC_{50} $2.6\pm1.8\times10^{-5}$M (n=4)) whereas the responses to the other three EETs were unchanged.

All four EETs were found to inhibit aggregation of washed platelets stimulated by either ADP or thrombin. Using ADP, IC_{50} values for 5(6)-, 8(9)-, 11(12) and 14(15)-EETs respectively were $4.2\pm1.0\times10^{-7}$ (n=4), $6.6\pm1.9\times10^{-6}$M (n=3), $6.7\pm2.0\times10^{-6}$ (n=3) and $5.7\pm1.1\times10^{-6}$M (n=3); and using thrombin, $6.7\pm1.9\times10^{-7}$M (n=4), $4.0\pm0.8\times10^{-5}$M (n=3), $2.2\pm0.3\times10^{-5}$M (n=3) and $1.6\pm0.5\times10^{-5}$M (n=3). Pre-incubation of the washed platelets with 3×10^{-6}M indomethacin for 30 seconds at 37°C prior to the addition of the EETs did not inhibit the anti-aggregatory effect of the EETs. However the aggregatory responses to AA were completely abolished by such treatment.

Carroll et al. [4] recently reported that the vasodilator action of 5(6)-EET was indirect and was dependent upon conversion via the cyclo-oxygenase pathway to other active products. The present results lend some support to this hypothesis but also demonstrate that the EETs, especially 5(6)-EET, have direct actions, particularly in blood platelets.

1) Oliw E.H. et al. (1982) J. Biol. Chem. 257, 3771
2) Finnen M.J. et al. (1986) Br. J. Parmacol. 88, 406P
3) Finnen M.J. et al. (1986) Br. J. Parmacol. 88, 407P
4) Carroll M.A. et al. (1988) Br. J. Parmacol. 96, 99P

MFC holds an SERC studentship.

COMPARISON OF CAPSAICIN AND OLVANIL AS ANTINOCICEPTIVE AGENTS *IN VIVO* AND *IN VITRO*

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Acute administration of capsaicin produces reversible antinociception by a selective interaction with peripheral polymodal nociceptive neurones (Hayes et al 1984). This suggests that capsacin-like compounds may offer a new class of analgesics. In keeping with this, a capsaicin analogue N-(3-methoxy-4-hydroxy benzyl) oleamide, NE-19550, olvanil) exhibited antinociceptive and antiinflammatory activity (Sietsema et al 1988). We have now compared olvanil with capsaicin in several antinociceptive tests *in vivo* and on peripheral nociceptive fibres *in vitro*.

At doses ranging from 5-20 μmol/kg s.c. (n=10 for each dose tested) each compound produced significant antinociception in the mouse tail flick, mouse acetic acid-induced writhing, rat paw pressure tests and also reduced yeast-induced inflammation in the mouse. The antinociceptive effect of olvanil was longer lasting (5 hours) than that of capsaicin (2 hours). Both compounds induced dose dependent hypothermia and sedation which was more marked in mice than in rats. Tolerance to the effects of each compound was observed in all tests except in the paw pressure test following daily injections (20 μmols/kg, s.c.) for 4 days.

Activity in vitro was compared using the isolated spinal cord with functionally connected tail, obtained from 1 day old rats following decapitation. The preparation was maintained as previously described (Dray et al 1989). Activation of peripheral fibres by noxious (capsaicin, bradykinin, serotonin, heat at 48°C, pinch) or innocuous (light brush) stimuli was assessed by measuring the depolarization produced in a spinal ventral root (L_3-L_5). Capsaicin (0.2-50μM) consistently activated peripheral fibres and following prolonged administration (5-20min, 0.2-2.0μM) induced desensitization without impairing sensitivity to other noxious stimuli. Superfusion with 20-50μM capsaicin, attenuated responses only to noxious stimuli. By contrast similar doses of olvanil did not evoke a measurable response but produced cross-desensitization to capsaicin without reducing the responses to other noxious stimuli.

These data show that olvanil and capsaicin possess similar in vivo antinociceptive activity but their effects on nociceptive neurones differ. Capsaicin-like compounds may be antinociceptive without producing overt pungency.

Dray, A. et al (1989) Neurosci Letts., 99, 50-54.
Hayes, A.G. et al (1984) Life Sci., 34, 1241-1248.
Sietsema, W.K. et al (1988) Life Sci 43, 1385-1391.

NK_3 TACHYKININ RECEPTORS ARE COUPLED TO INOSITOL PHOSPHOLIPID HYDROLYSIS IN NEONATAL RAT SPINAL CORD

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In the adult rat spinal cord, autoradiographic studies using ^{125}I-BH-eledoisin have revealed the presence of NK_3-like binding sites which appear to be discretely localised to the superficial laminae of the dorsal horn (Ninkovic et al, 1985; Hunter et al, 1987). In contrast, NK_1-like sites (labelled with ^{125}I-BH-substance P) are more diffusely distributed throughout the dorsal and ventral horns (Ninkovic et al, 1985; Yashpal et al, 1988). We have previously reported that the peripheral NK_3 receptor in the guinea-pig ileum longitudinal muscle-myenteric plexus preparation is linked to the hydrolysis of inositol phospholipids (Guard et al, 1988). In the present study, we have determined whether the NK_3-like binding sites observed in the spinal cord are similarly coupled to inositol phospholipid hydrolysis.

Experiments were performed on cross-chopped slices of neonatal rat spinal cord (1-3 day old, Sprague-Dawley) using previously described methodology (Guard et al, 1988). Concentration-response curves for the NK_3-selective agonist, senktide (succ-[Asp^6,MePhe^8]SP$_{6-11}$; Wormser et al, 1986) were constructed and results expressed as a percentage increase in total ^{3}H-inositol phosphates (IPs) relative to carbachol (1mM), which typically produced a 2.5-3.5 fold increase in total ^{3}H-IPs above basal. The senktide-induced increase in ^{3}H-IPs was maximal at 10μM producing 17.4 $\pm$ 5.0% of the response to carbachol. The half-maximal effect (EC_{50}) was 13.4 nM; -1.86, +2.16 (geometric mean; -sem, +sem; n = 4) which is in close agreement with the K_D value obtained for ^{3}H-senktide binding to guinea-pig cerebral cortex membranes (Guard et al, 1989). In comparison, substance P, neurokinin B and the NK_1-selective agonist, substance P methyl ester produced increases in ^{3}H-IPs equivalent to approximately 50% of those obtained with 1mM carbachol, suggesting the presence of other tachykinin receptor types in addition to NK_3. Given that senktide-evoked ventral root depolarisation of the hemisected neonatal rat spinal cord has been reported to be due to an indirect effect involving the release of excitatory amino acids (Ireland et al, 1988), we examined the effects of various ion channel blockers on senktide-induced ^{3}H-IP formation. Senktide alone (100nM) produced 15.4 $\pm$ 1.6% increase in ^{3}H-IPs (n = 3) which was not significantly affected by 300nM tetrodotoxin (15.5 $\pm$ 1.6%, n = 3), 1μM nifedipine (14.4 $\pm$ 3.4%, n = 3) or 100nM ω-conotoxin GVIA (16.9 $\pm$ 2.8%, n = 3).

It is concluded that the NK_3-like receptors reported to exist in the rat spinal cord represent functional receptors in the neonate which are coupled to the hydrolysis of inositol phospholipids.

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Ninkovic, M. et al (1985) Eur. J. Pharmacol. 106, 463-464.
Hunter, J.C. et al (1987) Neurosci. Letts. 78, 12-16.
Yashpal, K. et al (1988) Regulatory Peptides 22. 191.
Guard, S. et al (1988) Br. J. Pharmacol. 94, 148-154.
Wormser, U. et al (1986) EMBO J. 5, 2805-2808.
Guard, S. et al (1989) This meeting.
Ireland, S.J. et al (1988) Br. J. Pharmacol. 93, 85P.

CHRONIC LITHIUM TREATMENT DECREASES INOSITOL (1,4,5)-TRISPHOSPHATE LEVELS IN RAT CEREBRAL CORTEX SLICES

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Although the mechanisms by which lithium salts produce their therapeutic effects are unclear recent work has shown that chronic lithium treatment attenuates agonist-stimulated formation of inositol phosphate (IPs) in cerebral cortex (Kendall and Nahorski, 1987; Godfrey et al., 1989). Since these experiments measured [^{3}H]-IPs the results may be complicated by changes in specific radioactivity of the [^{3}H]inositol label. Recently a radio-receptor binding assay has been developed which permits direct assay of IP3 content in tissue slices (Challiss et al., 1988) and this assay has been utilised to further investigate the effects of lithium on agonist-stimulated IP3 formation in rat cerebral cortex.

Male Sprague-Dawley rats (200-300g) were used. 'Chronic lithium' rats were fed on a diet containing 0.1% lithium carbonate for 21-28 days prior to analysis; a control group was run in parallel. Cortical slices (350x350 μm) were prepared and incubated for 60 min in Krebs-HEPES buffer as described previously (Godfrey et al, 1989). 50 μl of packed slices were then incubated in 250 μl buffer for 10 min, in the absence or presence of 10 mM LiCl where appropriate, and then stimulated with agonist (10 μl) for the required period. Incubations were stopped with 0.31 ml ice-cold 10% perchloric acid and left on ice for 10 min. Following extraction using the FREON/ octylamine method described previously (Godfrey et al, 1989) an aliquot (25 μl) of the upper phase was assayed for IP3 essentially as described by Challiss et al (1988). In a final volume of 250 μl of 25 mM Tris-HCl/1 mM EDTA pH 8.5, 0.3-0.5 nM [^{3}H]-IP3 , binding protein (crude bovine adrenal membrane prep., 0.5-1.0 mg protein) plus test sample or IP3 standard were added. Non-specific binding was determined with 10 μM IP3. Samples were vortexed and incubated at 4^{o}C for 15 min, centrifuged and the pellets washed with distilled water. The pellet was then dissolved in 100μl of soluene and then added to 1 ml of scintillant; radioactivity was determined by scintillation counting.

The muscarinic agonist carbachol (1 mM) stimulated a rapid increase in IP3 formation which was maximal by 10s after stimulation (from a basal of 16 ± 4 to 45 ± 7 pmol/25μl tissue extract). This elevated IP3 concentration was maintained for 10 min before declining to basal levels by 30 min. 10 mM LiCl did not significantly alter the stimulation by carbachol, though the decline after 10 min was rather more rapid, basal levels being reached by 20 min. In chronic lithium-treated rats basal IP3 levels were only 40 % of that in controls and the response to 1 mM carbachol was attenuated (Table).

IP3 content (pmol/25 μl tissue extract)

Time (s)	control rats	lithium treated rats
0	18.7 ± 3.5	7.55 ± 1.4
5	20.5 ± 4.2	11.7 ± 2.2
10	32.7 ± 5.4	15.2 ± 2.8
60	30.2 ± 4.0	14.7 ± 1.5
300	28.5 ± 4.3	12.5 ± 2.1
1800	16.7 ± 1.9	7.6 ± 1.1

The results suggest that lithium does not alter the acute formation of IP3 in cerebral cortex slices, though chronic treatment markedly lowers both basal IP3 levels and stimulation by carbachol. These effects may be relevant to the therapeutic action of lithium.

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Challiss, R.A.J. et al. (1988) Biochem. Biophys. Res. Commun. 157, 684-691.
Godfrey, P.P. et al. (1989) J. Neurochem. 52, 498-506.
Kendall, D.A. and Nahorski, S.R. (1987) J. Pharmac. exp. Ther. 241, 1023-1027.

INHIBITION OF PROTEIN KINASE C DOES NOT PREVENT MUSCARINE DEPOLARISATION OF RAT SYMPATHETIC GANGLIA

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Activation of muscarinic receptors in the rat superior cervical ganglion (SCG) stimulates phosphoinositide (PI) turnover (e.g. Horwitz et al., 1985), with consequent production of diacylglycerol (DAG), a direct activator of protein kinase C (PKC). Phorbol esters, which can substitute for DAG as PKC activators, mimic the depolarizing actions of muscarine in sympathetic neurones by partially suppressing the outward M-current and activating a cation-selective inward conductance (Tsuji et al., 1987; Brown et al., 1989). We have tested the hypothesis that DAG mediates the depolarizing actions of muscarine by attempting to block muscarine-induced depolarization with the potent (but non-specific) PKC inhibitor staurosporine (see Ruegg and Burgess, 1989).

Population DC potential responses and compound transmitted action potentials of rat SCG cells were recorded from the intact tissue in vitro at 30°C, using a modification of the "grease-gap" method of Brown and Marsh (1978). The PKC activators β-phorbol 12,13-dibutyrate (PDBu; 20nM-10μM) and sapintoxin A (200nM-2uM; Ellis et al, 1987) caused a concentration-dependent depolarization that developed over at least 10 minutes. Recovery from PDBu responses was also slow, requiring 1-2 hours. The mean depolarization seen with 200nM PDBu (n=17) was 0.61 ± 0.04mV, probably reflecting a true membrane depolarization of about 6mV, allowing for shunting of the potential change across the grease-gap seal). Depolarizing concentrations of PDBu (threshold less than 20nM) and sapintoxin A correspond well with the concentration ranges over which the compounds activate PKC (Nishizuka, 1984; Ellis et al., 1987), suggesting that PKC activation is responsible for the phorbol ester response. In support of this, a phorbol ester that does not activate PKC (α-phorbol 12,13 didecanoate, 10μM) did not depolarize the ganglion.

We compared the ability of staurosporine to suppress either muscarine- or PDBu-induced depolarizations of equivalent size (routinely obtained with 100nM muscarine and 200nM PDBu). Consistent with mediation by protein kinase C activation, the depolarization produced by PDBu was reduced by staurosporine (≥100nM) in a concentration-dependent manner and was abolished by 1μM staurosporine. However, concentrations of staurosporine that completely blocked the PDBu response reduced the muscarine depolarization by only 26 ± 4% (n=5). Comparably small reductions in the size of muscarine responses, compound action potentials and GABA-induced depolarizations were observed. Thus, it is possible that the suppression of muscarinic depolarization is part of a cytotoxic or other non-specific effect of staurosporine.

In conclusion, the present findings do not support the hypothesis that PKC activation is a necessary transduction step in the mediation of muscarinic receptor-stimulated depolarization.

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Brown, D.A. & Marsh, S. (1978) Brain Res. 156, 187-191.
Brown, D.A. et al. (1989) J. Physiol. 413, 469-488.
Ellis, C.A. et al. (1987) Phytother. Res. 1, 187-190.
Horwitz, J. et al. (1985) J. Pharmac. Exp. Ther. 233, 235-241.
Nishizuka, Y. (1984) Nature 308, 693-698.
Ruegg, U.T. and Burgess, G.M. (1989) Trends Pharmac. Sci. 10, 218-220.
Tsuji, S. et al. (1987) Neurosci. Lett. 81, 139-145.

MUSCARINIC SUPERSENSITIVITY IN THE GLOBUS PALLIDUS OF MPTP-TREATED MONKEYS, REVEALED USING [^{3}H]-QNB AUTORADIOGRAPHY

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Muscarinic cholinergic receptors can be visualised autoradiographically using the radiolabelled ligand (3H)-QNB (Kuhar and Yamamura, 1976). This ligand was used to study the status of central muscarinic receptors in primates made parkinsonian by administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Nine monkeys (Macaca fascicularis) received systemic infusions of MPTP (1.8-27.0 mg/kg) (Burns 1983), causing an onset of parkinsonian symptoms. Six of these monkeys subsequently received dopamine (DA) replacement therapy (L-DOPA). Four monkeys received infusions of MPTP (0.5-2.5 mg/kg) into the right common carotid artery (Banciewicz et al., 1986), producing contralateral hemi-parkinsonism. Four naive monkeys were used as controls. The animals were killed by barbiturate overdose, after which the brains were rapidly frozen in isopentane at -30^{0}C. For muscarinic receptor binding studies cryostat-cut sections (20um) were preincubated in 50mM phosphate buffer (pH7.4) for 2x30 minutes, and then transfered to the incubation medium of the same buffer with 1nM (3H)-QNB, for 120 minutes. Specific binding was defined as that displaced by 1uM atropine. Sections were then washed in buffer, rinsed and dried. Sections were exposed to tritium-sensitive film (Hyperfilm, Amersham) for 3-6 weeks at -20^{0}C.

Histological staining using cresyl violet revealed a massive reduction in the number of cell bodies in the substantia nigra (SN) pars compacta of monkeys treated with MPTP, associated with depletion (>95%) of striatal DA levels (HPLC determination, Dr. R.G.Robertson of this laboratory). Analysis of (3H)-QNB autoradiographs revealed a distribution of muscarinic cholinergic receptors which was in agreement with that observed in human brain tissue by Palacios et al. (1986) and Cortes et al. (1987). Analysis of autoradiographs from animals treated unilaterally with MPTP revealed significant increases in (3H)-QNB binding to the lesioned side, in the putamen, medial and lateral pallidal segments (GPL and GPM) and SN. The greatest difference was in the GPM (47% increase). Analysis of autoradiographs from animals treated systemically with MPTP revealed a significant increase in (3H)-QNB binding to the GPM (89-96%), which was reduced by 16-36% after subsequent DA-replacement therapy. This result agrees with that of Boyce et al. (1989), and suggests a possible cholinergic supersensitivity in the GPM of parkinsonian brains. Of the major afferent pathways to the GPM, only that from the tegmental pedunculopontine nucleus is associated with cholinergic function. The present study would suggest that this pathway is underactive in parkinsonism.

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Kuhar, M.J. and Yamamura, H.I. (1976) Brain Res. 110, 229-243
Burns, R.S. et al. (1983) Proc. Natl. Acad. Sci. 80, 4546-4550
Bankiewicz, K.S. et al. (1986) Life Sci. 39, 7-16
Palacios, J.M. et al. (1986) T.I.P.S. Suppl. 2, 56-60
Cortes, R. et al. (1987) Neurosci. 20, 65-107
Boyce, S. et al. (1989) Neural mechanisms of movement disorder, Eds. Crossman, A.R. and Sambrook, M.A. John Libbey and Co. Ltd. London

THE TOXIC EFFECTS OF MPTP AND MPP+ IN MARMOSET AND RAT STRIATAL CELL CULTURES

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Primates have been shown to be more susceptible to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) than the rodent (Chiueh et al., 1984), although the species differences have not been fully explained. Thus the toxicity of MPTP may reflect its oxidation to 1-methyl-4-phenyl-pyridinium (MPP^+) and uptake into the dopamine neurone, but MPTP and MPP^+ are reported to have a similar affinity for dopamine uptake sites in marmoset and rat striatal synaptosomes (Barnes et al., 1989). In the present study we compare the susceptibility of rat and marmoset striatal cells to the toxic effects of MPTP and MPP^+ using the cell culture technique.

