IS THE EXCITATORY NEURONAL RESPONSE TO DOPAMINE MEDIATED BY D_1 OR D_2 DOPAMINE RECEPTORS IN THE CEREBRAL CORTEX?

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Single neurones in the cerebral cortex are sensitive to noradrenaline and dopamine applied by microelectrophoresis: the catecholamines can evoke both excitatory and depressant responses (Szabadi, 1979). Evidence in our laboratory indicates that these responses are mediated by at least three different receptors: the excitatory response to noradrenaline by α_1-adrenoceptors, the depressant response to noradrenaline by ß-adrenoceptors and the excitatory response to dopamine by an "excitatory dopamine receptor" which can be separated from the α_1-adrenoceptor by using selective α_1-adrenoceptor and dopamine receptor antagonists (Bevan et al, 1978; Bradshaw et al, 1983). In the present experiment we compared the effects of two selective dopamine receptor agonists (Kebabian et al, 1984): SKF 38393 (D_1 receptor agonist) and LY 171555 (the active l-enantiomer of LY 141865, a D_2 receptor agonist), in an attempt to identify the type of dopamine receptor involved in the excitatory response to dopamine.

Spontaneously active single neurones were studied in the somatosensory cortex of the halothane-anaesthetized rat. Our techniques for the extracellular recording of neuronal activity and for the microelectrophoretic application of drugs are described elsewhere (Bradshaw et al, 1983). All the drugs were applied by microelectrophoresis.

The effect of SKF 38393, applied with ejecting currents of up to 200 nA, was investigated on 17 cells which gave consistent excitatory responses to phenylephrine and acetylcholine. On none of these cells could any excitatory effect of SKF 38393 be detected. The effect of LY 171555 was tested on 58 cortical neurones: the drug evoked weak excitation on 31 cells which were also excited by phenylephrine and acetylcholine, and on 7 cells which were also excited by dopamine and acetylcholine. On 18 phenylephrine-sensitive cells and on 2 dopamine-sensitive cells, however, it was not possible to evoke any response to LY 171555 applied with ejecting currents of up to 125 nA.

Both the α_1-adrenoceptor antagonist, prazosin, and the dopamine receptor antagonist, haloperidol, could discriminate between excitatory responses to LY 171555 and phenylephrine: prazosin preferentially antagonized the response to phenylephrine (9 cells), whereas haloperidol preferentially antagonized the response to LY 171555 (3 cells); neither antagonist affected the response to the control agonist, acetylcholine. Since LY 171555 was a relatively impotent agonist, we examined whether LY 171555 itself could antagonize neuronal responses to the amines. LY 171555 could discriminate between excitatory responses to dopamine and phenylephrine, the response to dopamine being more susceptible to antagonism; responses to acetylcholine were not affected (14 cells). SKF 38393, when compared on excitatory responses to dopamine, phenylephrine and acetylcholine, showed no specific effect on the response to dopamine (9 cells).

These results suggest that D_2 dopamine receptors may play a role in mediating the neuronal excitation to dopamine, while D_1 receptors are unlikely to be involved.

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2P

ADRENERGIC INHIBITION OF SUBSTANCE P RESPONSES IN THE CINGULATE CORTEX: EFFECTS OF AGONISTS AND ANTAGONISTS

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Excitatory responses of cingulate cortical neurones to iontophoretically applied substance P (SP) can be reduced or abolished by noradrenaline (NA) applied concurrently with weak ejecting currents (Jones and Olpe, I983). Activation of the afferent NA-containing pathway to the cortex has a similar inhibitory effect on responses to SP (Jones and Olpe, I984). In the present study we have attempted to determine the nature of the adrenergic receptor involved in the interaction by studying the effects of a number of adrenergic agonists on neuronal responses to SP In addition we attempted to modify the inhibitory effect of NA on SP-responses by iontophoretic administration of a β-antagonist,practolol, or an α-antagonist, prazosin.

Conventional electrophysiological techniques were used to record the spontaneous activity of neurones in layers IV-V (approx) of the anterior cingulate cortex of rats anaesthetized with chloral hydrate and to apply substances iontophoretically.

In terms of the iontophoretic currents needed to reduce SP-responses the most effective agonists tested were NA (n=50), adrenaline (n=23) and the β-agonist, clenbuterol (n=8). With all 3, ejecting currents in the I-5 nA range were almost always effective and in many cases removal of the retaining current from the agonist was sufficient to completely abolish the SP-responses. The α_I-agonists, phenylephrine (n=I7) and methoxamine (n=9) were also found to reduce SP-excitations although threshold currents were higher (I5-40 nA). Dopamine (n=I4) and the α_2-agonist, clonidine (n=II) were relatively ineffective although reductions in SP-responses were seen with fairly high ejecting currents (30-80 nA). Finally, salbutamol (n=I5) and isoprenaline (n=I7) only reduced responses with high currents (80-I20 nA) but severe difficulties were experienced with their ejection so these results must be treated with great reservation.

NA and adrenaline evoked both increases and decreases in neuronal firing rate at the low ejecting currents tested but reduced SP-responses regardless of the direction of the change. Clenbuterol induced reduction of SP-excitations was associated with either a decrease or no change in firing rate. Phenylephrine and methoxamine consistently excited cells when low currents were applied (I-I5 nA) but at this level had little effect on SP-responses. When the ejecting current was increased baseline firing rate often plateaued or declined and reduction of SP-response became evident.The reduction of SP-evoked excitations by dopamine and clonidine was invariably associated with a decrease in baseline firing rate.

Iontophoretic application of the α_I-antagonist, prazosin (up to 60 nA) did not detectably modify the ability of NA to reduce responses to SP (n=5). However, the β-antagonist, practolol (I5-30 nA) largely prevented the inhibitory effect of the amine (n=6). These studies indicate that the receptor involved in the NA/SP-interaction is probably β in nature and this is supported by the marked effectiveness of clenbuterol. However the α_I-agonists were active in reducing SP-responses at only moderately higher currents so an α component in the interaction cannot be definitely ruled out yet.

Jones, R.S.G. and Olpe, H-R. (I983) Neurosci. Lett. Supp I4, SI84
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RELEASE OF ENDOGENOUS DOPAMINE AND ITS METABOLITES FROM THE NEUROINTERMEDIATE LOBE OF THE RAT HYPOPHYSIS IN VITRO

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There is good evidence that DA is a neurotransmitter in the NIL (Holzbauer et al, 1983). Little is known about the metabolism of DA in this tissue. In homogenates of rat NILs monoamine oxidase (MAO) activity has been detected, but no catechol-O-methyltransferase (COMT) activity (Saavedra et al, 1975). An analysis of the spontaneous-released ^{3}H-compounds from superfused NILs loaded with ^{3}H-DA showed, however, that more than 60 % of the ^{3}H-DA metabolites were O-methylated (Racké et al, 1984). When the release of ^{3}H-compounds evoked by electrical stimulation of the pituitary stalk was investigated it became obvious that ^{3}H-compounds, apparently released from an extraneuronal compartment confused the results.

Thus, experiments are now in progress in which the release of endogenous DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylacetic acid (HVA) and 3-methoxy-4-hydroxyphenylethanol (MOPET) are measured by HPLC with electrochemical detection. Isolated NILs with their stalk held in a platinum wire electrode were incubated in 40 µl Krebs-HEPES solution. The medium was changed every 10 min. DA and DA metabolites released either spontaneously or after electrical stimulation of the pituitary stalk (0.2 ms, 10 V, 15 Hz, 3 times 1 min with 1 min intervals) were analysed.

Table 1 shows the rates of spontaneous outflow of DA and DA metabolites. They remained constant throughout the experiment. When pargyline (PARG, 10 µM) was present DA outflow remained unaltered but that of DA metabolites decreased time-dependently: DOPAC $t_{1/2}$ = 24 min; HVA $t_{1/2}$ = 14 min. Table 1 shows also the values after 1 h of incubation with PARG. The DA uptake inhibitor GBR 12921 (100 nM, van der Zee et al, 1980) alone had no effect on the spontaneous outflow of DA and DA metabolites. When GBR 12921 plus PARG were present the spontaneous outflow of DA was increased to 346±20 fmol/10 min (n=7). At the end of incubation the tissue contents (pmol/NIL) were as follows: no drug: DA 18±0.8, DOPAC 0.96±0.08, HVA 0.88 ±0.88, MOPET < 0.2; presence of PARG: DA 27±1.6, DOPAC 0.2±0.1, HVA < 0.2.

Table 1: Effect of PARG on the spontaneous outflow of DA and DA metabolites

	DA	DOPAC	HVA	MOPET	n	
no drug	31±4	619±29	703±67	67±7	6	fmol/ NIL x 10 min
PARG	37±8	95±24	34±2	< 30	4	

Table 2 shows that during electrical stimulation in the absence of drugs which prevent the inactivation of DA, little DA escaped into the medium. PARG and GBR 12921 substantially increased the evoked DA release. The effect of both drugs were additive. In the presence of GBR 12921 the outflow of all DA metabolites increased during stimulation.

Table 2: Effects of PARG and GBR 12921 on the electrically evoked DA overflow

	no drug	PARG	GBR	GBR + PARG
DA (fmol/NIL)	83±14 (n=5)	359±63 (n=4)	457±58 (n=8)	1102±106 (n=7)

In conclusion: DA spontaneously released from the NIL in vitro is preferentially metabolised extraneuronally by MAO and COMT. During stimulation both reuptake and metabolism of DA may contribute to the inactivation of DA.

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4P

PIRENZEPINE BINDING TO SOLUBILIZED MUSCARINIC RECEPTORS FROM RAT CEREBRAL CORTEX AND MYOCARDIUM

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The selective antagonist pirenzepine (PZ) has been shown to bind with high affinity to a sub-population of muscarinic receptor sites in autonomic ganglia and forebrain. Muscarinic sites in other tissues eg. myocardium and smooth muscle, have a lower affinity for PZ (Hammer et al., 1980). Here we report that muscarinic receptors from rat cerebral cortex and myocardium retain some selectivity for PZ when removed from their membrane environment by solubilization in digitonin.

EDTA washed cortical membranes and KCl/pyrophosphate/EDTA extracted heart membranes were prepared as described by Hulme et al. (1983) and Berrie et al. (1984) respectively. Solubilization, assay and analytical methods and sucrose density centrifugation technique are described by Berrie et al. (1984). Solubilization was conducted at 4-5mg/ml protein in 1% digitonin. All assays were carried out in 20mM NaHEPES, 1mM Mg^{2+}, pH 7.5 at 4°C. Equilibration of ligands under these conditions is very slow, so binding experiments were incubated for 44h, including 24h preincubation with the non-radioactive ligand.

PZ binding to cortical membranes is heterogenous: 60-80% of sites have a high affinity for PZ ($K_H \sim 6 \times 10^7\ M^{-1}$), the remainder having a lower affinity ($K_L \sim 1 \times 10^6 M^{-1}$). This heterogeneity is retained in the solubilised preparation, the two sites having affinities of $\sim 6 \times 10^7 M^{-1}$ and $\sim 6 \times 10^6 M^{-1}$. The proportion of high affinity sites is variable (25-70%); this seems to be due to a selective instability of the high affinity site. The high affinity site can be labelled specifically with [^{3}H]PZ (1-10nM) and characterized: it has muscarinic pharmacology, behaves as a monomeric species in sucrose density gradient centrifugation and has low affinity for agonists.

In myocardial membranes PZ binds to a uniform population of low affinity sites ($K \sim 2 \times 10^6 M^{-1}$) and virtually no receptor specific [^{3}H] PZ binding is detectable. After solubilization PZ affinity is increased about tenfold to $\sim 2 \times 10^7 M^{-1}$ and the solubilized site can be labelled with [^{3}H]PZ. This site has muscarinic pharmacology and preliminary results show that it behaves as a monomeric species in sucrose density gradient centrifugation. Agonists inhibit [^{3}H]PZ binding to the myocardial site with high potency, and this binding is sensitive to guanine nucleotides.

The high affinity PZ site in cortex can be solubilized with very little change in affinity. The PZ affinity of the myocardial site increases about tenfold on solubilization. The solubilized [^{3}H]PZ sites from cortex and myocardium have similar affinities: in cortex $\log K = 7.81 \pm 0.058$ (mean ± s.e.mean, n=6) and in heart $\log K = 7.38 \pm 0.081$ (mean ± s.e.mean, n=4). However, $P < 0.001$ that these affinities are the same.

It is concluded that membrane constraints play a part in determining the myocardial receptors affinity for PZ. However, solubilization does not seem to abolish the cortex/heart selectivity of PZ completely, the cortical site having x3 the affinity of the myocardial site. The molecular basis for this heterogeneity remains to be elucidated.

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UPTAKE AND RELEASE OF [^{3}H]GABA FROM NERVE TERMINALS IN NEUROINTERMEDIATE PITUITARY GLAND

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Autoradiographic studies suggested that the uptake of [^{3}H] GABA in the posterior pituitary gland is exclusively into glial cells (Beart et al, 1974). More recently, immunohistochemical studies have demonstrated a dense GABAergic innervation of the intermediate lobe of the pituitary (Oertel et al, 1982), which may influence the release of αMSH (Tomiko et al, 1983). We have investigated the characteristics of [^{3}H] GABA uptake by the neurointermediate lobe (NI) in vitro and its subsequent release.

NI from male Wistar rats were homogenised in 0.32M sucrose using a teflon/glass homogeniser and diluted (1 in 10) in oxygenated physiological medium (pH 7.4) (Mitchell and Martin, 1978). Uptake studies were carried out at 25°C: after 20min preincubation, [^{3}H] GABA was added for 5 min before filtration and washing. For kinetic experiments, [^{3}H] GABA concentrations in the range 1 - 20μM were used. Release experiments were carried out at 37°C, and 10μM amino oxyacetic acid was included to inhibit [^{3}H] GABA catabolism. After 15 min of uptake ([^{3}H] GABA: 50nM), samples were introduced into superfusion chambers, and after 30 min of superfusion at 0.5ml/min, basal release was steady and the collection of 1 min fractions was begun.

The initial rate of uptake of 1μM [^{3}H] GABA (linear for 10 min) was 2.8 ± 0.3 pmol/mg protein/min, and this was reduced to 0.52 ± 0.18 and 0.30 ± 0.02 pmol/mg protein/min by incubation at 4°C and in Na^+-free medium respectively. β-alanine (1mM) inhibited uptake by 7% and 1-2,4-diaminobutyric acid (500μM) by 46%, suggesting that under these conditions uptake is predominantly neuronal. Kinetic analysis indicated a K_m of 5.4μM and a V_{max} of 17.2pmol/mg protein/min (mean of 4 experiments).

