RESERPINE-RESISTANT RESPONSES TO NERVE STIMULATION IN THE CAT NICTITATING MEMBRANE ARE DUE TO NEWLY SYNTHETISED TRANSMITTER

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A marked depletion of the noradrenaline stores in the cat nictitating membrane (NM) is observed by pretreatment with reserpine (Lee, 1967), however, in reserpine pretreated cats, the contractile responses of the NM to nerve stimulation are not abolished, and reach 50% of the maximum developed tension, both "in vivo" and "in vitro" (Langer and Pinto, 1976). The present study further examines the NM contraction in response to nerve stimulation and the tachycardia evoked by ansa-subclavia stimulation in anaesthetised reserpine pretreated cats.

Cats were pretreated with reserpine phosphate (1 mg/kg i.p. 24 hours before the experiment) anaesthetised with pentobarbitone (45 mg/kg i.p.) and ventilated with room air. In one series, the NM contraction was recorded under resting tension of 12 g in response to preganglionic sympathetic cervical trunk stimulation (1 msec - supramaximal voltage at 0.1 to 25 Hz). Animals received atropine and propranolol (1 mg/kg i.v.) 30 min. before starting the experiment. In the other series heart rate was recorded for measurement of the tachycardia in response to postganglionic ansa subclavia stimulation (1 msec. - supramaximal voltage at 0.1 to 25 Hz). Animals received atropine (1 mg/kg I.V.) 30 min. before starting the experiment. These procedures were also carried out in non-reserpinised anaesthetised cats.

In control cats, the maximum contractile response of the NM induced by electrical stimulation at 25 Hz was 20 $\pm$ 1.4 g (n=13). The maximum tachycardia was 113 $\pm$ 2 beats/min (n=5). Reserpine pretreatment significantly reduced (50-60%) the responses of the NM to all frequencies of stimulation but virtually abolished the neurally mediated tachycardia. In control cats, cocaine (2.5 mg/kg i.v., n=12) slightly potentiated the responses of the NM and the tachycardia (n=4) at low frequencies of stimulation. In reserpine treated cats, cocaine, produced a marked potentiation of the residual responses of the NM (n=12) at all frequencies of stimulation, but did not significantly influence the neural tachycardia (n=5). Pargyline (50 mg/kg i.v. ; 20 min infusion) did not modify the electrically induced contraction of the control NM (n=6), but markedly potentiated these respones in reserpine treated cats (n=5) at all frequencies of stimulation. In reserpine treated cats, treated with cocaine, the administration of α-methyl-p-tyrosine (300 mg/kg i.v.; 40 min infusion) reduced by 70-75% the residual responses of the NM after 90 min. In control cats, the NM responses were inhibited by only 25-35%. Phentolamine (1 - 3 mg/kg i.v.) or prazosin (0.1-0.3 mg/kg i.v.), significantly antagonised the responses of the NM, however, neither α-receptor antagonist completely blocked the responses. These findings suggest that, after depletion of the noradrenaline stores by reserpine pretreatment, a residual reponse can be obtained in the electrically stimulated NM, but not in the heart of the anaesthetised cat.

In the NM, these residual responses are markedly augmented by cocaine, or pargyline, and inhibited by treatment with α-methyl-p-tyrosine. These data indicate that a reserpine-resistant, neuronal pool of newly synthetized catecholamine maintains neuronally mediated responses in the NM but not in the cat heart.

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IN VITRO DUCTUS ARTERIOSUS AS A MODEL FOR ANTI-ISCHAEMIC DRUG STUDIES.

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Introduced by Dr. B. Pourrias

Ductus arteriosus isolated from foetal guinea pig was used as an experimental model to investigate the activity of drugs which are effective in clinical treatment of ischemic diseases. Availability of the model postulated that tissular oxygen concentration was the sole determinant of ductus tone. This followed from the fact that muscle tension was correlated to bath PO_2. Data from Roulet & Coburn (1981) supported the concept for an oxygen-sensitive decrease in cell membrane permeability to K^+ ions (P_K). The sequence of events coupling excitation to contraction was therefore modelled as :

oxygen $\longrightarrow \searrow P_K \longrightarrow$ membrane depolarization $\longrightarrow Ca^{++}$ influx $\longrightarrow$ contraction

The present study was performed on ductal strip placed under conditions of hypoxia in order to appreciate the anti-hypoxic activity of molecules, as it could be estimated from tension, and to attempt to locate the drug site of action within the model. Trimetazidine and diltiazem were selected for their antianginal properties (Toda et al, 1982 ; Cauvin et al, 1983). Eburnamonine was tested because of its cerebral anti-hypoxic activity in vivo (Linee et al, 1978). In addition papaverine was assayed for its known ability to relax smooth muscle (Bolton, 1979).

Strips from ductus were suspended in an organ-bath and perfused at 37°C with physiological salt solution bubbled with 5% CO_2 either in N_2 or in calibrated mixtures of O_2 and N_2 to attain the desired PO_2 in the bath.

The preparation equilibrated at PO_2 of 75 mm Hg (hypoxia) developed tension which amplitude averaged 30% of the oxygen-induced contraction ($PO_2 \geqslant 200$ mm Hg). Concentration-response curves to the drugs demonstrated that trimetazidine as well as eburnamonine elicited dose-dependent enhancement in hypoxic tension, whereas diltiazem induced dose-related relaxation of the preparation. ED_{50} for the drugs were 8.10^{-5} M, 3.10^{-7} M and 10^{-6} M, respectively. Papaverine at 10^{-5} M completely reversed the hypoxic tension. The contractile activities required the presence of Ca^{++} ions in the bath. In the absence of oxygen, the effect of trimetazidine at maximally effective doses of 10^{-3} M was dependent on external K^+ concentration causing contraction then relaxation as K^+ ions were raised.

Our data have disclosed the anti-hypoxic role for trimetazidine and eburnamonine inasmuch as both drugs significantly improved the efficiency of oxygen on ductus strip, as was evidenced from the leftward shift of the tension versus PO_2 relationship. It is concluded that these molecules interact with a mechanism by which oxygen controls the ductus arteriosus tension. In contrast, the antianginal properties of diltiazem are mainly due to the Ca-channel blocking activity of the drug. Electrophysiological work in progress will allow to confirm the specificity of the model.

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PRESSOR EFFECTS OF CENTRALLY ADMINISTERED SALMON CALCITONIN (sCT)

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We have previously demonstrated that sCT produces a dose dependent pressor response when administered peripherally to rats made hypotensive by haemorrhage, but is without effect in normotensive rats and those made hypotensive by pithing (Bates et al., 1983). An increase in sympathetic tone has been implicated as the mechanism underlying the pressor response of haemorrhaged rats to i.v. sCT (Bates et al., 1984), and of normotensive rats to intracerebroventricular (i.c.v) calcitonin gene-related peptide (Fisher et al., 1983). We have therefore determined the effect of i.c.v. sCT on mean arterial pressure (MAP) of normotensive rats and those rendered hypotensive by haemorrhage, and assessed the effect of chemical sympathectomy on the response observed in haemorrhaged animals.

Groups of Sprague-Dawley rats which were untreated or were pretreated with either 6-hydroxydopamine (6-OHDA, 100 $mg.kg^{-1}$ i.p. in 1 $mg.ml^{-1}$ ascorbic acid, 4,3, and 2 days prior to experimental use) or with appropriate vehicle, were cannulated for blood pressure recording under urethane anasthesia as previously described (Bates et al., 1983). The animals were then placed in a stereotaxic frame to facillitate i.c.v. injection and were given a single sham i.c.v. injection (needle entry without fluid ejection) followed by a 30 minutes stabilisation period. Where appropriate, arterial blood was removed to reduce MAP by approximately 20%, 10 minutes after the sham injection. After stabilisation, sCT (0.1, 1 or 10 $U.kg^{-1}$) or vehicle (50 mM Tris, 100 mM NaCl, in 1% bovine serum albumin, pH 7.4) was administered (i.c.v) in a volume of 10 µl and the change in MAP was monitored for 60 minutes thereafter. All animals received a single dose of sCT and of vehicle in varied order, statistical analysis was by comparison (Student's 't' test) of sCT induced effects with those of the vehicle treated internal controls.

In normotensive rats i.c.v. sCT produced a dose dependent pressor response with peak increases in MAP of 12.4 ± 1.4 and 17.7 ± 1.7 mm Hg for 1 and 10 $U.kg^{-1}$ respectively, (n=4-6, $P < 0.05$), whereas vehicle produced no significant change in MAP. A greater pressor response was produced by i.c.v. administration of sCT to rats rendered hypotensive by haemorrhage (peak increases in MAP; 14.6 ± 4.6 and 35.8 ± 3.9 mm Hg for 1 and 10 $U.kg^{-1}$. Furthermore, the pressor response of hypotensive animals to i.c.v. sCT (1 and 10 $U.kg^{-1}$) was not significantly altered by chemical sympathectomy. In contrast, the pressor effect of i.v. sCT (10 $U.kg^{-1}$) has been shown to be greatly attenuated by chemical sympathectomy (Bates et al., 1984).

These results provide further evidence that calcitonin or structurally related peptides might play a role in central cardiovascular regulatory systems, and indicate that the mechanisms underlying the haemodynamic effects of centrally and peripherally administered sCT may differ.

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DOPAMINE RECEPTOR SUBTYPES MEDIATING THE DILATION OF CEREBRAL PIAL ARTERIOLES IN SITU

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Dopamine receptors mediating relaxation have been demonstrated in vitro in major cerebral arteries only after the vessels have been tonically contracted with, for example, prostaglandins, and pretreated with phenoxybenzamine (Toda, 1976; Edvinsson et al. 1978). As there have been no systematic investigations of vasomotor responses to dopamine agonists of cerebral vessels in their normal microenvironment or in the absence of prior pharmacological manipulation, we have examined the in situ responses of the small pial arterioles on the convexity of the cerebral cortex of anaesthetised cats to local perivascular microinjection of dopamine agonists (Stoof and Kebabian, 1981), and sought to establish the dopamine receptor subtype involved.

The investigations were performed on mechanically ventilated cats anaesthetised with α-chloralose. Pial vessels were exposed by a parietal craniotomy. A pool of warmed mineral oil was used to maintain homeostasis after the dura was excised. The calibre of individual pial arterioles (diameter 30 to 200 μm) was measured by a video display - image splitting technique. The agents being investigated were dissolved in artificial cerebrospinal fluid at physiological pH, and were administered as subarachnoid microinjections (5 μl) close to the arteriole being examined. (For details, see McCulloch and Edvinsson, 1980).

The perivascular administration of dopamine (10^{-9} to 10^{-3}M) provoked dose dependent constriction of pial arterioles, with the maximum reduction in calibre (-22 ± 3%, mean ± SE) being observed with dopamine, 10^{-3}M. Cerebrovascular dilatation was never observed when dopamine was employed as the agonist. The constrictions elicited by dopamine could be significantly attenuated by the concomitant perivascular administration of either phentolamine (10^{-6}M) or methysergide (10^{-6}M). Neither phentolamine nor methysergide alone altered arteriolar calibre.

Perivascular microapplication of various dopamine receptor agonists provoked significant dilatation of the arterioles on the cortical surface. The putative dopamine D_1 receptor agonist, SKF 38393A (10^{-9} to 10^{-4}) resulted in pial arteriolar dilatation, with the maximum response (24 ± 3%) being observed with SKF 38393A, 10^{-7}M. The putative D_2 receptor agonist, LY 141865 (10^{-8} to 10^{-4}M) increased arteriolar diameter, but only at high concentrations (maximum response, 25 ± 6% with 10^{-4}M). Apomorphine (10^{-8} to 10^{-4}M), which can interact with both receptor subtypes, also increased arteriolar calibre markedly (31 ± 6% with 10^{-5}M). The arteriolar dilatations provoked by apomorphine could be markedly attenuated by perivascular injections of the putative D_1 antagonist, SCH 23390 (Cross et al. 1983). The administration of SCH 23390 (10^{-9} to 10^{-5}M) alone did not alter pial arteriolar calibre, nor the arteriolar dilatation provoked by acidic pH.

These data indicate that, in the intact animal, dopamine receptor agonists can directly elicit cerebrovascular dilatation, and that this effect is probably mediated via dopamine D_1 receptors. These cerebrovascular receptors provide a potential mechanism by which specific dopamine agonists may modify cerebral blood flow in vivo without alterations in cerebral metabolic activity (which are mediated predominantly via D_2 receptors).

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EFFECT OF APOMORPHINE ON NEUROGENIC VASOCONSTRICTION IN THE ISOLATED AUTOPERFUSED HINDQUARTERS OF THE RAT

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There is much interest in the possible use of dopamine agonists as antihypertensive agents but their mechanism of action in eliciting this effect is not clear (Cavero et al., 1982). The hypotensive effect of apomorphine in the rat has been claimed to be of central origin (Finch and Haeusler, 1973). More recently, Mugabo et al. (1983) attributed the hypotensive effect of apomorphine in the rat to stimulation of peripheral dopamine receptors. In the dog apomorphine is able to stimulate presynaptic dopamine receptors on the sympathetic nerve endings in the femoral bed (Buylaert et al., 1977). Therefore, we studied the effects of locally administered apomorphine in the isolated autoperfused hindquarters of the rat.

Experiments were done in normotensive Wistar rats weighing 240-420 g, anaesthetized with pentobarbital. After intravenous administration of heparine (5 mg/kg), the abdominal aorta was cannulated for constant flow perfusion of the hindquarters (Brody et al., 1963). The lumbar sympathetic chains were isolated and placed on a bipolar electrode. All animals were pretreated with atropine (1 mg/kg IV).

Apomorphine, infused locally into the hindquarters (1 µg/kg/min for 5 min), had no effect on the perfusion pressure per se, but reduced the pressor response to electrical stimulation of the lumbar sympathetic chains (supramaximal voltage, 1 msec, frequency 0.25 - 16 Hz; n = 5). To study the mechanism of this inhibitory effect, further experiments were done with a stimulation frequency of 4 Hz. Apomorphine diminished the pressor response elicited by electrical stimulation at 4 Hz by 45.2 $\pm$ 7.1 %, but did not modify similar increases of perfusion pressure produced by locally administered noradrenaline (n = 6). The inhibition by apomorphine of the pressor response at 4 Hz stimulation was completely antagonized by local administration of haloperidol (1 µg/kg, n = 6), but was not influenced by the α_2-antagonist rauwolscine (100 µg/kg, n = 6). The α_2-agonist UK-14,304 (Cambridge, 1981), infused locally in a concentration of 1 µg/kg/min for 5 min, did not influence the perfusion pressure per se, but produced an inhibition of the stimulation-evoked increase in perfusion pressure (42.7 $\pm$ 1.9 %, n = 6) similar to that by apomorphine. The inhibitory effect of UK-14,304 was completely antagonized by rauwolscine (100 µg/kg, n = 6) but was not influenced by haloperidol (1 µg/kg, n = 6). The local administration of haloperidol per se did not influence the pressor response to stimulation at 4 Hz, but rauwolscine increased this pressor response by 39.4 $\pm$ 5.0 % (n = 12).