Marmoset and rat foetal brains were taken at 2 days post-gestation and 15 days gestation respectively. The tissues obtained from marmoset caudate nucleus and rat striatum were dissociated by gentle pipetting in serum-supplement medium containing 2% Ultraser G, penicillin 100 units/ml and streptomycin 100μg/ml in equal parts of Ham F12 and Dulbecco's Modification of Eagle Medium (SSM). The cell suspension was plated in a 25cm^2 tissue culture flask and incubated at 37°C, 100% humidity and 5% CO_2 with the medium replaced every two days. On day 7, the cultures were sub-cultured in SSM and 0.15ml of the cell suspension was plated into cell wells and incubated for 24h. The medium was then replaced with the feeding medium with or without MPTP or MPP^+ and incubated for a further 48h. The live and dead cells were identified using ethidium bromide/acridine orange staining and fluorescent microscopy.

Table 1 The toxic effects of MPTP and MPP^+ on marmoset and rat striatal cell cultures.

	% live cells			
	MPTP		MPP^+	
Treatment	Marmoset	Rat	Marmoset	Rat
Control	89.7±1.0	92.9±2.4	84.6±3.4	92.9±2.4
10^{-9}M	87.3±2.0	N.T.	81.6±5.3	N.T.
10^{-8}M	84.2±1.4	N.T.	65.8±5.8	N.T.
10^{-7}M	80.1±4.2	85.4±2.0	72.7±3.1	85.4±1.3
10^{-6}M	81.4±1.7	86.9±1.7	60.8±2.9*	90.8±3.0
10^{-5}M	62.6±2.7**	85.7±1.6	51.2±3.7**	73.6±5.6*
10^{-4}M	48.1±3.1*	69.8±3.2**	25.2±4.8**	55.3±2.6***
10^{-3}M	15.2±3.1***	26.5±3.2***	24.1±3.3**	19.6±2.5***
	(4.19±0.25)	(3.57±0.10)	(4.97±0.18)	(3.88±0.06)

Each value represents the mean ± S.E., n = 3-4. Significant reductions compared to the control values indicated as *P<0.05, **P<0.01, ***P<0.001 (Student's t-test). N.T. = not tested. Value in parentheses indicate the -log molar concentrations ± S.E. required to cause 50% cell death.

The results indicate the marmoset caudate cell culture is approximately three and twelve fold more sensitive to MPTP and MPP^+ toxicity respectively than rat striatal cell culture. MPP^+ is approximately eight fold more potent to induce toxicity than MPTP in marmoset cell culture whilst it is only twice as potent in rat cell culture. It remains to be determined whether such difference in response to the toxic effects of MPTP and MPP^+ are relevant to the 'in vivo' toxicity of MPTP/MPP^+ on the dopamine neurone.

Barnes N.M. et al. (1989) Biochem. Pharmacol. (in press)
Chiueh C.C. et al. (1984) Pharmacology Bulletin 20(3): 548-553

MUSCARINIC RECEPTOR-LINKED CA^{2+} ENTRY IN A HUMAN NEUROBLASTOMA CELL LINE SH-SY5Y

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We have previously shown that the human neuroblastoma cell SH-SY5Y expresses an homogenous M_3 muscarinic receptor complement (Lambert et al. 1989) linked to both phosphoinositide hydrolysis and elevated intracellular Ca^{2+} ($[Ca^{2+}]_i$) (Lambert and Nahorski, 1989). Although this Ca^{2+} signal was in part dependent on extracellular Ca^{2+} the involvement of L- or N-type voltage-sensitive Ca^{2+} channels could be eliminated. Here we further characterise muscarinic agonist-stimulated Ca^{2+} influx in SH-SY5Y cells.

$[Ca^{2+}]_i$ measurements were made in Fura-2 loaded SH-SY5Y cells (5 μmol/l Fura-2 AM for 45 min). All incubations were carried out at 37°C in Krebs-Henseleit buffer (pH 7.4). The ratio of fluorescence at 340 and 380 nm excitation measured at 509 nm emission was used to calculate $[Ca^{2+}]_i$ according to Grynkiewicz et al. (1985), where R_{max} and R_{min} were determined using Triton-X-100 (0.1%) and EGTA (3 mmol/l) respectively.

Stimulation of SH-SY5Y cells with carbachol (0.1 mmol/l) causes a biphasic increase in $[Ca^{2+}]_i$ composed of a rapid transient increase, due to the release of internal stored Ca^{2+}, declining to a steady maintained plateau phase above basal. This biphasic profile is converted to a simple monophasic profile in the absence of external Ca^{2+} and reconstituted by Ca^{2+} (1.2 mmol/l) repletion. Ca^{2+} entry across the plasma membrane (occurring during the plateau phase) has been monitored using the divalent cation Mn^{2+} (0.1 mmol/l) which quenches Fura-2 fluorescence at all excitation wavelengths. Examination of the raw fluorescence intensities at 340 nm and 360 nm (the latter being the isosbestic point of Fura-2 which is insensitive to changes in Ca^{2+} but Mn^{2+} quenchable) reveals quenching (indicative of Ca^{2+} entry) occurring in parallel with the discharge of the internal stores. The observed Ca^{2+} entry occurs through a non-voltage-sensitive Ni^{2+} (5 mmol/l) blockable Ca^{2+} channel. Maintenance of Ca^{2+} entry is clearly dependent upon the continued presence of agonist since removal of carbachol from its receptor with atropine (1 μmol/l) abolished the plateau phase of $[Ca^{2+}]_i$. Discharge of the internal stores with carbachol under Ca^{2+} free conditions followed by atropine addition and Ca^{2+} repletion does not reveal a refilling transient increase in $[Ca^{2+}]_i$ suggesting that, if refilling of the internal stores is occurring in these cells, then Ca^{2+} is entering via a route not 'seen' by Fura-2.

In conclusion these data show in carbachol stimulated SH-SY5Y cells that the plateau phase $[Ca^{2+}]_i$ involves an early agonist dependent Ca^{2+} entry through a non-voltage sensitive Ca^{2+} channel. Whether this channel is controlled by second messengers and/or linked to the muscarinic receptor via a G-protein remains to be established.

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Grynkiewicz, G., Poenie, M. and Tsien, R.Y. (1985) J Biol Chem 260, 3440-3450
Lambert, D.G., Ghataorre, A.S. and Nahorski, S.R. (1989) Eur J Pharmac 165, 71-77
Lambert, D.G. and Nahorski, S.R. (1989) Brit J Pharmacol 96, 132P

EFFECT OF MUSCARINIC AGONISTS ON HIPPOCAMPAL THETA RHYTHM IN THE ANAESTHETISED RAT

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Muscarinic agonists have been reported to show a certain degree of functional selectivity in vivo (Van Charldorp et. al. 1985) which may be related to their efficacy on different systems with varying receptor density and response coupling efficiency. The purpose of these studies was to investigate the effects of a range of full and partial muscarinic agonists on hippocampal theta rhythm which, in the anaesthetised rat, can be modified by muscarinic drugs (Olpe et. al. 1987).

Male Lister-hooded rats (Glaxo), weighing 300-350g were anaesthetised with isoflurane and a bipolar recording electrode was located stereotaxically in the CA1 region of the hippocampus. The EEG signal was amplified through a Grass 7P511 amplifier, digitised by an analogue-to-digital converter (Microlink, Biodata) and subsequently stored on a computer. Signal sampling was carried out for 60 sec (at a sampling rate of 128 samples/sec, epochs of 2 sec duration and an inter-epoch gap of 3 sec). Spectral analysis on stored data was subsequently performed by fast Fourier transform. All drugs were administered i.v. and in each experiment, only 1 agonist was examined (n = 3-5). Increasing doses were administered 15 sec prior to sampling and at intervals of 15-30 min, depending upon the duration of each agonist response. At the start of all experiments, N-methylscopolamine (0.5mg/kg) and chlorisondamine (1mg/kg) were administered to prevent agonist-induced changes in blood pressure. Under these conditions and by maintaining constant anaesthesia, the frequency of theta rhythm ranged between 2-5Hz.

Administration of arecoline (0.03 - 0.3 mg/kg) produced a dose-dependent increase in the frequency of theta rhythm from 3.9±0.6Hz (control) to 5.1±0.2Hz (after 0.1mg/kg, $P<0.05$) and 6.1±0.2Hz (after 0.3mg/kg,$P<0.01$). Similarly, agonists such as oxotremorine (0.03 - 1mg/kg), arecaidine propargyl ester (APE, 0.01 - 0.3 mg/kg) and aceclidine (0.3 - 10 mg/kg) also produced dose-dependent increases in the theta frequency, with an order of potency of Oxotremorine≧APE≧Arecoline>Aceclidine. The highest frequency produced by these agonists ranged from 6.1 ± 0.2Hz (arecoline) to 8.0 ± 0.3Hz (oxotremorine), but higher doses could not be examined because of the toxicity of these drugs. Pilocarpine however, at doses of 3 and 10mg/kg increased the frequency to only 4.6±0.3Hz and 4.4±0.2Hz respectively ($P<0.05$ compared with saline: 2.8±0.5Hz). These responses were not maintained for more than 1 min and following 10mg/kg pilocarpine, there was a significant attenuation of the response to arecoline 0.3mg/kg. (Frequency after saline: 2.83±0.5Hz, arecoline: 5.95±0.2Hz, arecoline 10min after pilocarpine: 2.9±0.7Hz). No antagonist activity was seen with any of the other agonists tested.

We conclude that an increase in theta rhythm frequency can be produced by high efficacy agonists such as oxotremorine, aceclidine, APE and arecoline and that the weak agonist/antagonist action shown by pilocarpine may relate to its partial agonism on this system.

Olpe, H.R., Klebs, K., Kung, E., Campiche, P., Glatt, A., Ortmann, R., D'Amato, F., Pozza, M.F. and Mondadori, C. (1987) Eur.J.Pharmacol 142, 275-283
Van Charldorp, K., de Jonge, A., Thoolen, M.J.M.C. and van Zwieten, P.A. (1985) Naunyn-Schmeidebergs Arch Pharmacol 311, 301-306

DOPAMINE D_1-STIMULATED ADENYLATE CYCLASE IN RAT STRIATAL SLICES IS REDUCED BY CHRONIC ELECTROCONVULSIVE SHOCK

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Repeated electroconvulsive shock (ECS) enhances the behavioural responses of rodents to dopaminergic agonists (Green and Nutt, 1987). Recently we obtained evidence that this effect of ECS in rats may be attributed to a selective increase in central dopamine (DA) D1 receptor subtype function, as assessed using the the D1 agonist SKF 38393 (Sharp et al., 1989). In search of a biochemical correlate to these findings we have now studied the effect of repeated ECS on D1 receptor-stimulated adenylate cyclase in rat striatum in vitro.

Male Sprague-Dawley (200-300 g) were used throughout; ECS (90 V, 1s) was administered 5 times over a 10-day period under light halothane anaesthesia, and always resulted in a full tonic-clonic seizure; matched controls underwent the same protocol, though did not receive the shocks. Rats were used 24h after the last shock. Striatal slices (350x350 μm), from treated or untreated rats, were prepared and preincubated in Krebs-HEPES buffer for 60 min using the method of Godfrey et al. (1989). 30 μl aliquots of packed slices were then incubated in 250 μl of buffer containing 0.5 mM 3-isobutyl-1-methyl xanthine (IBMX) for 10 min. The appropriate agonist (10 μl)was then added and the incubation continued for a further 20 min; antagonists (10 μl) were added at the same time as the tissue. DA was dissolved in 1% ascorbic acid and experiments using DA were undertaken in the presence of 1 μM propranolol (Kelly and Nahorski, 1987). Reactions were stopped by addition of 300 μl of ice-cold 10% perchloric acid which was then extracted using the FREON/ octylamine method described in Godfrey et al. (1989). cAMP content was then determined using a commercial cAMP assay kit (Amersham).

Basal cAMP in striatal slices (in the presence of 0.5 mM IBMX) was 1.0-1.5 pmol/50 μl of tissue extract and this was dose-dependently increased by both DA (maximal response 86 ± 10% over basal; EC_{50} 20 μM) and SKF 38393 (max. response 159 ± 8% over basal; EC_{50} 300 nM). The selective D2 antagonist (-)-sulpiride markedly enhanced the stimulation of cAMP formation by 100 μM DA (the maximum response was increased to 175 ± 11% over basal, EC_{50} 5 μM), as has been previously found by Kelly and Nahorski (1987). Repeated ECS (5 shocks over 10 days) greatly reduced the maximal cAMP response to SKF 38393 (cAMP, % above basal; halothane 213 ± 13%, ECS 153 ± 9) though did not alter the EC_{50}. The response to 100 μM DA was also substantially reduced and the potentiation by (-)-sulpiride was almost completely lost (cAMP, % above basal; halothane, 100μM DA 80 ± 7, DA + 10 μM sulpiride 127 ± 18; ECS, 100 μM DA 29 ± 8, DA + 10 μM sulpiride 24 ± 13). A single ECS did not significantly affect the responses.

Thses data suggest that repeated ECS reduces the response of adenylate cyclase to D1 receptor activation. ECS would appear to alter receptor-effector coupling in this respect since ECS does not change the number of rat striatal D1 binding sites (Martin et al., 1988). Given that ECS increases the behavioural response to SKF 38393, it would appear that these behaviours are not mediated via D1 linked adenylate cyclase in striatum.

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Godfrey P.P. et al. (1989) J. Neurochem. 52, 498-506.
Green, A.R. and Nutt, D.J. (1987) in Handbook of Psychopharmacology, Plenum Press, 19, 375-419.
Kelly, E. and Nahorski, S.R. (1987) Naunyn-Schmied. Arch. Pharmac. 335, 508-512.
Martin, K.F. et al. (1989) Br. J. Pharmac. 95, 896P.
Sharp, T. et al. (1989) Psychopharmacol., in press.

MODIFICATION OF LIMBIC AND EXTRAPYRAMIDAL DOPAMINE METABOLISM FOLLOWING INJECTION OF DIME-C7 INTO THE RAT VENTRAL TEGMENTAL AREA

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DiMe-C7 [pGlu^5,MePhe^8,Sar^9]SP-(5-11) is a stable substance P analogue, infusion of which into rat ventral tegmental area (VTA) evokes a number of behavioural changes including a marked increase in locomotor activity (Eison et al. 1982). This behavioural change is associated with an increase in dopamine metabolism in the nucleus accumbens and pre-frontal cortex (Elliot et al. 1986; Hagan et al. 1987). The present studies expand these findings by demonstrating biochemical changes selective for the dopaminergic system of both limbic and extrapyramidal nuclei.

DiMe-C7, 3.0 μg in 0.5 μl 0.9% (wt/vol) sodium chloride, or vehicle, was injected bilaterally into the VTA over a 1 min period via stereotaxically placed chronically indwelling cannula. Immediately following the injection rats were placed in individual activity boxes equipped with photocell units for subsequent monitoring of behavioural hyperactivity for 90 min or for killing at 15 min (time of peak behavioural response) for neurochemical analyses. Brain nuclei were rapidly dissected, the samples were processed and assayed for catecholamine and indoleamine neurotransmitters and their respective metabolites by high performance liquid chromatography with electrochemical detection by the method of Barnes et al. (1988). Animals that received DiMe-C7 developed locomotor hyperactivity with peak responses typically falling between 10 and 30 min (Vehicle = 118 ± 12, DiMe-C7 = 537 ± 13 counts/5 min, mean ± S.E., n = 4-5, P<0.001, Student's t test). The changes in dopamine (DA) 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels in the striatum and nucleus accumbens are shown in Table 1. (No significant changes in 5-hydroxytryptamine or 5-hydroxyindoleacetic acid levels were detected.

Table 1 Biochemical correlates of DiMe-C7 injections into rat VTA

Brain Region	Treatment	DA	DOPAC	HVA
Nucleus accumbens	Control	5108 ± 372	1476 ± 121	655 ± 56
	DiMe-C7	5382 ± 387	2473 ± 276**	875 ± 56*
Striatum	Control	11316 ± 890	1323 ± 70	879 ± 75
	DiMe-C7	7916 ± 359**	1636 ± 91*	818 ± 108

Data represents the mean ± S.E., n = 10-12. All levels are expressed as pg mg^{-1} wet weight *P<0.05, **P<0.01. Significant change compared to control (Student's t test).

It is concluded that the increase in locomotor activity following intra-VTA injection of DiMe-C7 is accompanied by a selective increase in dopaminergic metabolism in both limbic and extrapyramidal areas.

Barnes J.M., Barnes, N.M., Costall, B., Naylor, R.J. & Tattersall, F.D. (1988) Neuropharmacol. 27(8): 783-790

Eison A.S., Eison, M.S. & Iversen, S.D. (1982) Brain Res. 238: 137-152

Elliot P.J., Alpert, J.E., Bannon, M.J. & Iversen, S.D. (1986) Brain Res. 363: 145-147

Hagan R.H., Butler, A., Hill, J.M., Jordan, C.C., Ireland, S.J. & Tyers, M.B. (1987) Eur. J. Pharmacol. 138: 303-305

ARE THE ELECTROPHYSIOLOGICAL EFFECTS OF MELATONIN IN THE RAT CNS MEDIATED *VIA* 5-HT RECEPTORS?

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Some mammalian species exhibit annual changes in reproductive function triggered by the seasonal variation in daylength. These seasonal changes appear to be, at least partially, mediated via nocturnal secretion of the indoleamine melatonin from the pineal gland; thus during the long-days of summer there will be a short period of melatonin secretion (Tamarkin et al, 1985). This melatonin signal is then believed to be read by central structures implicated in the regulation of seasonal reproductive cycles, in particular the suprachiasmatic nuclei (SCN) of the hypothalamus, (Groos et al, 1983; Mason & Rusak, 1989). The possibility that melatonin might exert its effects via serotonin (5-HT) receptors prompted a study of the responses evoked by these indoleamines on 5-HT responsive SCN and dorsal raphe neurones in the rat.

Extracellular recordings, using multibarrel electrodes, were obtained from SCN neurones in male Wistar rat hypothalamic slices maintained in vitro (Mason & Brooks, 1988). The effects of iontophoresed or pressure ejected 5-HT (2mM in 0.9% NaCl) and melatonin (2mM in 0.9% NaCl) were observed on SCN neuronal discharge activity. Ejection of 5-HT suppressed (n=45/80) or activated (n=3/80) SCN cells, the remaining cells (n=32/80) were unaffected. The predominant effect of melatonin was also to suppress SCN discharge activity (n=24/62 neurones); two SCN cells were activated, the remainder were unaffected. Five SCN neurones suppressed by melatonin were unaffected by 5-HT, while conversely twenty-four 5-HT responsive neurones were unaffected by melatonin. Melatonin-evoked suppressions were similar to those of 5-HT, showing an onset latency of 5-10s and recovery to basal firing within 10-20s. In contrast to 5-HT, the recovery from melatonin was not prolonged by ejection of the monoamine uptake inhibitor imipramine (20mM). Melatonin-induced suppression of firing was not affected by concurrent application of the 5-HT_2 receptor antagonist ketanserin (20mM) in five cells tested.

The effects of systemic injections of melatonin and the 5-HT_1 agonist 8-OH-DPAT were examined on dorsal raphe neuronal firing in halothane-anaesthetised Wistar rats. For nine raphe neurones tested, 8-OH-DPAT (10-100 ug/kg, i.v.) suppressed raphe firing in a dose-dependent manner. In contrast, injections of melatonin up to supraphysiological doses (140 ug/kg, i.v.) were without effect on raphe discharge activity. This regional difference in neuronal sensitivity to melatonin reflects the distribution of melatonin binding sites (concentrated within the SCN) rather than that of 5-HT receptors which are found in both areas (Vanecek et al, 1987).