Basal release of [^{3}H] GABA was approximately 0.5% of total tissue radioactivity/min, and was very sensitive to stimulation by low concentrations of K^+. 15mM K^+ caused 211 ± 8% increase in release which fell to 33 ± 5% in Ca^{2+}-free medium (n = 5). This is in marked contrast to glial [^{3}H] GABA release from isolated neural lobes of pituitary which was insensitive to K^+ below 30mM (Minchin and Nordmann, 1975). The response to 15mM K^+ was reduced by 100nM muscimol to 161 ± 15% ($p < 0.02$) and this effect was reversed to 229 ± 10% by 10μM bicuculline methiodide ($p < 0.005$, n = 5), demonstrating the presence of presynaptic GABA autoreceptors (Mitchell and Martin, 1978). Neither drug affected basal release at these concentrations.

These results indicate the presence of GABA nerve terminals in NI, (probably mainly in pars intermedia), with characteristics similar to those in the CNS, including their regulation by autoreceptors.

R.A.A. is a Houldsworth Scholar of the University of Edinburgh.

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6P

STUDIES ON THE EFFECTS OF CHOLECYSTOKININ ON CYCLIC AMP EFFLUX FROM RAT STRIATAL SLICES

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In the striatum it has been reported that dopamine both stimulates and inhibits cAMP production (Stoof and Kebabian, 1981). These effects are the result of interations with either a stimulatory or an inhibitory adenylate cyclase. The present study examines a possible interaction of dopamine with peptides located in the striatum on this adenylate cyclase system.

Rat striatal tissue was chopped on a McIlwain tissue chopper and the resulting blocks of tissue (2 x 0.3 x 0.3µm) were transferred to well-oxygenated Earles Balanced Salt Solution (EBSS). The slices were mounted in perspex chambers and superfused for one hour at 0.1 ml/min with EBSS fortified with 1 mM IBMX and 2.5 mg/ml BSA, before the introduction of drugs. 200 µg/ml bacitracin was included in experiments using peptides. Cyclic AMP in 1 ml fractions of superfusate was determined using radioimmunoassay (detection limit 2.5 fmol.100 µl; NEN). The amount of cAMP formed in the presence of drug was expressed as a percentage of the basal cAMP efflux. No drug was added to one superfusion channel in each experiment to serve as an additional control. Paired t-tests were carried out on untransformed data.

In the EBSS containing 2mM Ca^{++} the efflux of cAMP was enhanced by SKF 38393 (1-100 µM), 6,7,-ADTN (10 µM), CCK8sulphate (30 nM-10 µM), non-sulphated CCK8 (0.1-10 µM), VIP (0.5 µM) forskolin (100 µM) and KCl (10 and 20 mM).

In the presence of the dopamine D2 receptor antagonist, sulpiride (1 µM), the stimulation of cAMP efflux produced by CCK8 was abolished whilst that due to VIP orSKF38393 was unaffected. The inhibition produced by sulpiride was stereo specific. SCH23390 (R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol hemimaleate) the proposed dopamine receptor antagonist with D1 receptor selectivity inhibited the SKF38393-induced cAMP efflux, IC_{50} = 10 nM. 10 nM SCH 23390 completely abolished the effect of 1 µM CCK8 but had no effect on forskolin or VIP-stimulated cAMP efflux.

Removal of Ca^{++}(+1mM EGTA) significantly enhanced basal cAMP efflux to 265 ± 10% ($p<.01$ n=18). The effect of KCl was completely abolished but there was no significant effect on the response to SKF38393, CCK8sulphate or Forskolin.

In striatal homogenates adenylate cyclase activity was enhanced in a dose-dependent fashion by Forskolin (10^{-9} - 10^{-5} M), SKF38393 (10^{-7} - 10^{-5} M), VIP (0.2-2µM), and dopamine ($3x10^{-6}$ - 10^{-4} M). Sulphated CCK8 (10^{-13} - 10^{-5}M) enhanced AC activity in some experiments up to 25%, but in 6 experiments failed to produce a significant activation. Forskolin at 10^{-9} and 10^{-5} M did not influence the response to CCK.

These results suggest an interaction between the transmitters dopamine and CCK in rat striatum. The effect is specific in that VIP or forskolin-mediated adenylate cyclase activation do not appear to be modulated by dopamine receptor occupation.

At least a part of the effect of CCK in the striatum is postsynaptic in nature. However, the enhancement of cAMP production is unlikely to be the result of a direct activation of adenylate cyclase (cf dopamine-sensitive AC) but may be an inhibitor of receptor mediated mechanisms.

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EFFECT OF CCK PEPTIDES ON DOPAMINE RELEASE AND METABOLISM IN RAT STRIATUM IN VIVO

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Cholecystokinin (CCK) peptides exist in high concentrations in mammalian brain and may have a central neuroregulatory role (Emson, 1979). Various lines of evidence suggest an interaction between CCK peptides and nigrostriatal dopaminergic neurones in the rat brain. For example, immunohistochemical studies have demonstrated dopamine/CCK coexistence in certain dopaminergic neurones in substantia nigra, and CCK heptapeptide in the sulphated (CCK-7S) but not unsulphated (CCK-7U) form is a potent excitant of cells in this region (Skirboll et al, 1981). In the present study we have investigated the effect of CCK-7S and CCK-7U on dopamine release and metabolism in rat striatum in vivo using intracerebral dialysis (Zetterström et al, 1983). The actions of these peptides are compared with the effects of drugs known to act on dopamine neurones.

Rats were anaesthetized with halothane and dialysis loops (2 mm in length) were implanted into the striatum and perfused (2 µl/min) with physiological ringer solution. Perfusates were collected at 20 min intervals and analysed for dopamine and its metabolites DOPAC and HVA using HPLC with electrochemical detection. After an 80-100 min control period drugs were added to the perfusion medium for 20 min, and then ringer solution was perfused for a further 100 min.

The dopamine releasing agent amphetamine (10^{-5} M) caused a marked stimulation (+ 1721 %, N=5) of dopamine release during perfusion which was concomitant with a decline in DOPAC (- 36%). The dopamine agonist apomorphine (10^{-5} M)reduced dopamine and DOPAC to 40 % (N=3) and 80 % respectively of the control values over the time course while the dopamine antagonist cis-flupenthixol (10^{-5} M) caused a slight increase in dopamine (+ 40 %, N=4) during perfusion. CCK-7S (10^{-4}-10^{-8}M) caused a dose-dependent increase in dopamine release, the highest dose (10^{-4} M) increasing release to a maximum (+ 180 %, N=5) 40 mins following administration which lasted for the remainder of the experimental period. CCK-7S (10^{-4} - 10^{-6}M) also reduced DOPAC ($\bar{-}$ 10-25 %, N = 4-5) over the time course. In contrast to CCK-7S, CCK-7U (10^{-4} M) produced a small short-lasting increase in dopamine release (+ 40 %, N=4) during administration and had a weak effect on dopamine metabolism.

These results demonstrate that local administration of low doses of CCK-7S into the rat striatum causes a specific release of dopamine but a reduction in dopamine metabolism. This complex action of CCK-7S does not resemble the effects of a dopamine receptor agonist and antagonist or a dopamine releasing agent. We are currently investigating the possibility that CCK-7S is degraded to a biologically active form which has opposite actions to the parent peptide.

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8P

IODINATED SOMATOSTATIN BINDING TO HUMAN BRAIN MEMBRANES: A COMPARISON OF CONTROLS AND ALZHEIMER TYPE DEMENTIA PATIENTS

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Binding sites for the neuropeptide somatostatin (SST) can be readily characterised in the rat cerebral cortex using several different iodinated SST analogues as ligands. By contrast demonstration of SST binding in human brain has proved difficult because of the high proportion of non-specific binding in the total binding. In the present study we describe our attempts to optimise binding of SST to human brain membrane preparations, and the application of the technique to samples of post-mortem brain from patients who died with Alzheimer type dementia (ATD).

Four SST analogues were iodinated, (Tyr^{0})SST-14, (Tyr^{1})SST-14, (Tyr^{11})SST-14 and (Leu^{8}, D-Trp^{22}, Tyr^{25})SST-28, and their binding to a crude membrane preparation from human brain studied. Of the four analogues, (^{125}I-Tyr^{11})SST-14 had the lowest non-specific binding and this ligand was used in subsequent experiments. The effect of various additions to the incubation medium was investigated. 1% BSA, 1% gelatin, and 0.1% bacitracin were found to give maximum protection of the ligand against peptide adsorption and enzymatic degradation, whilst the addition of $MgCl_2$ did not increase binding and the cation chelators EDTA and EGTA both reduced specific (^{125}I-Tyr^{11})SST-14 binding. Incubations (1h at 37^{o}C) were terminated by the addition of 1 ml ice cold 50 mM Tris-HCl pH 7.4 followed by superficial washing of the pellet.

Despite these modifications specific (^{125}I-Tyr^{11})SST-14 binding to human brain membrane preparation comprised only 20% of the total binding compared to greater than 50% to rat brain preparations. To increase the reliability of the method therefore, all assays were carried out in quadruplicate. It is unlikely that delay between death and autopsy could account for the low binding in human tissue, because there was only a minimal decrease in binding to rat brain membrane preparations after maintaining the brains for 3h at 20^{o}C and 96h at 4^{o}C to simulate human post-mortem conditions.

Using the technique described, binding to human brain membrane preparations was found to be saturable, and Scatchard analysis indicated a single high affinity binding site, K_D = 1.1 ± 0.3 nM, Bmax 81 ± 20 fmol/mg protein (n = 4). (^{125}I-Tyr^{11})SST-14 (0.1 nM) was completely displaced by 1 µM SST-14 (IC_{50} 31 nM), SST-28 was also a potent displacer (IC_{50} 35 nM). Other peptides (VIP, CCK-8 and neurotensin) did not displace the ligand at µM concentrations.

SST binding (0.1 nM) final concentration) to membrane preparations from frontal and temporal cortices from 12 control and 12 ATD brains was then assessed. The results expressed as fmol/mg protein were as follows, frontal cortex - controls 7.2 ± 3.3, ATD 7.0 ± 3.1 and temporal cortex - controls 9.0 ± 2.7, ATD 7.6 ± 2.3. There were no significant differences in SST binding between the two groups.

Although there have been previous reports of decreased SST levels in both the csf (Wood et al, 1982) and post-mortem brain (Ferrier et al, 1983) of ATD patients, the initial results of the present study suggest that SST binding may be unchanged in brains from patients with ATD.

SMF is an MRC scholar.

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ACTIONS OF METHYLPHENIDATE ON NEUROGENIC RELEASE AND UPTAKE OF DOPAMINE IN VIVO

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CNS stimulants have been divided into two groups: those whose behavioural effects are inhibited by alpha methyl-p-tyrosine (e.g. amphetamine and phenylethylamine) and those whose actions are blocked by reserpine (amfonelic acid, nomifensine and methylphenidate: Braestrup, 1977). Amfonelic acid has been shown to increase stimulated dopamine (DA) release in vivo (Ewing et al, 1983). Nomifensine also increases DA release and blocks low affinity DA uptake (Stamford et al, 1984). This work describes the actions of methylphenidate on electrically stimulated DA release and reuptake in vivo.

DA release in the striatum of the chloral hydrate anaesthetised rat was monitored by high speed cyclic voltammetry following electrical stimulation of the median forebrain bundle (Kruk and Stamford, 1984). DA uptake was measured as the linear rate of decline in concentration of DA on cessation of stimulation. Three stimulations (50 Hz sine waves, 80-100 μA rms for 10s) were performed, twenty minutes apart. Saline or methylphenidate (50 or 100 mg/kg i.p.) was administered immediately after the first stimulation (S1). DA release and subsequent rate of reuptake on the second (S2) and third stimulations (S3) were expressed as a percentage of S1. Control peak DA release was 35.5 + 3.9 μM and the linear rate of DA clearance (uptake) was 4.9 + 0.3 μM/s.

	Control (saline)	Methylphenidate (50 mg/kg)	Methylphenidate (100 mg/kg)
RELEASE	(% of S1: mean + sem.)		
S2/S1	87.8 ± 3.8	124.8 ± 12.3**	182.5 ± 18.5**
S3/S1	91.8 ± 7.8	109.8 ± 6.8	121.3 ± 10.5
UPTAKE	(% of S1: mean + sem.)		
S2/S1	105.2 ± 7.7	97.5 ± 5.3	53.5 ± 12.2**
S3/S1	101.0 ± 9.9	85.0 ± 1.6	37.7 ± 7.3*
N	(5)	(4)	(4)

Significantly different from control, *($P<0.05$), **($P<0.02$) Mann Whitney U test

Methylphenidate increased DA release on S2 in a dose dependent manner. Low affinity DA uptake was blocked only by the higher dose of methylphenidate. The actions on release and uptake showed a different time course. Effects on release were seen only on S2 whereas uptake blockade was more marked on S3. This indicates that these actions are distinct from each other.

In conclusion, the actions of methylphenidate on release and uptake of DA in vivo appear similar to those previously reported for nomifensine (Stamford et al,1984). The results also show that the technique is capable of separately measuring effects on uptake and release of DA.

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THE EFFECTS OF NOMIFENSINE ON DOPAMINE RELEASE INTO A DIALYSIS FIBRE IMPLANTED IN THE NEOSTRIATUM OF ANAESTHETISED RAT

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The technique of "in vivo brain dialysis" developed by Ungerstedt and colleagues has been successfully employed to relate the release of dopamine(DA) to behavioural parameters of the response to drugs (Zetterstrom et al. 1983). In these experiments the drug was applied intraperitoneally or subcutaneously and DA and its metabolites measured in the dialysate from striatum. These experiments are not directly relevant to the mass of literature about the release of DA from striatal tissue "in vitro" since both the concentration of drug and its site of action are unknown. In our experiments we have applied drugs in the fluid passing through the 'fibre' and determined the response of the DA terminals nearby, from the amount of DA and its metabolites in the dialysate.

Male Sprague Dawley albino rats 150-200g were anaesthetised with halothane and fixed in a stereotaxic frame. The skull was exposed and the fibre introduced through burr holes in both temporal bones. The fibre was coated with impervious resin (Super-Epoxy) except where it passed through the striatum. Physiological salt solution (147mM Na^+, 2.3mM Ca^{++}, 4mM K^+, 155.6mM Cl^-) was perfused through the fibre at a rate of 2µl/min and samples collected every 20 min into a tube containing 10µl of 0.1M PCA. DA, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in the samples were assayed by reverse phase high performance liquid chromatography and electrochemical detection.