These results indicate that dopamine receptors are present on the sympathetic innervation of the vascular bed in the rat hindquarters. Stimulation of these receptors, leading to a decrease of noradrenaline release and consequently of sympathetic vasomotor tone, might - at least in part - explain the blood pressure lowering effect of intravenous apomorphine in the rat.

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NEUROMUSCULAR EFFECTS OF DIAZEPAM. INTERACTIONS WITH γ-AMINO BUTYRIC ACID AND METHOHEXITONE.

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It has been reported in the central nervous system that the action of benzodiazepines is mediated via gamma-aminobutyric acid(GABA) receptor (Niehoff, Mashal & Kuhar, 1983). Furthermore, a barbiturate site of action has also been linked to this receptor complex (Leeb-Lundberg, Snowman & Olsen, 1980).

In addition to its central effect, diazepam has also a peripheral action and it produces relaxation of skeletal muscle. Although at the neuromuscular junction there is no evidence for a diazepam, GABA or barbiturate receptor, these drugs may alter the contractile responses of skeletal muscle and modify neuromuscular transmission (Hamilton, 1967; Dretchen, Ghoneim & Long, 1971; Khan & Edman, 1983).

The aim of the present experiments was to study the neuromuscular effects of diazepam and its possible interactions with GABA or methohexitone to see if these drugs, alone or in combination, altered the contractile responses in the isolated chick biventer cervicis skeletal muscle.

The preparation was set up in an organ bath containing Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% CO_2 in oxygen. The contractile responses produced either by electrical or chemical stimulation were recorded isometrically using a force displacement transducer and a Washington pen recorder.

Diazepam (3.4-340 uM) increased the amplitude of the indirectly elicited twitch contractions (at 0.2 Hz with 5V maximum and 0.2 ms pulse duration) by 20-25% without producing an initial contracture in the chick skeletal muscle. However, diazepam (340 uM) had no significant effect on the tetanic contractions elicited at 1-100 Hz in the chick muscle. High concentrations of diazepam (500 uM) decreased the twitches and reduced the contractures produced by ACh (0.55 uM) or TEA (4.8 mM) by 42±2% and 55±4% respectively (mean±s.e., n=6, $P < 0.001$).
GABA (0.8-80 uM) had no significant effect either on the amplitude of the twitch contractions or on the ACh & TEA-induced contractures in the chick muscle. Furthermore, GABA (8 uM) did not alter the increase by diazepam (340 uM) or methohexitone (88 uM) of the twitch contractions.
Methohexitone (3.7-186 uM) increased the twitch contractions by 30-35% in the chick skeletal muscle. Methohexitone (88 uM) reduced the ACh-induced contracture by 51±6% whereas it increased the TEA contracture by 72±3%. Furthermore, there was no significant interaction between methohexitone and diazepam-induced potentiation of the twitch contractions. However, occasionally low concentrations of diazepam decreased the inhibition produced by methohexitone of the ACh-induced contracture in the chick skeletal muscle.

Although the effect of diazepam on the contractile responses of skeletal muscle is controversial and the mechanism by which diazepam potentiates the twitch contraction is not yet known, Khan & Edman (1983) had attributed the potentiation by diazepam to an action on intracellular calcium ions (release of activator calcium).

In conclusion, diazepam and methohexitone (but not GABA) potentiated the twitch contractions in response to indirect stimulation of the chick skeletal muscle. In addition, there was no significant interaction between the barbiturate methohexitone and the psychotropic drug diazepam.

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FUNCTIONAL SUBSENSITIVITY OF POST-JUNCTIONAL α_2 - BUT NOT α_1 - ADRENOCEPTORS IN THE DOG SAPHENOUS VEIN WITH REDUCTION IN TEMPERATURE

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In the dog saphenous vein vasoconstriction is mediated by both postjunctional α_1- and α_2-adrenoceptors (Timmermans and Van Zwieten, 1982). The present study examines the effect of reduction in temperature on the responses of this vessel to selective α_1-and α_2-adrenoceptor agonists.

Saphenous veins were cut into rings of 2-5mm in length and suspended in a 6ml organ bath under a tension of 500mg. Tissues were bathed in Krebs-bicarbonate solution at 37°C gassed with 5% CO_2 in O_2. After equilibration (30 min), responses to cumulative doses of NA were obtained and recorded via Grass FT03C force displacement transducers and model 7D polygraph. Tissues were then washed with agonist-free Krebs solution. The temperature of the circulating water bath was reduced to 27°C and after a further 30 min equilibration, new agonist concentration-response curves were constructed as described above. Similar procedures were followed at temperatures of 20°C and 15°C. Identical experiments were performed using the selective α_1-adrenoceptor agonists methoxamine and phenylephrine and the selective α_2-agonists B-HT 933 (Azepexole) and UK 14304 (5-Bromo-6-|2-imidazolin-2-ylamino|-quinoxaline). Clonidine was also tested in the absence and presence of the α_1-adrenoceptor antagonist indoramin (Rhodes and Waterfall, 1978). Responses were expressed as % of the maximum response obtained at 37°C and EC_{50} values calculated at each temperature are shown in Table 1.

Table 1. Effect of temperature on contractile responses to alpha-adrenoceptor agonists in the dog saphenous vein (n=4-8)

Agonist		EC_{50} (SEM)			
		37°C	27°C	20°C	15°C
Methoxamine	(μM)	2.51(0.32)	1.81(0.21)	2.28(0.30)	2.28(0.27)
Phenylephrine	(μM)	2.40(0.31)	1.15(0.42)	0.70(0.15)	0.56(0.08)
Noradrenaline	(μM)	2.67(0.32)	2.25(0.24)	1.80(0.31)	0.67(0.13)
B-HT 933	(μM)	1.09(0.23)	1.60(0.27)	2.91(0.47)	>9
UK 14304	(nM)	2.11(0.13)	2.55(0.18)	13.3(1.24)	>278
Clonidine	(nM)	9.31(0.47)	35.9(2.85)	62.1(5.87)	193(8.54)
Clonidine + Indoramin 10^{-6}M	(nM)	8.83(0.64)	42.9(2.57)	413(27.0)	>>1400

Within the temperature range 37-15°C there was an increase in tissue sensitivity to NA and phenylephrine, little change in the responses to methoxamine and reduced sensitivity to B-HT 933, UK 14304 and clonidine. The reduction in sensitivity to clonidine at temperatures below 37°C was more pronounced in the presence of indoramin (10^{-6}M), although this concentration did not block responses to clonidine at 37°C. This may indicate that, at high concentrations, clonidine also stimulates α_1-adrenoceptors post-junctionally.

In conclusion, reduction in temperature leads to a functional sub-sensitivity of post-junctional α_2 but not α_1-adrenoceptors, and suggests that at temperatures below 37°C, stimulation of α_1-adrenoceptors post-junctionally plays a greater role in vasoconstriction than stimulation of post-junctional α_2 adrenoceptors.

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AN INVESTIGATION OF THE NEGATIVE CHRONOTROPIC EFFECT OF ADENOSINE ON THE GUINEA-PIG ATRIUM

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The aim of this study was to determine the sites of action of adenosine as a negative chronotropic agent on guinea-pig isolated atria. Potential interactions of the purine with cell surface adenosine receptors, with the intracellular "P-site" (Londos and Wolff, 1977) and with the intracellular levels of s-adenosylhomocysteine (Ueland, 1982) were investigated.

Guinea-pig atrial pairs were mounted for isometric tension recording in organ baths containing Krebs solution (37°C, Atenolol 10^{-6}M present). Adenosine and its analogues were added cumulatively to the bath contents and the change in atrial rate measured after a 5 minute contact time.

Adenosine, and the cell surface receptor agonists 2-chloroadenosine, 5'-N-ethylcarboxamide adenosine (NECA), L-N^6-phenylisopropyl adenosine (L-PIA) and D-PIA evoked concentration related decreases in the rate of atrial contraction. The order of potency and - log IC_{50} values for these agonists were L-PIA (6.79±0.05) = 2-chloroadenosine (6.72±0.07) >NECA (6.58±0.05) >D-PIA (4.55±0.12) > adenosine (3.9±0.11). Responses evoked by adenosine were potentiated by the nucleoside transport inhibitor nitrobenzylthioinosine (NBMPR, 10^{-6}M) and were abolished by adenosine deaminase (1u/ml). The potency of the adenosine analogues was unaffected by either NBMPR or by adenosine deaminase. The adenosine receptor antagonist, 8-phenyltheophylline (10^{-6} to 10^{-5}M) blocked the effects of adenosine and its analogues in a competitive and equipotent manner.

There was little evidence for an intracellular site of action for adenosine. The "P-site" agonists 2'5' dideoxyadenosine and 9-β-D arabinofuranosyl adenine had minimal effects on the rate of atrial contraction. In addition, the negative chronotropic response to adenosine, in the presence of the adenosine deaminase inhibitor erythro-9-[2-hydroxy-3-nonyl] adenine (10^{-5}M), was not potentiated by l-homocysteine thiolactone (10^{-4}M). Homocysteine has been shown to enhance the intracellular levels of s-adenosyl homocysteine in cardiac tissue exposed to adenosine (Schrader et al, 1981). The lack of effect of homocysteine on the response to adenosine implies that an elevation of the intracellular levels of s-adenosylhomocysteine is unlikely to account for the negative chronotropic effect of the purine.

The results of this study lead to the conclusion that the negative chronotropic effect of adenosine is mediated via a cell surface adenosine receptor. The order of potency of adenosine analogues at this receptor suggest that it may be of the putative A_1 sub-type (Bruns, Daly and Snyder, 1980).

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FORSKOLIN AND POSITIVE INOTROPISM IN THE MAMMALIAN MYOCARDIUM

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De Souza et al (1983) have recently suggested that forskolin-induced positive inotropism in mammalian cardiac muscle is mediated by the cyclic AMP-dependent phosphorylation (inhibition) of sarcolemmal Na/K-ATPase activity and not by the phosphorylation mechanisms proposed for other drugs acting via cyclic AMP, e.g. β-adrenoceptor agonists. De Souza et al (1983) based their hypothesis on two sets of observations. First, forskolin, like the cardiac glycosides, caused potassium depletion from isolated perfused hearts without affecting the accumulation of either sodium or calcium, and second, forskolin was found to inhibit Na/K-ATPase activity subsequent to an increase in the active/inactive protein kinase ratio. Furthermore, these workers stated, in referring to the cardiac muscle twitch shape, that ".... forskolin did not change the velocity relaxation." We report here some observations from similar studies conducted in our laboratory that are wholly inconsistent with these authors' hypothesis.

Left atria from male Dunkin-Hartley guinea-pigs were set up for electrical stimulation using conventional methods and changes in isometric tension and atrial twitch shape characteristics subsequently recorded. Na/K-ATPase was purified from rabbit ventricular myocardium using a modification of that originally described by Pitts et al (1973), developed by Charnock et al (1980). Enzyme activity was measured using the pyruvate kinase/lactate dehydrogenase coupled enzyme assay of Schoner et al (1967). Specific enzyme activity is expressed as μmol ATP hydrolysed/hr/mg membrane protein. Values in the text represent the mean ± s.e.m. of 5 observations.

The results are summarised in table 1. Forskolin-induced positive inotropism was characterised by twitch shapes that were qualitively similar to those produced by isoprenaline. Thus, both drugs increased the rate of tension development (dT/dt), reduced the time to peak tension (TPT) and markedly enhanced the rate of relaxation (dR/dt). These later two events effected a significant ($P < 0.05$) abbreviation of the total twitch duration (TTD). In contrast the twitch shapes characterising ouabain-induced positive inotropism were markedly different from those produced by forskolin and isoprenaline. Thus, dT/dt, dR/dt and TPT were increased by ouabain. As a consequence of this latter effect TTD was significantly ($P < 0.05$) prolonged. Ouabain (100 nM-2 mM) produced a concentration-dependent inhibition of Na/K-ATPase activity. Neither forskolin (1-100 μM) nor isoprenaline (1 nM-10 μM), however, exerted any effect on the activity of the ouabain sensitive enzyme.

Table 1. Effect of forskolin, isoprenaline and ouabain on atrial twitch shape characteristics and on Na/K-dependent ATPase activity. (* 2 mM ouabain).

PARAMETER	FORSKOLIN (5.16 μM)		ISOPRENALINE (128nM)		OUABAIN (2 μM)	
	-drug	+drug	-drug	+drug	-drug	+drug
dT/dt (mN/msec)	0.26±0.04	0.69±0.06	0.23±0.03	0.77±0.05	0.22±0.01	0.59±0.03
TPT (msec)	65.20±3.60	55.00±1.70	66.10±2.00	53.40±1.50	63.20±5.60	71.46±5.30
dR/dt (mN/msec)	0.14±0.05	0.59±0.09	0.17±0.01	0.66±0.03	0.16±0.01	0.49±0.02
TTD (msec)	173.70±8.90	121.04±7.40	185.30±6.90	127.80±4.00	174.60±7.31	191.06±5.61
Na/K-ATPase	6.83±0.33	6.91±0.71	6.71±0.31	6.57±0.78	6.83±0.41	0.85±0.03*

In conclusion, and in contrast to that reported by de Souza et al (1983), the twitch shape characteristics provide evidence that forskolin exerts its positive inotropic action in the mammalian myocardium in a manner essentially the same as that established for β-adrenoceptor agonists. Whilst the inability of forskolin to inhibit Na/K-dependent ATPase is consistent with this hypothesis it should, nevertheless, be borne in mind that the microsomal sarcolemmal preparations containing the Na/K-dependent ATPase may lack adenylate cyclase and/or cyclic AMP-dependent protein kinase and so render forskolin ineffective.