Groos, G.A., Mason, R. & Meijer, J. (1983) Fed. Proc. 42, 2790-2795.
Mason, R. & Brooks, A (1988) Neurosci. Lett. 95, 296-301.
Mason, R. & Rusak, B. (1989) J. Physiol. 410, 53P.
Tamarkin, L., Baird, C.J. & Almeida, O.F.X. (1985) Science 227, 714-720.
Vanecek, J., Pavlik, A. & Illnerova, H. (1987) Brain Res. 435, 359-362.

ADDITIVE EFFECT OF DESIPRAMINE AND 8-OH-DPAT ON β–ADRENOCEPTOR DOWN-REGULATION

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Down-regulation of ß-adrenoceptors is a biochemical marker for antidepressant drug activity (Minneman *et al.*, 1982; Biegon, 1986) and the physiological process may be defective in human depression (Biegon & Israeli, 1988). The down-regulation of ß-adrenoceptors is also modulated by serotonergic neurons (Nimgaonkar *et al.*, 1985) and may involved $5HT_{1A}$ receptors (Dudley & Baron, 1989). We examined the effect of 8-OH-DPAT (8-Hydroxy-2-(di-n-propylamino)tetralin) on antidepressant-induced ß-adrenoceptor down-regulation in order to investigate the role of $5\text{-}HT_{1A}$ receptors in this process.

Groups of 6 adult male Sprague-Dawley rats (225 - 275 g) were injected twice daily for 4 weeks with either desipramine HCl (DMI; 10 mg/kg i.p.), 8-OH-DPAT HBr (0.5 mg/kg s.c.), saline (i.p.) or DMI (10 mg/kg i.p.) together with 8-OH-DPAT (0.5 mg/kg s.c.). Rats were anaesthetized and decapitated 12 h after the last injection. The brain was removed and 10 µm sections cut on a cryostat. These were mounted on microscope slides and preincubated for 30 min at 23°C in Krebs buffer containing GppNHp. Incubations were carried out for 150 min at 23°C in Krebs buffer with [^{125}I]-cyanopindolol (50 pM) and 5-HT (1 µM) in the presence or absence of propranolol (1 µM), ICI 118551 (erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol, 70 nM) and CGP 20712A (2-hydroxy-5-(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1H-imidazol-2-yl)-phenoxy)propyl)-amino)ethoxy)-benzamide monomethanesulphonate, 100 nM) to define non-specific and specific binding to $ß_1$ and $ß_2$-adrenoceptors. Following incubation the sections were air dried and apposed to [^{3}H]Ultrofilm (LKB). Quantitative autoradiography was performed using an MCID image analysing system (Imaging Systems, Ontario).

Table 1. Concentration of ß-adrenoceptors binding (fmol/mg protein) in brain regions of control and treated rats

		Cortex layer 1	Cortex layer 2	Cortex layer 3	Hippocampus	Thalamus	Caudate putamen
Control	$ß_1$	140 ± 5	79 ± 2	29 ± 5	67 ± 6	51 ± 1	102 ± 3
	$ß_2$	19 ± 1	14 ± 1	5 ± 2	10 ± 1	10 ± 1	35 ± 2
DMI	$ß_1$	86 ± 7**	33 ± 2***	26 ± 0	29 ± 2**	30 ± 1**	97 ± 1
	$ß_2$	15 ± 2	11 ± 2	5 ± 2	7 ± 2	9 ± 1	41 ± 3
8-OH-DPAT	$ß_1$	113 ± 8*†	79 ± 8	30 ± 5	51 ±10	45 ± 1	107 ± 3
	$ß_2$	21 ± 1	19 ± 1	11 ± 2	9 ± 2	12 ± 1	39 ± 1
8-OH-DPAT	$ß_1$	58 ± 7**†	41 ± 3***	26 ± 5	31 ± 7*	32 ± 1**	94 ± 16
+ DMI	$ß_2$	20 ± 3	20 ± 3*	8 ± 3*	4 ± 3	14 ± 3	56 ± 6*

(*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ relative to the control; † $P < 0.05$ relative to DMI alone; analysis of variance; values are the mean ±s.e.mean).

These results show a significant down-regulation of ß-adrenoceptor number following DMI treatment (Table 1), as has been found by others (Minneman *et al.*, 1982; Biegon, 1986), which was selective for $ß_1$-adrenoceptors in hippocampus, thalamus and layers 1 and 2 of the cortex. 8-OH-DPAT alone decreased $ß_1$-adrenoceptor number in layer 1 of the cortex only and combined treatment with DMI and 8-OH DPAT produced additive effects in this layer but not in others. Such an interaction suggests that there may be value in the combination of $5\text{-}HT_{1A}$ receptor agonists with other antidepressant agents in the therapy of depression.

Biegon, A.J. (1986) Neurochem. 47, 77-80.
Biegon, A. & Israeli, M. (1988) Brain Res. 442, 199-203.
Dudley M. & Baron B. (1989) Proc. XXII Winter Conf. Brain Res. (Snowbird, Utah) Abs 12.
Minneman, K. P., Wolfe B. & Molinoff, P.B. (1982) Brain Res. 252, 309-314.
Nimgaonkar, V., Goodwin, G., Davies, C. & Green, A.L. (1985) Neuropharmacol. 24, 279-283.

CHARACTERISTICS OF THE 5-HT-INDUCED SYNDROME IN THE MONGOLIAN GERBIL

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The reflex behaviour of the serotonin syndrome, which is mediated by brainstem structures (Bedard & Pycock, 1977), has not previously been described in the Mongolian gerbil.

Motor components of the serotonin syndrome were induced by 5-methoxy-N,N-dimethyltryptamine (5-MeODMT, 5 mg/kg, i.p.). Control gerbils received an injection of an equivalent volume of physiological saline. Behaviour of the animals was observed for 90 min following injection, with their motor acts and postures being recorded for 5 min. periods after 6 and 60 min. Behaviour was recorded as a spoken commentary by tape recorder using the check list of elements of the gerbil's normal non-social activity (Chapman & Cutler, 1989). The frequency of these along with "wet dog shake", "head shake", "head bob", "slow head turn" and "snake tail" were recorded as mean frequencies (±S.E.mean) within the 5 min. observation periods, while the occurrence of hindlimb abduction, extended digits, flattened body posture, fine body tremor and hyperlocomotion by "forward paddling" were rated as being absent (0), just present (1), definite (2) or severe (3).

Hyperlocomotion by forward paddling, a flat body posture, tremor, hindleg abduction, extension of the digits, head shake, wet dog shake, slow lead turn and snake tail were significantly increased during the initial 6-11 min. observation period. A delayed behavioural change also occurred after 1 h. This comprised vertical head bobs, increase of slow head turns, and increased occurrence of the normal behavioural acts, "scratch" and "self-groom". During the serotonin syndrome, components of normal motor activity other than "wash" were suppressed. At 12 h. after injection behaviour had returned to normal.

It can thus be seen that the serotonin syndrome has a longer duration of action in the gerbil than in the rat (Sloviter et al., 1978), and that it is associated with the behavioural changes of an immediate and a delayed response. Further studies of the metabolism of 5-MeODMT and of the psychopharmacological activities of metabolites formed in the gerbil is thus warranted.

Bedard, P. & Pycock, C.J. (1977) Neuropharmacology 16, 663-670.

Chapman, J.B. & Cutler, M.G. (1989) Neurotoxicol. Teratol. 11, 193-198.

Sloviter, R.S. et al. (1978) J. Pharmac. exp. Ther. 206, 339-347.

EVIDENCE FOR AN IMPORTANT SPECIES DIFFERENCE IN THE MECHANISM OF 8-OH-DPAT-INDUCED HYPOTHERMIA

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8-OHDPAT-induced hypothermia in the mouse appears to be mediated by inhibitory presynaptic 5-HT_{1A} autoreceptors (Goodwin et al., 1985). However, there is some controversy as to whether the 5-HT_{1A} receptors mediating 8-OHDPAT-induced hypothermia in the rat are located presynaptically (Goodwin et al., 1987) or postsynaptically (Hutson et al., 1987). Here we report the results of parallel series of experiments, in the mouse and the rat, to investigate the effects of facilitation or attenuation of 5-HT release on 8-OHDPAT-induced hypothermia.

Female Tuck T/O mice (20-27g) and male Sprague-Dawley rats (150-250g; Charles River) were used. Rectal (mice) or oesophageal (rats) temperatures were measured immediately before drug or vehicle pretreatments, and again immediately before, and 15 and 30 min. following, subcutaneous administration of 8-OHDPAT. Hypothermia was measured as the maximum decrease in temperature recorded in this latter period. Animals were allocated randomly to treatment groups (n=8) and experiments were performed 'blind'. Data were analysed by ANOVA followed by Dunnett's test.

The hypothermic responses to the submaximal doses of 8-OHDPAT used in these experiments (0.8mg/kg s.c. for mice; 0.3mg/kg s.c. for rats) ranged from -2.0 ± 0.3 to -3.0 ± 0.4 for mice and from -1.6 ± 0.3 to -2.3 ± 0.3°C for rats. In the mouse, the selective 5-HT uptake inhibitors citalopram, fluvoxamine, panuramine and Wy 27587 (Fletcher et al., 1988), administered p.o. 60 min. prior to 8-OHDPAT, dose-dependently antagonised 8-OHDPAT-induced hypothermia. The relative potencies (dose which attenuated hypothermia by 50%) of these compounds were 2.9, 8.0, 8.7 and 1.7mg/kg respectively. Administration of fenfluramine (20mg/kg i.p., 20 min. before 8-OHDPAT) or 5-hydroxytryptophan (5-HTP; 50 mg/kg s.c., 30 min. before 8-OHDPAT with carbidopa 25 mg/kg i.p. 60 min. before 5-HTP) also markedly attenuated 8-OHDPAT hypothermia by 76% ($p < 0.001$) and 93% ($p < 0.001$) respectively. In contrast, 5-HT uptake inhibitors or 5-HTP/carbidopa had no significant effect on 8-OHDPAT-induced hypothermia in the rat. Fenfluramine slightly attenuated (26%; $p < 0.05$) hypothermia only at a dose (5 mg/kg i.p.) which reduced body temperature significantly prior to 8-OHDPAT administration.

In the mouse, administration of para-chlorophenylalanine (pCPA; 750 mg/kg p.o. on days 1 and 2; tested on day 5) or 5, 7-dihydroxytryptamine (5,7-DHT; 50 µg i.c.v. on day 1; tested on day 5) depleted whole brain 5-HT levels by 54% and 61%, and respectively attenuated (by 59%; $p < 0.05$) or abolished 8-OHDPAT hypothermia. In the rat, neither pCPA (250 mg/kg p.o. on days 1 and 2; tested on day 5) or 5,7-DHT (150 µg i.c.v. on day 1; tested on day 10) attenuated 8-OHDPAT hypothermia despite depleting brain 5-HT levels by 90% and 70% respectively. Pretreatment with pCPA slightly enhanced 8-OHDPAT hypothermia in the rat. The behavioural syndrome response to 0.3 mg/kg s.c. 8-OHDPAT was also significantly increased ($p < 0.05$) by 5,7-DHT pretreatment.

These data strongly suggest that presynaptic 5-HT_{1A} autoreceptors mediate 8-OHDPAT-induced hypothermia in the mouse, whereas direct stimulation of postsynaptic receptors mediate this action of 8-OHDPAT in the rat. The enhanced responses to 8-OHDPAT in the rat following brain 5-HT depletion would be consistent with the development of postsynaptic 5-HT_{1A} receptor supersensitivity.

Fletcher,A., Forster,E.A., Bill,D.J. & Billingsley,A.J. (1988) Br. J. Pharmacol., 94, (Proc. Suppl.), 384P.
Goodwin,G.M., De Souza,R.J. & Green,A.R. (1985) Neuropharmacology, 24, 1187-1194.
Goodwin,G.M., De Souza,R,J., Green,A.R. & Heal,D.J. (1987) Psychopharmacology, 91, 506-511.
Hutson,P.H., Donohoe,T.P. & Curzon,G. (1987) Eur. J. Pharmacol., 143, 221-228.

SELECTIVE 5-HT-INDUCED REVERSAL OF CATALEPSY INDUCED BY FLUPHENAZINE

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Schizophrenic patients are treated predominantly with neuroleptic agents to reduce the symptoms of the disorder. A major side effect of such therapy is the development of extrapyramidal effects (Hornykiewicz, 1973). Drugs that produce this behavioural profile in man also induce catalepsy in rodents (Stanley & Glick, 1976). Dopamine antagonist induced catalepsy can be reversed with the dopamine precursor, L-DOPA, and directly acting dopamine agonists (Elliott et al., 1989). In man $5HT_2$ antagonists have been reported to reduce the tremor seen in Parkinsonian patients (Maertens de Noordhout & Delwaide, 1986), although in a recent report such drugs were unable to block haloperidol-induced catalepsy in rodents (Broekkamp et al., 1988). In the latter study, however, the selective $5HT_{1A}$ agonist, 8-OHDPAT, was shown to attenuate the neuroleptic-induced catalepsy. The aim of our experiments was to investigate the effects of the $5HT_2$ antagonists, ritanserin and ICI 170,809, and the $5HT_{1A}$ agonists, 8-OHDPAT, gepirone, BMY 7378 and NAN 190 on fluphenazine-induced catalepsy.

Male hooded rats (170-250g; Glaxo, Herts.) were injected subcutaneously (s.c.) with the dopamine antagonist fluphenazine (0.1-1.0mg/kg). The serotoninergic agents and vehicle control injections were all given intraperitoneally (i.p.) 4h later and catalepsy was assessed at 5h.

Results show that the catalepsy induced by fluphenazine (0.1-1.0mg/kg; s.c.) was dose dependent 5h after s.c. administration. Furthermore, ritanserin (20.0mg/kg) and ICI 170,809 (1.0 or 10.0mg/kg) were ineffective at reversing this catalepsy. However, the selective $5HT_{1A}$ agonists, 8-OHDPAT (0.3 or 1.0mg/kg) and NAN 190 (0.1 or 1.0mg/kg), were able to attenuate the fluphenazine-induced catalepsy by 50-90%.

Our data extend the previous findings (Broekkamp et al 1988) that $5HT_{1A}$ agonists, but not $5HT_2$ antagonists, reverse neuroleptic induced catalepsy. At present the exact mechanism through which this effect is mediated is unclear. It is possible that activation of $5HT_{1A}$ cell body autoreceptors, in the raphé nuclei, could play a significant role in the effect observed (Invernizzi et al 1988). This hypothesis is supported by anatomical studies which have shown that 5HT-containing cells in this area project to both ends of the nigro-striatal dopamine system (Conrad et al., 1974).

Broekkamp, C.L.E., Oosterloo, S.K., Berendsen, H.H.G., van Delft, A.M.L. (1988) Naunyn-Schmeideberg's Arch. Pharmacol. 338, 191-195.
Conrad, L.C.A., Lenoard, C.M., Pfaff, D.W. (1974) J. comp. Neurol. 156, 179-206.
Elliott, P.J., Walsh, D.M., Close, S.P., Hayes, A.G., Marriott, A.S. (1989) Brit. J. Pharmacol. In press.
Hornykiewicz, I. (1973) Brit. Med. Bull. 29, 172-178.
Invernizzi, R.W., Cervo, L., Samanin, R. (1988) Neuropharmacol. 27, 515-518.
Maertens de Noordhout, Delwaide (1986) Clin. Neuropharm. 9, 480-484.
Stanley, M.E. & Glick, S.D. (1976) Neuropharmacol. 15, 393-394.

THE INFLUENCE OF AN AVERSIVE ENVIRONMENT ON LOCOMOTOR RESPONSES TO 5-HYDROXYTRYPTAMINERGIC DRUGS

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Previous studies suggest that the psychostimulant effects of nicotine are more pronounced in an aversive environment (Vale & Balfour 1989). There is also evidence that behavioural responses to nicotine may be mediated through brain 5-HT mechanisms (Balfour 1989). The purpose of this study was to investigate the possible role of serotonergic systems in the differential locomotor responses to an aversive environment.

Male Sprague-Dawley rats (n=8) were given i.p. or s.c. injections of either saline or drug and spontaneous locomotor activity monitored for 15 min. using infrared photobeams on either an elevated open platform or a less aversive enclosed platform of the same dimensions (Vale & Balfour 1989). The rats from each treatment group were studied once on each platform using a counter-balanced design.

Exposure to the open platform resulted in a significant reduction in total activity compared to that on the enclosed platform ($F(1,42)=81.0;P<0.001$). The effects of the 5-HT uptake inhibitor paroxetine (parox {Beechams} 0.5 & 5.0 mg/kg i.p. injected 1.0h prior to the test) on locomotor activity are summarised in Table 1. The smaller dose of parox increased total activity on the open platform ($F(1,14)=5.2;P<0.05$) whereas the larger dose reduced activity on the enclosed platform compared to controls ($F(1,14)=4.8;P<0.05$). Analysis of the subtrial activity (sub 1-3) suggested that parox increased activity on the open platform during the first 5 min of the trial, and at the larger dose reduced activity on the enclosed platform during the last 10 min. In an attempt to characterise the receptor subtypes involved, the effects of various 5-HT receptor ligands were investigated using this model. The 5-HT_3 antagonist GR38032F ({Glaxo} 0.001-1.0 mg/kg i.p.), the 5-HT_{1A} agonist 8-OH-DPAT (0.0625-0.250 mg/kg s.c.) and the 5-HT_2 antagonist ritanserin ({Janssen} 0.5 & 5.0 mg/kg i.p.) had no effect on locomotor activity on either platform at any of the doses tested.

Table 1 Effects of Paroxetine on Locomotor Activity

	Open Platform			Enclosed Platform		
	Saline	0.5 Parox	5.0 Parox	Saline	0.5 Parox	5.0 Parox
Sub 1	61±6	83±9	84±7*	193±11	195±9	179±12
Sub 2	41±7	58±10	51±9	108±8	84±12	74±12*
Sub 3	33±6	56±13	37±9	97±8	70±17	59±15*
Total	135±15	197±23*	172±22	399±24	350±32	312±31*

Data are expressed as mean ± SEM and were analysed using ANOVA for repeated measures followed by the Duncans multiple range test. * $p<0.05$ versus saline.

These data suggest that paroxetine, like nicotine, can attenuate a stress induced fall in locomotor activity. However, at larger doses of the drug this response is masked by a reduction in activity which is more apparent on the less aversive enclosed platform.

Balfour, D.J.K. (1989) Pharm. Ther. In Press.
Vale A.L. & Balfour, D.J.K. (1989) Pharmacol. Biochem. Behav. In Press.