Nomifensine in concentrations of 10^{-7} - 10^{-4}M was introduced into the perfusate for 1 hr. (3 x 20 min samples) and the DA, DOPAC and HVA concentrations compared with those in the dialysate from the hour before and after the drug application. No animal received more than two concentrations of drug with never less than 1hr between applications. Another group of rats received 10^{-6}M Amphetamine similarly applied in the perfusate.

DA concentrations were 0.46 ± 0.07 (33) (mean ± SEM(n)) pmoles in control 40µl samples. Neither drug had significant effects on DOPAC or HVA efflux which remained at 17.6 ± 2.9(33) and 13.5 ± 1.2(33)pmoles per sample, respectively. Amphetamine perfused at 10^{-6}M caused a 500% increase in DA release. This increase was matched by Nomifensine at 5 x 10^{-5}M. In common with "in vitro" measurements Nomifensine 10^{-6}M had no significant effect on DA release although this dose is sufficient to increase the electrically stimulated release 'in vitro' approx. 400% (Kapoor and Arbuthnott, 1982).

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RETARDATION OF CEREBRAL DOPAMINE DEPLETION IN MICE TREATED REPEATEDLY WITH MORPHINE

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In rats withdrawn from chronic morphine treatment the α-methyl-p-tyrosine (αMPT)-induced depletion of dopamine (DA) is retarded in the limbic forebrain and after long enough treatment in the striatum, too (Attila & Ahtee, 1983 and 1984). There is some evidence that the cerebral DA turnover is also reduced in mice on withdrawal from morphine (Rosenman & Smith, 1972; de la Baume et al, 1979).

Male albino mice (24-31 g) were given either saline or morphine s.c. three times daily for 1-5 days increasing the single morphine dose from 100 to 200 mg/kg. After a withdrawal period of 1 to 7 days the mice were given αMPT i.p. and a challenge dose of morphine s.c. The DA concentrations in the hypothalamus, the striatum and the rest of the forebrain were measured by LCEC (Attila & Ahtee, 1984).

The αMPT-induced DA depletion was not altered in the hypothalamus but it was clearly retarded in the striatum and in the rest of the forebrain after discontinuation of the morphine treatment (Table 1).

Table 1 Effect of αMPT (250 mg/kg, 2 h) on cerebral DA concentration in mice 1 day after discontinuation of morphine treatment

Repeated treatment	DA concentration, µg/g±s.e.mean, n=5-15					
	Hypothalamus		Striatum		Rest of the forebrain	
	Control	αMPT	Control	αMPT	Control	αMPT
3-day treatment:						
Saline	0.34±0.02	0.19±0.02	3.93±0.17	2.05±0.13	1.13±0.04	0.52±0.02
Morph.	0.36±0.04	0.16±0.02	4.09±0.16	2.40±0.13	1.18±0.03	0.65±0.02*
5-day treatment:						
Saline	0.33±0.02	0.18±0.01	4.25±0.08	2.02±0.09	1.02±0.05	0.48±0.03
Morph.	0.32±0.04	0.21±0.03	4.44±0.24	2.87±0.08*	1.03±0.06	0.68±0.02*

*P<0.001 compared with corresponding repeated saline group.

10 mg/kg morphine challenge dose enhanced the αMPT-induced DA depletion by about 20% in the striatum and in the rest of the forebrain of both the control mice given saline repeatedly and the mice treated repeatedly with morphine. 30 mg/kg of morphine retarded the depletion of hypothalamic DA in control mice by 15% (P<0.05), but not in mice treated with morphine.

Our results indicate that repeated morphine treatment does not alter the hypothalamic DA turnover in mice but reduces the DA turnover in the striata and the rest of the forebrain. Tolerance seems to develop to the morphine-induced retardation of depletion of hypothalamic DA. However, repeated morphine treatment does not induce tolerance to the enhancement of DA depletion by morphine in the striatum and the rest of the forebrain.

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12P

DOES B.PERTUSSIS VACCINE INCREASE INSULIN SECRETION BY ANTAGONISM OF ENDOGENOUS CATECHOLAMINES?

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Bordetella pertussis vaccine and infection and a toxin from the organism (islet activating protein, pertussis toxin, pertussigen) have been shown to increase insulin secretion in vivo, in vitro and ex vivo and to prevent the inhibitory action of catecholamines on glucose-induced insulin release (Katada & Ui 1977, 1981, Furman et al 1981). The present work was undertaken to determine if the stimulating effect of B.pertussis vaccine on insulin secretion ex vivo can be explained by the prevention of the inhibitory action of endogenous catecholamines released from the noradrenergic nerves within the islets. Rats were injected with pertussis vaccine (2×10^{10} organisms i.p.) or saline. One week later the islets were isolated by collagenase digestion. Islets were incubated in batches of 5 in Krebs bicarbonate buffer (pH 7.4; 37°C; 95% O_2/5% CO_2) containing albumin (3 mg ml^{-1}) and various concentrations of glucose with or without adrenaline.

Glucose-induced insulin secretion was augmented in islets from pertussis vaccinated rats (e.g. Insulin secretion in 16.7 mM glucose: Control, 10.3±1.03 (n=9); pertussis 13.4±1.06 (n=10) ng $islet^{-1}$ h^{-1} $P<0.05$). In control islets adrenaline (2.7×10^{-9}M - 1.08×10^{-5}M) produced a concentration dependent inhibition of glucose-induced insulin secretion (e.g. Insulin secretion in 8 mM glucose: Control, 5.8±1.9; adrenaline (2.7×10^{-7}M) 0.83±0.3 ng $islet^{-1}$ h^{-1} $P<0.01$). Pertussis vaccine pretreatment prevented the inhibition of insulin secretion produced by adrenaline (2.7×10^{-9}M or 2.7×10^{-7}M) and attenuated the inhibition produced by a high concentration of adrenaline (1.08×10^{-5}M) (e.g. Insulin secretion in 8 mM glucose in control islets: Control, 5.5±0.7 ng $islet^{-1}$ h^{-1}; adrenaline (2.7×10^{-7}M) 1.84±0.26 ng $islet^{-1}$ h^{-1}; in islets from vaccine treated rats: control 7.7±1.3 ng $islet^{-1}$ h^{-1}; adrenaline (2.7×10^{-7}M)- 6.3±1.1ng $islet^{-1}$ h^{-1}).

In normal islets the α-adrenoceptor blocking drug phentolamine enhanced insulin secretion induced by 8 mM glucose (Control, 4.1±0.77; phentolamine (2.6 ± 10^{-6}M) 8.5±0.95 ng $islet^{-1}$ h^{-1} $P<0.01$). However, this is unlikely to be due to blockade of endogenous catecholamines since neither yohimbine nor idazoxan enhanced insulin secretion although each, like phentolamine, blocked the inhibitory effect of adrenaline. Prazosin neither stimulated insulin secretion, nor blocked the response to adrenaline. The above experiments using phentolamine were carried out using dilutions of standard phentolamine mesylate (Rogitine(R) injections. The experiments were repeated using freshly prepared solutions of phentolamine hydrochloride. In such experiments phentolamine had no stimulatory effect on insulin secretion in concentrations that prevented the inhibitory effect of a high concentration of adrenaline (1.08×10^{-5}M).

We suggest that the stimulatory effect of phentolamine on insulin secretion in vitro reported here and by Efendic et al (1975) is not due to α-adrenoceptor blockade and may be a peculiar property of the mesylate salt. Moreover, in view of the inability of various α-adrenoceptor blocking drugs to enhance glucose-induced secretion the enhancement produced by pretreatment with B.pertussis vaccine is unlikely to be due to blockade of the effects of endogenous islet catecholamines.

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IS THE MUSCARINIC RECEPTOR PRESENT ON THE VASCULAR ENDOTHELIUM ATYPICAL?

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Muscarinic receptors (mAChRs) have been classified into two subtypes (Hammer and Giachetti, 1982): M_1 (present in neural tissue and exhibits a high affinity for pirenzepine) and M_2 (present in the periphery and exhibits a low affinity for pirenzepine). It has also been reported (Furchgott and Cherry, 1984) that stimulation of the mAChR present on the vascular endothelium results in vasodilatation. The mAChR mediating this response has yet to be characterised. This study has compared the affinity of pirenzepine at the endothelial mAChR with that obtained at mAChRs present in other smooth muscle.

The following tissues were used: guinea-pig ileum, trachea and urinary bladder; dog femoral artery and rabbit thoracic aorta. All tissues were placed in modified Krebs bicarbonate physiological salt solution (pH 7.4, 30°C) under 1 g tension. Responses were measured isometrically, and concentration-response curves were constructed using carbachol as the agonist (Clague et al, 1984). Antagonist affinities were assessed using the method of Arunlakshana and Schild (1959). Three concentrations of pirenzepine were used, allowing 45 min equilibration at each concentration.

The antagonist affinities (pA_2) are shown in Table 1. The values obtained at mAChR present in the ileum, trachea and bladder were very similar and are in good agreement with values obtained by other workers (e.g. Barlow et al, 1981) The values obtained at mAChRs mediating vasodilatation were significantly ($p < 0.05$) higher than the values obtained at mAChRs present in the ileum, trachea or bladder. In addition, this value is lower than that reported by Brown et al (1980) for pirenzepine at ganglionic mAChRs (8.36)

We conclude that mAChR mediating vasodilatation is not easily accommodated into the current classification of mAChR subtypes.

Table 1 Antagonist affinities (pA_2) for pirenzepine at various types of smooth muscle

Aorta	Femoral Artery	Ileum	Trachea	Bladder
7.92# (7.81-8.03)	7.64 (7.31-7.97)	6.77 (6.67-6.87)	7.07 (6.95-7.19)	6.76 (6.64-6.88)

Values are mean with 95% confidence limits, n = 4. #Slope was significantly ($p < 0.05$) different from unity; the value, after imposing the unity constraint was 7.54.

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INHIBITION OF THE CHOLINERGIC TWITCH RESPONSE IN GUINEA PIG ILEUM: EVIDENCE FOR A RECEPTOR TO CLONIDINE DISTINCT FROM α_2 SITES

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There is a growing body of evidence to suggest that phenylethylamines and imidazolines may interact differently with alpha-adrenoceptors (see Ruffolo et al., 1983). In particular, it has been proposed that the alpha$_2$-adrenoceptor exhibits two sites or states, one recognizing the phenylethylamine structure and the other that of the imidazolines, such as xylazine and clonidine (Vizi et al., 1983; Mottram, 1983). The objective of the present experiments was to further investigate this proposal using noradrenaline and clonidine as agonists and rauwolscine, idazoxan (RX 781094) and benextramine as antagonists. Benextramine is a relatively specific irreversible alpha-adrenoceptor alkylating agent (Melchiorre et al., 1978). The test preparation used was the transmurally stimulated guinea pig ileum in which the cholinergically-induced "twitch" response is inhibited by alpha$_2$-adrenoceptor activation.

Segments of ileum (about 3cm) were set up in vitro in Kreb's solution of the following composition (mM): NaCl 118, $CaCl_2$ 2.6, KCl 4.9, $NaHCO_3$ 25, NaH_2PO_4 1, $MgSO_4$ 1.2, glucose 11.7 and propranolol 0.001. Electrical stimulation was done continuously at 0.1Hz with a pulse duration of 1msec at supramaximal voltage.

Both noradrenaline and clonidine inhibited the "twitch" response with mean IC_{50} values (nM) of 114 ± 4.1 (n=17) and 14.6 ± 0.8 (n=15), respectively. (The IC_{50} for noradrenaline was not altered by the addition of cocaine, 5μM, and corticosterone, 1μM, to the Kreb's solution). The intrinsic activities were: 1.0 for noradrenaline (100 percent inhibition) and 0.9 for clonidine. Following exposure of the ileum to benextramine (10μM for 30 min followed by 20 washes over a period of 20 min) the dose-effect curves to clonidine were virtually abolished whereas those to noradrenaline showed only a slight nonparallel shift to the right with a 10 percent reduction in the maximum response. In benextramine-treated preparations clonidine, even at 10μM, failed to inhibit the dose-effect curves to noradrenaline. Thus, noradrenaline and clonidine do not act at a common site. Other experiments with rauwolscine and idazoxan also revealed differences between noradrenaline and clonidine. Both inhibitors acted as competitive antagonists toward clonidine but not toward noradrenaline. The differences between clonidine and noradrenaline cannot be attributed to an alpha$_1$-adrenoceptor action of noradrenaline since prazosin (100nM) failed to alter the dose-effect curves to this agonist.

In conclusion, the present data indicate strongly that noradrenaline and clonidine act at different recognition sites. Thus, the distinct possibility of a separate imidazoline or imidazolidine receptor is raised.

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THE EFFECT OF NIFEDIPINE ON BRONCHOCONSTRICTION IN THE GUINEA-PIG IN VIVO

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We have previously shown (Rounding & Towart 1984) that the calcium antagonist nifedipine attenuates antigen-induced contractions of guinea-pig respiratory smooth muscle in vivo and in vitro. This was concluded to be due not to the inhibition of mediator release but to inhibition of smooth muscle contractility. To study this further we have examined the effect of nifedipine on broncho-constriction in the guinea-pig in vivo. Male Dunkin-Hartley guinea-pigs 400-500gms were anaesthetised using Fentanyl/Fluanisone and diazepam. The trachea was cannulated for the measurement of pulmonary air flow and a cannula inserted into the pleural cavity for the measurement of intrapleural pressure. From these values airways resistance (R_{aw}) and dynamic compliance (C_{dyn}) were calculated (Amdur & Mead 1958). The carotid artery (for measurement of BP) and the jugular vein were also cannulated. Bronchoconstriction was induced by histamine (8µg/kg i.v.) until a repeatable response was obtained. Nifedipine (500µg/kg i.v.) or DMSO (0.5ml/kg i.v.) as vehicle control were given 1 minute before the histamine. Nifedipine completely inhibited the histamine-induced bronchoconstriction but also caused a large fall in BP (Table 1). To try to prevent this hypotensive effect, we administered nifedipine by aerosol. Reproducible contractions were induced to aerosol histamine (100µg/ml for 30 secs). Nifedipine (10mg/ml 2.5 mins) or DMSO control (2.5mins) were administered 5mins before. No inhibition of the aerosol histamine-induced bronchoconstriction was seen even though there was a slight fall in BP (Table 1). Brugman et al (1982) have shown that aerosol nifedipine will inhibit the bronchoconstriction induced by aerosol citric acid in hyperreactive dogs. Responses were therefore obtained to aerosol citric acid (10% for 30secs, 40mins apart). Nifedipine (1-10mg/ml) or DMSO were given 5mins before the citric acid. Nifedipine dose-dependently inhibited the citric acid-induced constriction with nearly maximal inhibition at 10mg/ml (Table 1). There was a slight fall in BP at this higher concentration, indicating that at least some nifedipine was acting systemically.