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EFFECT OF OXYPERTINE ON DOPAMINE RECEPTOR STIMULATION IN THE CARDIOVASCULAR SYSTEM

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Oxypertine's behavioural effects in rats at a dose of 4 mg/kg i.p. have been shown to be associated with a selective depletion of the reserpine-resistant pool of monoamines without affecting the reserpine-sensitive pool (Palomo & Reid, 1984). This study was undertaken to investigate whether oxypertine also antagonizes dopamine receptors in vivo like other antipsychotic drugs. That such an action might occur was suggested by in vitro receptor binding studies (Nakahara & Uchimara, 1980) and by the demonstration of antagonism of dopamine receptors by oxypertine on the isolated rat vas deferens preparation (Miranda, 1978).

Male Sprague-Dawley rats were anaesthetized with urethane (1.5 g/kg i.p.). The trachea was cannulated to facilitate respiration. The right external jugular vein was cannulated for intravenous drug administration. Systemic arterial blood pressure was recorded continuously using a pressure transducer connected to a cannula inserted into the left carotid artery. 2 mg/kg phentolamine and 1 mg/kg propranolol were infused together over a 10 min period and in all animals (n=11) antagonized the pressor and depressor effects of 0.1 mg/kg i.v. noradrenaline and isoprenaline respectively. In the presence of these α- and β-adrenoceptor antagonists 50 μg/kg i.v. dopamine induced a depressor response, the opposite effect to that obtained when dopamine was administered before infusing the antagonists. Treatment with 4 mg/kg i.p. oxypertine alone caused a sustained fall in mean arterial blood pressure of 28.0±7.38 mmHg (mean ± S.E., n=7). In another 8 animals treated with the adrenoceptor antagonists 50 μg/kg i.v. dopamine was administered before and again 10 min after the oxypertine treatment. The reduction of blood pressure of 10.50±1.05 mmHg before oxypertine was not significantly different from the reduction of blood pressure of 8.00±2.40 mmHg after oxypertine (mean ± S.E., paired t-test, $p>0.2$).

The depressor effect induced by dopamine in the presence of α- and β-adrenoceptor antagonists has been observed previously in various species of animals including the rat and has been prevented by treatment with dopamine receptor antagonists such as haloperidol and sulpiride (Sampson et al, 1974; Chapman et al, 1980). Therefore the above result indicates that 4 mg/kg i.p. oxypertine does not antagonize dopamine receptors, unlike other antipsychotic drugs. This result is in agreement with observations made by Palomo et al (1984) who have found that 1 mg/kg i.p. haloperidol, but not 4 mg/kg i.p. oxypertine, antagonizes the behavioural effects of apomorphine, a dopamine receptor agonist. The effects of oxypertine on brain monoamines are currently being investigated.

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β-ADRENOCEPTOR BINDING CHARACTERISTICS OF CULTURED HUMAN CARDIAC CELLS

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Studies of the β-adrenoceptor characteristics of primary animal heart cell cultures suggest that functional β-adrenoceptors are maintained in primary culture (Kaumann, 1982). The use of binding techniques on cultured cells allows the investigation of receptor characteristics of tissue of human origin. This study compares the β-adrenoceptor characteristics of the Girardi human heart cell line with those of human atrial cells, which have mixed population of β_1 and β_2-adrenoceptors and with guinea pig left ventricular cells which have an almost homogenous population of β_1-adrenoceptors.

The Girardi heart cell line was established in 1956 from the right atrial appendage of a 41 year old male. Cells were obtained from Flow Laboratories and cultured in a roller system in minimal essential media (MEM) with 10% foetal calf serum (FCS). Scatchard binding assays were performed using a modification of the assay described by Engel et al (1981) to determine that the β-adrenoceptors on the cultured cells demonstrated high affinity (Kd = 8.7 pmol) for the ligand (-)-[^{125}I]Iodocyanopindolol ([^{125}I]cyp) and saturability (Bmax = 24 fmol.mg protein^{-1}). Aliquots (100 µl) of cell homogenate were incubated with [^{125}I]cyp 9-300 pM. Total binding was determined in triplicate and non-specific binding (nsb) in duplicate in the presence of 200 µM (-)-isoprenaline. The characteristics of the β-adrenoceptor binding sites on cultured human cardiac cells were assessed by examining the ability of ICI 118,551 (β_2-selective antagonist) and betaxolol (β_1-selective antagonist) to displace [^{125}I]cyp binding. Aliquots (100 µl) of cell homogenate were incubated with 50 µl of ligand and 50 µl competing antagonist in a total volume of 250 µl for 60 min at 37°C.

Table 1 pK_I values for ICI 118,551 and betaxolol. The values for the β_1 and β_2 populations in normal human atrial cells are taken from Heitz et al (1983).

Compound	Girardi Heart Cell Line		Guinea Pig Left Ventricle	Normal Human Atrial Cells	
	β_1 (40%)	β_2 (60%)	β_1	β_1 (65%)	β_2 (35%)
ICI 118,551	6.82 (± 0.16)	5.15 (± 0.12)	8.20 (± 0.09)	7.36 (± 0.02)	5.05 (± 0.26)
Betaxolol	6.35 (± 0.09)	8.40 (± 0.02)	6.30 (± 0.08)	5.42 (± 0.11)	7.20 (± 0.12)

The results show that the proportion of β_1-adrenoceptors on the cultured human atrial cells is less than on normal human atrial cells. Stiles et al (1982) demonstrated a comparable number of β-adrenoceptors (Bmax = 25 fmol.mg protein^{-1}) on normal human cardiac tissue and found that at > 6 h post mortem the β_1-adrenoceptors are more labile than β_2-adrenoceptors. We have shown this may also be true for long-term cultured human cardiac cells.

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SELECTIVE PROTECTIVE ACTION OF α-TOCOPHEROL ON NORADRENALINE-MEDIATED RESPONSES IN VASCULAR SMOOTH MUSCLE

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α-Tocopherol enhances noradrenaline mediated responses in vascular smooth muscle under hypoxic conditions, but the mechanism of this protective action is unknown (Kelly & Richardson, 1981; Kelly et al, 1982). In the present study, we have examined the selectivity of α-tocopherol against a range of agonists, each having a different way of activating the tissue. In addition, we have considered the possibility that α-tocopherol has the ability to inactivate noradrenaline uptake systems.

Spirally cut guinea-pig portal veins were suspended in sucrose-Krebs-Henseleit solution at 37°C and bubbled with 5% carbon dioxide in oxygen (normoxic conditions). After 30 min equilibration, the gas was changed to 5% carbon dioxide in nitrogen (hypoxic conditions). Full concentration-effect curves to the agonists were obtained in both normoxic and hypoxic conditions. All responses were expressed as a percentage of the maximal response obtained with each agonist under normoxic conditions. When present, α-tocopherol (41.8 μM) was added to the incubating solution at the start of the normoxic period.

Responses to acetylcholine (10 nM-100 μM), histamine (10 nM-10 μM) and potassium chloride (11.6-55 mM) were reduced by 35-64% when the tissue was transferred from normoxic to hypoxic conditions. The responses were unaffected when α-tocopherol was included in the incubation medium. In contrast, responses to noradrenaline (10 nM-100 μM) were markedly reduced in hypoxia, for example, responses to 10 μM noradrenaline were reduced by 84.8 ± 5.5%, but in the presence of α-tocopherol, the responses were only reduced by 54.8 ± 9% ($P = <0.05$; $n = 5$).

One possible mechanism whereby the responses to noradrenaline, but not to acetylcholine, histamine or potassium, could be increased is by the blockade of noradrenaline uptake. We investigated this possibility. In sucrose-Krebs solution, normoxic responses to noradrenaline were significantly ($P = <0.05$) increased by either cocaine (10 μM), or cocaine (100 μM) plus hydrocortisone (20 μM), but remained unaffected by α-tocopherol. The same concentrations of cocaine, or cocaine plus hydrocortisone, had no effect on noradrenaline responses under hypoxic conditions; whereas responses were significantly ($P = <0.05$) increased in the presence of α-tocopherol. Thus, α-tocopherol showed a different pattern of activities to those of the noradrenaline uptake blocking agents, cocaine and hydrocortisone. It was therefore concluded that the protective action of α-tocopherol was not mediated through the blockade of noradrenaline uptake. In support of this conclusion, α-tocopherol failed to affect responses to tyramine (100 μM every 3 min); whereas cocaine (10 μM), or cocaine (100 μM) plus hydrocortisone (20 μM), completely blocked these responses.

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EFFECTS OF δ-AMINOLAEVULINIC ACID IN ISOLATED PREPARATIONS OF RABBIT SMALL INTESTINE

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We have previously reported that delta-aminolaevulinic acid (ALA) at concentrations above 0.2 mM inhibits contractile activity in isolated preparations of rabbit small intestine and that phentolamine reduces this inhibitory effect, and also that the inhibition is followed by heightened contractions which can be blocked by pretreating preparations with indomethacin (Cutler, et al 1982). These pharmacological effects qualitatively resemble actions of GABA in rabbit intestine (Girdhar et al, 1981) although responses to GABA vary considerably from one preparation to another. These pharmacological actions of GABA have been attributed to release of noradrenaline and also of prostaglandin-like material.

In the present experiments, effects of pretreatment with prazosin and yohimbine followed by ALA administration have been examined. Inhibitory actions of ALA have been compared with effects of the α_1 and α_2 agonists, cirazoline and guanoxabenz. Effects of ALA have also been examined after pretreatment of preparations with guanethidine and with 6-hydroxydopamine to prevent noradrenaline release. Preparations of rabbit small intestine were bathed in oxygenated Ringer-Locke solution at 37°C and contractions of the preparations were recorded by isotonic transducer and displayed on a calibrated Washington 400 MD2R oscillograph.

Inhibitory effects of ALA on tone and amplitude of contractions were dose-related. Pretreatment of preparations with prazosin (10^{-7} M) reduced the duration of the inhibitory effects due to ALA at 7.6 mM concentrations from 220 ± 33 sec to 81 ± 29 sec (mean ± s.e. mean, $P < 0.05$) and effects due to ALA at 3.8 mM concentration from 108 ± 13 sec to 43 ± 4.9 sec (mean ± s.e. mean, $P < 0.01$). Pretreatment with yohimbine (10^{-6} M) did not significantly alter ALA's inhibitory action. Cirazoline (10^{-5} M) caused a transient inhibition of contractile activity whereas guanoxabenz (10^{-5} M) in the presence of prazosin (10^{-7} M) to block any residual α_1 effects did not significantly alter contractions of the preparation. There is thus no evidence that α_2-receptors can affect contractile activity under these circumstances.

Pretreatment of preparations with either 6-hydroxydopamine (10^{-3} M) or with guanethidine (1.5×10^{-5} M) did not prevent inhibitory actions from ALA (3.8 mM). Therefore ALA appears to be directly affecting α_1 receptors, and the inhibitory actions cannot solely be ascribed to noradrenaline release consequent on GABA receptor activation.

These findings might be relevant to the gastrointestinal features of episodes of acute porphyria in which blood concentrations of ALA may increase many fold over normal values (Laiwah et al, 1983), but this cannot be substantiated until concentrations of ALA in the gastrointestinal tract have been measured during acute attacks.

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CHARACTERISATION OF A 5-HYDROXYTRYPTAMINE RECEPTOR MEDIATING DEPOLARISATION OF MURINE NEUROBLASTOMA CELLS

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Cells of the neuroblastoma clonal line N1E-115 (Amano et al, 1972) respond to microperfused and ionophoretically applied 5-hydroxytryptamine (5-HT) with a membrane depolarisation arising from an increase in membrane permeability to sodium and potassium ions (Guharay & Usherwood, 1981; Peters & Usherwood, 1983). The pronounced tachyphylaxis exhibited by this response renders the construction of dose response curves by bath application methods impractical. We have therefore utilized ionophoretic techniques in the examination of the 5-HT evoked response.

The sensitivity of N1E-115 cells to 5-HT was found to be 9.86 ± 9.97 (S.D.) mV nC^{-1} (n = 28) (Miledi, 1960). Double logarithmic plots of ionophoretic ejection current against the 5-HT evoked depolarisation and conductance increase yielded limiting slopes of 2.88 ± 0.79 (n = 20) and 3.0 ± 1.09 (n = 8) respectively. This suggests that the binding of a minimum of 3 molecules of 5-HT is required for activation of the 5-HT receptor-ionophore complex. In order to determine whether a high facilitation or independent binding site receptor model best fitted the data, a 'least squares' regression analysis (Constanti, 1977) was performed on each of 5 individual dose/conductance curves. In the analysis the interaction coefficient was allowed to take on integral values between 1 and 5. In all cases, the best fit was provided by the high facilitation model with an interaction coefficient of 3. A 5-HT receptor present on autonomic neurones displays similar properties (Higashi & Nishi, 1982).

A number of possible agonists were tested for potency at this receptor. All agonists were initially applied to the cells by microperfusion at a concentration of 1mM. Those demonstrating agonist potency were then further evaluated by ionophoresis and assigned an equipotent dose ratio (EPDR) taking the EPDR for 5-HT as 1. 5-methoxytryptamine, 5-methyltryptamine, 5-hydroxyindole acetic acid and RU 24969 all failed to elicit depolarisations on cells shown to be responsive to 5-HT (10 µM). 5-fluorotryptamine (5-FT) and tryptamine induced depolarising responses which displayed cross-tachyphylaxis with responses to 5-HT. Dose response curves for 5-HT, 5-FT and tryptamine were parallel and displayed similar maxima suggesting that 5-FT and tryptamine behave as full agonists with calculated EPDR's of 8.8 ± 7.2 (n = 5) and 31.5 ± 11.6 (n = 6) respectively.

Dopamine (DA) also elicited a depolarising response that displayed cross-tachyphylaxis with 5-HT. The reversal potentials of the 5-HT and DA-induced responses were virtually identical, being -7.0 ± 0.8 mV (n = 3) and -7.7 ± 1.0 mV (n = 3) respectively, indicating a common ionic basis. Antagonists which abolished the response to 5-HT (e.g. quipazine; Peters & Usherwood, 1983) were also effective against DA. The dose response curve for DA was of shallower slope and smaller maximum than that for 5-HT. The EPDR for DA, calculated at the ED_{25} of the 5-HT dose response curve was 30.3 + 17.7 (n = 3). It is suggested that DA may act as a partial agonist at this 5-HT receptor (cf Kato & Narahashi, 1982).

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NOVELTY MODIFIES THE STIMULANT EFFECTS OF β-PHENYLETHYLAMINE ON LOCOMOTION AND REARING IN THE RAT.