STEREOSPECIFICITY OF THE ACTIONS OF PN 200-110 ON THE CHANGES DUE TO ETHANOL WITHDRAWAL IN HIPPOCAMPAL FIELD POTENTIALS

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Long term intake of ethanol causes a withdrawal syndrome on cessation of intake. In man, this includes tremors, hyperreflexia, and potentially fatal convulsions. The neurophysiological basis of this is uncertain, but dihydropyridine calcium channel antagonists, such as nitrendipine, protect against the behavioural syndrome in rodents. We have previously reported a pattern of changes in the field potentials in the isolated hippocampal slice, following a chronic ethanol treatment *in vivo* that caused a clear withdrawal syndrome in behavioural tests (Whittington & Little, 1989; Whittington & Little, 1988).

Nitrendipine prevented these electrophysiological changes, when added to the bathing medium *in vitro* (Whittington & Little, 1988). The concentration required was in the range needed for actions on central neurones (e.g. Jones & Heinemann, 1987) and it had no effect on control tissues. However, the concentration was higher than those required for actions of dihdyropyridine calcium channel antagonists on tissues other than neurones. We have therefore determined the stereospecificity of the dihydropyridine PN 200-110 in protecting against the electrophysiological manifestations of the ethanol withdrawal syndrome.

Male mice, C57 strain, 25-30g, received ethanol, 24% v/v, as sole fluid, for fifteen weeks; ethanol intake was 12-16 g/kg/day. Controls drank water. Hippocampal slices, made without prior withdrawal of the animals from ethanol, were perfused with standard Ringer solution. Extracellular recording was from CA1 area, using orthodromic stimulation of Schaffer collateral/commissural fibres. PN 200-110 isomers were dissolved in DMSO, then diluted in Ringer solution to 2 μM, with a final DMSO concentration of 0.02%. Every 15 min, the thresholds for production of first and multiple population spikes and paired pulse potentiation (PPP, % 2nd/1st reponses; 70 msec interval, 1.25 x threshold) were measured. Results (mean ± s.e.m.) were compared by analysis of variance (n=5 each group).

As reported previously, the thresholds for elicitation of single population spikes were lower in slices from ethanol treated animals (Eth) than from controls, after the first 2h of recording. This change was significantly ($P<0.001$) decreased by 2 μM (+)-PN 200-110 (PN), but not by (-)-PN 200-110 ($P>0.1$). Thresholds for multiple spikes were lower in slices from ethanol treated animals, an effect prevented by 2 μM (+)-PN 200-110 ($P<0.001$), but not by (-)-PN 200-110 ($P>0.1$). In slices from ethanol treated animals paired pulse potentiation increased, compared with controls, from the beginning of recording, reached a peak at 2h 15 min, then returned to normal, a change decreased (+)-by PN 200-110. The PN 200-110 isomers had no effects in control tissues (Con).

Thresholds, μA	Controls	Ethanol	Eth+(+)PN	Eth+(-)PN	Con+(+)PN	Con+(-)PN
Single spike (5h)	27 ± 1	20 ± 1	24 ± 1	21 ± 2	27 ± 1	27 ± 2
Mult. spikes (5h)	190 ± 6	120 ± 17	178 ± 7	132 ± 12	189 ± 5	178 ± 13
PPP, % (2h)	202 ± 7	325 ± 12	222 ± 15	315 ± 25	200 ± 10	199 ± 13

These results show the stereopecificity of the actions of PN 200-110 in preventing hyperexcitability following ethanol withdrawal followed that on calcium channels in other tissues (Hof et al, 1986), providing evidence that the action was mediated through calcium channels. This is agreement with results from behavioural studies (Littleton & Little, 1987)

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Hof,R.P.,Hof,A.,Ruegg,U.T., Cook,N.S. & Vogel,A. 1986 J.Cardiovasc.Pharmacol., 8, 221-226
Littleton, J. M. & Little, H. J. Br. J. Pharmacol., 92, 663P,1987
Whittington, M. A. & Little, H. J. 1988 Br. J. Pharm., 94, 385P
Whittington, M. A. & Little, H. J. 1989 Br. J. Pharm., 96, 326P
Jones, R. S. G. & Heinemann, U. 1986 Brain Res., 416, 257 - 266

STUDIES ON THE ACTION OF CHLORMETHIAZOLE IN ETHANOL WITHDRAWAL

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Chlormethiazole has been used successfully for many years as a treatment for acute ethanol withdrawal (see Evans et al, 1986). The current study investigated whether it would attenuate the convulsive behaviour associated with alcohol withdrawal in an animal model being used extensively to examine the effects of dihydropyridine administration on the ethanol withdrawal syndrome (Whittington & Little, 1988).

Male mice (TO strain), 30 - 35g, were exposed to ethanol vapour (10mg/L) in an inhalation chamber for two weeks. They were then withdrawn from the ethanol and the withdrawal syndrome measured by ratings of convulsive behaviour on handling, by an observer unaware of treatment conditions (Whittington & Little, 1988). Control animals received no ethanol.

Mice withdrawn from ethanol showed an increase in convulsive behaviour between 2h and 10h from ethanol withdrawal, compared with control animals. Immediately following the 5h measurement, mice were injected with either chlormethiazole (100mg/kg) or saline. Chlormethiazole did not alter the control response, but almost totally attenuated the convulsive behaviour in the ethanol group 30 min later. Ethanol withdrawal median score [interquartile range] was: 3 [3-2]; ethanol withdrawal plus chlormethiazole: 0.5 [0-1]; controls: 1 [1-1]. Sixty minutes after chlormethiazole the median score was still decreased, compared with the ethanol withdrawn only group, but this was not statistically significant. Chlormethiazole at 40 mg/kg did not alter the withdrawal behaviour.

Chlormethiazole, 100 mg/kg, significantly increased the seizure threshold to bicuculline, 30 min after injection, as measured by infusion into the tail vein. Seizure threshold was still increased 60 min after chlormethiazole, 100 mg/kg, but was less than that measured 30 min after 40 mg/kg chlormethiazole (Table 1). Chlormethiazole, 100 mg/kg, also decreased the spontaneous locomotor activity (measured by light beam interruption meters) in the first 30 min after injection but not in the period 31-60 min after injection (see Table 1).

Table 1 Effects of chlormethiazole on seizure threshold and on locomotor activity

	Seizure threshold mg/kg		Locomotor activity (counts x 10^2)	
Treatment	30 min	60 min	0-30 min	31-60 min
Saline	0.59 ± 0.06		131 ± 22	81 ± 15
Chlormethiazole, 100 mg/kg	0.96 ± 0.1*	0.74 ± 0.15	52 ± 16*	71 ± 21
Chlormethiazole, 40 mg/kg	0.78 ± 0.14*		86 ± 16*	59 ± 18†

Compared with control group: *$P < 0.01$; †$P < 0.05$, Student's t-test

These data therefore demonstrate further the validity of the withdrawal model, insofar as a drug used in clinical withdrawal states attenuated the syndrome. The data also show that the efficacy of chlormethiazole parallels the sedative and convulsive action. It is uncertain whether these effects are directly related, since chlormethiazole, 40 mg/kg, had no action on withdrawal, but had significant effects on both locomotor activity and seizure threshold.

Evans, J. G., Feuerlain, W., Glatt, M. M., Kanowski, S. & Scott, D. B. (1986) Acta Psychiat. Scand., 73, Suppl. 329
Whittington, M. A. & Little, H. J. (1988) Br. J. Pharmacol., 94, 385P

CHARACTERISATION OF PROSTANOID BINDING SITES ON NG108-15 CELLS

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NG108–15 cells are a hybrid between the N18TG2 mouse neuroblastoma line and a rat glioma line. These cells have been used extensively to study receptor binding and receptor–coupled adenylate cyclase. For studies of receptors which inhibit adenylate cyclase, the enzyme has almost without exception been first stimulated with PGE_1. This has led to the assumption that the prostanoid receptors on the NG108–15 cell are of the PGE (EP) type. However, there is little firm evidence to support this assumption. The aim of this study was therefore to characterise the prostanoid receptors on the NG108–15 cell.

A crude membrane fraction was prepared by standard methodology. All experiments were conducted in HEPES buffer containing 10^{-5}M indomethacin. Binding experiments using [3H]PGE_1 (58.1 Ci.mmol^{-1}, 2.5nM), [^{3}H]PGE_2 (185 Ci.mmol^{-1}, 1nM) or [^{3}H]iloprost (14.7 Ci.mmol^{-1}, 2nM) were run for 60 minutes at 25°C. Non–specific binding was defined as the portion which was not displaced by 10^{-5}M PGE_1.

No specific binding of [^{3}H]PGE_2 was detectable under these conditions. In contrast, significant PGE_1 binding (800 cpm) was observed, of which 58% was displaceable by an excess of the unlabelled compound. [^{3}H]Iloprost binding was also readily detectable, but in this case the proportion of specific binding (47%) was lower. A range of compounds were examined for their ability to displace [^{3}H]PGE_1 binding. IC_{50} values (with 95% confidence limits) were: PGE_1 33.3 (26.5 – 41.9)nM, iloprost 2.37 (1.83 – 3.07)nM, PGE_2 1.91 (1.42 – 2.57)μM and 16,16–dimethyl PGE_2 887 (443 – 1780) nM. The PGE analogue sulprostone and the EP_1 receptor antagonist SC–19220 had IC_{50} values in excess of 10^{-5}M. The Hill slope for the displacement of [3H]PGE1 by iloprost was somewhat shallow (0.70 ± 0.04). In all other cases the Hill slope was not significantly different from unity.

The lack of specific PGE_2 binding indicates that the prostanoid receptors on the NG108–15 cell are not of the EP type. Moreover, the order of potencies observed for the displacing agents is entirely different from the reported potencies of these agents at the EP_1, EP_2 and EP_3 receptors (Coleman et al., 1987). A more likely explanation is that the NG108–15 cell possesses prostacyclin (IP) receptors. The low potency of PGE_2 compared to PGE_1 and iloprost is entirely consistent with the potencies of these agents in isolated tissue assays for IP–receptor activity such as the bovine coronary artery (Omini et al., 1977; Schror et al., 1981), and in IP–receptor binding assays (Town et al., 1982).

Although the possibility cannot be excluded that the assay conditions used in this study were inappropriate for the detection of EP–receptors, it is clear that the NG108–15 cell possesses IP–receptors.

Coleman, R.A., Kennedy, I., Sheldrick R.L.G. & Tolowinska, I.Y. (1987) Br. J. Pharmacol. 91, 407P.
Omini, C., Moncada, S. & Vane J.R. (1977) Prostaglandins 14, 625–632.
Schror, K., Darius, H., Matzky, R. & Ohlendorf, R. (1981) Naunyn–Schmiedeberg's Arch. Pharmacol. 316, 252–256.
Town, M–H., Schillinger, E., Speckenbach, A. & Prior, G. (1982) Prostaglandins 24, 61–72.

EFFECTS OF CROMAKALIM ON POTASSIUM AND RUBIDIUM EFFLUX RATE FOLLOWING DUAL ISOTOPE LABELLING

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Recent studies have shown that low concentrations of cromakalim (CK, ≤ 1 μM) produce a concentration-dependent increase in the ratio of K : Rb efflux (Bray & Weston,1989; Quast & Baumlin,1988). However, in the presence of a relatively high concentration of CK (10 μM) the numerical value of the K : Rb ratio falls (Bray & Weston,1989). Simultaneous measurement of the efflux of these two ions, following dual labelling, might therefore provide additional information about the types of potassium channel at which CK or other potassium channel openers are effective. This study was designed to determine whether K efflux is modified when tissues are loaded with both K and Rb. Using this method, the effect of a second challenge with CK on rat dual-labelled portal vein or bladder was examined to determine whether the tachyphyllaxis to CK- stimulated Rb efflux (Coldwell & Howlett, 1988) is also associated with a K-selective Rb-impermeable channel.

Rat portal veins or bladder strips were incubated at 37°C for 3h with one or both isotopes in Krebs physiological salt solution (PSS) in which all the potassium was ^{42}K or to which ^{86}Rb had been added (final concentrations 1.74 μCi/ml or 5 μCi/min respectively). Efflux was monitored by transferring the tissues, at 4 min intervals, into tubes containing non- radiolabelled PSS. The tissues were exposed to 1 μM or 10 μM CK, or to an equivalent amount of vehicle, at least 20 min after the start of the efflux period.

There was no difference between the maximum (10μM) CK - stimulated Rb efflux from veins labelled with either Rb alone (181.7 ± 8.5%, n=5) or with the Rb/K mixture (180.8 ± 8.8%, n=5). Although K efflux was greater than Rb efflux, the higher concentration of CK produced similar increases in K efflux from single- or from dual-labelled tissues (299.9 ± 10.7% and 280.1 ± 19.2 % respectively). However, following dual labelling the stimulated K efflux in the presence of the lower concentration of CK (1μM) was only 85.5 ± 4.1% of that from tissues labelled with K alone ($p<0.05$).

In further experiments the effect of a second exposure to 10 μM CK on Rb and K efflux from rat dual- labelled bladder and portal vein was determined. In the rat portal vein there was no difference between the K efflux stimulated by the first (283.6 ± 9.8%) or second (283.8 ± 36.9 %) exposure to CK. Similarly, stimulated Rb efflux resulting from an initial challenge with CK (181.3 ± 5.7%) was not significantly different from the second challenge (167.8 ± 4.02%). In contrast, although there was no difference in the maximum K efflux associated with each exposure of rat bladder to CK, there was a significant reduction ($p<0.05$) in the Rb efflux following the second CK challenge.

With the higher concentration of CK the Rb and K efflux rates were similar for tissues labelled with single or dual isotopes, indicating that no inhibition of either basal or stimulated efflux had resulted from the presence of the other ion. The presence of Rb may modify the K efflux associated with low concentrations of CK. However, although significant, the reduction in K efflux in the presence of Rb was slight and qualitatively similar results were obtained using single- or dual-labelled tissues.

We were unable to detect inhibition of CK- stimulated K efflux resulting from a prior exposure to CK. Tachyphyllaxis to CK-stimulated Rb efflux was not observed in rat portal vein but occurred in the bladder and is reported to occur in rat mesenteric artery (Coldwell & Howlett, 1988). Since there appear to be no reports of tachyphyllaxis to the mechano-inhibitory effects of CK in these tissues, the data presented here suggest that the relaxant effects of CK are associated with an action at Rb-impermeable K channels. Simultaneous dual labelling with K and Rb should therefore provide important information on the mode of action of K-channel openers.

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Bray, K. M. & Weston, A.H. (1989) Br. J. Pharmacol. Proc. Suppl., this meeting.
Coldwell, M.C. & Howlett, D.R. (1988) Biochem. Pharmacol. 37, 4105 - 4110
Quast, U. & Baumlin, Y. (1988) Naunyn-Schmiedeberg's Arch. Pharmacol. 338, 319 - 326

DIFFERENTIAL EFFECTS OF PENTYLENETETRAZOL ON CYCLIC AMP-DEPENDENT SODIUM CURRENT IN NEURONES OF *LYMNAEA STAGNALIS*

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(Introduced by C.T. O'Shaughnessy)

The convulsant drug pentylenetetrazol (PTZ) has opposing effects on two identified neurones in the snail Lymnaea stagnalis. Application of PTZ leads to intense paroxysmal bursting in a buccal neurone (B1), whereas it inhibits spontaneous firing activity in a parietal neurone (RPD1). McCrohan & Gillette (1988) suggested that PTZ's convulsant action was due in part to enhancement of a cyclic AMP-dependent persistent inward current (I_{Na}), and that PTZ slows degradation of cyclic AMP by inhibition of phosphodiesterase. This study compares the effects of PTZ and the phosphodiesterase inhibitor isobutyl methylxanthine (IBMX) on cyclic AMP-dependent I_{Na} in neurones B1 and RPD1.

Intracellular recordings were made from neurones in the isolated central nervous system. For injection with cyclic AMP under voltage clamp, cells were penetrated with a single-barrelled voltage electrode and a double-barrelled current electrode, one barrel of which contained 0.2 M cyclic AMP, buffered to pH 7.5. Cyclic AMP was iontophoresed using 5 s, 1-10 nA current pulses. PTZ and IBMX were applied to the bath to give final concentrations of up to 40 mM and 0.1 mM respectively.

Intracellular injection of cyclic AMP elicited a slow inward current (I_{Na}) in both B1 and RPD1 neurones. In both cell types, the current was abolished in zero Na^+ saline, and was voltage-independent over a range of holding potentials from -100 to -20 mV. In B1, at a holding potential of -60 mV, PTZ caused an increase in amplitude of I_{Na} from 1.41 ± 0.22 to 2.24 ± 0.47 nA (mean ± s.e.mean, $P<0.005$, paired t-test; n=6), whereas in RPD1, PTZ caused a decrease in amplitude, from 2.19 ± 0.39 to 1.33 ± 0.30 nA ($P<0.005$; n=9). In B1, IBMX caused an increase in amplitude of I_{Na} from 1.81 ± 0.29 to 3.07 ± 0.49 nA ($P<0.05$; n=6). In RPD1, IBMX had no significant effect on the amplitude of I_{Na} (n=6).

The effects of PTZ on amplitude of I_{Na} in B1 and RPD1 correspond with its opposing effects on firing activity. This suggests that differential responses of identified neurones to PTZ may be partly due to alterations in the cyclic AMP-dependent persistent inward current. IBMX mimics the effects of PTZ in B1, but has no effect on the amplitude of I_{Na} in RPD1. This suggests that at least part of PTZ's action in B1 may be explained by inhibition of phosphodiesterase, but that other actions must be proposed for RPD1.

McCrohan, C.R. & Gillette, R. (1988) Brain Res. 452, 21-27.

SUBTHALAMIC EFFERENT TRANSMISSION IN THE RAT IS MEDIATED BY AN EXCITATORY AMINO ACID (EAA)

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The subthalamic nucleus is intimately involved in the control of movement by the basal ganglia and in the pathophysiology of many movement disorders. In the rat, subthalamic efferents emanate as collaterals of single output cells, and are distributed predominantly to the globus pallidus, endopeduncular nucleus and the pars reticulata of the substantia nigra (Van der Kooy and Hattori, 1980). These fibres have been considered to be GABAergic (Nauta and Cuenod, 1982). 2-deoxy glucose studies in a variety of experimentally induced dyskinesias (Crossman et al, 1987) and the production of chorea following intrapallidal injection of kynurenate suggest an excitatory nature (Robertson et al., 1989). We have investigated the possibility that subthalamic efferents might utilise an EAA rather than GABA as transmitter by assaying the uptake of [^{3}H]-D-aspartate and [^{14}C]-GABA using a dual labelling technique. We investigated these uptake mechanisms in the globus pallidus, substantia nigra and striatum of control rats and following lesion of the right subthalamic nucleus with the excitotoxin quinolinic acid (12 μg).

The regions of interest were dissected out from fresh tissue and washed once in an artificial CSF (0.28 M sucrose, 10 mM D-glucose, 21.6 mM Tris acetate pH 7.2, 1 mM Mg SO_4) and a further three times in NaCl (140 mM)/ Tris Ac buffer (12.5 mM, pH 7.8) to produce a crude synaptosomal preparation. Incubation in 100 nM [^{3}H]-D-aspartate and 1 μM [^{14}C]-GABA for 45 minutes at 30 °C was terminated by filtration. The time course and pharmacological characteristics of the uptake site are consistent with the assay being for an EAA and GABA uptake sites. Differences in the specific uptake of [^{3}H]-D-aspartate between right and left basal ganglia regions following subthalamic lesion with quinolinic acid are shown in the table. In unlesioned rats or in those lesioned in the zona incerta adjacent to the subthalamic nucleus no side to side differences were noted in EAA uptake. In no group of animals was a change in GABA uptake observed.