These results indicate that nifedipine inhibits some types of bronchoconstriction in the guinea-pig in vivo. Some workers have suggested that aerosol administration of nifedipine may circumvent the cardiovascular effects of this drug when tested as an anti-asthmatic. However the limited effects of nifedipine observed in this study do not support this view.

Table 1 Effect of nifedipine on lung function changes in the anaesthetised spontaneously-breathing guinea-pig

Challenge	nifedipine administration	inhibition of R_{aw} rise	inhibition of C_{dyn} fall	
histamine 8µg/kg iv	500µg/kg iv	100%	100%	n=4
histamine 100µg/ml aerosol	10mg/ml aerosol	0%	0%	n=2
citric acid 100mg/ml aerosol	1mg/ml aerosol	0%	0%	n=3
citric acid 100mg/ml aerosol	5mg/ml aerosol	37% ± 7	10% ± 8	n=3
citric acid 100mg/ml aerosol	10mg/ml aerosol	77% ± 9	70% ± 10	n=4

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CARDIOVASCULAR SYNERGISTIC ACTION BETWEEN DILTIAZEM AND ALFUZOSIN UNDER IN VIVO AND IN VITRO CONDITIONS

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Alfusozin (SL 77.499) (N-[3-[(4-animo-6,7-dimethoxy-2-quinazolinyl)methylamine] propyl]tetrahydro-2-furancarboxamide hydrochloride) is a selective α-adrenoceptor antagonist which lowers bood pressure in animals (Cavero et al., 1984a,b) and man. This study was designed to evaluate whether the α-receptor blocking actions of alfuzosin could be enhanced by diltiazem.

Adult male SHR (5-6 months old) were anaesthetized with pentobarbitone (60 mg/kg i.p.) and prepared for blood pressure measurement (carotid artery) and drug administration (femoral vein). Three groups of animals were used, in which the antihypersensive effects of a) diltiazem (10 µg/kg/min infused for 30 min), b) alfuzosin (10 µg/kg given over 5 min) or c) the combination of these two drugs were studied.

Male rabbits (3-4 Kg) were sacrificed and pulmonary artery spirals were mounted in isolated organ baths in Krebs' bicarbonate at 37 °C. Concentration contractile-response curves were evoked by phenylephrine, before and after 30 min incubation with either alfuzosin (0.01-30 µM), diltiazem (300 nM), or the combination of these two drugs. The pA_2 for alfuzosin was calculated according to the method of Schild.

An i.v. infusion of diltiazem caused a small sustained fall in blood pressure of 12.6 ± 2.1 mmHg (n=15) from an initial value of 192 ± 2 mmHg. In contrast, alfuzosin induced at the end of its administration a maximum hypotensive effect of 19.7 ± 4.7 mmHg (n=6) which 15 min later was only 4.2 ± 1.5 mmHg. The injection of the same dose of alfuzosin to diltiazem-treated SHR, resulted in a profound fall in blood pressure of 58.4 ± 7.8 mmHg (n=7) at the end of the administration. This antihypertensive effect was still present (48.7 ± 8.7 mmHg, n=7) 15 min later.

Alfuzosin, but not diltiazem (300 nM), antagonized phenylephrine-induced contractions in the rabbit pulmonary artery; the calculated pA_2 was 7.6 (slope 0.87, r=0.9, n=15). The potency of alfuzosin against phenylephrine was markedly increased (pA_2=8.5; slope 0.9, r=0.94, n=20) in diltiazem-bathed strips.

These results demonstrate a synergistic interaction between diltiazem and alfuzosin in terms of their antihypertensive effects in SHR and against phenylephrine-induced contractions in rabbit pulmonary artery. The mechanism underlying this observation remains to be clarified, but is unlikely to be pharmacokinetic in origin, since it also occurred in vitro.

This pharmacological interaction between alfuzosin and diltiazem may have clinical potential in several cardiovascular situations in which a decrease in peripheral resistance is desirable.

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EFFECTS OF THE DIHYDROPYRIDINE BAY K8644 ON GUINEA PIG ISOLATED TRACHEALIS

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Calcium antagonists (such as the dihydropyridine nifedipine) have characteristic inhibitory effects on respiratory and other smooth muscle. In general, contractions induced by calcium entry through potential-dependent channels (PDC) are more sensitive to inhibition than those induced by either calcium entry through putative receptor-operated channels (ROC) or by mobilisation of intracellular calcium stores (Triggle, 1983). A novel dihydropyridine calcium agonist (BAY K8644) has recently been described which increases calcium ion flux through the PDC and thus has positive inotropic and hypertensive effects (Schramm et al, 1983). It has been shown (Towart et al, 1983) for aortic smooth muscle that BAY K8644 potentiates K^+ (spasm mainly involving PDC) but has no effect on noradrenaline-induced contractions (release of intracellular Ca^{2+} or Ca^{2+} entry through ROC). We have now examined the effects of BAY K8644 on airways smooth muscle.

Segments of trachea from guinea-pigs of either sex were prepared for isometric tension recording (Foster et al, 1983a). The Krebs solution contained 0.8 μM indomethacin to inhibit prostaglandin formation. Cumulative concentration-response curves were constructed for acetylcholine (ACh) and then to either KCl, TEA or histamine. This latter concentration-response curve was reconstructed in the presence of BAY K8644 or its solvent (concurrent controls). Finally the concentration-response curve to ACh was reconstructed, again in the presence of BAY K8644 or solvent.

BAY K8644 had a modest spasmogenic action (0.1 μM, 0% ACh max; 0.1 μM, 13±6% (n=6); 1 μM, 17±2% (n=9) but did not affect concentration-response curves of ACh or histamine. In contrast BAY K8644 1 μM caused two-fold potentiation of K^+ (n=9) and 3.1 fold potentiation of TEA (n=8). In preliminary experiments measuring $^{45}Ca^{2+}$ uptake by the lanthanum method (Foster et al, 1983b) BAY K8644 1 μM significantly increased calcium influx into the trachealis.

These results indicate that BAY K8644 is active on respiratory smooth muscle and provide additional evidence that ACh and histamine cause spasm of trachealis by a mechanism which differs from that utilised by KCl or TEA.

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18P

PHOTODESTRUCTION OF NIFEDIPINE REVEALS MULTIPLE MECHANISMS OF ANTAGONIST ACTION

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Voltage-activated calcium channels, present in the membranes of cardiac and smooth muscle cells, provide an important link in excitation-contraction coupling. Organic 'calcium antagonists' inhibit ion flow through these channels and are effective in the treatment of several cardiovascular disorders. Nifedipine, a potent and widely used calcium antagonist, contains an *o*-nitrobenzyl moiety and is photolabile; irradiation destroys its activity, the reactions being complete within 100 μs (Morad et al, 1983).

Whole-cell currents through Ca^{++} channels of single, cultured rat ventricular cells were evoked by stepping the membrane potential from a holding level of -50 mV to values more positive than -20 mV. Nifedipine suppressed these currents, $IC_{50} \simeq$ 0.5 μM, and this effect was reversed by light flashes of 1 ms duration produced by a xenon flashlamp. Blockade was independent of the test potential and the drug did not affect the kinetics of current activation. In the presence of 0.5 μM nifedipine, with 140 mM Cs^{+} in the recording pipette to block outward currents, and either Ca^{++} or Ba^{++} ions (5 -10 mM) as the current carrier, flashes increased the current amplitude (ca. 2 fold) within a few milliseconds. When a flash was presented before, or at the peak current, blockade was completely reversed and the flash-induced current increased with the same rate constant as the normal, voltage-activated current (Ba^{++}: 0.42 ± 0.03 ms^{-1}; Ca^{++}: 1.90 ± 0.02 ms^{-1}, 10mV, 20°C). These results suggest that, before the peak current, nifedipine binds mainly to the resting, closed state of the channel and prevents it from opening in response to depolarisation. Nifedipine also appeared to interact with the open Ca^{++} channel: it accelerated the rate of current inactivation (4 fold, 0.5 μM nifedipine, Ba^{++}, 20°C) and current increases induced by flashes presented after the peak current (during its decaying phase) appeared to have a small step-like component, suggesting that some unblocked channels were able to conduct instantly. Presenting flashes after the peak current revealed a third mechanism of nifedipine blockade: flashes became increasingly less effective at reversing blockade when delivered later and later during a depolarising voltage step. We interpret this to mean that photodestruction of nifedipine, at long times after the peak current, unblocked Ca^{++} channels that could not conduct i.e. inactivated channels. The latter two effects were, however, more pronounced when Ba^{++}, rather than Ca^{++}, carried the current.

Thus like other types of organic calcium antagonist, nifedipine appears to interact with the cardiac Ca^{++} channel in at least three kinetic states: a closed state, the open and an inactivated state, and our results were well modelled by the 'modulated receptor' hypothesis described by Hille (1977) and Hondeghem and Katzung (1977). Under our experimental conditions, however, block of closed channels appeared to be the most significant mechanism when Ca^{++}, the physiological ion, carried the current, suggesting that this effect could be the most important therapeutically.

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THE RELAXANT EFFECT ON THE RABBIT AORTA OF THE INHIBITORY FACTOR FROM THE BOVINE RETRACTOR PENIS IS NOT ENDOTHELIUM DEPENDENT

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We have previously described some of the properties of a smooth muscle inhibitory factor (IF) extracted from the bovine retractor penis muscle including its ability to relax arterial smooth muscle (Gillespie et al., 1981; Bowman & Gillespie, 1983). Furchgott and his colleagues have demonstrated that smooth muscle relaxation of blood vessels by several drugs is dependent on the release from the endothelium of a relaxant factor (EDRF). It is possible that the IF is also dependent on endothelium for its relaxant effect. On the other hand both agents have properties in common. The relaxant effect of both is abolished by borohydride or by haemoglobin and both appear to be mediated by the activation of guanylate cyclase (Furchgott, 1984; Bowman & Drummond, 1984). It is possible therefore that both compounds are related. If so the relaxant effect of the IF should be independent of the presence of endothelial cells.

We have tested this point by comparing the relaxant effect of carbachol (endothelium dependent), caffeine (endothelium independent) and the IF on rubbed and unrubbed strips of rabbit abdominal aorta contracted with either noradrenaline or 5HT. We have also examined the dependence of each of the three stimuli together with sodium nitroprusside on guanylate cyclase by measuring the ability of methylene blue, which inhibits guanylate cyclase, to abolish the relaxant effect.

In confirmation of the work of several other authors carbachol caused the expected dose-related relaxation of unrubbed aortic strips but had no relaxant effect on rubbed strips, causing only contraction at higher doses. In contrast the IF from the bovine retractor penis caused relaxation in both rubbed and unrubbed strips. Methylene blue (10^{-5}M) blocked completely the inhibitory response to carbachol but only reduced that to both sodium nitroprusside and the IF by about 20%. The response to caffeine was unchanged.

These results show that the IF does not relax arterial smooth muscle by releasing EDRF. The differential inhibition by methylene blue of relaxation by carbachol and by the IF suggest the latter is not identical with the EDRF.

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20P

MODIFICATION OF THE EFFECTS OF BAY K 8644 IN RAT AORTA BY THE ENDOTHELIUM

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Endothelial derived relaxant factor (EDRF; Furchgott, 1983) can be liberated following increases in intracellular Ca^{++} in the endothelial cells. Reduced contractility to α_2-adrenoceptor stimulants in the presence of endothelium has been ascribed to increased release of EDRF by these agents (Miller et al., 1984). While the increased release of EDRF probably follows increases in cytoplasmic Ca^{++}, the role of Ca^{++} channels in this effect is controversial. We have therefore tested whether Ca^{++} channel activation by Bay K 8644 can release EDRF.

Pairs of rat aorta rings from male rats (200-350g) were opened and one ring was lightly rubbed to remove the endothelium. The rings were mounted between two clips in 10 ml organ baths filled with Tyrode solution at 35°C, gassed with 95% O_2: 5% CO_2. Tissues were contracted with phenylephrine (1 µM) and challenged with acetylcholine (1 µM), which induced a relaxation of 66.4 ± 5.3% (n=12) in the control strips and of 1.6 ± 0.8% (n= 12) in the rubbed strips: the relaxation was abolished by atropine (1 µM).

Bay K 8644 (0.01-1 µM) did not contract aorta preparations in normal Tyrode solution (2.7 mM K^+) but when the K^+ was increased to 12 mM subsequent addition of Bay K 8644 caused sustained contractions, sometimes with superimposed spontaneous contractions. Both maximum developed tension and the pEC50 for Bay K 8644 were significantly greater in the rubbed preparations than in those with endothelium. These findings might indicate that Bay K 8644 contracted smooth muscle cells but also released EDRF at the same time. Nifedipine (0.1 µM for 25 min) shifted the curves to Bay K 8644 to the right; the displacement was identical in the presence and absence of endothelium. Verapamil (1 µM for 25 min) was only effective in shifting the Bay K 8644 curve in the absence of endothelium. In contrast, cinnarizine (1 or 10 µM for 90 min) reduced the maximum effects of Bay K 8644 without changing the EC50 values, indicating that cinnarizine has a site of action beyond that of the Ca^{++} channel activator.

EDRF increases cyclic GMP in smooth muscle cells. Cyclic GMP levels were measured (Miller et al., 1984): there was a 2-3 fold increase in the basal cyclic GMP level in the preparations with endothelium, but Bay K 8644 (1 µM + 12 mM K^+) did not increase this level. Thus either the effects of EDRF are independent of cyclic GMP in the presence of Bay K 8644 or EDRF is continually released and reduces the sensitivity of the preparations to Bay K 8644, perhaps by hyperpolarizing the smooth muscle cells. In support of the latter possibility, increasing K^+ to 15 mM increased responsiveness to Bay K 8644, although the rubbed preparations were still more sensitive. Nevertheless, it is difficult to envisage how a single layer of cells can continually release a substance having such marked effects on contractility when EDRF has a claimed half life of 6 s (Griffith et al., 1984).