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Robbins & Sahakian (1981), in their review of determinants of drug-induced locomotion and stereotyped behaviour, point out that exposure to novel test situations can bring about qualitative changes in behavioural responses to psychostimulant drugs. Recently we showed that β-phenylethylamine (PEA), administered in a moderate dose (12.5 mg/kg), increased the locomotion of thirsty rats tested in a novel environment (Cooper & Dourish, 1984). The aim of the present study was to establish if environmental novelty is an important factor in determining the qualititative nature of PEA's effects on spontaneous motor activity.

The subjects were 24 naive, adult male Wistar rats (200-250 g). The animals were allocated to 4 equal groups which received 0, 6.25, 12.5 and 25.0 mg/kg PEA hydrochloride respectively. Injections were given i.p., using a saline vehicle, immediately prior to testing. On Day 1, each rat was placed individually after injection into an activity-measuring apparatus (a Perspex cage positioned in an Opto-Varimex Minor, Columbus Instruments) for a 15 min test under novel conditions. Locomotion and rearing were measured automatically by photobeam interruptions which were recorded by a microcomputer. Direct behavioural observations supplemented the automated recordings. After an interval of 7 days, each animal was exposed for 15 min each day to the test apparatus for 6 successive days. On Day 14, each rat was injected again according to the Day 1 injection scheme and re-tested in the apparatus for a 15 min test, under familiar conditions. A 2 factor ANOVA was used to analyse activity scores.

For locomotion scores, there was a significant effect of PEA dosage ($p < 0.01$), of test condition ($p < 0.05$), and a significant interaction between the two ($p < 0.01$). There were also significant effects of PEA dosage and test condition on rearing ($p < 0.01$ in each case). Under conditions of familiarity PEA significantly increased locomotion when administered in doses of 12.5 or 25.0 mg/kg, but did not affect rearing at any dose level tested. In strong contrast, when the environment was novel, only 12.5 mg/kg PEA increased locomotion whereas 12.5 and 25.0 mg/kg significantly enhanced rearing.PEA (25.0 mg/kg) elicited stereotyped headmovements in both novel and familiar test conditions.

Behavioural responses to PEA treatments appear to be modulated by the novelty or familiarity of the test situation. Whereas in the familiar condition, PEA selectivity enhanced locomotion, a different response emerged in the novel condition. PEA, in the latter case, significantly enhanced rearing. These data provide a striking instance therefore of a qualitative change in the behavioural response to a psycho-stimulant compound which is associated with the relative novelty of the test environment (Robbins & Sahakian, 1981).

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AMINE OXIDASE ACTIVITIES IN DISSOCIATED CELL FRACTIONS FROM RAT SKELETAL MUSCLE

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Rat skeletal muscle homogenates contain predominantly the A-form of monoamine oxidase (MAO), with little or no MAO-B being present (Meltzer & Arora, 1979); Kwatra & Sourkes, 1980). Also, a semicarbazide-sensitive amine oxidase (SSAO), distinct from MAO-A or B has been described in this tissue (Lewinsohn et al, 1978). Possible differences in the cellular localization of these enzymes have been studied here in dissociated cell fractions from rat skeletal muscle.

Adult Wistar rat gastrocnemius muscle, sliced longitudinally, was shaken in buffer containing 1 mg/ml collagenase and dissociated cells were harvested by filtration through nylon mesh. Skeletal muscle cells were sedimented by centrifugation (3 g/5 min) and further purified by settling through 4% (w/v) bovine serum albumin (BSA). Light microscopy showed short sections of single muscle fibres, with occasional adherent blood vessel fragments. A non-myocyte pellet (of connective tissue cells e.g. fibroblasts, as well as smooth muscle and endothelial cells from the vasculature) was isolated by centrifugation (800 g/10 min) of the myocyte-depleted filtrate, followed by resuspension and re-centrifugation (50 g/ 10 min) through BSA. Final cell fractions were washed and homogenized in 1mM potassium phosphate pH 7.8 for enzyme assays. Corresponding homogenates of undissociated tissue were also prepared for comparative purposes.

Amine oxidase activities were assayed with ^{3}H-5-hydroxytryptamine (5-HT) and ^{14}C-benzylamine (BZ). 1mM 5-HT metabolism was completely inhibited by 10^{-7}M clorgyline, whereas 1 μM BZ metabolism was unaffected by 10^{-3}M clorgyline, showing that these substrates were metabolized by MAO-A and SSAO, respectively, under these conditions. Little deamination of 1mM BZ above blank values could be detected in homogenates of this muscle, suggesting that it was largely devoid of MAO-B activity. Alkaline phosphatase (AP) was assayed by following spectrophotometrically the release of p-nitrophenol from p-nitrophenylphosphate.

Specific enzyme activities (nmol/h/mg protein) in myocyte (M), non-myocyte (NM) and undissociated tissue (U) homogenates were MAO-A, 16.5 $\pm$ 2.7 (M), 36.5 $\pm$ 8.5 (NM) and 12.9 $\pm$ 2.5 (U) (n = 5 preparations); SSAO, 0.11 $\pm$ 0.04 (M), 3.8 $\pm$ 1.1 (NM), and 0.26 $\pm$ 0.06 (U) (n = 7); AP (μmol/h/mg protein), 0.24 $\pm$ 0.04 (M), 7.1 $\pm$ 1.7 (NM) and 1.5 $\pm$ 0.29 (U) (n = 7).

These results suggest that MAO-A may be found in both skeletal muscle as well as non-myocyte cells. In contrast, the markedly higher activities of SSAO and AP in non-myocyte fractions suggest a preferential localization to cellular components found there. This supports histochemical evidence for AP being found in the vasculature but not on skeletal muscle cells in this tissue (Beckett & Bourne, 1972). Also, our preliminary use of a histochemical method for SSAO has localized this enzyme to the smooth muscle layers of larger blood vessels in this tissue.

It would appear that vascular smooth muscle cells may be a major source of SSAO in some tissues, whereas the activity, if any, in skeletal muscle (this work) and cardiac muscle cells (Lyles et al, 1984) is much lower.

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SODIUM IONS INCREASE THE AFFINITY OF IDAZOXAN FOR CENTRAL α_1-BINDING SITES LABELLED BY [^{3}H]-PRAZOSIN.

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Idazoxan has been described as a selective antagonist at peripheral and central α_2-adrenoceptors (Dettmar et al., 1983; Doxey et al., 1983). The binding of [^{3}H]-idazoxan to rat brain α_2-binding sites was modified by sodium, calcium and magnesium ions, (Lane et al., 1983), whereas [^{3}H]-prazosin was unaffected by these ions (Hornung et al., 1979). However, the imidazoline α-adrenoceptor agonists naphazoline and clonidine displaced [^{3}H]-prazosin with higher affinity in the presence of sodium ions (Glossmann and Hornung, 1980). It was of interest therefore to study the metal ion dependency of the imidazoline α_2-antagonist idazoxan at displacing [^{3}H]-prazosin from central α_1-binding sites.

Binding experiments were performed as described previously (Doxey et al., 1984). The rat brain membranes after initial differential centrifugation and washing were resuspended either in TRIS-buffer, 0.05M, pH 7.8 with or without added metal ions at physiological concentration, or in a physiological salt solution (PSS) as described previously (Lane et al., 1983).

The data (Table 1) show that the affinity of idazoxan was higher in the presence of sodium ions than in TRIS. No increases in affinity were observed with potassium, magnesium or calcium ions, but when sodium ions were mixed with each of these ions, or in a mixture of ions at physiological concentrations (PSS), the increase in affinity was still observed. The selective effect by sodium ions was not due to changes in [^{3}H]-prazosin binding characteristics: in TRIS, B_{max} = 142 ± 13 fmole/mg, K_D = 0.71 ± 0.09 nM; in PSS, B_{max} = 158 ± 16 fmole/mg, K_D = 0.69 ± 0.21 nM.

Table 1. The displacement of [^{3}H]-prazosin by idazoxan.

Medium	Ki (nM)	Hill Slope
TRIS	399±55	0.95±0.02
TRIS + KCl(6mM)	443±52**	0.95±0.01
TRIS + NaCl (143mM)	146±18**	0.87±0.01
TRIS + $MgCl_2$ (1.2mM)	557±34	1.01±0.02
TRIS + $CaCl_2$ (1.3mM)	527±40**	0.89±0.05
TRIS + NaCl + $MgCl_2$	177±13**	0.90±0.04
TRIS + NaCl + $CaCl_2$	171±38**	0.86±0.04
TRIS + NaCl + KCl	112±23**	0.81±0.03
PSS	142±27**	0.92±0.09

** P< 0.01 vs TRIS (Dunnett's Test): Mean ± SEM, n=3.

In conclusion, it seems likely that sodium ions increase the affinity of imidazoline structures such as idazoxan, naphazoline and clonidine for the α_1-site labelled by [^{3}H]-prazosin.

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INVOLVEMENT OF ACETYLCHOLINE IN THE HYPOTHERMIC RESPONSE OF MICE TO Δ^9-TETRAHYDROCANNABINOL.

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We have tested the possibility that cholinergic pathways could be involved in the production of hypothermia by Δ^9-tetrahydrocannabinol (THC) by investigating firstly whether atropine can reduce THC hypothermia and secondly whether animals showing tolerance to oxotremorine hypothermia are also tolerant to the hypothermic effect of THC. Experiments were conducted with unrestrained adult male albino mice at an ambient temperature of 22°C. Body temperature (Tr) was measured with a rectal thermistor probe. Intracerebroventricular (i.c.v.) injections (0.5µl) were made into the third ventricle (Nyemitei-Addo et al, 1980). THC was mixed with 2 parts of Tween 80 by weight, dispersed in a 0.9% (w/v) NaCl solution (saline) and administered at a submaximal hypothermic dose (20 mg/kg i.p. or s.c.). Oxotremorine sesquifumarate was dissolved in saline and given by s.c. injection (0.5mg/kg) or at submaximal hypothermic doses by i.p. (0.175mg/kg) or i.c.v. (5µg) injections. Atropine sulphate dissolved in saline was given 30 min before THC either subcutaneously at a dose (2mg/kg) known to abolish the hypothermic response to oxotremorine (0.175mg/kg i.p.) or by i.c.v. injection (5µg).

The hypothermic response to i.p. THC was significantly reduced by s.c. pretreatment with atropine. Over the first 20 min after THC administration Tr fell from 37.3 ± 0.2 to 32.9 ± 0.4 (mean ± s.e., n=6) in mice pretreated with saline and from 37.0 ± 0.2 to 34.6 ± 0.4 in atropine pretreated animals. Similar results were obtained after i.c.v. pretreatment with saline or atropine suggesting that the interaction between atropine and THC occurs in the brain. Twice daily s.c. administration of oxotremorine for 4 days significantly attenuated the hypothermic responses both to oxotremorine itself and to THC when these were administered on day 5. Over the first 40 min. after i.p. administration of oxotremorine, Tr fell from 37.8 ± 0.2 to 33.0 ± 0.6 in the group which had been pretreated subcutaneously with oxotremorine and from 37.8 ± 0.2 to 29.6 ± 0.4 in the saline pretreated group. THC caused Tr to fall from 37.3 ± 0.3 to 35.2 ± 0.6 in a second oxotremorine pretreated group and from 37.4 ± 0.2 to 33.5 ± 0.3 in saline pretreated mice. Subcutaneous oxotremorine pretreatment also significantly reduced the hypothermic response on day 5 to i.c.v. oxotremorine. Interestingly, a 3-day pretreatment with THC (20mg/kg s.c. once daily) producing tolerance to the hypothermic effect of i.p. THC on day 4 did not alter the hypothermic response to i.p. oxotremorine suggesting that onset of tolerance to THC does not depend on the development of tolerance to endogenously released ACh. Our results do however support an involvement of cholinergic pathways in the hypothermic effect of THC in mice.

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THE EFFECT OF NITROUS OXIDE ON BEHAVIOURAL THERMOREGULATION IN MICE

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In each experiment an adult male albino mouse was placed in an apparatus consisting of two narrow semi-circular tunnels joined together to form a complete circle. The apparatus was mounted in a pressure chamber. The two tunnels were kept at different temperatures (21.6^{o} and 27.5^{o}C) and behavioural thermoregulation was monitored by measuring the length of time spent in each tunnel. Deep body temperature (Tr) was recorded continuously with a rectal thermistor connected to a swivel device. Locomotor activity was observed through a window and recorded as the number of times a mouse moved between tunnels. Carbon dioxide was absorbed by soda lime placed beneath the mesh floor of each tunnel. For the first 50 min each mouse was subjected to 1 atmosphere absolute (atm) of oxygen. The pressure chamber was then flushed for 5 min with a known mixture of O_2 and either N_2O (test gas) or N_2 (control gas) and the mouse kept in the new gas mixture (N_2O or N_2 plus 1 atm O_2) for a further 120 min.

N_2O produced significant reversible falls in Tr of 0.3 $\pm$ 0.1^{o}C at 0.25 atm, 1.8 $\pm$ 0.4^{o}C at 0.5 atm and 2.9 $\pm$ 0.3^{o}C at 0.75 atm (mean $\pm$ s.e.). Tr fell over a period of about 30 min. and thereafter remained steady. The hypothermia was associated with a significant decrease in the length of time spent in the warm, the N_2O-treated mice spending most time either in the cooler tunnel (0.75 and 0.5 atm) or at one of the two junctional positions between the tunnels (0.25 atm). In experiments with 0.25 atm of N_2O the time spent in the warmer tunnel was 1545 $\pm$ 48 s (mean $\pm$ s.e.) in the final 30 min before the addition of N_2O and 1028 $\pm$ 180 s in the first 30 min after N_2O (n=10). The corresponding values at 0.5 atm were 1437 $\pm$ 26 s and 279 $\pm$ 117 s whereas at 0.75 atm they were 1522 $\pm$ 153 s and 246 $\pm$ 123 s (n=6). Neither N_2 (0.75 atm) nor 0.1 atm of N_2O had any significant effect on Tr or behaviour and none of the treatments produced any detectable change in locomotor activity. The results suggest that N_2O can produce behavioural changes directed at lowering body temperature. They also show that the gas is active at doses far below those required to produce light anaesthesia in mice, the ED50 for the abolition by N_2O of the mouse righting reflex being 1.5 atm (Miller et al, 1967). Whether subanaesthetic pressures of other gases can produce similar behavioural changes remains to be determined. Of particular interest is the possibility that the diving gases N_2 and He can alter behavioural thermoregulation in divers and experiments with these gases are now in progress.