TABLE Side to side differences in D-aspartate uptake following subthalamic nucleus lesion with quinolinic acid

AREA	SPECIFIC UPTAKE fmol/mg protein/min (SEM) Unlesioned	Lesioned	% DIFFERENCE (unlesioned - lesioned)
Striatum	591 (± 36)	551 (± 29)	- 7 %
Globus Pallidus	267 (± 77)	37 (± 19)	-76 %*
Substantia Nigra	619 (± 109)	119 (± 35)	-81 %*

(an * denotes $p < .05$ with a paired t-test)

These results are suggestive of the presence of EAA uptake sites on the terminals of subthalamic efferents to the pallidum and substantia nigra. It is suggested that these data form the basis of the first neurochemical evidence of excitatory subthalamic efferents, and augment the increasing body of data that may alter our appreciation of the role of the subthalamic nucleus in the functioning of the basal ganglia.

Crossman A.R., Sambrook M.A., Mitchell I.J. (1987) In Basal Ganglia II Eds. Carpenter MB, Jayaraman A, pp.377-394
Robertson R.G., Farmery S.H., Sambrook M.A., Crossman A.R. (1989) Brain Res. 476: 317-322
Nauta H.J.W, Cuenod M. (1982) Neuroscience 7: 2725-2734
Van der Kooy D., Hattori T. (1980) Jnl. Comp Neurol 192: 751-768

MICROINJECTIONS OF EXCITATORY AMINO ACIDS CAUSE LONG-TERM KINDLED EPILEPSY INVOLVING NMDA RECEPTORS

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Electrical kindling of the rat amygdala is a widely accepted animal model of temporal lobe epilepsy (Löscher et al, 1986). Selective, competitive NMDA receptor antagonists (e.g. AP5, AP7) block fully kindled limbic seizures produced in this manner (Peterson et al, 1983). In addition, daily focal injection of NMDA antagonists prior to electrical stimulation inhibits the development of the kindling response (Croucher et al, 1988).
Recently, we have demonstrated that repeated, bi-daily microinjections of glutamate (Glu) or aspartate(1.5μmol), or a mixture of these, cause kindling of the rat amygdala in the absence of histological damage (Croucher & Bradford, 1989). These seizures closely resemble those seen after electrical kindling of the amygdala. We now report that this Glu-induced kindling can be prevented by co-injection of AP7 with the agonist. This is achieved in the absence of any overt signs of gross behavioural impairment.

Male Sprague - Dawley rats (260-340g) were implanted with a combined guide cannula and stainless steel bipolar electrode unit into the right basolateral amygdala (for details see Croucher & Bradford, 1989). At least one week after surgery, intra - amygdaloid injections were delivered every second day. Animals were given either Glu, 1.5μmol (n=5); Glu, 1.5μmol plus AP7, 70nmol (Glu/AP7; n=5); or vehicle (phosphate buffer) alone (n=4). Electroencephalographic (EEG) and motor seizure responses (graded stage 0-5) were recorded post-injection for 1 h.

Following initial Glu injections all animals showed clear limbic seizure activity (Stage 1-3 seizures). With subsequent injections this progressively developed into full (Stage 5) limbic seizures. The time - course of development of this kindling response was similar to that observed in a previous study (Croucher & Bradford, 1989). In contrast, after the first Glu/AP7 injection animals showed only weak or no seizure response (Stage 0-1). After a mean of 4.0 $\pm$ 1.5 (mean $\pm$ s.e.m.) injections this eventually developed into Stage 2 focal seizures. However, even after 14 injections no animal showed any sign of generalization of this seizure activity (i.e. Stage 3-5 seizures). Moreover, there was no development of prolonged afterdischarges as seen after Glu administration. No clear EEG or motor responses were observed at any time following repeated (14) vehicle injections.
These results show that NMDA receptors serve an important functional role in the development of Glu-induced kindling. The many similar properties of EAA-induced and electrical kindling suggest that these models of epilepsy may share common neurochemical mechanisms.

Croucher, M.J. et al (1988) Eur. J. Pharmac. 152, 29-38.
Croucher, M.J. & Bradford, H.F. (1989) Brain Res. (in press).
Löscher, W. et al (1986) Exp. Neurol. 93. 211-226.
Peterson, D.W. et al (1983) Brain Res. 275, 169-172.

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THE EFFECT OF A BENZODIAZEPINE, MIDAZOLAM, ON NMDA-MEDIATED EPILEPTIFORM ACTIVITY *IN VITRO* AND *IN VIVO*

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A wide range of compounds have been investigated for their antagonism of excitatory amino acid activity using the in-vitro cortical wedge preparation (Harrison and Simmonds,1985). In addition, the in-vivo administration of the excitatory amino acid receptor agonist N-methyl-D-aspartate induces a typical behavioral pattern often culminating in a form of clonic seizure (Czuczwar et al,1983). We have investigated the water soluble benzodiazepine, midazolam, for both its anticonvulsant activity against NMDLA in-vivo, and its action on both spontaneous activity and exogenously applied NMDA in-vitro, using the cortical wedge model.

Male or female mice (MF-1, Manchester Medical School), all aged between 20 and 30 days were used for both studies. For the in-vitro experiments, brain slices were taken and mounted in a two chamber bath by the method of Harrison and Simmonds,1985. After equilibration in the absence of Mg^{2+}, spontaneous epileptiform activity appeared. The number of bursts were quantified before, during, and after addition of midazolam (100μM for 30 mins). In other experiments, NMDA 10-80μM was applied, in the presence and absence of midazolam (100μM) with 0.02μM TTX present throughout.
For the in-vivo experiments, animals received midazolam 10 or 20mg/kg i.p. followed 15 mins later by NMDLA 250 or 350mg/kg s.c. The animals were then observed for 45 mins and the time to salient behavioral points recorded.

Midazolam had no effects on spontaneous epileptiform activity (n=4). NMDA produced a dose dependent depolarisation of cortical wedges (μM $\log_{10}EC_{50}$=1.36±0.03 ; n=10). In the presence of midazolam (100μM) the μM $\log_{10}EC_{50}$ was reduced to 1.08±0.13 (P=0.021, students t test ;n=6). The maximal response to NMDA (40μM) was enhanced, but not significantly (P=0.067). Complete recovery was achieved on midazolam washout. In-vivo, NMDLA induced hyperventilation 2.25mins (±0.27) after s.c. administration in control animals (n=10) which progressed to a full clonic seizure 10.75mins (±1.33) later. Midazolam 20mg/kg delayed the onset of hyperventilation by 2min (±0.76) and onset of full seizure by 11.2min (±6.01). However, the variability of the final value precluded this change from being statistically significant. Midazolam 10mg/kg did not affect the onset of NMDLA-induced excitation or affect the overall incidence of seizures. Both doses of midazolam produced obvious sedation. 2-Amino-5-phosphonovaleric acid, a NMDA receptor antagonist, inhibited responses to NMDLA in-vitro and in-vivo (100μM and 33mg/kg respectively).

These results suggest that the post-synaptic NMDLA receptor modulation is not important in the anticonvulsant activity of midazolam. The apparent potentiation of NMDLA responses in the presence of midazolam requires further investigation.

JDW is a Frederick Craven Moore scholar.

Harrison, N. & Simmonds, M.A. (1985). Br. J. Pharmac., 84, 381-391.
Czuczwar, S.J., Frey, H-H. & Loscher, W. (1983). Eur. J. Pharmac., 108, 273-280.

DIFFERENTIAL EFFECTS OF BENZODIAZEPINE INVERSE AGONISTS ON ELECTROSHOCK SEIZURE THRESHOLD

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Benzodiazepine-receptor inverse agonists of the β-carboline class e.g. β-CCE and DMCM, lower seizure thresholds to both GABA-antagonists and electroshock (Petersen et al, 1983). Recently, inverse agonists with benzodiazepine structure e.g. Ro 15-4513 (Lister & Nutt, 1988) and Ro 19-4603 (Pieri, 1988) have also been characterised as proconvulsant to chemical convulsants. We have now evaluated the effects of these compounds on seizure threshold induced by electroshock.

Electroshock seizure thresholds were assessed in groups of ten 30-40g male TO mice (Bantin and Kingman) using a constant current generator (0.5s, unipolar, via ear-clip electrodes) by the up and down method and probit analysis (Finney, 1971). Drugs were administered in vehicle (one drop Tween 80/10 ml 0.9% saline) i.p. 15 min before testing using a dose volume of 10 ml/kg.

Table 1 Electroshock seizure thresholds.

Drug (mg/kg i.p.)	CD50(mA)	Drug (mg/kg i.p.)	CD50(mA)
Vehicle	11.1(10.2-12.1)	Vehicle	11.6(10.9-12.3)
Ro 19-4603(0.3)	9.6(9.0-10.3)*	Ro 19-4603(3.0)	9.4(8.9-10.0)*
Ro 19-4603(1.0)	10.1(9.7-10.6)*	Ro 19-4603(3.0) +	
Ro 19-4603(3.0)	9.4(8.8-10.0)*	Ro 15-4513(10.0)	10.0(9.6-10.5)*
Ro 15-4513(0.3)	11.5(10.9-12.2)	Ro 19-4603(3.0) +	
Ro 15-4513(1.0)	11.0(10.1-11.9)	Flumazenil(10.0)	10.8(9.8-11.9)*@
Ro 15-4513(3.0)	11.0(10.1-11.9)		
		Flumazenil(10.0)	11.0(10.3-11.7)
Vehicle	10.3(9.8-10.8)		
Ro 15-4513(10.0)	10.2(9.7-10.7)		

Data expressed as current required to induce tonic seizures in 50% of sample (95% fiducial limits); * P<0.05-v-vehicle; @ P<0.05-v-Ro 19-4603(3.0).

Ro 19-4603 was proconvulsant at each dose, consistent with its action against bicuculline (Lister & Durcan, 1989). This effect was mediated by the benzodiazepine receptor as it was blocked by concurrent administration of flumazenil, a selective antagonist, which was by itself without effect. Surprisingly, Ro 15-4513 had no activity in either direction, even at high doses which are very proconvulant against GABA-antagonists (Lister & Nutt, 1988). Furthermore, it was not an antagonist since 10 mg/kg Ro 15-4513 (5 min pre-testing), did not modify the proconvulsant action of 3 mg/kg Ro 19-4603. These unexpected findings may indicate involvement of different subtypes of benzodiazepine receptor in chemical- and electroshock-seizure models.

We thank Roche (Basel) for Ro 19-4603, Ro 15-4513 and flumazenil.

Finney, D.J. (1971) Probit Analysis, Cambridge University Press.
Lister, R.G. & Durcan, M.J. (1989) Brain Res. 482,141-144.
Lister, R.G. & Nutt, D.J. (1988) Br.J.Pharmac. 93,210-214.
Petersen, E.N. et al (1983) in Advances in Biochemical Psychopharmacol. Vol 38 Eds Biggio G. & Costa E. Raven Press pp 57-64.
Pieri, L. (1988) Br.J.Pharmac. 95,477P.

BEHAVIOURAL EFFECTS OF IDAZOXAN GIVEN CONTINUOUSLY BY OSMOTIC MINIPUMP IN THE RAT

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Acute treatment with idazoxan, a selective α2-adrenoreceptor antagonist, in the rat, enhances noradrenaline (NA) turnover and release in the cerebral cortex (Walter et al. 1984) by an action at presynaptic α2-receptors located on NA-neurones in terminal regions (Dennis et al. 1987). Furthermore, in fully habituated animals, idazoxan leads to slight behavioural activation (Dickinson et al. 1987). Due to species differences in plasma clearance of idazoxan (rat is 10 times greater than human, Muir, unpublished observations), it is uncertain whether the long term effects of idazoxan can be investigated using sub-acute methods in rodents. The present studies have therefore investigated the effects of idazoxan, continuously administered by osmotic minipump, on behaviour, body weight and food consumption over a ten day period in the rat.

Male Sprague-Dawley rats (Olac) 230-320g were singly housed in circular perspex activity monitors on a 12hr:12hr light:dark cycle (lights on 0600), temp. 20±2°C with free access to food and water. They were allowed to acclimatise for 7 days. During this period, locomotion was continuously recorded using infra-red detectors (2 per cage) between the hours of 1400 and 1000 the following day. At 1030 each day measures of body weight, faecal output (number of pellets) and food consumption (g) were made. On the 8th. day, rats were implanted subcutaneously, under halothane anaesthesia, with 'primed' osmotic minipumps (Alzet, mean output, 5.38 μl/hr containing either idazoxan HCL or sterile saline, pH 4.5), allowed to recover from surgery, and returned to their respective cages. Recordings continued for a further 10 days. The rate of idazoxan infusion was approx. 0.84 mg/kg/hr. In a separate group of identically treated animals, the ability of the α2-agonist guanoxabenz to induce mydriasis under pentobarbitone anaesthesia (Berridge et al. 1983) was determined 12 & 24 hr. after removal of idazoxan.

Before treatment, rats displayed regular patterns of circadian activity together with consistent gains of body weight and food consumption. Post surgery, all animals showed short-lasting but significant reductions of body weight, food consumption and faecal output. However, the reduction of body weight in rats given idazoxan (steady state plasma level 67.2±6.4 ng/ml, brain level 952±78 ng/ml, n=5, not detectable in vehicle treated, n=4) was significantly greater on the 1st day following surgery (vehicle, -1.1±1.2g, idazoxan, -11.8±2.0g, $P<0.01$ ACOVAR). All surgical disturbances returned to pre-implantation baselines by the second (idazoxan treated rats) or third day (vehicle treated). Idazoxan significantly increased locomotor activity during the first 4 hrs. of infusion on the 1st day only (end of light phase, vehicle 127±28 counts, idazoxan 389±69, $P<0.02$ ACOVAR). There was some evidence that idazoxan also enhanced activity during the early dark phase of the circadian cycle for several nights ($P<0.01$ ACOVAR). There was no evidence that idazoxan altered the phase timing of circadian activity cycles. Removal of the minipumps and clearance of idazoxan for 24hr (but not 12hr) led to a leftward shift of the guanoxabenz dose response curve (agonist ED50, vehicle 66.4±2.7 μg/kg,iv; idazoxan 42.6±4.7, n=5, $P<0.01$, t-test) possibly indicating idazoxan-induced supersensitivity of CNS α2-adrenoreceptors.

The results indicate that idazoxan induced moderate behavioural activation for several hours following commencement of infusion, possibly during a time of dynamically changing NA function. After this initial effect, behaviour returned to and remained largely normal for up to 10 days during which the drug was well tolerated. Some evidence of α2-adrenoreceptor supersensitivity was also obtained.

Berridge,T.L. et al. (1983) Br.J.Pharmac. 78, 507-515
Dennis,T. et al. (1987) J.Pharmac.Exp.Ther. 241, 642-649
Dickinson,S.L.,Gadie,B., & Tulloch,I.F. (1987) Br.J.Pharmac. 91, 415P
Walter,D.S. et al (1984) Biochem. Pharmac. 33, 2553-2557

FURTHER INVESTIGATION OF THE EFFECTS OF HISTAMINE H1 ANTAGONISTS ON NOCICEPTION IN THE MOUSE

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Histamine H1 antagonists have been reported to be clinically effective analgesics and to potentiate the anti-nociceptive actions of opioids in some animal models (Dews & Graham, 1946; Bluhm et al, 1982). Initial studies suggested that these effects are mediated centrally rather than peripherally (Freeman & Sturman,1989) and the present study investigates this further.

Groups of ten female albino mice (Triangle, Devon) were pre-treated with anti-histamine or vehicle and tested for response to nociceptive stimuli before and after morphine injection as previously described (Freeman & Sturman, 1989).
In these experiments diphenhydramine alone demonstrated short-lived dose-related anti-nociceptive activity and potentiated the effects of morphine in the tail pinch but not the hot-plate test (Table 1).
In contrast, acrivastine, a non cns-penetrating H1 anti-histamine (Cohen et al, 1985), had no anti-nociceptive activity, and antagonised the activity of morphine in the tail pinch and hot-plate tests.

Table

		Median Latency (S)			
Pretreatment	Morphine SO_4	Tail Pinch		Hot Plate	
($mg.kg^{-1}$ s.c.)	($mg.kg.^{-1}$ s.c.)	30min.	90min.	30min.	90min.
saline	5	4.5	2	12	6
diphen (15)	5	6	3	7	5
diphen (30)	5	25*	12.5	9	9
saline	10	30	6.5	20	16
diphen (15)	10	30	24	17.5	16
diphen (30)	10	30	30*	20	18
saline	5	15.5	1.5	11.75	6.75
acrivastine (1)	5	3.5	1.75	6.00**	5.0*
acrivastine (10)	5	5.5	1.25	8.75	4.5*
saline	10	30.0	24.5	13.5	10
acrivastine (1)	10	5.5**	2.5*	10	8.25
acrivastine (10)	10	4.25**	2 **	10.25	8

significantly different from control: *$p < 0.05$, **$p < 0.01$

These results suggest that diphenhydramine-induced reduction in central H1 histaminergic function can both induce anti-nociception and potentiate opioid-induced analgesia as previously shown for mepyramine (Freeman & Sturman, 1989). However, results with acrivastine suggest that blockade of peripheral H1 receptors may inhibit some opioid-mediated anti-nociceptive effects.

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Bluhm, R., Zsigmond, E.K., & Winnie, A.P.(1982) Life Sci. 31, 1229-1232.
Cohen, A.F., Hamilton, M.J., Philipson, R. & Peck, A.K.(1985) Clin.Pharmac.Ther., 38, 381-386.
Dews, P.B. & Graham, J.D. (1946) Br.J.Pharmac. 1, 278-286.
Freeman, P. & Sturman, G. (1989) Br.J.Pharmac. (in press).

DPDPE, THE STANDARD DELTA OPIOID AGONIST, INTERACTS WITH MU RECEPTORS *IN VIVO*

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The widespread use of [D-Pen^2, D-Pen^5]enkephalin (DPDPE) as a selective delta agonist seems justified, at least in vitro, based on both bioassay and receptor binding data (Mosberg et al., 1983). It is often assumed that DPDPE acts in vivo as a selective delta agonist. We question the generality of this assumption. Specifically, we report that moderate doses of DPDPE induce a classic morphine-like effect in mice - stiffening of the tail musculature - which suggests the involvement of mu, rather than delta, opioid receptors.

Male ICR albino mice (20-25 g) were adapted to the laboratory overnight, and to their individual Plexiglas chambers for 2 h before experimentation. The animals were anesthetized lightly with ether then given DPDPE (Peninsula; 3-30 µg), morphine SO_4 (Mallinckrodt; 10 µg) or distilled water (5 µl) by i.c.v. injection (Murray et al., 1988). The mice were observed (in pairs) for five 2-min periods: 5-7, 11-13, 20-22, 30-32 and 40-42 min post-injection. The observer was unaware of the test agent administered. During each 2 min period, the mouse under study was assigned a tail position score of 0, 1, 2 or 3, with the top score being given when the tail was either 90° from the horizontal or "S" shaped. The DPDPE-induced stiffening effect on the mouse tail is shown in Figure 1. DPDPE also increased spontaneous locomotion and stereotyped pacing in circles.