In conclusion these findings indicate that the presence of endothelial cells can markedly influence sensitivity to Bay K 8644, but Bay K 8644 may not stimulate release of EDRF.

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THE EFFECT OF CAPTOPRIL ON THE BARORECEPTOR REFLEX IN THE CAT

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There are indications that angiotensin converting enzyme inhibition (CEI) may modulate the baroreceptor reflex. It has been noted that the hypotensive action of CEI is not accompanied by a reflex tachycardia (e.g. Clough, Hatton & Conway, 1981). Experiments designed to examine the effects of CEI on baroreceptor reflexes have yielded conflicting results (Clough et al, 1981; Lee & Lumbers, 1981). In the present study, two different techniques were used to assess the effects of the CEI, captopril, on the baroreceptor reflex.

Cats were anaesthetised with α-chloralose (70 mg kg^{-1}) and artificially ventilated. The animals' temperature, level of ventilation and the pH, PCO_2 and PO_2 of arterial blood were maintained within normal limits.

In four cats, both carotid sinuses were perfused via the common carotid arteries with the animals' own blood taken from the left common carotid artery. The perfusate was returned to the jugular vein via the external carotid arteries. The internal carotid artery and any branches arising from the sinus were tied off. Pressure within the carotid sinus was measured by means of a cannula inserted into the sinus through the lingual artery. Both vagi were cut in the neck. Carotid sinus pressure (CSP) was maintained at a holding pressure for at least 5 min and then the CSP was either raised or lowered by differing amounts and the reflex change in systemic blood pressure (B.P.) recorded. Between interventions CSP was returned to the holding pressure for 5 min to eliminate effects due to resetting of the baroreceptors. Recordings were made during an initial control period, and 30 min after the start of infusion of captopril (3 mg kg^{-1} bolus), followed by 200 μg kg^{-1} h^{-1} i.v. at a rate of 40 μl min^{-1}.

Captopril produced a significant reduction in the slope of the line relating the fall in B.P. to the rise in CSP when this slope was compared with that found in the control period. A mean value of -0.31 ± 0.04 mm Hg fall in B.P. per mm Hg rise in CSP (Mean ± S.D.) was found during captopril infusion compared to a value of -1.28 ± 0.01 during the control period. Captopril abolished the response to a fall in CSP in all four cats.

In a further four cats, the effect of captopril on the relationship between heart period and the rise in blood pressure produced by the bolus injection of phenylephrine (4-8 μg kg^{-1} i.v.) was assessed (Smyth, Sleight & Pickering, 1969). Captopril had no consistent effect on the slope of this relationship but in three cats did result in a shift to the left of this relationship.

It is concluded that captopril does attenuate the baroreceptor reflex and that this modulation is particularly pronounced in the reflex responses to falls rather than to rises in CSP. However, these changes in the baroreceptor reflex cannot be consistently detected using the technique of Smyth et al. (1969).

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22P

SK&F 94120, A NOVEL AGENT WITH POSITIVE INOTROPIC AND VASODILATOR ACTIVITY

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Treatment of cardiac failure is based on the use of diuretics, positive inotropic agents and vasodilators used either separately or in combination.

SK&F 94120, 5-(4-acetamidophenyl)-pyrazin-2(1H)-one, is a novel agent possessing both positive inotropic and vasodilator activity.

SK&F 94120 increased the force of contraction in isolated ventricular preparations from guinea-pig, cat, dog and marmoset hearts, threshold concentration about 1×10^{-7}M. SK&F 94120 did not exhibit positive inotropic activity in isolated hearts from rats.

In anaesthetised cats treated with mecamylamine, 5 mg/kg i.v. to prevent reflex stimulation of the heart, and propranolol, 1 mg/kg i.v. to prevent β-adrenoceptor responses, SK&F 94120 administered intravenously increased left ventricular dp/dt max at doses in excess of 30 µg/kg with minimal changes in heart rate.

Vasodilator activity of SK&F 94120 was measured in rat hindquarters autoperfused at constant pressure. SK&F 94120 increased hindquarters blood flow over the dose range 1-5 mg/kg i.v.

In anaesthetised cats, SK&F 94120, 100 µg $kg^{-1}min^{-1}$ by intravenous infusion caused increases in dp/dt max, cardiac output and stroke volume and decreased total peripheral resistance; heart rate increased minimally and changes in blood pressure were small and inconsistent. In animals in which heart rate was maintained constant by atrial pacing, the increase in cardiac output caused by SK&F 94120 was similar to that which occurred in animals without atrial pacing.

In conscious dogs, SK&F 94120, 5 mg/kg orally, caused large increases in left ventricular dp/dt max (measured from a solid state transducer implanted in the left ventricle) with minimal changes in blood pressure and heart rate (measured from a cannula implanted in the descending aorta).

These studies provide evidence that SK&F 94120 has both positive inotropic and vasodilator activity and may be a useful drug for the treatment of cardiac failure.

BIDIRECTIONAL FLUXES AND FACTORS AFFECTING THE MOVEMENT OF LITHIUM IN ISOLATED JEJUNAL MUCOSA OF GUINEA PIG

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Oral lithium is widely used in the prophylactic treatment of manic-depressive psychoses (Birch et al 1982). Lithium (Li^+) absorption from small intestine mucosal to serosal (M to S) is passive both in vitro, (Birch et al 1983, 1984a; Karim et al 1984) and in vivo (Ehrlich et al 1983) and is not affected by temperature, substrate depletion and metabolic inhibitors (Birch et al 1984b). We report now transport studies in a three compartment system in contrast to the multicompartment model previously employed.

Isolated jejunal mucosa of guinea pig was prepared devoid of underlying muscle and connective tissue layers and mounted to form a membrane separating two flux chambers (Lauterbach 1977). The tissue was bathed on both sides with oxygenated Krebs-Tris buffer at 37°C. Lithium replaced Na^+ to a maximum of 20mM.

Bi-directional transfer of Li^+ across the epithelium was measured using the stable isotopes ^6Li and ^7Li (Birch et al 1978). Lithium was determined using either an IL Video 22 or IL 357 atomic absorption spectrometer. The former, a dual channel instrument, was used in all isotopic experiments. Viability was tested by observation of active transport, potential difference and histological integrity. Bi-directional fluxes of ^6Li and ^7Li showed no assymetry suggesting that there was no active component. Both the luminal and basolateral surfaces handled Li^+ isotopes similarly. Unlike Na^+, Li^+ movement was independent of glucose transport and there appeared to be no significant interaction between Li^+ and either Ca^{2+} or Mg^{2+}.

Acidification of the serosal side alone (pH 5.4) stimulated Li^+ absorption ($P<0.012$) whereas mucosal acidification alone had no effect on transport. Neither treatment affected tissue uptake. Lithium, therefore, might be substituting for Na^+ in the Na^+/H^+ exchanger (Benos 1982). The pH gradient dependent increase in absorption was inhibited by 1mM Amiloride ($P<0.0004$).

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24P

THE FATE OF EUGENOL IN THE RAT AND ITS VARIATION WITH DOSE

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The allylbenzenes are an important group of food flavours which occur in the essential oils of many herbs and spices, certain of which have been shown to be carcinogenic in the mouse, albeit at doses far in excess of human dietary exposure levels (1). These compounds are unreactive, but undergo metabolic activation by hydroxylation at the 1'-position in the side chain (1). Eugenol (4-hydroxy-3-methoxyallylbenzene) is the major component of Oil of Cloves and is used as a food flavour, fragrance agent and in dental therapeutics. Human dietary exposure has been estimated to be 0.6mg/capita/day. Despite its structure, it is apparently not a carcinogen at doses at which the related compounds safrole and estragole induce liver tumours in rodents. To further our knowledge of the relationships between chemical structure, dose size, metabolic fate and toxic effects amongst the allylbenzenes, we now give a preliminary account of the fate of eugenol in the rat at doses ranging from levels close to human dietary exposure to those used in toxicity tests.

[ring-^{14}C]Eugenol was administered by stomach tube in trioctanoin to female Wistar albino rats (b.w. 200g) at dose levels of 0.5,5,50 and 1000mg/kg (10-15μCi/rat). The animals were kept in metabolism cages, urine and faeces collected for 3 days and excretion of ^{14}C monitored by scintillation counting. Urinary metabolites were separated by solvent extraction, TLC and HPLC before and after treatment of the urine with β-glucuronidase or sulfatase, and characterized by GC-MS as such and as methyl or TMS derivatives.

The elimination of ^{14}C by these animals was rapid, and occurred predominantly in the urine (75-80% of dose in 24h), with some 10% in the faeces. Both rate and route of elimination were independent of dose size. At all dose levels the major excretion product was conjugated eugenol (ca. 50% of dose) but the nature of the conjugate was dependent upon dose. At low doses, eugenol sulphate predominated, but at 1000mg/kg, its glucuronide was the major metabolite. In addition there were present at the lower doses reduced metabolites, 4-hydroxy-3-methoxy and 3,4-dihydroxy-propylbenzene which were excreted conjugated with glucuronic acid or sulphate. Some 10% of the dose at all dose levels is excreted in the form of a number of uncharacterized acidic metabolites. Anaerobic incubation of eugenol with rat caecal contents has shown the ability of the gut flora to effect its reduction and O-demethylation.

The metabolism of the structurally related rodent hepatocarcinogens safrole and estragole involves in part oxidation of the side chains to the presumed proximate carcinogenic metabolites, the importance of which increases with increasing dose (2). In contrast, the major routes of metabolism of eugenol involve conjugation and C=C reduction, reactions generally associated with a reduction in chemical reactivity. This may account for the lack of carcinogenicity of this particular allylbenzene.

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INOSITOL PHOSPHOLIPID HYDROLYSIS AND Ca^{2+} FLUX IN THE RESPONSES OF RAT PLATELETS TO ADP AND THROMBIN

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Blood platelets contain receptors for stimulatory and inhibitory agonists. Inhibitory agonist (e.g. PGI_2)-receptor interaction is coupled to adenylate cyclase activation and consequent cAMP formation. Stimulatory agonist (e.g. Thrombin)-receptor interaction is coupled to phosphoinositide hydrolysis and consequent formation of 1,2-diacylglycerol (DAG) and elevation of cytosolic free calcium (Caf) (Feinstein et al., 1981; Nishizuka, 1984).

Although there exist species variations in platelet responses to stimulatory and inhibitory agonists, it is assumed that the transduction processes that modulate platelet reactivity are similar in all species. As a prelude to investigating the ontogeny of the enzymes involved in stimulus-response coupling in (rat) promegakaryocytes and (rat) platelets, we sought to establish the relationship, if any, between phosphoinositide hydrolysis and Ca^{2+} flux in the response of rat platelets to the stimulatory agonists Thrombin and ADP.

Plasma-free suspensions of rat platelets prepared essentially as described for human platelets (Moncada et al., 1982), were used in all studies. As an index of phosphoinositide hydrolysis, formation of [^{32}P]-phosphatidate (PA) was measured in platelets labelled with [^{32}P]-PO_4 (150μCi; 37°C; 90 min). Aliquots of pre-labelled platelets, suspended in Ca^{2+}-free, phosphate-free buffer were incubated with agonist or vehicle as appropriate, prior to extraction of lipids, separation by t.l.c. and quantification of [^{32}P]-PA by liquid scintillation counting (Pollock et al., 1984). For estimation of Caf, rat platelets (~ 10^8/ml) in modified Tyrodes solution were incubated (37°C; 3 min) with Quin-2-acetoxy methyl ester (20μM) prior to centrifugation and resuspension (~ $5x10^8$/ml) in fresh buffer. Extracellular [Ca^{2+}]-free was restored to 1mM 60s before the addition of agonists and measurement of resultant changes in fluorescence (Tsien et al., 1982).

ADP (0.1-30μM) and Thrombin (0.01-1u/ml) induced concentration-dependent formation of [^{32}P]-PA which was maximal (~ 2.5 fold and ~ 9 fold above basal, respectively) within 30-60s of agonist addition. When added to Quin 2 loaded platelets, ADP (0.3-3μM) and Thrombin (0.01-0.3u/ml) elicited a rapid, concentration-dependent elevation of Caf to around 0.8μM and 1.1μM respectively, from a basal level of 76±11nM.

These results indicate that following interaction with their receptors on rat platelets, the physiological stimuli thrombin and ADP initiate rapid phosphoinositide hydrolysis and concomitant elevation of Caf. For both agonists, maximum elevation of Caf occurs at lower concentrations than maximum phosphatidate formation, perhaps indicative of the receptor reserve of the system. In general, these findings are compatible with those observed in platelets of other mammalian species, although ADP-induced phosphoinositide hydrolysis is more readily demonstrable in rat than in human platelets. Thus the transduction processes of stimulatory agonists would appear to be similar in platelets of different species. Whether similar transduction processes exist in the platelet progenitor cells remains to be established.

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THE EFFECTS OF PROPRANOLOL ON ^{86}Rb EFFLUXES IN RAT THYMOCYTES AND HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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There is evidence that β-adrenoceptor antagonists may affect potassium homeostasis. Propranolol was shown to adversely affect the ability of human subjects to handle an acute KCl load (Rosa et al, 1980). The Medical Research Council Working Party on Mild to Moderate Hypertension (1981) reported that serum potassium increased significantly in patients on propranolol treatment. We have previously reported that propranolol stimulated ^{86}Rb (used as an analogue of K) influx in rat thymocytes (Murphy & Ryan, 1983). The stimulation by propranolol of ^{86}Rb influx in rat thymocytes was 1) independent of β-adrenoceptor antagonism, 2) detectable in the presence of ouabain and 3) dependent on extracellular calcium. We have now extended our investigations to the effects of propranolol on ^{86}Rb efflux in rat thymocytes and human peripheral blood lymphocytes and have also examined the effect of propranolol on cellular K concentrations in rat thymocytes.

Thymus glands were removed from male Wistar rats (100-150g) and thymocytes were isolated at 4°C by mincing, aspiration and separation through gauze. Human peripheral blood lymphocytes were isolated by centrifugation on a ficoll-metrizoate gradient. Cells were loaded with ^{86}Rb by incubation at 37°C in medium containing ^{86}Rb. Cells were washed twice by centrifugation in radioactive-free medium. For measurement of efflux rates, cells were resuspended in radioactive-free medium and triplicate samples of cell suspensions were removed at time intervals up to 90-115 min. After sampling, cells were rapidly separated from medium by centrifugation in an Eppendorf microcentrifuge. Flux rates were calculated by the procedure of Segel & Lichtman (1976).