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NON-SELECTIVE INHIBITION OF GABA AND 5-HT UPTAKE IN RAT BRAIN BY MOLECULES POSSESSING LONG ALKYL SIDE CHAINS

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We have previously synthesized a series of N-n-alkyl hydroxybenzylamine derivatives which inhibit the uptake of [^{3}H]-GABA into brain synaptosomes (Breckenridge et al., 1981). To examine further their pharmacological specificity, the above compounds, together with several newly synthesised N-n-alkyl phenylethylamine derivatives, have been examined for their ability to inhibit [^{3}H]-5-hydroxytryptamine (5-HT) uptake into rat brain synaptosomes. These data were compared to those obtained for the inhibition of brain [^{3}H]-GABA uptake. [^{3}H]-5-HT and [^{3}H]-GABA uptake were measured in synaptosomes prepared from rat hypothalamus and cerebral cortex, respectively, using previously described techniques (Breckenridge et al.1981).

Table 1 Inhibition of [^{3}H]-GABA uptake and [^{3}H]-5-HT uptake into rat brain synaptasomes by various compounds.

Compound	IC_{50} value (μM) [^{3}H]-GABA uptake	[^{3}H]-5-HT
GABA	7.0 ± 1.3	>1000
Nipecotic Acid	4.6 ± 0.6	>1000
5-HT	>1000	0.33 ± 0.04
Imipramine	23 ± 2.4	0.86 ± 0.06
N-n-dodecyl-2-hydroxybenzylamine	42 ± 0.3	43 ± 6.1
N-n-octyl-3-hydroxybenzylamine	33 ± 4.6	11 ± 3.1
N-n-dodecyl-3,4-dihydroxybenzylamine	33 ± 3.2	7.1 ± 3.0
N-n-octyl-2-methylbenzylamine	75 ± 5.6	12.8 ± 1.8
N-n-octyl-4-hydroxyphenylethylamine	48 ± 3.1	14.7 ± 1.7
N-n-octyl-3,4-dihydroxyphenylethylamine	29 ± 2.2	6.9 ± 1.2
N-n-octyl-3,4-dimethoxyphenylethylamine	55 ± 2.9	2.8 ± 0.8

100% inhibition of [^{3}H]-GABA or [^{3}H]-5-HT uptake was defined using 1mM GABA and 1mM 5-HT respectively. IC50 values (concentration of test compound causing 50% inhibition of uptake) were obtained from inhibition curves involving 4-6 different concentrations of test substance, and are the means of 3-6 separate experiments.

The results (Table 1) indicated that [^{3}H]-5-HT uptake was potently inhibited by 5-HT itself and the classical 5-HT uptake blocker imipramine, while GABA and the GABA uptake inhibitor nipecotic acid were inactive. Predictably, whereas GABA and nipecotic acid both inhibited [^{3}H]-GABA uptake, imipramine was less active while 5-HT was without effect. In marked contrast, the various N-n-alkyl hydroxybenzylamine or N-n-alkyl hydroxyphenylethylamine derivatives were all moderately active at inhibiting both [^{3}H]-5-HT and [^{3}H]-GABA uptake. It is suggested that the ability of molecules possessing long alkyl side chains to inhibit GABA uptake systems in the brain does not reflect their specific interaction with GABA uptake sites, but rather a non-specific interaction with neurotransmitter uptake systems in general. This non-selective action almost certainly relates to the detergent/surfactant-like properties of these highly lipophilic compounds, and may reflect a non-selective interaction with neuronal cell membranes.

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CENTRAL 5-HT MECHANISMS MAY MEDIATE 5-HYDROXYTRYPTOPHAN-INDUCED HYPOTHERMIA IN GUINEA PIGS BUT NOT RATS

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L-5-Hydroxytryptophan (5HTP)-induced hypothermia in the rat appears due to an action of 5-hydroxytryptamine (5HT) in the periphery (Carter & Leander, 1980). Using the rat for comparison we have investigated the role of 5HT in thermoregulation in the guinea pig. We find that 5HTP-induced temperature changes in the guinea pig are predominantly mediated through central 5HT mechanisms.

Peripheral and central actions of 5HT were differentiated by treatment of animals with either the peripheral decarboxylase inhibitor carbidopa (α-methyldopahydrazine; 25 mg/kg i.p. 1h prior to 5HTP) or the peripheral 5HT antagonist xylamidine tosylate (1 or 5 mg/kg i.p. immediately prior to 5HTP), or by the systemic administration of 5HT creatinine phosphate (1-36 mg/kg i.p.) which does not cross the blood-brain barrier. The rectal temperatures of female Wistar rats (140-210 g) or female Dunkin Hartley guinea pigs (250-500 g) were measured at hourly intervals before and after 5HTP or 5HT administration. All experiments were performed at 20 ± 1°C.

5HTP (45-200 mg/kg sc) induced a dose-dependent hypothermia in both rats and guinea pigs. Pretreatment of rats with carbidopa (25 mg/kg i.p. 1h previously) reduced both the intensity and duration of 5HTP-induced hypothermia. The combination of 5HTP and carbidopa in rats caused a weak incidence of "wet dog" shakes. Carbidopa pretreatment also prevented the hypothermia evoked in guinea pigs by 5HTP (45 & 75 mg/kg) and induced hyperthermia in combination with 125 mg/kg 5HTP. However, the combination of 5HTP and carbidopa caused rhythmic myoclonic jerking which might explain the reversal of 5HTP-induced hypothermia.

Xylamidine tosylate (1 or 5 mg/kg i.p.) reversed the hypothermia evoked in rats by administration of 5HTP (90 mg/kg) but did not reverse 5HTP (75 mg/kg)-induced hypothermia in guinea pigs (Table 1). The temperature changes evoked in carbidopa pretreated rats and guinea pigs by 200 and 100 mg/kg 5HTP respectively were unaffected by administration of xylamidine tosylate (1 or 5 mg/kg i.p.). Administration of 5HT (1-36 mg/kg i.p.) induced a dose-dependent hypothermia in rats but not in guinea pigs.

Table 1 Effect of xylamidine tosylate (1 mg/kg) on 5HTP-induced hypothermia in rodents

Treatments		Mean rectal temperature (±) 1 SEM (°C) Rat (5HTP : 90 mg/kg i.p.) +1h	+3h	Guinea pig (75 mg/kg i.p.) +3h
Vehicle	Saline	37.48±0.21	37.00±0.12	38.36±0.09
Vehicle	5HTP	34.80±0.29*	35.65±0.24*	37.52±0.12*
Xylamidine	5HTP	35.87±0.28+	36.90±0.17+	37.40±0.15

$p < 0.05$ vs saline* or 5HTP+ : Student's t-test

The attenuation of 5HTP-induced hypothermia by administration of carbidopa or xylamidine, and the hypothermic effect of 5HT each suggest that peripheral 5HT systems play an important role in rat thermoregulation. In the guinea pig, however, we suggest that central 5HT thermoregulatory systems are largely responsible for 5HTP-induced temperature changes.

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DEPLETION OF STRIATAL DOPAMINE BY MPTP DOES NOT ALTER SPONTANEOUS OR DOPAMINE-DEPENDENT BEHAVIOUR IN ALBINO OR PIGMENTED MICE

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N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces Parkinsonism in man and primates (Langston et al, 1983; Burns et al,1983) but apparently not in albino rats (Boyce et al, 1984). To investigate whether the inactivity in albino rodents was due to a lack of melanin pigmentation or an inadequate dose of MPTP we have chronically administered MPTP to both albino and pigmented mice and now report the effect on spontaneous and dopamine-dependent behaviours.

Male albino CD1 mice (24-27g) or male pigmented C57 BL/10 mice (23-26g) were administered MPTP (25 or 10mg/kg i.p.) or vehicle (0.3% v/v acetic acid i.p.) daily for 16 days. The following behaviours were assessed during drug withdrawal (day of testing): Spontaneous (withdrawal days 1 and 8) and d-amphetamine-induced (withdrawal day 8) locomotor activity, and spontaneous and apomorphine-induced gnawing (withdrawal day 4). Brains were removed for biochemical analysis on withdrawal day 11 (Buckett, Diggory & Luscombe, 1984).

During chronic MPTP (25mg/kg) treatment body weight gain was attenuated only in albino mice although both strains of mice showed a transient reduction in body weight gain during MPTP (10mg/kg) administration. Chronic MPTP administration did not alter the spontaneous locomotor activity of either albino or pigmented mice (Table 1). d-Amphetamine (1mg/kg i.p.)-induced locomotor activity was increased for 0.5h following administration to MPTP (25 and 10 mg/kg) pretreated albino mice compared to the activity of vehicle pretreated mice. This potentiation was not observed in pigmented mice. Spontaneous and apomorphine (50mg/kg i.p.)-induced gnawing in both albino and pigmented mice were each unchanged by chronic MPTP treatment. For example, the number of holes (mean±SEM) gnawed following apomorphine administration to mice pretreated with vehicle, 25 or 10mg/kg MPTP were respectively: Albino 52±9, 53±12 and 45±12; Pigmented 15±3, 19±4 and 20±4.

Table 1 Spontaneous locomotor activity of albino (Alb) and pigmented (Pigm)mice pretreated with MPTP (25 or 10 mg/kg x 16 days) or vehicle.

Withdrawal day	Locomotor activity (counts in 2h; mean ± SEM)					
	Alb(Veh)	Alb(25)	Alb(10)	Pigm(Veh)	Pigm(25)	Pigm(10)
1	3943±588	4870±461	4303±341	3166±347	3860±271	3722±243
8	4471±856	3484±374	3235±365	2979±300	2855±355	2458±220

Chronic MPTP (25 mg/kg) administration depleted striatal dopamine levels by 29% and 50% in albino and pigmented mice respectively (Buckett et al, 1984). The lack of behavioural effect of this treatment in mice is therefore in agreement with the observations in primates that Parkinsonism is observed only after 80-90% depletion of striatal dopamine content (Burns et al, 1983).

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NOVEL SELECTIVE RADIOLIGAND FOR ONE SUBPOPULATION OF RAT CORTEX SOMATOSTATIN RECEPTORS

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The cortex of the rat brain is known to have a high concentration of somatostatin (SS) and of SS receptors. Using an SS receptor binding assay (Reubi et al., 1981) with ^{125}I-Tyr^{11}-SS as radioligand, it can be shown there that a stable, cyclic octapeptide somatostatin analog, SMS 201-995, [H-(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr(ol)] (Bauer et al., 1982), is binding to a subpopulation of SS receptors (Reubi, 1984). In the cortex and hippocampus SMS 201-995 binds with high affinity to 75%, respectively 68% of the SS receptors labelled with ^{125}I-Tyr^{11}-SS, whereas in pituitary and pancreatic β-cells (insulinoma) it binds with high affinity to all SS receptors.

The biologically active Tyr^3 analog of SMS 201-995 code-named 204-090 shares with SMS 201-995 the above characteristics. It labels 62% and 65% of SS receptors in cortex and hippocampus but 100% of SS receptors in a pancreatic β-cell preparation. This peptide has been successfully iodinated (chloramine T method) and purified on HPLC (^{125}I-204-090) in order to investigate its characteristics as selective radioligand for a part of the rat SS receptors. Using above-cited methodology, it is shown to have saturable and high affinity binding to CNS, pituitary and pancreatic β-cell membrane preparations. Dissociation constant (K_D) for 204-090 in cortex is 0.19 ± 0.07 nM. Peptidase inhibitors (Bacitracin and $MgCl_2$) are necessary for optimal binding. The ratio specific: non specific binding of 7.2 using ^{125}I-204-090 is a considerable improvement over that found with ^{125}I-Tyr^{11}-SS. The number of binding sites in the cortex measured with ^{125}I-204-090 (B_{max} = 360 ± 80 fmoles/mg protein) is greater than with ^{125}I-Tyr^{11}-SS as radioligand. This is probably due to technical improvement (less degradation of the radioligand) rather than to the fact that ^{125}I-204-090 labels a type of receptor not detected by ligands with the full SS structure such as ^{125}I-Tyr^{11}-SS. Indeed, in cortex, hippocampus, striatum, hypothalamus, pituitary and insulinoma, SS can displace with high affinity ^{125}I-204-090 from all its binding sites. Number of binding sites in these regions are respectively 342 ± 35, 368 ± 62, 210 ± 17, 80 ± 17, 97 ± 13 and 7129 ± 1385 fmoles/mg protein (mean ± SEM, n = 3).

SMS 201-995 inhibits completely and with high affinity ^{125}I-204-090 binding in all preparations including hippocampus and cortex, contrasting though with the results using ^{125}I-Tyr^{11}-SS and suggesting selective labelling of one SS receptor subtype. That ^{125}I-204-090 binds indeed to true SS receptors is indicated in competition experiments by the pharmacological selectivity of various SS analogs, which correlate either with the selectivity seen in in vitro bioassay for GH or cortical receptor binding assay using ^{125}I-Tyr^{11}-SS. Active analogs such as D-Trp^8-SS and SS-28 are highly potent (5.1 ± 0.3 resp. 1.58 ± 0.08 times the potency of 204-090; n = 3) whereas biologically inactive analogs such as Des-Trp^8-SS or SS-28 (1-12) are inactive in this assay, as well as unrelated peptides such as Leu-enkephalin.

This novel radioligand should be of great value for further investigations on SS receptor populations, in particular in CNS.

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CLONIDINE DOES NOT ANTAGONISE SEIZURES INDUCED BY INTRAVENOUS INFUSION OF LEPTAZOL IN RODENTS

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Several recent reports suggest that central α_2-adrenoceptors are involved in seizure mechanisms. Clonidine was shown to be anticonvulsant in DBA/2 mice (Horton et al., 1980) and to protect against leptazol-induced seizures in rats (Papanicolaou et al., 1982a; Lazarova and Samanin, 1983a). In contrast, pro-convulsant properties of yohimbine (Lazarova and Samanin, 1983b) and the more selective α_2-antagonists idazoxan and Wy 26392 have been described (Fletcher and Forster, 1984). In this report we describe the effects of clonidine on seizures induced by intravenous infusion of leptazol in rats and mice.

Clonidine hydrochloride was administered i.p. as a solution in isotonic saline to male Sprague-Dawley rats (100-140g). Fifteen minutes later leptazol (12 $mg.ml^{-1}$ in isotonic saline) was infused via a tail vein at a rate of 0.35 $ml.min^{-1}$, and the times to onset of clonic and tonic seizures were measured. The results of this experiment are shown in Table 1.