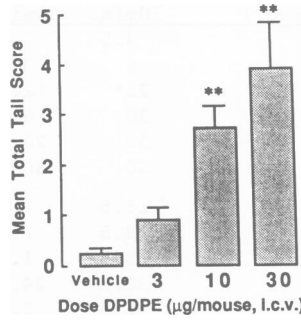


Figure 1. Effect of DPDPE on the mouse tail (n=8-17; error bar = s.e. mean; **P<0.01, compared to vehicle group by Dunnett's test). A score of 5.2 ± 1.5 was obtained with 10 µg of morphine.

More mice (n=8) were pretreated s.c. (at -5 min) with vehicle, naloxone (0.5 mg/kg) or the delta antagonist ICI 174864 (2 mg/kg) before challenge with 10 µg of DPDPE. Only naloxone antagonized DPDPE-induced tail stiffening. Additionally, pretreatment with beta-FNA (10 µg i.c.v. at -4 h), the mu-selective antagonist, attenuated DPDPE-induced tail stiffening and locomotion.

In this study, doses of DPDPE have been identified which probably interact with mu receptors in the mouse and for which claims of delta selectivity need to be justified.

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Mosberg, H.I. et al (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 5871-5874
Murray, C.W. et al (1988) J. Pharmacol. Meth. 20, 175-186

EFFECTS OF INTRACEREBROVENTRICULAR CALCITONIN ON GASTRIC ACID SECRETION IN RATS

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Many of the brain-gut peptides are thought to play intermediary roles as neurotransmitters or neuromodulators in the regulation of gut function. Calcitonin is a peptide composed of 32 amino acid residues whose principal peripheral action is regulation of plasma calcium and phosphate concentrations. It has been reported that intracerebroventricular (icv) administration of calcitonin has little effect on plasma calcium concentrations but causes inhibition of gastric acid secretion (Morley et al., 1981; Morimoto et al., 1985). However the neurohumoral pathways mediating this antisecretory effect are not fully elucidated (Tache, 1987).

In the present study the central effects of calcitonin on gastric acid secretion and stress ulceration were investigated in rats. Sprague-Dawley rats of either sex were anaesthetized with urethane. The stomach was perfused with unbuffered 0.9% physiological saline and acid secretion measured by titration of the perfusate with 0.05N NaOH. Gastric perfusion was done 5 days after icv cannulation. The volume of icv injection was 10 µl.

ICV calcitonin (0.001-0.1 IU) suppressed the basal gastric acid secretion up to 66.8% in a dose dependent manner: it was 250 times more potent when given icv than when given iv. Gastric acid secretion induced by histamine (1.8 mg/kg/h), bethanechol (150 µg/kg/h) or pentagastrin (10 µg/kg/h) was not suppressed by icv calcitonin (0.1 IU). However gastric acid secretion induced by icv TRH (5 µg) was markedly inhibited by icv calcitonin (0.1 IU). The effects of calcitonin were influenced little either by bilateral vagotomy or by metyrosine (80 mg/kg i.p. every 3h for 24h) pretreatment. Stress ulcers (evaluated by severity x depth x number) induced by cold restraint and icv TRH (5 µg) administration were diminished by icv calcitonin (1.5 IU).

Based on these results it is clear that calcitonin can suppress gastric acid secretion by a central mechanism. In particular, suppression of icv TRH-induced gastric acid secretion suggests that calcitonin may play a role in the central nervous system as an inhibitory neuromodulator of gastric acid secretion.

Morimoto, T., Ikamoto, M., Koida, M., Nakamuta, H., Stahl, G.L. & Orlowski, R.C. (1985) Jpn.J.Pharmacol. 38:21-29.
Morley, J.E., Levine, A.S. & Silvis, S.E. (1981) Science 214:671-673.
Tache, Y. (1987) in 'Physiology of the gastrointestinal tract': Johnson, L.R., Christensen, J., Jackson, M., Jacobson, E.D. & Walsh, J.H. (ed.), 2nd ed. pp911-930. New York, Raven.

CENTRAL EFFECTS OF PGE_2 AND PGF_{2A} IN LEAN AND GENETICALLY OBESE RATS AND MICE

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Interleukin-1ß is an endogenous cytokine with potent pyrogenic actions. In normal rodents, 1L-1ß increases body temperature, metabolic rate and brown adipose tissue (BAT) thermogenesis by a central action which is dependent on eicosanoids and corticotrophin releasing factor (CRF) (Dascombe et al., 1989a; Rothwell, 1989). Genetically obese rats and mice show impaired thermogenic responses to central injections of IL-1ß (Carnie et al., 1989; Dascombe et al., 1989b). In the present study we have therefore tested the effects of two prostaglandins which have been implicated in the development of fever, on BAT thermogenesis in obese mutants.

Genetically obese Zucker rats (fa/fa), obese (ob/ob) mice and their lean littermates (+/?) were stereotaxically implanted with guide cannulae in the third ventricle of the brain under halothane anaesthesia. The effects of icv injections of sterile saline (0.9%), CRF (2µg), PGE_2 or $PGF_{2\alpha}$ (0.1µg) were tested on brown fat activity and oxygen consumption (VO_2). Colonic temperature was measured by insertion of a plastic coated thermocouple. VO_2 was assessed by indirect calorimetry and BAT activity was estimated from purine nucleotide binding to mitochondria isolated from the interscapular BAT depot of animals killed 60 min after injections.

Injections of CRF elicited significant ($P<0.01$, n=6-8) increases in GDP binding in lean (35±4%, mean ±SEM) and obese mice (50±10%), and in lean (71±6%) and obese rats (50±8%). Central injections of PGE_2 also produced significant increases in BAT activity which were greater in lean (74±4%) than obese (31±7%) animals. $PGF_{2\alpha}$ stimulated BAT activity by (52±8% , $P<0.01$) in lean animals, but produced only a 23±5% (non-significant) increase in the obese mutants. Increases in colonic temperature were recorded in mice in response to prostaglandins. (Temperature increase ^{o}C; PGE_2 lean 1.8, obese 0.9; $PGF_{2\alpha}$ lean 1.1, obese 1.3). In vivo oxygen consumption responses to PGE_2 and $PGF_{2\alpha}$ were attenuated in obese compared to lean rats (% increase in VO_2:PGE_2 lean 26±4, obese 13±3; $PGF_{2\alpha}$: lean 27±2, obese 17±2).

These data demonstrate that the obese animals do exhibit thermogenic responses to PGE_2 and $PGF_{2\alpha}$, but the responses are reduced compared to their lean littermates and thus may contribute to the impaired responses to interleukin-1.

Carnie, J.A., Johnston, J.A., Busbridge, N., Dascombe, M.J. & Rothwell, N.J. (1989). Proc. Nutr. Soc. In press.

Dascombe, M.J., Rothwell, N.J., Sagay, B.O. & Stock, M.J. (1989a). Am. J. Physiol. 256, E7-E11.

Dascombe, M.J., Hardwick, A.J., LeFeuvre, R.A. & Rothwell, N.J. (1989b). Int. J. Obesity. In press.

Rothwell, N.J. (1989). Am. J. Physiol. 256, E111-E155.

EFFECTS OF THE CHOLECYSTOKININ RECEPTOR ANTAGONIST MK-329 AND (+)-FENFLURAMINE ON GASTRIC EMPTYING IN THE RAT

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We reported that (±)-fenfluramine decreased the rate of gastric emptying in freely fed rats, and showed that its effect could be antagonized by xylamidine, a peripherally acting, 5-HT receptor antagonist (Baker et al., 1988). By this means, fenfluramine may prolong the satiating effect of absorption from the gut, and hence contribute to a reduction in food consumption. Endogenous cholecystokinin (CCK) may reduce food intake in part by reducing gastric emptying rate as a result of action on peripheral CCK receptors (Moran et al., 1988, Smith et al., 1988). Recently, a potent and selective CCK receptor antagonist MK-329 (3S(-)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1-H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide) has been described (Chang & Lotti, 1986), and which provides a means to investigate whether endogenous CCK influences gastric emptying and feeding. Here, we report experiments which indicate a possible interaction between the effects of MK-329 and those of (+)-fenfluramine.

Individually housed, adult male hooded rats were adapted to a reversed lighting cycle (lights on between 22.30h and 10.30h), with ad lib. access to food and water. On the test day, food was removed for 4h during the first part of the dark period. Each rat was then given 3.0g standard food pellets to eat in a 15-min period. Drugs and their vehicles were administered immediately after the meal, the animals were left without further food for 2h, and then they were killed by pentobarbitone injection. The contents of the stomach were rapidly washed into a centrifuge tube, spun at 2000 rpm for 10 min, and dried to constant weight.

First, an effective dose of (+)-fenfluramine was determined. Three groups of animals (N=9 per group) were injected i.p. with 1.0 $mg.kg^{-1}$ or 3.0 $mg.kg^{-1}$ of (+)-fenfluramine, or vehicle (distilled water), respectively, after the standard meal. In the control condition, the stomach contents dry weight was 0.74 ± 0.14g (mean ± S.E.M.) and this was significantly increased to 1.51 ± 0.18g after the larger dose of (+)-fenfluramine ($p<0.01$). In a second study, MK-329 (3.0-30 $\mu g.kg^{-1}$, s.c., 0.05% methylcellulose vehicle) dose-relatedly reduced stomach contents; at 30 $\mu g.kg^{-1}$ MK-329, they were reduced from a control value of 1.01 ± 0.23g to 0.36 ± 0.10g ($p<0.05$). The final study investigated the effects of 3.0 $mg.kg^{-1}$ (+)-fenfluramine and 100 $\mu g.kg^{-1}$ MK-329, alone and in combination. Dry-weight stomach contents in the control condition were 0.99 ± 0.15g; (+)-fenfluramine increased them to 1.60 ± 0.11g ($p<0.05$), and MK-329 reduced them to 0.44 ± 0.15 ($p<0.05$). In the drug-combination condition, considerable retention occurred and contents were 1.76 ± 0.10g ($p<0.05$). Hence, the effect of MK-329 on gastric emptying was overriden by (+)-fenfluramine.

These results indicate that endogenous CCK retards gastric emptying and hence promotes retention of food in the stomach; that (+)-fenfluramine decreases gastric emptying rate in the rat, as the racemate does; that (+)-fenfluramine counteracts the enhanced gastric emptying produced by MK-329.

MK-329 was donated by Merck, Sharp & Dohme, and (+)-fenfluramine by Servier Laboratories.

Baker, B.J. et al. (1988). Eur. J. Pharmacol., 150, 137-142.
Chang, R.S.L. & Lotti, V.J. (1986). Proc. Natl. Acad. Sci.,USA, 83, 4923-4926.
Moran, T.H. et al. (1988). Am. J. Physiol., 255, R1059-R1063.
Smith, G.P. et al. (1988). Am. J. Physiol., 255, R113-R116.

CEREBRAL 2-DEOXYGLUCOSE UPTAKE IN EXPERIMENTAL TARDIVE DYSKINESIA IN THE *CEBUS* MONKEY

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Long-term treatment of psychiatric illness with neuroleptic drugs, is known to result in the appearance of abnormal movements (tardive dyskinesia) in a number of patients. Little is known about the pathophysiological mechanisms which underlie this condition. Similar dyskinesia can be induced experimentally by long-term exposure of monkeys to neuroleptics. We have investigated the neural mechanisms that mediate experimental tardive dyskinesia in the monkey using the 2-deoxyglucose (2-DG) metabolic mapping technique. This technique permits the autoradiographic localisation and quantification of local cerebral 2-DG uptake which can be used as an index of synaptic activity (Schwartz et al., 1979)

Two Cebus monkeys received chronic neuroleptic treatment (3 monthly depot injections of fluphenazine decanoate, 4-10 mg/kg) for seven years. This treatment regime resulted in the appearance of tardive dyskinesia in one animal whilst the other monkey showed no motor abnormalities. The 2-DG procedure was performed after the drug administration had been discontinued for 3 months. At this time the abnormal movements in the dyskinetic animal were maximal. 5 mCi of tritiated 2-DG (N.E.N.) was administered intravenously following an overnight fast. The animals were left unrestrained for a 45 minute period and then killed by i.v. barbiturate overdose. Autoradiographs were prepared from the brains as described in Mitchell et al., 1985. The data are expressed in terms of regional optical density ratios (Mitchell and Crossman 1984).

Comparison of the optical density ratios from the dyskinetic and non-dyskinetic animals revealed a reduction in 2-DG uptake in the medial segment of the globus pallidus of the dyskinetic animal, compared with the equivalent structure in the non-dyskinetic animal (11.3%). Reductions in 2-DG uptake were also seen in the lateral pallidal segment (4.5%) and the ventral anterior/ventral lateral thalamus (8.4%), but increased uptake was observed in the subthalamic nucleus (11.9%). On the basis of evidence that changes in 2-DG uptake reflect changes in nerve terminal activity, the data suggest that experimental tardive dyskinesia is characterised by decreased activity in the subthalamopallidal and pallidothalamic pathways and increased activity in the pallidosubthalamic pathway. We have previously reported that both experimentally-induced chorea and ballism are characterised by a similar neural mechanism (Mitchell et al., 1985). This implies that all three forms of dyskinesia may be mediated by a common pathophysiological mechanism.

Supported by the Medical Research Council.

Schwartz W.J. et al., (1979) Science, 205, 723-725.
Mitchell I.J. et al., (1985) Brain Res., 339, 346-350.
Mitchell I.J. and Crossman A.R. (1984) Brain Res., 298, 191-192.

SINGLE CHANNEL PATCH-CLAMP STUDIES OF THE APICAL MEMBRANE IN INTACT EPITHELIUM (CHOROID PLEXUS AND PLACENTA)

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Fluid and electrolyte secretion by epithelia are largely governed by changes in the anion conductance of the apical membrane of the tissue (Case et al, 1989). In many epithelia, access to this membrane is obstructed by mucus in an inhospitable environment (e.g. intestine and trachea), or by the small size of the apical compartment (e.g. kidney and exocrine glands). These anatomical features make patch-clamping difficult without resort to enzymatic treatments or use of cells in tissue culture. However, a few epithelia interface with a large and hospitable environment, notably choroid plexus (cerebrospinal fluid in the ventricles) and ciliary body (aqueous fluid in the eye). It is possible to make patch-clamp recordings directly from these epithelia.



Figure 1. Records from a channel in an inside-out patch excised from choroid plexus. The patch was held at a membrane potential = -10 mV with a k^+ rich electrode solution and a Na^+ rich bath with 1mM $CaCl_2$.

Seal resistances of greater than 20 Gohms can be obtained on intact choroid plexus tissue without any use of enzymes. Figure 1 shows the activity of a single calcium-activated potassium channel in the apical membrane of tissue from the third ventricle of the frog (*Rana temporaria*). The channel was highly selective to potassium over sodium and increasingly active at depolarising membrane potentials. These channels have a conductance of 218 ± 12 pS (n=3, mean ± S.E.M.) and are similar to channels observed in *Necturus maculosa* (Brown et al, 1988).

This technique is now being applied to record single channel activity in rat choroid plexus and human placental tissue. The pharmacological modification of ion transport across these barriers may be studied in this way.

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Brown, P.D., Loo, D.D.F. & Wright, E.M. (1988). J. Membrane Biol. 105, 207-219.
Case, R.M., Lau, K.R., Steward M.C., Brown, P.D. & Elliott, A.C. (1989). Biochem. Soc. Trans. (in press).

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A COMPUTER PROGRAM TO TEACH THE PRINCIPLES OF THE LABORATORY DETERMINATION OF RED BLOOD CELL INDICES

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Computer-assisted learning (CAL) programs are increasingly being used in teaching and may be particularly useful in augmenting or in some cases even replacing certain practical classes in physiology and pharmacology. For some groups of students computer simulations of practical classes which use animals or require expensive equipment can be a realistic and often more economic alternative. Recently a number of practical classes which depend on using human blood have been abandoned due to the risk of viral cross-infection. Here we describe an interactive computer simulation of the laboratory determination of the common physiological indices of red blood cells: red cell count, packed cell volume and haemoglobin concentration.

The program runs on the BBC microcomputer and is suitable for students on a range of biology or health-related courses which feature introductory physiology. It is written in a combination of BASIC and assembly language, is menu driven, and makes extensive use of colour and high-resolution graphics.

The principles of each method are explained simply using a combination of text and imaginative grapics. Students then select from a menu to determine the haematological profile of one of the following subjects: normal male, normal female, an anaemic subject or an unknown. For each subject students determine the red cell count by counting cells on a simulated haemocytometer grid on the monitor, the packed cell volume using a simulated haematocrit reader and the haemoglobin concentration using a simulated spectrophotometer and constructing a standard curve with haemoglobin standards. The program then calculates the mean cell volume and mean cell haemoglobin and displays a haematological profile of the chosen subject which may be output to an Epson-compatible printer.

THE USE OF AN INTERACTIVE STATISTICS PACKAGE IN TEACHING DATA HANDLING AND ANALYSIS IN PHARMACOLOGY

Patricia Freeman & Gillian Sturman, Division of Physiology & Pharmacology, Department of Medical Sciences & Health Studies, Polytechnic of East London, Romford Road, London E15 4LZ.

Although fundamental to research methodology, the teaching of statistics in biomedical courses is often relegated to a minor role. As a consequence,students fail to appreciate how application of statistical techniques can aid the interpretation of data they obtain in the laboratory. To overcome the reluctance of the less-numerate to tackle exploratory data analysis we have introduced the use of a user-friendly interactive statistics package (Minitab, Minitab.Inc.) which facilitates data manipulation. We have on file a number of data sets obtained as a result of research within the department but, wherever possible, use data generated in class practicals. Using the computer to reduce time spent on calculations and to improve the accuracy of exploratory data plots has made it possible to increase the time available for detailed discussion of the application of statistical techniques to the interpretation of pharmacological data. This approach has proved successful with both post-graduate and undergraduate students.

The demonstration will include examples of workshops based on small animal data, derived both from research and from class practicals (Webb & Jakobson, 1980).

Webb,G.P. & Jakobson,M.E. (1980). J.Biol. Ed., 14, 318-324.

A SIMPLE, INTERACTIVE PROGRAM FOR DEMONSTRATING THE METHOD OF RESIDUALS

Robin Whelpton, Department of Pharmacology, London Hospital Medical College, Turner Street, London E1 2AD.

A series of "on-screen" curve fitting programs for solving unknown pharmacokinetic data has been demonstrated (Whelpton, 1988). These programs started life as short routines to teach students the method of residuals (Riggs, 1970). The routines have been combined into a single, menu driven, program that shows how to apply the graphical method.