In rat thymocytes, control efflux rates were 7.17 ± 0.63 f mol cell^{-1} h^{-1}. (±)-Propranolol (50 μM) significantly increased the efflux rates to 11.23 ± 0.65 ($p<0.001$). In human peripheral blood lymphocytes, the control efflux rates were 2.38 ± 0.10. (±)-Propranolol (50 μM) also significantly increased the efflux rates in those cells to 3.51 ± 0.17 ($p<0.025$). The percentage stimulation by propranolol of ^{86}Rb efflux rates was similar in both cell types. To assess the effect of propranolol on cell K concentration; rat thymocytes were incubated for 30 mins at 37°C in control and (±)-propranolol (50 μM)-containing media. After separation by centrifugation, cell K was measured by flame emission spectrophotometry. Control values for rat thymocyte cell K were 152 ± 7 m mol/l.cell H_2O and (±)-propranolol (50 μM) significantly reduced cell K to 129 ± 8 ($p<0.005$).

These results show that (±)-propranolol (50 μM) stimulated ^{86}Rb efflux rates in both rat thymocytes and human peripheral blood lymphocytes. The effect, as shown in rat thymocytes, can result in a reduction in cell K. Though the dose of propranolol used in these in vitro experiments is higher than those likely to occur with therapeutic use of propranolol, these findings may be relevant to the report by Pedersen et al. (1979) of hyperkalaemia in hypertensive patients on propranolol therapy without any alteration in total exchangeable K.

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12-O-TETRADECANOYL-PHORBOL-13-ACETATE (TPA) INHIBITS AGONIST-INDUCED PHOSPHOINOSITIDE METABOLISM AND Ca^{2+} FLUX IN HUMAN PLATELETS

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Platelet activation is regulated by the actions and interactions of two second messenger molecules: cytosolic free Ca^{2+} (Caf; via stimulation of Ca^{2+}-calmodulin dependent protein kinases) and 1,2-diacyl glycerol (DAG; via stimulation of protein kinase C) that are produced as a consequence of receptor-mediated phosphoinositide hydrolysis (Nishizuka, 1984).

The effects of DAG can be mimicked by tumour-promoting phorbol esters (e.g. 12-O-tetradecanoyl-phorbol-13-acetate, TPA) (Castagna et al., 1982). Although TPA-induced platelet activation is independent of elevation of Caf, it augments platelet responsiveness to modest (subthreshold) elevation of Caf induced by Ca^{2+} ionophores (Rink et al., 1983). to investigate the role of protein kinase C in modulating platelet responses to agonists, we examined the effects of TPA on agonist-induced phosphoinositide metabolism and Ca^{2+} mobilisation.

All studies were performed using plasma-free suspensions of human platelets. TPA, the inactive 4β-phorbol or their vehicle (DMSO) were pre-incubated with platelets for 1-2 min at 37°C before the addition of agonists. Caf was measured using platelets pre-labelled with the fluorescent Ca^{2+} indicator Quin 2, and changes in inositol phospholipid metabolism were monitored as [^{32}P]-phosphatidic acid (PA) formation in platelets pre-labelled with [^{32}P]-PO_4 (Pollock et al., 1984).

TPA (<1.6μM) alone exerted no significant effect on platelet Caf or [^{32}P]-PA levels. Thrombin (0.1u/ml), PAF (10nM) and Vasopressin (VP; 100nM) induced formation of [^{32}P]-PA (respectively ~5 fold, ~5 fold and ~3 fold over basal) and elevation of Caf (from 90±3nM = basal, to around 200-400nM in different experiments). These effects of Thrombin, PAF and VP were inhibited in a concentration-dependent manner, by TPA (10nM-1.6μM) but not by 4β-phorbol (<1.6μM).

Inhibition of agonist-induced phosphoinositide metabolism and Ca^{2+} mobilisation by TPA suggests that, besides its role in mediating so-called 'Ca^{2+}-independent' pathways of platelet activation, protein kinase C might also be involved in the mechanism(s) whereby the transduction processes initiated by agonist-receptor interaction at the surface of platelets, (and of other cells) are terminated.

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DIRECT AND INDIRECT EFFECTS OF ADP ON PLATELET CYTOSOLIC FREE CALCIUM

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Platelet responses to agonists are modulated by the opposing actions of intracellular second messenger molecules: diacylglycerol and cytosolic free calcium ions (Caf) mediate platelet activation whereas elevation of the cAMP concentration ([cAMP]) mediates inhibition of platelet activation (Kawahara et al., 1980; Haslam et al., 1978). This effect of cAMP may be due, in part, to promotion of Caf sequestration (Feinstein et al., 1981). There is some controversy as to whether a reduction in the basal [cAMP], perhaps in a specific pool, leads to platelet activation (Haslam et al., 1978). It has been claimed that in platelets exposed to adenylate cyclase stimulants (e.g. PGI_2), ADP and adrenaline, via inhibition of adenylate cyclase, indirectly promote Ca^{2+} mobilisation and enhance platelet activation (Zavoico & Feinstein, 1984). Other agonists, including vasopressin, which do not inhibit adenylate cyclase also enhance platelet activation (Grant & Scrutton, 1980).

To evaluate the role of basal and elevated [cAMP] in modulating human platelet Caf we examined the effects of ADP, adrenaline and vasopressin on platelet responses to PAF in the presence and absence of PGI_2. Using gel-filtered platelets suspended in a modified Tyrodes solution, platelet aggregation was monitored photometrically, Caf was monitored using quin 2 (Pollock et al., 1984) and cAMP was estimated by radioimmunoassay (Harper & Brooker, 1975).

In control platelets, adrenaline (50nM), ADP (100nM) and vasopressin (10nM) augmented PAF-induced platelet aggregation. ADP (0.1-5μM) and vasopressin (0.1-2μM) but not adrenaline (<5μM) directly induced elevation of Caf. Adrenaline and sub- or supra-threshold concentrations of ADP or vasopressin did not augment PAF (9-900nM)-induced elevation of Caf. In platelets pretreated with PGI_2 (10nM), adrenaline (5μM) and ADP (0.1-5μM) attenuated the elevation of cAMP and potentiated PAF-induced aggregation and elevation of Caf. However, vasopressin (<2μM) did not affect the [cAMP] or the responses to PAF in PGI_2-treated platelets.

These results indicate that adrenaline exerts an indirect effect (via adenylate cyclase inhibition), vasopressin a direct effect (presumably via phosphoinositide hydrolysis) and ADP a dual effect (direct and indirect) on platelet Caf. The failure of ADP or adrenaline to potentiate agonist-induced Ca^{2+} flux in the absence of PGI_2 suggests that platelet Caf is not regulated by the basal [cAMP]. Moreover, the potentiation by ADP, adrenaline or vasopressin of aggregation observed under these conditions is independent of changes in Caf.

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EFFECTS OF Ca^{2+}-ENTRY BLOCKERS ON SPONTANEOUS AND ELECTRICALLY-EVOKED RELEASE OF TRANSMITTER FROM GUINEA-PIG VAS DEFERENS NERVES

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While Ca^{2+}-entry blockers are effective antagonists both postsynaptically against K^+-induced contractions and presynaptically against K^+-evoked transmitter secretion, they are comparatively ineffective against those Ca^{2+} channels involved in transmitter release from the nerve terminal varicosities (Alberts, Bartfai & Stjarne, 1981). High concentrations (circa 10^{-4}M) of these agents, however, reduce the release of transmitter evoked by the nerve action potential. In the present study, we have further investigated (Beattie, Cunnane & Muir, 1984) the mechanism of this action in guinea-pig vas deferens using conventional techniques by comparing the effects of nifedipine, verapamil, diltiazem, nicardipine and cobalt on (a) action potential conduction in vas deferens nerve, (b) spontaneous and electrically-evoked release of tritiated noradrenaline, in the presence of normetanephrine, 10 uM desmethylimipramine, 0.6 uM, atropine 2.6 uM and ascorbic acid, 114 uM,. Under these conditions more than 90% of the released tritium is intact ^{3}H-noradrenaline (Alberts et. al., 1981), (c) Intracellularly-recorded excitatory junction potentials (EJPs, SEJPs).

Effects on spontaneous transmitter release: Nifedipine (10^{-4}M) had no effect on SEJPs or on tritium overflow; verapamil, diltiazem and nicardipine each (10 uM-300 uM) produced a dose-dependent increase in spontaneous tritium overflow (metabolite studies in progress) with no corresponding increase in SEJP frequency. Further examination of the effect of verapamil on spontaneous tritium release showed that the presence of external Ca^{2+} in the bathing medium was not required. The increased tritium overflow was abolished however by pretreatment with 6-OHDA (150 mg/kg days 1 & 2, sacrificed day 3) to destroy sympathetic nerves.

Effects on electrically-evoked release: Nifedipine at concentrations up to 10^{-4}M was ineffective against any of the parameters measured. Verapamil, diltiazem and nicardipine had no effect on electrically- evoked tritium release or EJPs in concentrations (up to 10 uM) which did not increase spontaneous tritium overflow. These drugs blocked EJPs but only at concentrations (circa 100 uM) which blocked action potential conduction in vas deferens nerve. They also appeared to block the electrically evoked rise in tritium efflux (superimposed on the increased background). The inorganic Ca^{2+}-entry blocker cobalt (2 mM) blocked electrically-evoked tritium release at concentrations which did not affect nerve conduction in preterminal fibres or spontaneous tritium overflow. These results indicate that organic Ca^{2+}-entry blockers inhibit transmitter release by nerve stimulation only by preventing the nerve action potential from reaching the secretory varicosities.

In conclusion, Ca^{2+}-entry blockers reduce electrically-evoked transmitter release by preventing the action potential from reaching the secretory varicosities rather than by blocking calcium channels in invaded varicosities. Unlike their antagonism of K^+-induced contractions and K^+-induced transmitter secretion, which occur at much lower concentrations (less than 10 uM, Alberts et. al., 1981) the effects of organic Ca^{2+}-entry blockers on nerve action potential evoked release require high concentrations indicative of a 'local anaesthetic' effect.

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30P

THE EFFECTS OF NICERGOLINE AN α-ADRENOCEPTOR BLOCKING DRUG ON ISCHAEMIA AND REPERFUSION-INDUCED ARRHYTHMIAS IN ANAESTHETISED DOGS

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Both α-adrenoceptor blocking drugs (Sheridan et al., 1980) and inhibition of thromboxane synthesis (Coker, 1984) have been shown in various animal models to reduce the incidence of ventricular fibrillation which results from myocardial reperfusion. The aim of this study was to investigate the action of nicergoline, a drug with α-adrenoceptor blocking (Arcari et al., 1968) and platelet phospholipase inhibiting properties (Largarde et al., 1980) in a canine model of reperfusion-induced arrhythmias.

Greyhounds were anaesthetised with chloralose after induction with sodium thiopentone and prepared as previously described (Coker, 1984). The left anterior descending coronary artery (LAD) was prepared for occlusion and a catheter placed in a coronary vein draining the region of myocardium rendered ischaemic by occlusion of the LAD. Plasma concentrations of TXB_2 (the stable metabolite of TXA_2) and 6-keto $PGF_{1\alpha}$ (a metabolite of prostacyclin) were measured by radioimmuno-assay. Nicergoline was administered as an intravenous infusion (50 μg kg^{-1} min^{-1}) commencing 30 min prior to occlusion of the LAD and maintained for the duration of the experiment. A 40 minute period of coronary artery occlusion was followed by reperfusion.

The effect of nicergoline on arrhythmias occurring during occlusion and following reperfusion is shown in Table 1.

Table 1. The effect of nicergoline on the number of ischaemia-induced ventricular ectopic beats (VEB) and on the incidence of ventricular fibrillation (VF) during occlusion and following reperfusion.

Treatment	VEB	VF (occlusion)	VF (reperfusion)
Control	490 ± 122	4/12	6/8
Nicergoline	357 ± 148	1/10	4/9

The difference between the control and nicergoline-treated animals was not statistically significant at $P<0.05$. The local release from the ischaemic myocardium of TXB_2 may have been delayed but the release of 6-keto $PGF_{1\alpha}$ was not influenced by administration of nicergoline. Nicergoline did however reduce mean arterial blood pressure (from 154 ± 9 to 96 ± 5 mmHg), cardiac output (from 2.84 ± 0.19 to 2.01 ± 0.11 l min^{-1}) left ventricular dP/dt max (from 2510 ± 250 to 1520 ± 94 mmHg s^{-1}) and heart rate (152 ±15 to 132 ±6 beats min^{-1}). 50% of the nicergoline-treated animals survived ischaemia and reperfusion compared to 17% of controls. Nicergoline was therefore less effective than thromboxane synthetase inhibitors in this model.

F.M. Williams is an SERC (CASE) award student.

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PHARMACOLOGICAL PROFILE OF CICLOPROLOL, A COMPOUND WHICH BEHAVES AS AN AGONIST AND AN ANTAGONIST AT β_1-ADRENOCEPTORS

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Cicloprolol is (±)-1-{4-[2(cyclopropylmethoxy)ethoxy]phenoxy}-3-[(1-methylethyl) amino]-2-propanol hydrochloride. It differs from betaxolol (Boudot et al., 1979) by the presence of an oxygen between the aromatic ring and the cyclopropylmethoxy-ethyl chain. This communication deals with some pharmacological properties of this new compound which, in contrast to betaxolol, behaves as a partial agonist at β_1-adrenoceptors.

In isolated spontaneously beating guinea pig atria, cicloprolol, like betaxolol and pindolol, antagonized the positive chronotropic effects of isoprenaline. Their pA_2 values calculated according to the method of Schild were 7.91 ± 0.3 (slope 0.99), 8.89 ± 0.4 (slope 0.93) and 9.63 ± 0.3 (slope 1.09), respectively.

Cicloprolol, in contrast to pindolol, was a weak antagonist of the isoprenaline-induced relaxation in isolated guinea pig tracheal chains contracted with carbachol. The ratio between the pA_2 values obtained in the atria and the tracheal chains (selectivity ratio) is 56 and 4, respectively.