Table 1 Effect of clonidine on leptazol seizure thresholds in rats

Dose of clonidine HCl ($mg.kg^{-1}$)		vehicle	0.001	0.005	0.025	0.125	0.625	3.125
Seizure thresholds ($mg.kg^{-1}$ leptazol i.v.) (mean ± s.e.m. n=10)	clonic	25±1	25±1	27±1	27±1	25±1	22±1*	18±1***
	tonic	58±4	58±4	48±7	50±6	54±6	50±6	41±4**

*p<0.02 **P<0.01 ***p<0.001

Up to a dose of 0.125$mg.kg^{-1}$ clonidine did not modify seizure thresholds whereas at higher doses the clonic seizure threshold was decreased significantly. The top dose also significantly reduced the tonic seizure threshold. Similar results were obtained with male Olac MFI mice (26-32g) using clonidine in the dose range 0.008-5.0$mg.kg^{-1}$ s.c. administered 30 min. before i.v. leptazol infusion (12$mg.ml^{-1}$ at 0.15$ml.min^{-1}$).

The negative results we have obtained with clonidine at low α_2-selective doses contrast with reports describing a potent anticonvulsant action of the drug, with a maximal effect occurring at 1$\mu g.kg^{-1}$. (Papanicolaou et al., 1982 a,b). This discrepancy may be due to the different seizure model employed i.e. the latter authors measured duration of seizures following a bolus injection of leptazol. Lazarova and Samanin (1983a) measured latency to first seizure and the incidence of seizures in groups of rats following a bolus injection of leptazol. They obtained a significant protective effect of clonidine on tonic seizures at the relatively high dose of 0.5$mg.kg^{-1}$ i.p. although this effect was not clearly dose-related.

We conclude that any anticonvulsant properties which clonidine may possess are not as marked or as readily demonstrable as those of standard anticonvulsant drugs.

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THE EFFECTS OF THE THROMBOXANE SYNTHETASE INHIBITOR, UK-37248, ON ANAPHYLACTIC BRONCHOCONSTRICTION IN GUINEA PIGS

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One of the constituents of Rabbit Aorta Contracting Substance which is released from guinea-pig lungs following anaphylaxis has been found to be Thromboxane A2 (TxA2 : Svensson et al, 1975) which is a potent bronchoconstrictor in the guinea-pig (Svensson et al, 1977). Thus, the production of TxA2 could be partly responsible for the intense bronchoconstriction which characterises anaphylaxis in the guinea-pig. The claimed specific inhibitor of thromboxane synthetase, UK-37248 (Randall et al, 1981), has therefore been compared with antagonists of other chemical mediators, against anaphylactic bronchoconstriction in conscious guinea-pigs.

It can be seen from Table 1 that pretreatment with UK-32748 significantly increased the time to onset of dyspnoea and cough of sensitised guinea-pigs exposed to an aerosol of antigen (1% ovalbumin). No further protection was afforded by a larger dose but the combination of UK-37248 and mepyramine gave an additive effect. Phenidone gave similar protection to that of UK-37248 but Indomethacin gave no protection.

Table 1. Effects of UK-37248 and other drugs against time to onset of dyspnoea evoked by aerosolised antigen in conscious guinea-pigs (Mean ± S.E.M. : n = 6).

Drug Treatment (i.p.)	Normal Predyspnoea Time (s)	Drug-Treated Predyspnoea Time (s)
UK-37248 (1mg kg^{-1})	68 ± 9	162 ± 22
UK-37248 (5mg kg^{-1})	62 ± 6	142 ± 16
Mepyramine (1mg kg^{-1})	67 ± 9	194 ± 17
Indomethacin (5mg kg^{-1})	60 ± 4	60 ± 4
Phenidone (10mg kg^{-1})	55 ± 7	104 ± 11
UK-37248 (1mg kg^{-1}) + Mepyramine (1mg kg^{-1})	67 ± 8	365 ± 32
Phenidone (10mg kg^{-1}) + Mepyramine (1mg kg^{-1})	67 ± 5	348 ± 54

UK-37248, Phenidone and Indomethacin all gave no protection against bronchoconstriction evoked by Histamine aerosol (1%) whereas Mepyramine (1mg kg^{-1}) increased the time to onset of dyspnoea by a factor of 6.3.

The similarity between the patterns of the protective effects of UK-37248 and Phenidone are compatible with the suggestion that, in the guinea-pig, leukotrienes released in anaphylaxis owe much of their bronchoconstrictor activity to their ability to release TxA2 (Piper and Samhoun, 1982).

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EFFECT OF UNILATERAL NEPHRECTOMY ON RENAL PROSTANOID BIOSYNTHESIS IN THE RAT

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Unilateral nephrectomy in rats causes compensatory changes in the structure and function of the remaining kidney. Functional changes include increased GFR and renal blood flow and reduced tubule electrolyte absorption. Since indomethacin pretreatment antagonises these changes in renal function in uninephrectomised rats (Hahne et al, 1983) it is possible that renal prostanoids are involved in some or all of these modifications to renal function. Although the identity of the prostanoid responsible is not known, both PGI_2 and its enzymatic metabolite 6 oxo prostaglandin E_1 (6 oxo PGE_1) are candidates. In this study we have measured the release of PGE_2, $PGF_{2\alpha}$, 6 oxo $PGF_{1\alpha}$ and TxB_2 from incubated kidney slices of uninephrectomised rats and sham-operated control rats and determined the conversion of PGI_2 to 6 oxo PGE_1 by 100,000g supernates prepared from the kidneys of these animals.

Unilateral nephrectomy was performed under ether anaesthesia. Animals were sacrificed either 2 days or 7 days after surgery. Renal medulla slices (approx. 400mg) were incubated in Krebs' solution (1ml, 37°C, 30min) and the incubation medium acidified with 1M formic acid and extracted twice into 2 vol. ethyl acetate. Dried residues were resuspended in 1ml water of which 2µl and 5µl aliquots were radioimmunoassayed for prostanoids by a double antibody method. In separate experiments, kidneys were removed, homogenised in 4 vol. phosphate buffer (K_2HPO_4 40mM, KH_2PO_4 10mM, pH 7.4) and centrifuged twice at 4°C (3000g, 10min; 100,000g, 45min). Cytosolic supernates obtained were incubated (37°C, up to 240min) with 0.4 µCi 98 - ^3H PGI_2 methyl ester and the formation of 6 oxo PGE_1 determined by a loss of radioactivity method as described by Griffiths & Moore (1983).

Renal biosynthesis of PGE_2, $PGF_{2\alpha}$ and 6 oxo $PGF_{1\alpha}$ was significantly reduced 7 days (but not 2 days) after uninephrectomy (experiments on 8 rats). No TxB_2 was detected. No change in renal prostanoid biosynthesis was observed in sham-operated rats. In contrast, renal supernates from uninephrectomised rats 2 days after surgery avidly oxidised PGI_2 to 6 oxo PGE_1. Similar (but reduced) enzyme activity was still present in renal supernates from animals sacrificed 7 days after operation. Cytosolic supernates prepared from animals which had been sham-operated either 2 days or 7 days previously did not convert PGI_2 to 6 oxo PGE_1.

We conclude that alterations in renal function which occur after uninephrectomy in rats are not dependent on increased renal biosynthesis of PGE_2 or PGI_2. However, the induction of the enzyme 9-PGDH which converts PGI_2 to 6 oxo PGE_1 suggests that the latter prostanoid may have some part to play in the changes in renal blood flow and tubule fluid absorption in the first few days after operation.

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REDUCTIONS IN THROMBOXANE B_2 PRODUCTION AS A CONSEQUENCE OF THROMBOXANE RECEPTOR BLOCKADE IN HUMAN PLATELET RICH PLASMA

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AH23848, a highly specific thromboxane (TP-) receptor blocking drug (Brittain et al., 1984) has been studied for effects on thromboxane B_2 (TxB_2) production during platelet aggregation to compare the data with that reported previously for the thromboxane synthetase inhibitor UK34787 (Hornby & Skidmore, 1982).

Platelet aggregation was measured in an optical aggregometer (Born, 1962). Collagen (1 µg/ml) was used as the agonist and concentration-effect curves for inhibition of aggregation and TxB_2 production were generated for AH23848 (1×10^{-8}M - 1×10^{-5}M). The effect of the thromboxane synthetase inhibitor UK34787 (1×10^{-4}M), was studied in the same way (Cross et al., 1981). In addition, AH23848 (1×10^{-4}M) and UK34787 (1×10^{-4}M) were studied for effects on TxB_2 production in a microsomal preparation of thromboxane synthetase (Hornby & Skidmore, 1982). Inhibition of TxB_2 production by UK34787 (1×10^{-9} - 1×10^{-5}M) and AH23848 (1×10^{-8}M - 1×10^{-4}M) was also examined in clotting blood in vitro (Cross et al., 1981).

AH23848, at concentrations up to 1×10^{-4}M had no significant effect on TxB_2 production in microsomal preparations or during blood clotting in vitro. AH23848 is therefore devoid of inhibitory activity against the enzymes prostaglandin cyclo-oxygenase or thromboxane synthetase. In contrast, UK34787 (1×10^{-4}M) caused approximately 90% inhibition of TxB_2 production in a microsomal preparations of thromboxane synthetase and in clotting blood, UK34787 caused almost total inhibition of TxB_2 production at 1×10^{-5}, with an IC_{50} of $5 \pm 2 \times 10^{-7}$M (mean ± s.e. mean, n=4).

In platelet rich plasma, AH23848 (1×10^{-8}M - 1×10^{-5}M) caused a concentration-related inhibition of collagen induced aggregation with an IC_{50} at $1.4 \pm 0.4 \times 10^{-7}$M (mean ± s.e. mean, n=4) and almost total inhibition at 1×10^{-5}M. TxB_2 production was also inhibited (IC_{50} $9.4 \pm 3.6 \times 10^{-7}$M) with approximately 70% inhibition at 10^{-5}M AH23848. In contrast, UK34787 (1×10^{-9}M - 1×10^{-5}M) only weakly inhibited platelet aggregation with approximately 45% inhibition at 1×10^{-5}M while TxB_2 production was inhibited by almost 90%.

We conclude that AH23848 has no effect on prostaglandin cyclo-oxygenase or thromboxane synthetase. Data has been presented elsewhere showing that AH23848 is a specific thromboxane receptor blocking drug (Brittain et al., 1984). Using AH23848 we have demonstrated a reduction in TxB_2 production during platelet aggregation despite the observation that AH23848 is not an enzyme inhibitor. This suggests that a component of platelet TxA_2 production in response to collagen results from either platelet aggregation induced by thromboxane or some event resulting from TP-receptor stimulation.

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INHIBITION OF DRUG METABOLISM BY MEFLOQUINE

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Mefloquine (MQ) is a new antimalarial drug, structurally related to quinine, which has been developed for the prevention and treatment of chloroquine-resistant strains of falciparum malaria. MQ is thought to act on the malaria parasite in a similar way to quinine and is an effective blood schizonticidal compound. It is important to establish if antimalarial drugs interact with other drugs which may be used concurrently. In a previous study we have shown that primaquine (PQ) and to a lesser extent chloroquine, inhibit hepatic drug metabolism both *in vitro* and *in vivo* in the rat (Back et al., 1983a) and that PQ inhibits antipyrine metabolism in man (Back et al., 1983b). The aim of the present work was to compare the effects of MQ and PQ on drug metabolism in the rat *in vitro* and *in vivo*.

In vitro, the demethylation of aminopyrine (2.5 mM) was studied in the presence of MQ (0.001 - 2.0 mM) or PQ (0.001 - 0.1 mM) and Lineweaver-Burk plots constructed using substrate concentrations ranging from 0.25 - 2.5 mM and antimalarial concentrations of 0.01 and 0.1 mM. Also the microsomal metabolism of the synthetic oestrogen ethinyloestradiol (EE_2; 1 μCi; 0.01 mM) was studied in the presence of MQ (0.01, 0.1, 0.5 mM) or PQ (0.5 mM). Radiolabelled metabolites present were analysed by h.p.l.c. *In vivo*, twelve rats were divided into three groups and individually housed in metabolism cages. Rats were either controls or were given MQ or PQ (25 mg kg^{-1}) 30 min before i.p. administration of tolbutamide (50 mg kg^{-1}). Urine was collected at intervals to 24 h. Urinary hydroxy-tolbutamide (OHTOL) concentrations were measured by h.p.l.c.

Both MQ and PQ inhibited aminopyrine N-demethylase activity *in vitro* and the concentration required to produce 50% inhibition was 0.2 mM for MQ and approximately 0.1 mM for PQ. Lineweaver-Burk plots indicated inhibition by both antimalarials to be non-competitive. Both MQ and PQ produced comparable inhibition of EE_2 metabolism, with the percentage recovery of the major metabolite 2-hydroxyEE_2 being reduced from 49.3 ± 10.8 to 5.1 ± 3.1 (0.5 mM MQ) and 1.5 ± 0.4% (0.5 mM PQ; mean ± S.D.). Following acute administration of MQ and PQ to rats, urinary recovery of OHTOL, the major metabolite of tolbutamide was markedly reduced. In the period, 0-8 h, MQ caused a reduction in recovery from, 54.4 ± 3.1 to 9.3 ± 3.4% and PQ from the control level to 32.2 ± 14.1%.

We have used in this study three substrates which undergo microsomal oxidative metabolism. There is clear evidence that MQ inhibits microsomal enzymes both *in vitro* and *in vivo*, with some indications that inhibition may be more marked than that produced by PQ.

J.H.R. is in receipt of an MRC studentship.

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THE BILIARY ELIMINATION OF INDOCYANINE GREEN AND NITRAZEPAM IN GERMFREE AND CONVENTIONAL RATS

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Previous studies (Hewick & Wilson, 1983) have shown that bile flow and biliary amaranth excretion rate tend to be reduced in germfree (GF) rats. The biliary elimination of two further xenobiotics has now been tested: the dye indocyanine green (IG, excreted unchanged) and the nitrobenzodiazepine hypnotic nitrazepam (extensively metabolised).