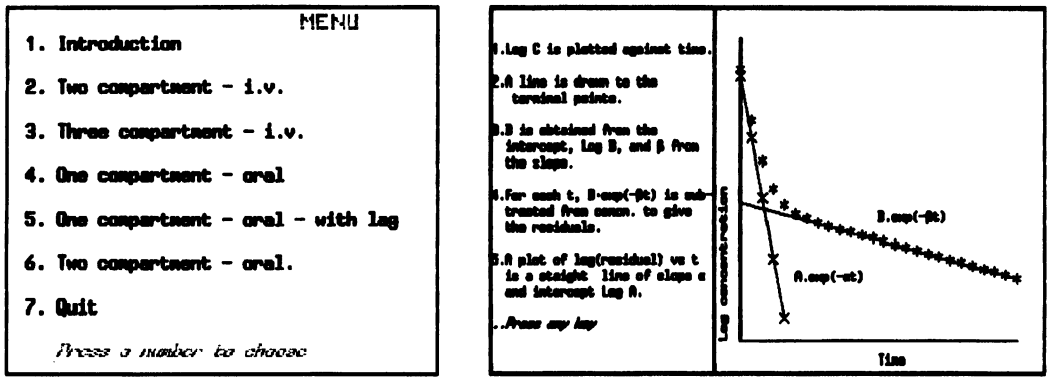


Figure 1.Menu screen (left) & one of the Introduction screens (right)

Using the elimination of drug from a two compartment model as an example the program works as follows. One window is used as a text screen to give simple instructions and make comments. The other, a graphic screen, is used as a piece of graph paper to plot model data (log C versus t). Using the mouse, students can draw a construction line to the terminal data points. From this line, which represents B.exp(-ßt), the computer calculates and plots the residuals. From the shape of the residual line the student can see how successful they have been in correctly positioning the construction line. There is the facility to plot the residuals while the construction line is being drawn and the student can position the line to obtain a straight line plot of log(residual) against time. Incorrect positioning produces a curved residual plot.

The program is written in Locomotive Basic2 for IBM PC's and compatibles. Because of the screen handling facilities and simple mouse commands available with this interpreter the task was relatively simple. The program will run with CGA, EGA or Hercules adaptors.

Riggs R. (1970) "The Mathematical Approach to Physiological Problems" MIT Press, Cambridge, Massechusetts
Whelpton R. (1988) Br. J. Pharmacol. 95 907P

CHOREA IN HUNTINGTON'S DISEASE: RELATIONSHIP TO PALLIDAL GABA DEFICITS IN POST-MORTEM BRAIN TISSUE

S.J. Pearson & G.P. Reynolds, Department of Pathology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

TITLE ONLY

944P

EARLY LIFE UNDERNUTRITION CHANGES THE SUSCEPTIBILITY OF RATS TO AVERSIVE SITUATIONS AND TO ANXIOLYTIC DRUGS

Graeff FG, Almeida SS & de Oliveira LM (introduced by JFW Deakin). Laboratory of Psychology FFCLRP-University of Sao Paulo, Campus USP, 14049 Ribeirao Preto, SP, Brazil.

TITLE ONLY

MEASUREMENTS OF INTRACELLULAR pH IN MAMMALIAN EXOCRINE CELLS BY DUAL WAVELENGTH FLUORESCENCE MICROSCOPY

A.C.Elliott, K.R. Lau & P.D. Brown. Department of Physiological Sciences, University of Manchester. Manchester M13 9PT

946P

COMPUTER SIMULATIONS OF *IN VIVO* AND *IN VITRO* PREPARATIONS: AN AID TO TEACHING PRACTICAL AND THEORETICAL ASPECTS OF PHARMACOLOGY

I.E. Hughes, Department of Pharmacology, Worsley Medical & Dental Building, The University of Leeds, Leeds. LS2 9JT

COMPUTER-ASSISTED LEARNING PROGRAMS IN PHARMACOKINETICS

R.W. Foster, [1]L. Aarons, M, Hollingsworth and [2]C.H. Morgan, Department of Physiological Sciences and [1]Pharmacy and [2]Staff Teaching Workshop, University of Manchester, Oxford Road, Manchester M13 9PT

948P

STRUGGLES WITH STATISTICS: A TEACHING PROGRAM FOR LARGE OR SMALL GROUPS OF STUDENTS

R.B. Barlow, Department of Pharmacology, University of Bristol, Medical School, University Walk, Bristol BS8 1TD.

COMPUTER SIMULATIONS OF UNDERGRADUATE PHYSIOLOGY PRACTICAL CLASSES

Brown G.J.,Dewhurst D.G.,Johnstone S. & Meehan A.S. Department of Biomedical Sciences, Sheffield City Polytechnic, Sheffield S1 1WB.

950P

COMPUTER SIMULATIONS OF UNDERGRADUATE PHARMACOLOGY PRACTICAL CLASSES

Brown G.J.,Dewhurst D.G.,Howells C.M. & Meehan A.S. Department of Biomedical Sciences, Sheffield City Polytechnic, Sheffield S11WB.

COMPUTER-ASSISTED INSTRUCTION IN PHARMACODYNAMICS

H. van Wilgenburg, Department of Pharmacology, University of Amsterdam, Academic Medical Center, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

952P

COMPUTER SIMULATIONS OF ORGAN BATH EXPERIMENTS

R.G. Stevens and M.A. Shaw, Welsh School of Pharmacy, University of Wales College of Cardiff, Cardiff CF1 3XF

GETTING THE MOST OUT OF PRACTICALS: COMPUTER SIMULATION IN THE TEACHING OF QUANTITATIVE PHARMACOLOGY

Keighley AC, Noras JM* and Wood D**, Departments of Computing, *Electrical Engineering and ** Pharmacology, University of Bradford, Bradford, West Yorkshire, BD7 1DP

COMMUNICATIONS

The author who intended to present the work is indicated by an asterisk (*) on the abstract.

606P **COLLINS P*, BROEKKAMP CLE, JENNER P & MARSDEN CD**
Both D_1 and D_2 receptors appear to be involved in the induction of purposeless chewing in rats

607P **SCHAPIRA AHV*, COOPER JM, DEXTER D, JENNER P, CLARK JB & MARSDEN CD**
Substantia nigra complex I deficiency in Parkinson's disease

608P **DOURISH CT, WILLIAMS AR*, NARUSE T, MIYAJI M & IVERSEN SD**
A comparison of locomotor stimulation in 6-hydroxydopamine-lesioned mice induced by L-Dopa and the L-Dopa prodrug NB-355

609P **RIVEST R*, JOLICOEUR FB, CRESPI F & MARSDEN CA**
Neurotensin reverses the effects of amphetamine on *in vivo* dopamine metabolism in the accumbens but not the striatum

610P **BOULTER N*, SERRAO JM, GOODCHILD CS & GENT JP**
Chlordiazepoxide causes spinally mediated analgesia in the rat

611P **WHITWORTH P* & KENDALL DA**
Lithium enhances the inhibitory effects of receptor agonists on inositol phospholipid hydrolysis in brain slices

612P **TREHERNE JM, STERN JS, FLACK WJ & YOUNG JM***
Na^+ ions distinguish between the binding of a tertiary and a quaternary [^{3}H]-antagonist to the histamine H_1 receptor

613P **BONSER JC*, THOMAS KL, GENT JP & DEAN HG**
The relevance of the distribution and metabolism of octanoate to its anticonvulsant activity in the mouse

614P **BADAWY AA-B*, MORGAN CJ & GORDON CA**
Effects of carbamezepine on tryptophan metabolism and disposition in the rat

615P **ADAMSON P*, BRAMMER MJ, CAMPBELL IC & MELDRUM BS**
Increases in intrasynaptosomal free $[Ca^{2+}]_i$ elicited by glutamate are mediated via a quisqualate-type receptor

616P **STEELE JE*, ROBINSON TN, CROSS AJ, BOWEN DM & GREEN AR**
[^{3}H]-MK-801 labels more NMDA receptor sites than [^{3}H]-TCP in human cortical membranes

617P **GIBSON A* & MIRZAZADEH S**
Does an endogenous nitrovasodilator mediate NANC relaxations of the mouse anococcygeus?

618P **MALLARD NJ*, MARSHALL RW & SPRIGGS TLB**
Suramin, a selective inhibitor of NANC transmission in rat vas deferens: comparison with nifedipine

619P **HENRION D, CHILLON JM, CAPDEVILLE C & ATKINSON J***
Vascular endothelium relaxant properties in a rat model of calcium overload

620P **WALDER CE*, THOMAS GR & VANE JR**
The effects of acetylcholine, bradykinin, sodium nitroprusside and AP-II on renal cortical blood flow in the rat

621P **PARKINGTON HC*, TARE M, COLEMAN HA, NEILD TO & DUSTING GJ**
Endothelium-derived nitric oxide is responsible for hyperpolarization and relaxation in arterial smooth muscle

622P **PLANE F*, BRUCKDORFER KR & JACOBS M**
The inhibition of endothelium-derived nitric oxide (EDNO) by native and oxidatively modified low-density lipoproteins

623P **GARDINER SM*, COMPTON AM, BENNETT T, PALMER RMJ & MONCADA S**
Haemodynamic effects of N^G-monomethyl-L-arginine (L-NMMA) in conscious Long Evans rats

624P **GARDINER SM, COMPTON AM* & BENNETT T**
Haemodynamic effects of endothelin-1 and endothelin-3 in conscious Long Evans rats

625P **DUSTING GJ*, DUBBIN P, KUDO K, MACDONALD PS & MAKRIDES M**
Endothelin potentiates vasoconstriction and antagonises vasodilatation selectively in the presence of a thromboxane analogue

626P **GARDINER SM, COMPTON AM, BENNETT T*, PALMER RMJ & MONCADA S**
The effect of N^G-monomethyl-L-arginine (L-NMMA) on the haemodynamic actions of endothelin-1 in conscious Long Evans rats

627P **FUNG AYP, WARNER TD*, THOMAS GR & VANE JR**
Endothelin-1 modulates the inotropic effects of isoprenaline in rabbit isolated papillary muscle

628P **GUC MO*, FURMAN BL, STOCLET JC & PARRATT JR**
Endotoxin depresses vascular reactivity to phenylephrine, clonidine and Bay K 8644 but not to endothelin

629P **HILL DR* & WOODRUFF GN**
Differentiation of central CCK receptor binding sites using the non-peptide antagonists MK-329 and L-365,260

630P **KEMP JA*, MARSHALL GR & WOODRUFF GN**
Antagonism of CCK-induced excitation of rat ventromedial hypothalamic neurones by a new selective CCK-B receptor antagonist

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Enhancement of morphine analgesia and prevention of morphine tolerance by the selective CCK-B antagonist L-365,260

632P **EBENEZER IS*, DE LA RIVA C & BALDWIN BA** (introduced by **S. ARKLE**)
Intravenous administration of the cholecystokinin (CCK) antagonist MK-329 increases operant responding for food in pigs

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The cholecystokinin receptor antagonist MK 329 blocks the anorectic effect of (+)-fenfluramine in the rat

634P **RODGERS RJ* & SHEPHERD JK**
Anxiolytic 5-HT_3 antagonist, GR38032F, potently blocks the analgesic consequences of social defeat in male mice

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Evidence that the amygdala and not the area postrema is involved in the putative anxiolytic effects of 5-HT_3 receptor antagonists

636P **COSTALL B, COUGHLAN J*, KELLY ME, NAYLOR RJ & TYERS MB**
Scopolamine-induced deficits in a T-maze reinforced alternation task are attenuated by 5-HT_3 receptor antagonists

637P **COSTALL B, DOMENEY AM*, GERRARD PA, JONES DNC, NAYLOR RJ & TYERS MB**
Effects of GR38032F on performance of common marmosets (Callithrix Jacchus) in an object discrimination reversal task

638P **GARRATT JC* & MARSDEN CA**
Effects of the 5-HT_2 agonist DOI on DRN 5-HT neuronal firing and intake of food in freely feeding rats

639P **SHEPHERD JK* & RODGERS RJ**
Evidence to suggest that 8-OH-DPAT, but not GR38032F, specifically enhances feeding behaviour in mice

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Failure of ketanserin to selectively block the excitatory action of 5-HT on rat sympathetic preganglionic neurones

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The effects of central administration of 8-OH-DPAT and GR43175 on autonomic outflow in the anaesthetised cat

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Mechanism of 8-OH-DPAT-mediated inhibition of plasma insulin in spontaneously hypertensive rats

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Investigation of the *in vivo* cardiovascular effects of ICI 169,369 in anaesthetised rats

644P **LUMMIS SCR*, NICOLETTI G, JOHNSTON GAR & HOLAN G**
[^{3}H] diazepam binds to a peripheral-type, GABA-modulated benzodiazepine binding site in *E. Coli*

645P **PANJEHSHAHIN MR*, BOWMER CJ & YATES MS**
A comparison of drug binding sites on human and rat albumins

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Consequences of a steady-state ternary complex model

647P **MACKAY D*, LATHE GH, GRIFFIN I & WORMALD A**
Evidence in favour of the steady-state ternary complex mode

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Potent inhibition of receptor-linked PGI_2 synthesis by NSAIDs in the rat aorta: evidence for effects on calcium mobilisation

649P **BAXTER GS*, CLAYTON JK, COLEMAN RA & SENIOR J**
Prostanoid receptors mediating contraction and relaxation of human isolated uterine artery

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Renal vascular responses to arachidonic acid in experimental diabetes

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A single α_1-adrenoceptor, distinct from the α_{1A} and α_{1B} subtypes, mediates contraction of the rat aorta to phenylephrine

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Heterogeneity of α_1-adrenoceptor binding sites in the rat heart - subtype: selective agonists and antagonists

653P **AUSTIN CE*, BRECKENRIDGE AM & CHESS-WILLIAMS R**
Effect of duration of diabetes on cardiac adrenoceptor-mediated inotropic responses

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(introduced by **MJ LEWIS**)
Smooth muscle proliferation from rabbit aorta is inhibited by 8-Br-cAMP but not 8-Br-cGMP

655P **AKERS I*, COATES J, DREW GM & SULLIVAN AT**
Antagonist profile of SK&F104078 at pre- and post-junctional α_2-adrenoceptors

656P **EL-MAS M* & HUGHES IE**
Differential effect of cocaine on electrically evoked tritium overflow from mouse vas deferens and rat brain cortex slices

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Responses to catecholamines in rat distal colon may be mediated, in part, by 'atypical' β-adrenoceptors.

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Comparative inhibitory effects of FPL 63547 and enalapril on rat tissue converting enzyme

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Effects of K^+-channel openers on bovine tracheal smooth muscle

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Comparison of the effects of gastrin/cholecystokinin-related peptides in rat gastric mucosa and guinea-pig ileum

662P **EGLEN RM*, MONTGOMERY WW, MACHADO CA & WHITING RL**
Interaction of parafluorohexahydro-siladifenidol (pFHHSiD) at muscarinic receptors *in vitro*

663P **ELLIOTT P*, SEEMUNGAL BM & WALLIS DI**
Antagonism by metoclopramide and BRL 43694 of 5-HT-induced responses in rabbit isolated vagus nerve

664P **KAUMANN AJ*, MURRAY KJ, BROWN AM, SANDERS L & BROWN MJ**
A receptor for 5-HT in human atrium

665P **PURCELL WM*, COHEN DL & HANAHOE THP**
Contribution of amine re-uptake to observed differences in histamine and 5-HT secretion in rat mast cells

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The stimulatory effect of 5-HT on lhuman osteoblasts is antagonised by ketanserin tartrate and imipramine

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Heterogeneity of the regulation of phospholipase C in T lymphocytes

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Dissociation of 5-lipoxygenase inhibition and methaemoglobin-aemia induction by indazolinones

669P **JEREMY JY*, MIKHAILIDIS DP, BARRADAS MA, THOMPSON CS & DANDONA P**
Effect of nabumetone and its principal metabolite on rat and human gastric mucosal prostanoid synthesis and platelet function

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The effect of non-steroidal anti-inflammatory drugs on IL-1 secretion from murine macrophages

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The potentiation of human neutrophil superoxide generation by sodium meclofenamate

672P **KEERY RJ*, PIKE NB, WATTS IS & LUMLEY P**
Operation of a Na^+/H^+-exchange mechanism is not a prerequisite for human platelet activation

673P **McKECHNIE K*, KILLINGBACK PG, NAYA I, O'CONNOR SE, SMITH GW, WATTAM DG, WELLS E, WHITEHEAD YM & WILLIAMS GE**
Calcium channel activator properties in a novel non-dihydropyridine - FPL 64176

674P **MERRITT* JE, ARMSTRONG WP, HALLAM TJ, JAXA-CHAMIEC A, LEIGH BK, MOORES KE & RINK TJ**
SK&F 96365, a novel inhibitor of receptor-mediated calcium entry and aggregation in quin2-loaded human platelets

POSTER COMMUNICATIONS

675P **LAWRENCE KM, CRANE JA & HORTON RW**
[^{3}H]-imipramine and [^{3}H]-paroxetine binding to fresh and frozen human platelet membranes

676P **WILSON JI, FEELY M & GENT JP**
Apparent separation of benzodiazepine tolerance and dependence: flumazenil does not abolish the withdrawal effect

677P **LITTLE HJ, ANDREASON M & LAMBERT JDC**
Chronic benzodiazepine agonist treatment increases the actions of the inverse agonist FG7142 on the hippocampal slice

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The central administration of the GABA-B receptor agonist induces feeding in the satiated pig.

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Actions of 5-HT$_{1A}$ ligands and standard anxiolytics on mouse exploratory behaviour in a two-compartment light:dark arena

680P **GARRATT JC, KIDD EJ & MARSDEN CA**
The differential effects of RU24969 under halothane compared to chloral hydrate anaesthesia

681P **BROWN CM, KILPATRICK AT, MACKINNON AC, KENNY BA, BROUDISCOU A, CLAGUE RU, CLARK RD, WEINHARDT KK & SPEDDING M**
RS-30199, an azepine with affinity for the 5-HT$_{1A}$ site

682P **REDFERN WS, CLAGUE RU & SPEDDING M**
Anxiogenic-like properties of RS-30199 in the rat

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Acute effects of centrally-acting drugs on the behaviour of rats in an elevated X-maze and a partially-shaded holeboard

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Which 5-HT receptors are involved in the inhibition of object burying behaviour by 5-HT uptake inhibitors?

685P **PRYKE JG, KEY BJ & MARTIN GR**
The effect of 5-HT on rat pial arterioles *in vivo*.