In intact or reserpinized pithed rats, isoprenaline, cicloprolol, xamoterol and pindolol, produced dose-related increases in heart rate. The curves obtained with the first three compounds were monophasic sigmoids whereas pindolol curve was biphasic. The maximal response was 138 ± 4, 86 ± 6, 102 ± 3 and 73 ± 4 beats/min for isoprenaline, cicloprolol, xamoterol and pindolol, respectively. The dose producing an increase of 50 beats/min were 0.02 ± 0.001, 7.7 ± 0.5 and 0.4 ± 0.02 µg/kg i.v. for the first three compounds, respectively. The corresponding value for pindolol (which is difficult to calculate due to the biphasic shape of the dose-response curve) was about 100 µg/kg i.v.

The increase in heart rate produced by isoprenaline, cicloprolol and xamoterol was inhibited by propranolol in an apparent competitive manner. However, propranolol antagonized the effects of the lower doses of pindolol more than those of the higher doses.

Dose-response curves to isoprenaline were studied in pithed rats pretreated with either saline, cicloprolol or pindolol. Since the latter compounds raised baseline heart rate, the control dose-response to isoprenaline was obtained in saline-pretreated rats in which the heart rate was elevated through electrical stimulation of spinal cord or by infusing isoprenaline to values similar to those measured in cicloprolol and pindolol-pretreated rats. These dose-response curves to isoprenaline were expressed as a percentage of the maximum. In this *in vivo* study cicloprolol and pindolol were found to be competitive antagonists of β_1-adrenoceptors, pindolol being about 26 times more potent than cicloprolol.

In conclusion, cicloprolol behaves as an agonist and antagonist of cardiac β_1-adrenoceptors. Its β_1-adrenoceptor agonist properties are relatively more important than those of pindolol due to the fact that it is less potent than pindolol as an antagonist of β_1-adrenoceptors. Thus, cicloprolol may have a clinical potential as a modulator of myocardial activity.

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EFFECTS OF CHRONIC LOW LEVEL LEAD TREATMENT AND HYPERTENSION ON THE SEVERITY OF CORONARY ARTERY LIGATION INDUCED ARRHYTHMIAS

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Rats chronically exposed to subtoxic (5 ppm) levels of lead in their drinking water have impaired cardiac conduction, a factor which may make the heart more susceptible to cardiac arrhythmias (Kopp et al., 1980). The aim of this study was to investigate whether chronic (for 1,3,6,12 or 16 months) low level lead (5 or 25 ppm), administered as lead acetate in the drinking water, commencing either after weaning or from conception, altered the susceptibility of the heart to arrhythmias induced by coronary artery ligation in anaesthetised male Sprague-Dawley rats. The cardiac effects of chronic (3 or 12 months) administration of lead (25 ppm) were also examined in spontaneously hypertensive rats (Okamoto strain) since high blood pressure is recognised as a risk factor in sudden cardiac death (Kannel & Thomas, 1982).

Control and lead treated rats were anaesthetised with sodium pentobarbitone, the left coronary artery ligated and the severity of the arrhythmias that occurred within 30 min were assessed. Blood and bone lead concentrations were measured by atomic absorption spectrophotometry. Treatment from weaning of normotensive rats with 5 or 25 ppm lead for periods of 1,3 or 6 months had no statistically significant effect on the severity of coronary artery ligation induced arrhythmias. Each of the groups treated from conception with 5 or 25 ppm lead for periods of 1,12 or 16 months exhibited a higher incidence of ventricular tachycardia than the appropriate control group, but the difference was only statistically significant in the case of animals treated with 25 ppm lead for 12 months (100 vs 69%). The incidence of ventricular fibrillation was significantly higher (92 vs 50%) in rats treated for 16 months with 5, but not with 25 ppm lead. Neither the number of ectopic beats nor the durations of arrhythmias were significantly altered by any of the dosing regimens commencing from conception. Spontaneously hypertensive rats (SHR) treated with 25 ppm lead for 3 months after weaning had significantly more ectopic beats than control Sprague-Dawley rats (1289 ± 298 vs 359 ± 83). Normotensive animals given this dose of lead and control SHR rats had slightly more arrhythmias than control normotensive rats. By 12 months after weaning, the number of ectopic beats following ligation in control Sprague-Dawley rats had risen to 1022 ± 294; at this time there were no significant differences in any of the arrhythmic indices between control normotensive, control hypertensive and lead-treated animals.

Lead accumulated in the bone of all animals whether treated from conception or from weaning. Treatment with 5 or 25 ppm lead for 16 months from conception caused a significant accumulation of lead in the blood. Lead treatment did not alter mean arterial blood pressure nor heart rate in normotensive rats and did not influence the time course of the development of high blood pressure in spontaneously hypertensive rats.

We conclude that chronic exposure to low levels of lead, either alone or in combination with high blood pressure, does not consistently alter the susceptibility of the heart to ischaemia-induced arrhythmias in anaesthetised rats.

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Acute inflammation was induced by injection of zymosan (Z, grade A from S. cerevisiae yeast) or by carrageenan (CAR, type 1, 80% kappa, 20% lambda) in one-day-old air-pouches in WAG rats. Inflammatory exudates were collected from the air-pouches at various times. The number of leucocytes in the exudates was counted and differential cell counts were also assessed. Concentrations of the cyclo-oxygenase products, PGE_2 and PGI_2 (measured as 6-oxo-$PGF_{1\alpha}$) and the lipoxygenase product, LTB_4, were measured by radioimmunoassay.

The following were the main findings:

1. Z-CMC (zymosan suspended in carboxymethyl cellulose) induced a continuous infiltration of polymorphonuclear (PMN) leucocytes into the air-pouches during the period of investigation (t = 1h, 4h, 1 and 8 days), with cell viability $> 90\%$. The cell number on day 8 was $59.4 \pm 11.2 \times 10^6$/ml. By contrast, CMC alone and CAR stimulated the leucocyte infiltration temporarily (from 1 to 24h) but towards the end of investigation (day 8) the leucocyte number returned to the control level similar to that seen in air-pouches in which saline was injected.

2. LTB_4, 6-oxo-$PGF_{1\alpha}$ and PGE_2 levels in exudates from air-pouches treated with Z-CMC increased to reach a peak of 3.2 ± 1.3m 7.3 ± 2.5 and 10.8 ± 5.2 ng/ml respectively within the first day. By day 8, these levels had fallen to 0.9 ± 0.2, 0.7 ± 0.2 and 6.4 ± 1.1 ng/ml respectively.

3. The PMN leucocytes from 8 day-old air-pouches in which Z-CMC had been injected were capable of producing PGE_2, PGI_2 and LTB_4 ex vivo when incubated in culture medium for 24h. The production of PGE_2 was 3.2 times higher than that of PGI_2. The PGE_2 and PGI_2 production by the cells was inhibited by indomethacin and BW755C.

These results indicate that during the early phase of the reaction (1-24h) the eicosanoids are acting synergistically as inflammatory mediators resulting in vascular changes and migration of PMN leucocytes. In the late phase (8 days) there is a reduced formation of arachidonic acid metabolites, in spite of a continued increase in the number of PMN leucocytes. The most recent experiments indicate the presence of an inhibitor of arachidonic acid metabolism during this late phase and its nature is being examined.

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DEVELOPMENT AND CHARACTERISATION OF A RADIOIMMUNOASSAY FOR LEUKOTRIENE B_4

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We previously reported the measurement and characterisation of immunoreactive LTB_4 present in calcium ionophore stimulated human blood (Forder and Carey 1984) and now describe the properties of the LTB_4 radioimmunoassay used in these studies. An immunogenic conjugate of LTB_4 was prepared by a reaction of LTB_4 with bovine serum albumin in the presence of 1,1-carbonyldiimidazole. Following dialysis and lyophilisation, rabbits were immunised with this conjugate and antibody titres determined by incubation of serial dilutions of plasma with [^{3}H]LTB_4. Dextran coated charcoal was used to separate antibody bound from free [^{3}H]LTB_4 and non-specific binding, determined by incubation with normal rabbit serum, was less than 5%.

Four of six rabbits generated antibodies of adequate titres and one of these antibodies has been extensively characterised. Significant displacement of antibody bound [^{3}H]LTB_4 was obtained with 2.5pg of synthetic LTB_4 and 20pg produced 50% displacement of the [^{3}H] ligand. Under radioimmunoassay conditions, which included overnight incubation, analytical studies (tlc) revealed no evidence of LTB_4 d-lactone formation. The specificity of this antibody has been measured with several eicosanoids. Cyclic pathway eicosanoids, including 12-hydroxy heptadecatrienoic acid all produced less than 0.2% cross reactivity, while cross reactivity with the platelet lipoxygenase product 12-HETE was 0.24%. Cross-reactivity with other racemic mono-HETEs was as follows: 11-HETE (0.03%), 9-HETE (0.1%), 8-HETE (2.1%) and 5-HETE (0.07%). The double lipoxygenase product 5S,12S di-HETE gave 3.4%, 6 trans LTB_4 (all trans isomer I) 60% and isomer II, 7.5%. The omega side chain metabolite of LTB_4, 20-OH LTB_4, cross reacted 41% and the omega COOH 0.1%.

Measurement of immunoreactive LTB_4 in human plasma showed that basal levels did not exceed 100pg ml^{-1} and this immunoreactivity diluted in a non-linear fashion. In contrast, plasma immunoreactive LTB_4 obtained from blood stimulated with A23187 assayed either directly or following extraction, revealed a linear relationship between the volume assayed and the level of immunoreactive LTB_4. Analytical HPLC studies of this immunoreactive material showed that 85-90% was due to authentic LTB_4 and a variable proportion to 6-trans LTB_4. Incubation of [^{3}H]LTB_4 in human blood up to 30 mins in the presence of ionophore revealed no evidence of metabolism of this material to more polar substances. In conclusion this sensitive radioimmunoassay has utility in the evaluation of lipoxygenase inhibitors in blood based systems and in bioavailability studies in experimental models of disease and in clinical studies.

Forder and Carey (1984), IUPHAR Satellite Symposium, Leukotrienes and Other Lipoxygenase Products.

FREE RADICALS, LIPID PEROXIDATION AND PROSTAGLANDINS DURING THE DEVELOPMENT AND MAINTENANCE OF FOOT-PAD OEDEMA IN THE KOCH MODEL

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In the Koch model, the inflammatory response induced by the sub-plantar inoculation of Freund's complete adjuvant (FCA) involves a role for re-circulating T lymphocytes, and is associated with the presence in the extravascular space of fibrin, macrophages and PMN leucocytes (Bullock et al, 1983).

PMNs generate free radicals (Halliwell, 1982), T lymphocytes release arachidonic acid and the macrophages form prostaglandins (Goldyne & Stobo, 1983). Therefore the possible occurrence of free radicals, lipid peroxidation and prostaglandins during the evolution of foot-pad inflammation in this model was investigated in the present study.

Foot-pad inflammation was induced in male Wistar rats (200-250g) previously immunised to FCA (Bullock et al, 1983). Oedema was measured as the increase in fresh weight of the challenged compared with unchallenged paws. Presence of free radicals was measured as chemiluminescence using luminol (Dowling et al; Proceedings this meeting). The extent of lipid peroxidation was quantified by measurement of malondialdehyde (MDA) using thiobarbituric acid (Gutteridge, 1982). The prostaglandin content was measured using standard radioimmunoassay techniques. Drugs were administered (p.o.) 1 h before challenge using procedures previously described (Jasani et al, 1983).

Foot-pad oedema developed in a bi-phasic manner over the initial 24 h. It then persisted at around the 24 h level. Tissue MDA content increased in a similar manner, but luminol-amplified chemiluminescence and the prostaglandin levels changed differently. Peak levels of chemiluminescence were observed before each of the two phases of oedema and increases in MDA content. PGE_2 rose to a maximum in association with the first phase of oedema, whereas TXB_2 and 6-oxo-PG-$F1_\alpha$ rose to a maximum before the second phase of oedema.

Prednisolone (10 mg/kg) was found to inhibit tissue MDA content, and in association with it the first phase of oedema; both by around 50%. Indomethacin (3 mg/kg) was found to be without effect on both these measurements during the initiation phase only. The findings contrast with those obtained when the two agents were administered, 6h post-challenge (Jasani et al, 1983). In that event indomethacin (2 mg/kg) consistently inhibits oedema to almost the same extent as prednisolone (10 mg/kg).

The data presented indicate that in this model the initiation of foot-pad oedema occurs in association with the production of free radicals. Prostaglandins appear to contribute to initiation of the second phase only. Indomethacin and other known cyclooxygenase inhibitors moderate to a limited extent the severity of this second phase. The significance of lipid peroxidation requires to be further investigated.

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A RE-EXAMINATION OF THE ACTIONS OF PROSTAGLANDINS E_1, E_2, $F_{1\alpha}$ AND D_2 ADMINISTERED INTRACEREBROVENTRICULARLY TO CATS

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Prostaglandins are natural constituents of and can be synthesised by mammalian brain (Wolfe & Coceani, 1979) but there have been only restricted reports on their actions upon the central nervous system. Horton (1964) described the effects of exogenous prostaglandins (PG) E, administered intracerebroventricularly (icv), on the behaviour of unanaesthetised cats and Milton & Wendlandt (1971) reported the effects of central prostaglandin administration on body temperature. The present study has examined the effects of PGE_1, E_2, $F_{1\alpha}$ and D_2, administered centrally, on gross behaviour and body temperature of unanaesthetised cats.

Male cats (2.5-3 kg) had a cannula implanted through the skull so that the tip was in the left lateral cerebral ventricle under general anaesthesia as described by Feldberg & Sherwood (1953). At least one week was allowed after surgery before animals were used experimentally. Prostaglandins (Sigma) were dissolved in sterile artificial cerebrospinal fluid and icv injections were of a standard volume of 0.3 ml. Cats were observed for gross behavioural effect and rectal temperatures were taken at hourly intervals. The observer was unaware of the treatment given to each cat.

PGE_1, 12.5-100 $\mu g.cat^{-1}$ icv, caused immobility, viscous salivation, ptosis, relaxation of the nictitating membranes and the assumption of a characteristic pose with the head lowered and the ears flattened. There was no evidence of catatonia. These effects became apparent within 15 min of dosing and persisted for between 2 and 24 h depending on the dose. The intensity of effect was also dose dependent. PGE_2, 12.5-100 $\mu g.cat^{-1}$ icv, caused similar depressant effects and tremor and ataxia but no catatonia or immobility. $PGF_{1\alpha}$, 50-100 $\mu g.cat^{-1}$ icv, caused an initial excitation which reversed to depression within 1 h of injection. PGD_2, 25-100 $\mu g.cat^{-1}$ icv, caused an initial mild excitatory phase which was quickly followed by a hypnotic effect with increased somnolence from which the animals could be easily aroused.