Four littermate pairs of inbred GF and conventional (CV) BDIX rats (10-12 weeks old) of each sex were compared in the IG study. Five age-matched pairs (11-22 weeks) of GF and CV male WAG/Rij rats were compared in the nitrazepam study. Biliary excretion in bile duct-cannulated anaesthetised rats was determined essentially as previously described (Hewick & Wilson, 1983; Klaasen & Strom, 1978). Bile was collected every 10 min for 120 min after i.v. IG (10 mg ml^{-1}, 10 mg kg^{-1}) or every hour for 6 h after i.v. ^{14}C-nitrazepam (10 mg kg^{-1}, 11µCi kg^{-1}). Biliary radio-activity was separated by silica gel thin-layer chromatography (solvent: n-butanol, ethanol, NH_4OH (0.88), water; 40, 10, 1, 9 by volume) and quantitated by liquid scintillation counting. All paired data were significantly different unless indicated (Student's t-test, $P < 0.05$). Results are given means ± s.e. mean.

For GF rats of both strains bile flow was lower (eg. 60 min values: male BDIX, 25.8 ± 4.0 versus 39.4 ± 5.1; female BDIX, 33.7 ± 3.8 versus 57.2 ± 5.0; male WAG/Rij, 42.6 ± 2.8 versus 72.0 ± 5.4 µl min^{-1} kg^{-1}). IG and ^{14}C-nitrazepam-derived radioactivity excretion rates were also lower (60 min values: male, 57.0 ± 7.9 versus 82.0 ± 6.5; female 75.2 ± 7.2 versus 99.5 ± 8.7 µgIG min^{-1} kg^{-1}; 14.2 ± 0.9 versus 24.2 ± 2.0 µg nitrazepam equivalent min^{-1} kg^{-1}). Half the dose of IG had been excreted by about 85 and 60 min (GF and CV males) and 65 and 45 min (GF and CV females). By 4 h about 33 and 46% of the nitrazepam had been excreted in the bile by the GF and CV rats respectively.

Chromatographic analysis of bile revealed that about 95% of the radioactivity comprised three major loci, which were more polar than nitrazepam, 3-hydroxynitrazepam and the putative nitroreduced metabolites, aminonitrazepam and acetamidonitrazepam. The relative proportions of the three main loci were similar in GF and CV rat bile.

Our present quantitative findings (as well as those described previously with amaranth) may possibly be explained on the basis of the reported lower hepatic blood flow in GF rats (Gordon, 1968). Therefore, although GF rats are useful in qualitatively assessing the involvement of the gastro-intestinal microflora in xenobiotic metabolism (Goldman, 1982), their physiological "abnormalities" may make quantitative assessment difficult.

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INHIBITION OF METOPROLOL OXIDATION BY DEBRISOQUINE AND OTHER DRUGS IN RAT LIVER MICROSOMES

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The polymorphic oxidation of debrisoquine is thought to be catalysed by a single form of hepatic cytochrome P-450 which, in poor metabolisers, is functionally inactive or present in lowered amounts (Davies et al, 1981). The α-hydroxylation of metoprolol has been shown to display polymorphism of the debrisoquine-type in man and evidence suggests that other routes are similarly affected (Lennard et al, 1983). Boobis et al (1983) have recently proposed the use of in vitro inhibition studies as a means of defining whether one or more forms of cytochrome P-450 are involved in the monogenically-controlled metabolism of two substrates. We have therefore tested the ability of debrisoquine and other drugs to inhibit metoprolol oxidation in rat liver microsomes.

Hepatic microsomes prepared from male Wistar rats were incubated with substrate and inhibitor at 37°C and pH7.25. Metoprolol and α-hydroxymetoprolol were assayed by HPLC. Under conditions that were linear with respect to time and protein concentration, values for the apparent K_m and V_{max} for α-hydroxylation were 18μM (range 17-21) and 0.30 nmole/mg protein/min (range 0.25-0.39)(n=3), respectively.

Debrisoquine and also guanoxan were found to be potent competitive inhibitors of α-hydroxylation (Table). The K_i values are in close agreement with respective spectral dissociation constant values of 8.5 and 1.0 μM obtained for these compounds in Sprague-Dawley rat liver microsomes (Küpfer et al, 1982). The metabolism of metoprolol by all routes (measured by substrate disappearance) was also strongly inhibited by debrisoquine and guanoxan (Table). Cimetidine and ranitidine, drugs which appear to impair the clearance of metoprolol in man (Spahn et al, 1983) showed an inhibitory action comparable to that of debrisoquine, in rat liver microsomes (Table).

Antipyrine, a drug whose metabolism is not impaired in poor metabolisers of debrisoquine was found to be only a weak, non-competitive inhibitor of metoprolol metabolism (Table).

Table Effect of various drugs on the oxidative metabolism of metoprolol.

K_i= inhibitor constant for α-hydroxylation. IC_{50} = concentration of inhibitor required to impair elimination of metoprolol (20μM) by 50%.

Drug	K_i(μM)	Mechanism	IC_{50}(μM)
Debrisoquine	8	Competitive	93
Guanoxan	4	Competitive	6
Cimetidine	9	Competitive	30
Ranitidine	35	Competitive	86
Antipyrine	4,000	Non-competitive	17,500

The data suggest that the oxidation of metoprolol is closely linked to that of debrisoquine, guanoxan, cimetidine and ranitidine but not of antipyrine in the rat.

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α_2-ADRENOCEPTOR ANTAGONISTS: A NEW APPROACH TO THE TREATMENT OF HYPERGLYCAEMIA

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Evidence exists to indicate that the level of circulating glucose is related to α_2-adrenergic receptor modulation of insulin release from pancreatic islet cells. The rise in plasma glucose, following administration of adrenaline or phenylephrine, was blocked by the α_2-adrenoceptor antagonist, yohimbine, but not by the α_1-adrenoceptor antagonist, prazosin (Nakadate et al, 1980; Nakaki et al, 1980). This study examines the effect of imiloxan (RS-21361), a selective α_2-adrenoceptor antagonist (Michel & Whiting, 1981) on plasma insulin levels in the rat and compares it to tolbutamide, an established hypoglycaemic agent.

Male Sprague-Dawley rats (150 g) were fasted overnight and pre-dose blood samples taken. Animals were orally dosed for 7 days with either imiloxan (30 or 100 $mg.kg^{-1}$ twice a day) or tolbutamide (30 $mg.kg^{-1}$ twice a day). Control animals received distilled H_2O alone. At the end of the study animals were fasted overnight and terminal blood samples collected 1 h after drug treatment. Serum insulin was measured by radioimmunoassay (Diagnostic Products Coporation).

The effect of treatment on the level of circulating insulin is shown in Table 1. A significant increase in serum insulin was observed following 100 $mg.kg^{-1}$ imiloxan or 30 $mg.kg^{-1}$ tolbutamide but not at 30 $mg.kg^{-1}$ imiloxan.

Table 1
Chronic administration of imiloxan or tolbutamide on insulin levels in the rat

Group	Serum Insulin ($\mu IU.ml^{-1}$) Pre-dose	Post-dose	Significance relative to pre-dose
Control	15.0 ± 1.29	13.7 ± 3.38 (8)	NS
Imiloxan (30 $mg.kg^{-1}$ bid)	13.5 ± 1.79	13.9 ± 1.46 (8)	NS
Imiloxan (100 $mg.kg^{-1}$ bid)	15.1 ± 0.98	23.9 ± 3.56 (9)	$p < 0.05$
Tolbutamide (30 $mg.kg^{-1}$ bid)	14.6 ± 0.76	55.5 ± 7.76 (9)	$p < 0.001$

Each value represents the mean ± SE of the number of determinations in brackets. Paired Student's 't' test was used to assess significance.

Alteration in insulin levels by imiloxan suggests that α_2-adrenoceptor antagonists may offer an alternative therapy to the treatment of hyperglycaemia.

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CARDIOSELECTIVE CALCIUM ENTRY BLOCKING PROPERTIES OF A NICARDIPINE METABOLITE

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Nicardipine has been shown to possess vasodilator activity with the following order of potency: cerebral > coronary > peripheral vasculature (Takenaka et al, 1976). This action has been shown to be due to inhibition of Ca^{2+} influx into vascular smooth muscle (Terai et al, 1980). The profile of nicardipine has made it clinically useful in cerebral insufficiency and essential hypertension.

Nicardipine has been shown to block calcium channels in the mammalian myocardium (Patmore & Whiting, 1984) although like nifedipine it possesses a vascular selective profile (Clarke et al, 1983). This accounts for the clinical effectiveness of these compounds as antihypertensives.

Nicardipine is extensively metabolised in all species studied. One human plasma metabolite, DHM9, is itself a dihydropyridine and is, therefore, likely to possess calcium entry blocker (CEB) activity. However, DHM9 has substantially lower (1×10^3 fold) peripheral vasodilator activity in comparison to nicardipine (Takenaka & Maeno, 1981) and is inactive as an antihypertensive agent.

We have evaluated the negative inotropic and vasodilator potency of DHM9 and nicardipine using the techniques described by Clarke et al (1983). The CEB potencies on mesenteric artery of the metabolite, DHM9, in comparison with that for nicardipine is substantially reduced (5×10^3) fold whilst on cardiac and coronary artery tissues, the decrease is only a factor of 10 fold (see Table 1).

Thus, DHM9, unlike nicardipine or nifedipine, is cardiac selective rather than vascular selective. This cardiac selective profile may provide some benefits in comparison with nifedipine and help to explain the results of Alps et al (1983). These authors have shown that nicardipine, unlike nifedipine, is active in models of cardiac ischaemia.

Table 1
Comparative effects of nicardipine, DHM9 and nifedipine on cardiac muscle and mesenteric and coronary vascular smooth muscle

Compound	pIC_{50} values			Ratio
	Papillary Muscle (1)	Mesenteric Artery (2)	Coronary Artery	(1:2)
Nicardipine	7.19 (7.33-7.09)	8.22 (8.36-8.12)	8.58 (8.67-8.51)	10.72
DHM9	6.16 (6.25-6.07)	4.52 (4.55-4.50)	7.61 (7.72-7.52)	0.02
Nifedipine	7.23 (7.36-7.13)	7.88 (7.94-7.83)	7.91 (7.98-7.84)	4.47

Values shown are mean with SD range (n = 3-6) calculated using an iterative curve fitting programme.

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DIFFERENTIAL EFFECTS OF RYANODINE ON CALCIUM ENTRY AND CONTRACTION IN CARDIAC MUSCLE

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Ryanodine, an intracellular calcium antagonist, has been suggested to act by inhibiting the release of calcium from intracellular stores (Sutko & Willerson, 1980). Mitchell et al (1984) have shown that ryanodine inhibits contractility and the late phase of the action potential of rat ventricular cells, attributed to a second inward calcium current activated by intracellular calcium release. The present study investigates the effects of ryanodine on the slow inward current (Isi) and contractility in rat and guinea pig ventricular tissues. Isi has been measured indirectly from the recording of voltage-induced, calcium-dependent slow action potentials (Ca APs) from 20 mM K^+ depolarised rat and guinea pig papillary muscles as described by Patmore & Whiting (1982).

The inotropic effects of ryanodine are shown in Fig. 1a. In normal (6 mM K^+) and 20 mM K^+ salt solution ryanodine has a marked negative effect on rat papillary muscle with IC_{50} values of 1.3×10^{-7} and 8.3×10^{-8} M respectively. Ryanodine only reduced contractility to 42% and 65% of control (6 and 20 mM K^+ respectively) in guinea pig fibres. In both rat and guinea pig tissues at concentrations which produced maximal inotropic effects (1×10^{-5} and 3×10^{-4} M respectively), ryanodine increased Ca AP magnitude and duration. Ca AP area was also increased from 1.42 ± 0.30 to 3.85 ± 1.03 mV.s in rats and from 9.65 ± 0.70 to 13.65 ± 0.85 mV.s in guinea pig (Fig. 1b). In both species a residual ryanodine insensitive component of the contraction remains (Fig. 1a). This component and the associated potentiated electrical responses were inhibited by 1×10^{-6} M verapamil (Fig. 1b).

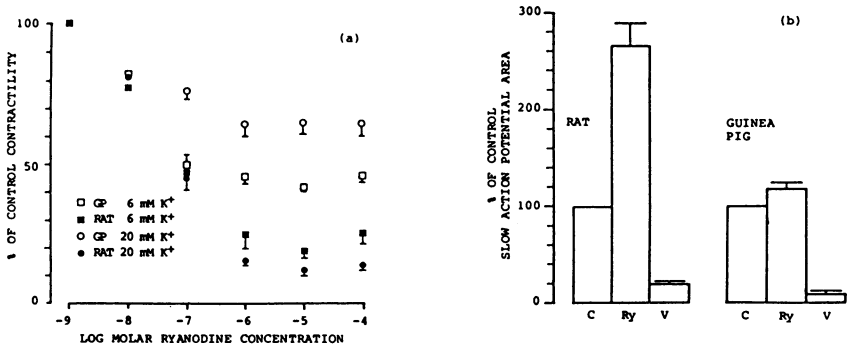

Fig. 1 Effects of ryanodine on (a) contractility and (b) Ca AP area. Results in (b) expressed as % of control [C] values; ryanodine [Ry] 1 x 10^{-5} M for rat and 3 x 10^{-4} M for guinea pig; verapamil [V] 1 x 10^{-6} M.

The results show that ryanodine is an intracellular calcium antagonist which either directly or indirectly potentiates the slow inward current. The results also suggest that when intracellular release of calcium is inhibited by ryanodine, Isi may contribute directly to contraction. Whilst the negative inotropic efficacy of ryanodine varies greatly between rat and guinea pig, the effects have similar concentration-dependence. These factors suggest that rat and guinea pig have similar sensitivity but underline the different contributions from inward calcium currents and intracellular release to contraction in these tissues.

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DOES THROMBOXANE A_2 DIRECTLY OR INDIRECTLY STIMULATE BRONCHIAL SMOOTH MUSCLE IN GUINEA-PIGS ?

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11.9 epoxymethano PGH_2 (U46619) is a stable prostaglandin endoperoxide analogue that has been classified as a thromboxane A_2 (TxA_2) receptor agonist. It produces vasoconstriction, bronchoconstriction and aggregates platelets.

Guinea-pigs 400-800 gm were anaesthetized with a mixture of urethane 280 mg/kg and diallylbarbituric acid 70 mg/kg i.p. The trachea was cannulated for ventilation and a transducer inserted into the ventilation system to measure lung inflation pressure (LIP). All injections were given i.v. via a jugular vein and BP was recorded via a carotid artery.