686P **KING BH, BRAZELL C, DOURISH CT & MIDDLEMISS DN**
Activation of the 5-HT$_2$ receptor subtype increases rat plasma ACTH concentration

687P **HEATON JCP & HANDLEY SL**
Comparison of DOI-induced scratching and head-twitch behaviours: differential action of 8-OH-DPAT

688P **TWIST EC, HAJIMOHAMADREZA I, BRAMMER MJ, BRAZELL C, CORN T & CAMPBELL IC**
Chronic administration of the 5-HT$_2$ antagonist ritanserin produces elevation of 5-HT-mediated PI hydrolysis

689P **KING BH, BRAZELL C & PRESTON GC**
5-HT-induced platelet shape change in the rat: potency of inhibition correlates with 5-HT$_2$ binding affinity

690P **XIANG J-Z, ADAMSON P, BRAMMER MJ & CAMPBELL IC**
α_2-Adrenoceptors and K-opiate receptors decrease ^{45}Ca entry into rat cortical synaptosomes by closure of N-type Ca^{2+} channels

691P **BOESS FG, BALASUBRAMANIAN MK, BRAMMER MJ & CAMPBELL IC**
Presynaptic muscarinic cholinoceptors increase $[Ca^{2+}]_i$ by protein kinase C-dependent opening of L-type Ca^{2+} channels

692P **REYNOLDS DJM, ANDREWS PLR, LESLIE RA, HARVEY JM, GRASBY PM & GRAHAME-SMITH DG**
Bilateral abdominal vagotomy abolishes binding of [^{3}H]-BRL 43694 in ferret dorsovagal complex

693P **BARNES JM, COSTALL B, KELLY ME, NAYLOR RJ, ONAIVI ES, TOMKINS DM & TYERS MB**
GR38032F improves performance in a mouse habituation test, and inhibits cholinergic-linked deficits

694P **BAGETTA G, CONSTANTI A & NISTICO G**
Surface N-wave inhibition by oxotremorine-M is mediated by pre-synaptic M1 receptor stimulation in guinea-pig olfactory cortex

695P **WHELPTON R**
Anti-cholinergic and anti-histaminic properties of chlorpromazine and five metabolites determined on guinea-pig ileum

696P **SMITH KM & JOSEPH MH**
Regionally selective effects of chronic nicotine on rat brain tyrosine hydroxylase activity: a time-course study

697P **BACHELARD H & MARSDEN CA**
Identification of the hypothalamic receptors involved in the increase in blood pressure during stimulation of the C_1 area.

698P **GROSS G, ZILLES K, HANFT G, BAHRO M, SCHWENDEMANN G & MEHDORN HM**
Subtypes of α_1-adrenoceptor binding sites in human brain: radioligand binding and receptor autoradiography

699P **YAKUBU MA, HAMILTON CA & REID JL**
Chronic guanabenz treatment down-regulates α_2-adrenoceptors in rabbit brain

700P **RUCK A, KENDALL DA & HILL SJ**
α- and β-adrenoceptor regulation of cyclic AMP accumulation in a mixed primary astroglial cell culture

701P **KOSHIKAWA N, MARUYAMA Y, KOBAYASHI M & CAMPBELL IC**
Brain β-adrenoceptor loss after a phenelzine injection does not involve receptor internalisation

702P **FORD APDW, KENDALL DA & MARSDEN CA**
Effect of dopamine on neurotensin-stimulated inositol phosphate accumulation in rat striatal slices

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Effect of N-ethylmaleimide on dopamine D_2 receptor regulation of striatal adenylate cyclase activity and [^{3}H]-acetylcholine release

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Agonist-induced desensitization of the D_1 dopamine receptor expressed by a human astrocytoma cell line

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Regional changes in [^{3}H]-SCH 23390 binding to striatum following L-DOPA treatment of 6-OHDA lesioned rats

706P **RUPNIAK NMJ, TYE SJ, NARUSE T, MIYAJI M & IVERSEN SD**
NB-355: a prodrug for L-DOPA with reduced risk for dyskinesias in MPTP-treated primates

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Evidence to suggest a differential involvement of corticosteroids in the expression of opiate and opioid analgesia

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Interleukin 1β and lipopolysaccharide effects on prostaglandin E_2 release from rat brain slices

709P **McAULEY MA, MACRAE IM & REID JL**
Haemodynamic responses to endothelin after peripheral and central administration in the conscious rat

710P **MACHADO CN, ANDERSON AJ, SHARIF NA, EGLEN RM & PATMORE L**
Pharmacological actions of endothelin (ET-1) on cardiac tissue *in vitro*

711P **EGLEME C, CRESSIER F & WOOD JM**
Influence of endothelium on the contractile response of isolated rat aorta to synthetic tetradecapeptide renin substrate

712P **JACOBS M, PLANE F, TURNER MT & BRUCKDORFER KR**
LDL inhibits relaxations evoked by thimerosal in rabbit aorta

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Comparative effects of lignocaine and electromagnetic induction on impulse conduction in frog isolated nerve-muscle preparation

714P **GREENIDGE E & WALI FA**
Noradrenaline-induced depolarization and contraction in the rat isolated smooth muscle of seminal vesicle

715P **BURLEIGH DE & FURNESS JB**
Interactions of galanin with vasoactive intestinal peptide on human colonic motility *in vitro.*

716P **CALCUTT NA, TOMLINSON DR, WILLARS GB**
Effect of ganglioside treatment on substance P-like immunoreactivity on axonal transport in rats with experimental diabetes

717P **HAGAN RM, IRELAND SJ, JORDAN CC, BAILEY F, STEPHENS-SMITH M, DEAL M & WARD P**
Novel, potent and selective agonists at NK-1 and NK-2 receptors

718P **MOORE BA, ELWOOD WE, GATER PR & ALABASTER VA**
The muscarinic receptor sub-type mediating airway responses to acetylcholine in the guinea-pig

719P **WATSON N, BARNES PJ & MACLAGAN J**
Comparison of the *in vivo* potency of methoctramine for pre- and post-junctional muscarinic receptors in guinea-pig lung.

720P **PACIOREK PM, COWLRICK IS, PERKINS RS, TAYLOR JC, WILKINSON GF & WATERFALL JF**
Actions of Ro 31-6930, a novel potassium channel opener, on guinea-pig tracheal smooth muscle preparations

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Comparison of the effects of cromakalim (BRL 34915) on rabbit isolated basilar artery and thoracic aorta.

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Ro 31-6930, a novel potassium channel opener with smooth muscle relaxant and antihypertensive properties

723P **WILSON C, HAMILTON TC & CAWTHORNE MA**
Antagonism of vasorelaxation to cromakalim and BRL 38227 by non-sulphonylurea hypoglycaemic agents

724P **MATHIE A, OBEN JA & FOREMAN JC**
Potassium channels of the T-lymphocyte hybridoma cell line DO-11.10.

725P **NIMMO AJ, WHITAKER EM, KARIM F, MORRISON JFB & CARSTAIRS JR** (introduced by **H DEAN**)
Localization of β-adrenoceptor subtypes in the coronary arteries and gracilis arteries of the dog and pig

726P **RICHARDSON AG, STEIN WK, DOWNING OA & WILSON KA**
Initial calcium release in rat aorta does not appear to contribute to the sustained contraction

727P **KELLY J, ROBERTS SP & SENNITT MV** (introduced by **JRS ARCH**)
The effects of SKF 104078 at pre- and post-junctional α_2-adrenoceptors *in vivo*.

728P **DUNN WR, MCGRATH JC & WILSON VG**
Isolation of post-junctional α_2-adrenoceptors in rabbit isolated distal saphenous artery: influence of angiotensin-II

729P **HEREPATH ML & BROADLEY KJ**
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Dose-related effects of cocaine hydrochloride on canine nasal vascular and airway resistances

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Dose-related effects of adenosine 5'-triphosphate on canine nasal vascular and airway resistances

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738P **ALEXANDER SPH, HARDING SM, RATCLIFFE KM & HILL SJ**
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770P **ARMSTRONG FH & LOWE KC**
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772P **WHITE RD & WOOTTON R**
589C80, a potent long-acting left-shifter of the oxygen saturation curve of haemoglobin *in vivo* in the rat

CONTRIBUTION BY TITLE ONLY

773P **BARNES JM*, BARNES NM, COSTALL B, NAYLOR RJ & TYERS MB**
5-HT_3 receptors mediate inhibition of [^{3}H]-acetylcholine release from rat entorhinal cortex *in vitro*

COMMUNICATIONS

The author who intended to present the work is indicated by an asterisk (*) on the abstract

775P **Brock JA, Cunnane TC & Ziogas J***
Local application of ω-conotoxin GVIA to sympathetic nerve terminals in the guinea-pig isolated vas deferens

776P **Musgrave IF*, Foucart S & Majewski H**
Do phorbol myristate and phorbol dibutyrate enhance noradrenaline release by the same mechanisms?

777P **Pendry YD* & Maclagan J**
Evidence for pre-junctional muscarinic receptors on pulmonary sympathetic nerves in the guinea-pig

778P **Barlow RB* & Veale MA**
Variation in estimates of selectivity at muscarinic receptors in guinea-pig ileum and atria

779P **Good DM* & Small RC**
Muscarinic agonists and guinea-pig perfused tracheal tubes: effects of epithelial removal and route of administration

780P **Stretton CD*, Belvisi MG & Barnes PJ**
Sensory nerve depletion potentiates non-adrenergic, non-cholinergic inhibitory nerves in guinea-pig trachea

781P **Stretton CD*, Belvisi MG & Barnes PJ**
Neuropeptide Y modulates non-adrenergic, non-cholinergic neural bronchoconstriction *in vivo* and *in vitro*

782P **Stretton CD*, Belvisi MG & Barnes PJ**
The effect of sensory nerve depletion on cholinergic neurotransmission in guinea-pig airways

783P **Pique JM, Esplugues JV & Whittle BJR***
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784P **Farmer SG* & Togo J**
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785P **Raeburn D***
Do phorbol esters produce relaxation of tracheal muscle by generation of arachidonic acid from airway epithelium?

786P **Bramley AM*, Samhoun MN & Piper PJ**
Effect of a bradykinin B_2 antagonist on responses of intact and rubbed guinea-pig trachea *in vitro*

787P **Chilvers ER*, Challiss RAJ, Barnes PJ & Nahorski SR**
Characterisation of stereospecific binding sites for [^{3}H]-inositol (1,4,5)-trisphosphate in airway smooth muscle

788P **Hall IP* & Hill SJ**
Inhibition by salbutamol, forskolin and IBMX of AlF_4-induced inositol phosphate formation in bovine airway smooth muscle

789P **Burrows LJ*, Piper PJ, Lindley I, Baglolini M & Westwick J**
Human recombinant neutrophil-activating factor/interleukin-8 is a spasmogen of airway smoooth muscle *in vitro*

790P **Yeadon M* & Payne AN**
Ascorbic acid prevents ozone-induced bronchial hyperreactivity in guinea-pigs

791P **Blaney LM* & Newby AC**
Calcium permeability of pig aortic microsomes is increased by histamine and a guanyl nucleotide

792P **Allen MJ* & Carpenter JR**
Mechanical responses to inositol (1,4,5)-trisphosphate in permeabilised strips of guinea-pig ileum longitudinal smooth muscle

793P **Newgreen DT*, Carter CM, Weston AH & Souness JE**
Characterisation of the intracellular calcium response to vasopressin in A7r5 smooth muscle cells

794P **Wood BE*, Squire A, O'Connor SE & Leff P**
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795P **Allsup DS* & Boarder MR**
Comparison of P_2 receptors of aortic endothelial cells with those of adrenal medulla non-chromaffin cells

796P **Lawrence RA*, Jones RL & Wilson NH**
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797P **Perren MJ*, Feniuk W & Humphrey PPA**
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798P **Guard S*, Watling KJ & Watson SP**
Characterisation of [^{3}H]-senktide binding to NK_3 tachykinin receptors in guinea-pig ileum and cerebral cortex

799P **Cutler MG*, Doyle P & Moore MR**
Pharmacological effects of haem biosynthetic intermediates: actions of δ-aminolaevulinic acid in the rat jejunum

800P **Reeves JJ*, Bunce KT, Humphrey PPA & Gunning SJ**
Further characterisation of the 5-HT receptor mediating smooth muscle relaxation in rat oesophagus

801P **Wardle KA* & Sanger GJ**
Atypical 5-HT receptors in guinea-pig isolated ileum

802P **Nicholson CD*, Cottney J, Marsden G & Shahid M**
A comparison of the inotropic activity of ORG 30029, IBMX, milrinone and rolipram in rat papillary muscles

803P **De Souza RN*, Gater PR & Alabaster VA**
Bronchodilator and tracheal relaxant effects of potassium channel openers in the guinea-pig

804P **Longmore J* & Weston AH**
Effects of cromakalim and glibenclamide on isolated strips of bovine tracheal smooth muscle

805P **Bowring NE, Taylor JF & Arch JRS***
Evaluation of inhaled cromakalim and its active enantiomer BRL 38277 as bronchodilators in the guinea-pig

806P **Bray KM*, Duty S & Weston AH**
Effects of glibenclamide on cromakalim-induced responses in rabbit isolated aorta

807P **Piper I* & Hollingsworth M**
Cromakalim, RP49356, pinacidil and minoxidil sulphate in the rat uterus and their antagonism by glibenclamide

808P **Perkins RS*, Paciorek PM & Waterfall JF**
Glibenclamide and procaine, but not apamin, inhibit the relaxant responses to Ro 31-6930 in guinea-pig *taenia caeci*

809P **Newberry NR***
5-HT_{1A} receptors mediate a hyperpolarisation of rat ventromedial hypothalamic neurones *in vitro*

810P **Connor HE* & Higgins GA**
Cardiovascular effects following injection of 8-OH-DPAT and flesinoxan into the dorsal raphe nucleus of conscious rats

811P **Bogle RG* & Ramage AG**
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812P **Lawrence KM*, De Paermentier F, Cheetham SC, Crompton MR, Katona CLE & Horton RW**
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813P **Watling KJ*, Beer MS & Stanton JA**
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814P **Murray AM* & Waddington JL**
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815P **Collins P*, Broekkamp P, Jenner P & Marsden CD**
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816P **Rose S*, Thomas K, Jenner P & Marsden CD**
Fluctuations in response to L-DOPA in Parkinson's disease may be explained by changes in striatal dopamine turnover

817P **Lange KW*, Wells FR, Rossor MN, Jenner P & Marsden CD**
Cortical muscarinic receptors in demented patients with Alzheimer's disease or Parkinson's disease

818P **De Paermentier F*, Cheetham SC, Crompton MR, Katona CLE & Horton RW**
Lower cortical β-adrenoceptor binding sites in post-mortem samples from depressed suicide vicitms

819P **Graham WC*, Robertson RG, Sambrook MA & Crossman AR**
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824P **Barclay PL*, Peters CJ, Bennett JA, Danilewicz JC, Samuels GMR & Shepperson NB**
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Involvement of excitatory amino acid receptors in epileptiform activity in the rat entorhinal cortex and dentate gyrus *in vitro*

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870P **Knox AJ, Ajao P, Cragoe EJ, Tattersfield AE**
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871P **Berry JL, Elliott KRF, Foster RW &1Small RC**
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Effects of aqueous onion and garlic extracts on contractions of guinea-pig airways *in vitro*

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Ionophore A23187 causes PAF release in the isolated perfused lungs of oxygen-treated rats

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Bradykinin B_1 and B_2 receptor activation stimulates chloride transport in isolated rat colon

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Effects of propranolol and dimethylquaternary propranolol on K^+ influx in guinea-pig heart ventricle slices

881P **Taylor SG, Foster KA, Shaw DJ & Taylor JF**
BRL 31660 inhibits the effects of cromakalim and its enantiomer BRL 38277 on tension and $^{43/42}K^+$ efflux in guinea-pig trachea

882P **Sweetland J & Raeburn D**
Effect of potassium channel openers on Ca^{2+}influx, Na^+/Ca^{2+} exchange and Na^+/K^+ pumping in guinea-pig trachea *in vitro*

883P **Underwood SL & Raeburn D**
Studies on the bronchodilator effects of β_2-agonists administered by dry powder or by aerosol to the anesthetised guinea-pig

884P **Longmore J, Newgreen DT & Weston AH**
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885P **Bray KM & Weston AH**
Differential concentration-dependent effects of K channel openers on ^{42}K and ^{86}Rb efflux in rabbit isolated aorta

886P **Downing SJ & Hollingsworth M**
Cardiovascular tolerance to cromakalim in the rat *in vivo*

887P **Sadrei H, Downing SJ & Hollingsworth M**
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888P **Longman SD**
Potassium channel activation in smooth muscle cells may be associated with decreases in intracellular ATP

889P **Wilson C & Howlett DR**
Comparative effect of glibenclamide on responses to cromakalim and hypoxia *in vitro*

890P **Sadri R, Nicholls PJ & Smith HJ**
Tolerance to the oestrogen-lowering effect of aminoglutethamide in the rat

891P **Hallam A, Mansel RE, Nicholls PJ & Smith HJ**
Comparison of aminoglutethimide and two novel analogues as inhibitors of aromatase in human breast tumours

892P **Jackson SM, Alcock P, Jamieson A & Tuffin DP**
Dependence of the fibrinolytic response on plasma tPA and PAI-1 levels in the laboratory rat

893P **Campbell KB, Jackson SM, Jamieson A, Johnstone D & Tuffin DP**
Plasminogen activator inhibitor levels in an arthritic rabbit model - effects with the anabolic steroid stanozolol

894P **Kelly MJ & Kowalska AZ**
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Potentiation of adrenaline-induced human platelet aggregation by ATP

898P **Rimoy GH, Horn EH & Rubin PC**
Potentiation of human platelet aggregation by dynorphin

899P **Gilani, A-ul-H, Saeed, SA & Surla A**
Effect of adrenoceptor-blocking drugs on platelet aggregation induced by various aggregating agents

900P **Martin GN, Hair WM, Hughes AD, Schachter M, Thom SMcG & Sever PS**
The response of isolated human resistance arteries to endothelin-1: effect of calcium channel blockers

901P **Warren JB & Brady AJ**
Vascular smooth muscle factor causes vasoconstriction followed by slow onset and prolonged EDRF release

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Effects of hypoxia on sheep isolated intrapulmonary artery

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Effects of dimethylthiourea and dizocilpine on infarct size following middle cerebral artery occlusion in the rat

904P **Gibson A, Mirzazadeh S, al-Swayeh OA, Chong NWS & Moore PK**
L-N^G-nitro-arginine is a potent, L-arginine reversible, inhibitor of NANC relaxations in the mouse anococcygeus

905P **Moore PK, al-Swayeh OA, Chong NSW, Evans R, Mirzazadeh S & Gibson A**
L-N^G-nitro arginine (NOARG) inhibits endothelium-dependent vasodilatation in the rabbit aorta and perfused rat mesentery

906P **Clegg MF, Finnen MJ & Williams KI**
Biological activities of the epoxides of arachidonic acid

907P **Campbell EA, Dray A & Perkins MN**
Comparison of capsaicin and olvanil as antinociceptive agents *in vivo* and *in vitro*

908P **Guard S, Watling KJ & Watson SP**
NK_3 tachykinin receptors are coupled to inositol phospholipid hydrolysis in neonatal rat spinal cord

909P **Godfrey PP, Varney MA, O'Callaghan K, Taghavi Z & Watson SP**
Chronic lithium treatment decreases inositol (1,4,5)-trisphosphate levels in rat cerebral cortex slices

910P **Grove EA & Caulfield MP**
Inhibition of protein kinase C does not prevent muscarine depolarisation of rat sympathetic ganglia

911P **Mellor SJ, Clarke CE, Boyce S, Sambrook MA, Crossman AR**
Muscarinic supersensitivity in the globus pallidus of MPTP-treated monkeys, revealed using [^{3}H]-QNB autoradiography

912P **Barnes NM, Cheng CHK, Costall B & Naylor RJ**
The toxic effects of MPTP and MPP^+ in marmoset and rat striatal cell cultures

913P **Lambert DG & Nahorski SR**
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