Consistant with published observations rectal temperature was elevated significantly ($p<0.05$) by PGE_1 and E_2 (1.0°C at 12.5 $\mu g.cat^{-1}$ to 3.0°C at 100 $\mu g.cat^{-1}$) and $F_{1\alpha}$ (1.5°C at 50 and 100 $\mu g.cat^{-1}$) but not PGD_2 (100 $\mu g.cat^{-1}$).

These results confirm the previous work of Horton (1964) that the primary central effects of prostaglandins in the cat are depressant. However, they differ in detail in that, unlike Horton, we found no evidence of catatonia following the injection of either PGE_1 or E_2. Also these two prostaglandins had qualitatively different actions with PGE_1 causing increased muscle tone while PGE_2 was muscle relaxant. The observation that PGD_2 caused increased somnolence was consistent with the claim of Veno et al (1983) that PGD_2 induced sleep in rats.

It is concluded that whilst PGE_1, E_2, $F_{1\alpha}$ and D_2 were all depressant when administered centrally to cats, each prostaglandin had activities distinct from the others which have not been reported previously.

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VASOCONSTRICTOR ACTIVITY OF LEUKOTRIENE C_4 AND EPOXY-METHANO PGH_2 IN THE RAT GASTRIC SUBMUCOSA

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Studies on the gastric microcirculation are of importance since an adequate vascular perfusion is essential for the maintenance of mucosal function and integrity. In the present study, the gastric vascular actions of the arachidonate lipoxygenase product, leukotriene C_4 (LTC_4) and the prostanoids, $PGF_{2\alpha}$ and the epoxy-methano PGH_2 analogue which acts as a thromboxane mimetic (Coleman et al., 1981) have been investigated in vivo in the submucosal microcirculation of the anaesthetised rat using direct microscopy techniques.

The in vivo microscopy technique described previously in detail by Guth and Moler (1982) was used to study gastric submucosal arteriolar and venular responses in the pentobarbital anaesthetized rat. A fibre-optic light carrier rod was inserted into the gastric lumen via an incision in the forestomach to transilluminate the stomach wall. After removal of the serosal and muscle layers, a shallow disk with a 5 mm orifice was sealed over the exposed submucosa for the local application of Krebs' solution and compounds under investigation. This system allows direct microscopic visualization and measurement of the submucosal vascular networks, which were video-recorded via a T.V. camera.

Topical application of $PGF_{2\alpha}$ (1-100 µM) to the exposed submucosal vascular bed reduced vessel diameter of the venules, reaching peak vasoconstriction within 1 min of application and which remained during the 3 min period of administration. Vasoconstriction in the arterioles by $PGF_{2\alpha}$ was less pronounced. Epoxy-methano PGH_2 (U-46619, 1-1000 nM) induced vasoconstriction in both arterioles and venules, reaching plateau responses within 1.5-2 min of application, which were well-maintained. With the highest concentration of U-46619 (1 µM) the maximal reduction in vessel diameter was 74 ± 4 % (n=5; $P < 0.05$) and 70 ± 10% (n=7; $P < 0.05$) of control for the arterioles and venules respectively. Application of leukotriene C_4 (25-400 nM) induced significant ($P < 0.05$ for each concentration) vasoconstriction in the venules, which was more pronounced than in the arterioles, reaching peak responses within 1-1.5 min. The maximal reduction in vessel diameter with LTC_4 (200 nM) was 18 ± 10% (n=10; $P < 0.05$) and 59 ± 7% (n=10; $P < 0.05$) of control for arterioles and venules respectively. With both LTC_4 and the thromboxane mimetic, intense focal vasoconstriction in the venules was clearly demonstrated, leading to sluggish blood flow or stasis within the vessel. The potent gastric venoconstrictor activity of these arachidonate products contrasts with the actions of noradrenaline which has no significant action on submucosal venules in concentrations (1-5 µM) substantially reducing arteriolar vessel diameter.

Previous studies have indicated that both the potent vasoconstrictors, epoxy-methano PGH_2 as well as thromboxane A_2 generated in situ, can rapidly lead to extensive gastric mucosal necrosis and ulceration when a topical irritant such as acidified bile salts bathes the gastric mucosa (Whittle et al., 1981; Whittle & Moncada, 1983). The present demonstration of the potent vasoconstrictor actions of the LTC_4 in the gastric submucosal microcirculation identifies this 5-lipoxygenase metabolite as a further potential endogenous pro-ulcerogenic agent. The current findings thus suggest that both cyclo-oxygenase and lipoxygenase arachidonate products could play a role in microcirculatory events accompanying gastric ulcer formation.

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DIFFERENTIAL RELEASE OF EICOSANOIDS BY VARIOUS STIMULI IN GUINEA-PIG ISOLATED LUNG

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The metabolism of arachidonic acid (AA) in isolated, perfused lungs from male guinea-pigs was studied after infusion of AA through the pulmonary circulation (exogenous AA) or after liberation of endogenous AA stimulated by infusion of bradykinin (Bk) or calcium ionophore A23187 in normal lungs or by antigen challenge in ovalbumin sensitized lungs.

The lungs were perfused via the pulmonary artery with warmed ($37^{o}C$) and oxygenated (95% O_2-5% CO_2) Krebs' bicarbonate solution, pumped at 5 $ml.min^{-1}$ (Bakhle et al. 1969). Each stimulus was given as a 5 min infusion and the metabolites of AA in lung effluent were assayed by radioimmunoassay (RIA) of TXB_2, 6-oxo-$PGF_{1\alpha}$, (as a measure of TXA_2 and PGI_2 formation respectively) PGE_2, LTB_4 and LTC_4 or by bioassay after superfusion over a variety of isolated muscle strips.

Infusion of AA (4 $\mu g.ml^{-1}$) led to the synthesis of TXA_2. This synthesis reached a plateau in the second minute and was maintained until the end of the infusion falling immediately after the infusion had finished. With A23187 (250 ng ml^{-1}) the release of TXB_2 steadily increased during the infusion period and only reached a maximum after the end of the infusion. Both Bk (250 $ng.ml^{-1}$) and ovalbumin (100 $ng.ml^{-1}$) induced synthesis of TXB_2 which peaked during the first 2 min and declined thereafter. The release of eicosanoids detected by RIA is shown in the following table:

Stimulus	6-oxo-$PGF_{1\alpha}$	TXB_2	LTB_4	LTC_4	PGE_2
AA	6.8 ± 1.37	19.1 ± 1.6	< 0.1	< 1	< 1
A23187	10.3 ± 0.6	122.4 ± 10.7	0.9 ± 0.1	< 1	1.8 ± 0.3
Bk	7.3 ± 0.7	2.8 ± 1.1	< 0.1	< 1	< 1
Ovalbumin	31.25 ± 9.35	123.2 ± 8.7	2.3 ± 0.6	8.5 ± 3.0	3.9 ± 0.5

n = 3-5 observations; all values are in ng ml^{-1}

Bioassay studies revealed the release of a leukotriene-like substance following a bolus injection of A23187 (1.5-3.0 µg). This substance was stable in Krebs solution for at least two minutes, caused contractions of guinea-pig tracheal strips (between 10-40 ng LTC_4 equivalents) which were antagonised by FPL 55712 (1 µg ml^{-1}) and its release was not inhibited by indomethacin (2 µg ml^{-1}) perfused through the pulmonary circulation. These observations together with the much lower amounts of immunoreactive LTC_4 released by the A23187, suggest that LTD_4 may be the leukotriene like substance formed.

Our results indicate that the metabolism of exogenous and endogenous AA in guinea-pig lungs was both qualitatively and quantitatively different. The finding that Bk produced more PGI_2 than TXA_2 probably reflects a selective stimulation of the vascular compartment whereas the release induced by A23187 indicates an effect on other compartments. The observation that neither Bk nor AA released any leukotrienes confirms the specific requirements for the stimulation of the 5'lipoxygenase pathway.

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CYTOCHROME P450-DEPENDENT ARACHIDONATE METABOLITES IN RENOMEDULLARY CELLS FROM THE THICK ASCENDING LIMB OF HYPERTENSIVE RABBITS

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Cells isolated from the thick ascending limb of Henles' loop (TALH) have been shown to have low cyclo-oxygenase activity . However, they selectively convert arachidonic acid (AA) to two types of biologically active oxygenated metabolites (P_1 and P_2) via a cytochrome P450-dependent mechanism. P_2 exerts a marked inhibition of purified Na^+K^+ATPase and P_1 relaxes blood vessels. Since ATPase activity has been shown to be decreased in the renal outer medulla of hypertensive animals and a circulating Na^+K^+ATPase inhibitor has been reported in patients with essential hypertension, we have studied changes in AA metabolism in TALH cells obtained from the outer medulla of rabbits made hypertensive by aortic constriction. New Zealand White male rabbits (3.0-3.2 kg) were anaesthetized and the aorta, between the coeliac and anterior mesenteric artery, was constricted by > 75% using a stainless steel clamp. Sham-operated rabbits were used as controls. On the eighth post-operative day when the BP had stabilized, the mean arterial pressure under anaesthesia ranged from 64-75 mmHg for controls and 90-130 mmHg for hypertensive rabbits, respectively. The kidneys were flushed *in situ* with cold saline and the inner stripe of the outer medulla excised. Following trypsinization and mechanical disruption of the tissue, two cell fractions: TALH cells and outer medullary cells depleted of TALH cells (OMC), were isolated by centrifugal elutriation, as previously described (Ferreri et al., 1984).

Exogenous AA metabolism was determined by incubating $3x10^6$ cells with 7μM ^{14}C-AA for 30 min at 37°C. The radioactive products obtained after extraction were separated using TLC (ethyl acetate : iso-octane : acetic acid : water : 55:25:10:50) and radioactivity counted after visualization with autoradiography. The results from four pairs of rabbits are summarized in the following table:

	TALH cells		OMC	
	μg AA converted/mg protein/30 min			
	P_1	P_2	P_1	P_2
Control	1.27(1.12-1.42)	0.63(0.56-0.70)	0.43(0.18-0.56)	0.28(0.1-0.51)
Hypertensive	2.34(1.85-2.83)	1.28(0.75-1.81)	0.32(0.11-1.21)	0.30(0.08-0.66)

Results shown as medians with semi-quartiles values in parentheses.

There was a marked increase in P_1 and P_2 formation by TALH cells from rabbits made hypertensive compared to TALH cells obtained from sham-operated rabbits. In contrast other renal cells, the OMC fraction, produced less products than TALH cells and there was little change in AA metabolism by the OMC between control and hypertensive.

These results show that during hypertension the metabolism of AA to two oxygenated metabolites is selectively increased in TALH cells and may indicate a role for these metabolites in hypertension, e.g., mediation of enhanced natriuresis to volume expansion in hypertensive animals.

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EVIDENCE FOR AN INDOMETHACIN SENSITIVE α_1-ADRENOCEPTOR MEDIATED MODULATION OF RESPONSES IN THE PITHED RAT HEART

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Alpha$_1$ adrenoceptors have been demonstrated to moderate the responses of the pithed rat heart to electrical stimulation (Docherty, 1983), although their anatomical location has recently been questioned. (Van de Berg et al, 1984). The aim of this study was to evaluate further the location of these α_1 adrenoceptors, and the mechanism involved in the modulation of responses in the pithed rat heart.

Diastolic blood pressure (DBP) and heart rate (HR) were recorded from pithed vagotomised rats. All agents were administered via a jugular vein. Increases in HR were produced by the cumulative administration of isoprenaline (ISOP) or by electrical stimulation of the spinal sympathetic outflow (C_7-T_1). Stimulation parameters were 1Hz, 20v, 1msec, for 10 sec periods at 45 sec intervals. Results presented are the mean ± SEM of 4-6 experiments. Statistical significance was determined by nested analysis of variance.

None of the treatments employed in this study significantly increased basal HR above that of the vehicle control (HR = 315 ± 16 bmin^{-1}). In the presence of saline vehicle (1.0 mlkg^{-1}), ISOP (10-10^{3}ngkg^{-1}) produced a dose related tachycardia of 36 ± 10-178±7 bmin^{-1}. Methoxamine (MET, 200 µgkg^{-1}) administered 5 min prior to ISOP, significantly ($p<0.05$), reduced the maximum response evoked by ISOP to 103 ± 9 bmin^{-1}. Prazosin (0.1 mgkg^{-1}) had no effect on the peak tachycardia to ISOP (173 ± 7 bmin^{-1}), but when administered 10 min prior to MET, significantly ($p<0.05$) reduced its inhibitory effect, resulting in a peak tachycardia to ISOP of 166 ± 10 bmin^{-1}.

In the presence of the vehicle employed to dissolve indomethacin (phosphate buffered saline, pH8, and alcohol) ISOP produced a dose related tachycardia of 26 ± 5 - 176 ± 9 bmin^{-1}. Predosing (5min) with MET (200 µgkg^{-1}), significantly ($p<0.05$) reduced the peak tachycardia to ISOP to 112 ± 5 bmin^{-1}. Following pretreatment of the preparation with the prostaglandin synthetase inhibitor indomethacin (Flower, 1974) at a dose of 2.0 mgkg^{-1}, 25 min prior to MET, the inhibitory effects of MET were significantly ($p<0.05$) reduced resulting in a peak ISOP tachycardia of 148 ± 5 bmin^{-1}. Administration of indomethacin alone, had no significant effect on the peak tachycardia to ISOP (183 ± 2 bmin^{-1}).

Electrical stimulation of the cardiac sympathetic nerves produced a tachycardia of 72 ± 3 bmin^{-1} (n=20). MET produced a 50% inhibition of this response (ED_{50}) at a dose of 139 ± 24 µgkg^{-1}. Pretreatment (15 min) with prazosin (0.1 and 1.0 mgkg^{-1}) produced rightward shifts in the MET dose-response curve. (ED_{50} = 525 ± 24 and 1288 ± 159 µgkg^{-1} respectively). Neither phosphate buffered vehicle nor indomethacin (2 mgkg^{-1}), pretreatment had any effect on either the peak response to nerve stimulation or the inhibitory effects of MET (ED_{50} = 148 ± 23 and 151 ± 11 µg kg^{-1} respectively).

In conclusion, the α_1 adrenoceptor agonist MET reduced the response of the pithed rat heart to ISOP and nerve stimulation. Only the inhibition of the ISOP induced tachycardia was partially reduced by indomethacin, and this may suggest the existence of two separate inhibitory α_1-adrenoceptor mechanisms in the rat heart.

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