0.35 µg/kg and 0.7 µg/kg of U46619 caused dose-dependent reproducible increases in LIP when given at 15 min intervals over a period of 2 hr. The response to 0.7 µg/kg U46619 was biphasic, the secondary phase peaking 3-4 min after injection. U46619 also produced an immediate dose-dependent vasoconstriction. Previous studies with the Tx receptor antagonists EPO45 (Armstrong, Jones, Peesapati, Wilson & Smith, 1983) and EPO92 (Jones, Smith & Wilson, 1984) showed that both the LIP and BP responses to U46619 could be completely inhibited by Tx receptor antagonism. FPL 55712 has been defined as a leukotriene antagonist with a short plasma half-life of less than 5 min. When U46619 was given 1 min after FPL 55712 (1-10 mg/kg) a dose-dependent inhibition of both primary and secondary phases of the LIP response was obtained but the vasoconstriction was unaffected. Indomethacin (0.5 mg/kg) had no effect on the primary phase of U46619-induced increase in LIP but significantly potentiated the secondary phase ($P<0.05$). BW755C (5 mg/kg) produced similar effects to indomethacin and neither drug affected the vasoconstriction. Dazoxiben (10 mg/kg), ketotifen (1 mg/kg) and ketanserin (5 mg/kg) did not affect the LIP or BP responses to U46619.

FPL 55712 has been shown to inhibit Tx synthetase and to inhibit histamine release at high *in vitro* concentrations (Welton, Hope, Tobias and Hamilton, 1981). Since FPL 55712 inhibited both phases of U46619-induced bronchoconstriction, a leukotriene involvement in both phases is possible but Kennedy, Whelan and Wright (1983) showed that FPL 55712 inhibited U46619-induced increased perfusion pressure and reduced contractility of the isolated guinea-pig heart which questions its specificity in the guinea-pig. Indomethacin and BW755C only potentiated the second phase probably acting as cyclooxygenase inhibitors indicating that the secondary phase is due to the products from lipoxygenase metabolism of arachidonic acid. Further work is required to explain these results.

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NONSTEROIDAL ANTI-INFLAMMATORY AGENTS MAY ACTIVATE LIPOXYGENASE BY RELEASING CALCIUM FROM MITOCHONDRIA

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Arachidonic acid is metabolised by the cyclo-oxygenase pathway to prostaglandins (Vane, 1978) and by the lipoxygenase pathway to leukotrienes (Samuelsson, 1980). The former pathway is most sensitive to nonsteroidal anti-inflammatory agents. Inhibition of prostaglandin synthesis has therefore been proposed as a contributory factor in the action of the nonsteroidal anti-inflammatory agents. Since activation of the lipoxygenase pathway would produce a similar effect, we have conducted preliminary experiments to test this possibility.

15-Lipoxygenase activity was measured polarographically using a Clark-type oxygen electrode coupled to a Servoscribe potentiometric recorder. The oxygen electrode chamber contained 380 µmol MOPS buffer, pH 7.4 and 0.8 µmol arachidonic acid at 32 °C. The reaction was initiated by the addition of 200 µg soybean 15-lipoxygenase, to give a final volume of 4 ml. Anti-inflammatory agents or calcium chloride were added to the chamber 2 min before the addition of enzyme. Calcium ion movements in mitochondria isolated from rat liver were measured as described previously (McDougall et al, 1981).

Both flufenamic acid (EC50 = 3.6 ± 0.9 µM; n = 5) and diflunisal (EC50 = 10.1 ± 1.8 µM; n = 5) promoted the release of calcium from isolated mitochondria that had been allowed to accumulate the cation. In contrast, purified 15-lipoxygenase was relatively insensitive to these agents (the IC50 value for flufenamic acid was 4.8 ± 0.09 mM and for diflunisal was 5.5 ± 0.29 mM; n = 5).

Since the action of flufenamic acid and diflunisal on mitochondria would be likely to result in an increase in the concentration of free calcium in the cytosol, we investigated the effect of calcium on the extra-mitochondrial enzyme, 15-lipoxygenase. In the absence of calcium ions, arachidonic acid was metabolised at a rate of 1.61 ± 0.04 µgatom oxygen/min/mg enzyme protein (n = 10). When calcium ions were included in the reaction medium, there was a concentration dependent stimulation of enzyme activity. Maximal activity (3.61 ± 0.28 µgatom oxygen/min/mg enzyme protein; n = 5) was observed at 32.5 µM calcium (EC50 = 14.5 ± 2.4 µM; n = 5).

The results of these experiments suggest that, in addition to the direct action of nonsteroidal anti-inflammatory agents on arachidonic acid metabolism via inhibition of cyclo-oxygenase, an indirect action could be mediated through the ability of anti-inflammatory agents to release calcium from mitochondria, thus increasing the level of calcium in the cytosol, and so promoting the activity of lipoxygenase enzymes, an action that could divert arachidonic acid metabolism away from prostaglandin production.

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EVIDENCE FOR THE OCCURRENCE OF HISTAMINE H1 RECEPTORS IN HUMAN VAS DEFERENS

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There is much evidence for the presence of histamine receptors in the vasa deferentia of many laboratory mammalian species. Apart from the mouse vas deferens which appears to be unique in displaying exclusively H2 receptor mediated events, vasa deferentia from other species (rat, rabbit, guinea pig), all display varying proportions of H1 and H2 receptor mediated effects (Marshall 1978; Vohra 1981).

To date the occurrence of histamine receptor populations in the human vas deferens has not been reported. Accordingly, the action of histamine and H1 and H2 antagonists on the field stimulated human vas deferens (HVD) has been investigated.

Human vasa (2cm portions surplus to histological requirement) were obtained from 4 patients undergoing elective vasectomy. The HVD were investigated essentially by the method of Hayes and Sloan (1983). They were suspended in oxygenated magnesium-free Krebs solution at 37^{0}C under 1g tension and stimulated by trains of 2 pulses (2ms, 20Hz, 64V) at a rate of 0.05Hz. The addition of histamine to the bath resulted in a potentiation of the contractile response in all tissues investigated. This potentiation was dose-dependent over a dose range from 1μM, potentiation 16 ± 4.5% (mean ± SEM), to 100μM, 84 ± 3%, which was maximal. This potentiation was unaffected by naloxone (100nM), cimetidine (100μM) or yohimbine (300nM).

However, mepyramine (100nM) having no effect alone, competitively antagonised the histamine potentiation producing an 8-fold parallel shift to the right in the dose response curve to histamine.

These preliminary studies suggest that histamine H1 receptors are present in human vasa.

We are grateful to the staff at the Soho Hospital for Women, London W1 for obtaining human vasa.

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THYROIDAL UPTAKE OF RADIOACTIVITY FOLLOWING THE ADMINISTRATION OF [^{14}C]3-METHYL-2-THIOHYDANTOIN TO RATS

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3-Methyl-2-thiohydantoin (3-MTH), a metabolite of the antithyroid drug methimazole (MMI), has been detected in the blood, urine and thyroid tissue of patients receiving MMI (Skellern et al, 1977). Recently it has been shown that 3-MTH inhibits human thyroid peroxidase in vitro (Paterson et al, 1984) and that this action may contribute to the antithyroid effect of MMI. The aim of the present study was to determine whether ^{14}C 3-MTH accumulates in the thyroid gland and other tissues after i.p. administration and to compare its distribution with that of an equivalent dose of ^{14}C MMI.

Two groups of 8 male Sprague Dawley rats (200 - 300g), maintained on a No. 1 Expanded and Modified Rat and Mouse Maintenance Diet, were given either a single i.p. dose of (4-^{14}C) 3-MTH (0.140mg, 19.9 μCi mg^{-1}, dissolved in 0.2 ml distilled water) or (2-^{14}C) MMI (0.134 mg, 21.5 μCi mg^{-1}, dissolved in 0.2 ml distilled water). Four animals from each group were killed at 8 and 24h after administration of drug and the percentage of the injected dose of radioactivity determined per gram of tissue for the pooled thyroid glands, liver and lung tissue. Plasma samples were also taken for determination of radioactivity.

The results shown in Table 1 indicate that there is an accumulation of radioactivity by the thyroid gland following the i.p. administration of labelled 3-MTH at both 8 and 24h, and that the thyroid to plasma ratios of radioactivity were considerably greater than those after an equivalent dose of MMI. The nature of the thyroidal radioactivity for both 3-MTH and MMI is currently under investigation. The tissue to plasma ratios of radioactivity obtained for liver (2.0) and lung (2.0) tissue after administration of labelled 3-MTH indicates that there is some accumulation of the radiolabel by these tissues 24h post administration. The accumulation of radioactivity by the thyroid gland following the administration of radiolabelled antithyroid drugs has previously been reported for MMI, propylthiouracil, methylthiouracil and thiouracil (Marchant et al, 1972). Thus, 3-MTH appears to produce this effect in common with those other antithyroid drugs.

Table 1 The thyroidal uptake of radioactivity following the i.p. administration of 3-MTH (4-^{14}C) or MMI (2-^{14}C) in the rat

Thyroidal Radioactivity (% injected dose g^{-1} tissue)				Thyroidal Radioactivity (ratio to plasma)			
8h		24h		8h		24h	
3-MTH	MMI	3-MTH	MMI	3-MTH	MMI	3-MTH	MMI
17.1	0.951	12.4	1.018	46	4.2	155	20.8
10.4	0.854	12.5	0.683				

The mean plasma values used to calculate the tissue to plasma ratios were obtained from 4 animals. The thyroid glands from 2 animals were pooled and the values for two pairs of animals given. Thyroid to plasma radioactivity ratios were calculated using the mean result from the two thyroid results.

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THE IN VITRO METABOLISM OF PSORALENS USED IN PHOTOCHEMOTHERAPY

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Photochemotherapy using the phototoxic properties of furanocoumarin compounds such as 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and trimethyl psoralen (TMP) is an established treatment of psoriasis and other dermatoses (Wolff and Honigsmann, 1981). Both 8-MOP and 5-MOP are less effective than TMP when applied topically but produce greater photosensitization after oral administration. The inference is drawn that TMP may give rise to non-phototoxic metabolites while those of 8-MOP and 5-MOP retain the phototoxic potential of the parent substance (Pathak et al, 1983). Pharmacokinetic studies have shown that these compounds are metabolised and excreted rapidly (Pathak et al, 1975; Schmid et al, 1980) but in vitro metabolic studies have proved difficult.

Using rat and mouse liver preparations and thin layer chromatographic analyses we have demonstrated that the in vitro metabolism of 8-MOP and 5-MOP and of TMP is favoured by low substrate concentrations. These concentrations are at the lower limit of sensitivity in a standard test for phototoxicity using Candida Albicans and any metabolite phototoxicity is therefore difficult to detect. However, using a test with a repair-deficient strain of Escherischia coli (E coli, BS-1: Ashwood-Smith et al, 1983) we have shown that 1 of the metabolites from both 5-MOP and 8-MOP is phototoxic while 4 of the TMP metabolites are phototoxic. Further studies are required to identify the metabolites, to assess the clinical importance of the 8-MOP and 5-MOP metabolite phototoxicity and to determine why TMP is less effective even though its major metabolites in vitro are unequivocally phototoxic.

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PROPERTIES OF [^{3}H]-NITRENDIPINE BINDING TO HOMOGENATES OF CHICK HEART

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^{3}H-nitrendipine (^{3}H-NIT) has recently proved to be a useful tool for studying the binding site, believed to exist on the calcium channel, for the dihydropyridine class of calcium entry blockers (CEB) (Glossman and Ferry, 1983). A previous report of the effects of a non-dihydropyridine CEB, methoxyverapamil, upon ion flux and twitch-tension measurements in the embryonic chick heart, has suggested that developmental changes in sensitivity to the drug may occur in this tissue (Galper and Catterall, 1978). Consequently, we have studied some characteristics of ^{3}H-NIT binding in the developing chick heart, with a view to examining whether or not ontogenic changes in the binding site for this ligand may be apparent.

Whole hearts (chicks aged less than 12 embryonic days) or ventricular tissue (from older embryos or hatched chicks) were homogenized in a buffer containing (mM): NaCl (120), KCl (5), Na_2HPO_4 (1.5), NaH_2PO_4 (0.5), pH 7.4. The homogenate was filtered through nylon mesh and then centrifuged (40,000 g/20min) at 4°C. The resulting pellet was washed and centrifuged twice more to provide a final membrane pellet which was resuspended to a final volume corresponding to 100mg wet wt. of original tissue per ml of homogenization buffer. Homogenate aliquots were incubated with ^{3}H-NIT for 60min at 25°C under sodium vapour lighting, and bound ^{3}H-NIT was determined by liquid scintillation counting of radioactivity trapped on Whatman GF/B filters after rapid vacuum filtration. Specific ^{3}H-NIT binding was defined as the binding displaceable by the related dihydropyridine, nicardipine (1μM), and was approximately 50-80% of total binding with 0.1 - 0.3 nM ^{3}H-NIT.

In initial experiments with (day 5) hatched chick ventricles, specific ^{3}H-NIT binding with ligand concentrations of 0.03 to 0.6 nM was studied. A single binding component was indicated by Scatchard analysis with a K_D of 0.16 $\pm$ 0.003 nM and a B_{max} of 192 $\pm$ 12 fmol mg prot.$^{-1}$ (n = 3) (mean $\pm$ s.e.). Displacement of ^{3}H-NIT (0.3 nM) by verapamil (10^{-9} to 10^{-5} M) was also studied. In contrast to nicardipine, a plateau at 55% inhibition by verapamil (10 μM) of total specific ^{3}H-NIT binding was obtained. Another CEB, bepridil, in low doses (around 5 x 10^{-8}M) potentiated ^{3}H-NIT binding whereas inhibition of ^{3}H-NIT binding occurred with higher bepridil doses (greater than 5 x 10^{-6} M).

In preliminary studies, kinetic constants for ^{3}H-NIT binding have been determined in hearts from embryonic (at days 5, 12 and 18) and hatched chicks (at days 1, 5 and 10). The resulting values for K_D (0.14 - 0.19 nM) and B_{max} (172 - 242 fmol mg prot.$^{-1}$) followed no obvious developmental trend.

In conclusion, the ^{3}H-NIT binding site in chick heart appears to be similar to that described in other tissues (Glossman and Ferry, 1983). Similarly, our data also provide evidence that the dihydropyridines may not necessarily share the same binding site on the calcium channel as other CEB drugs. It would appear from our developmental studies that the dihydropyridine site is present from a very early age in the embryonic chicken heart.

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POTENT BUT SELECTIVE INHIBITION OF CYTOCHROME P-450 BY CANNABIDIOL

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Cannabidiol (CBD), a psychically inactive constituent of cannabis may be a potentially clinically useful, relatively non-toxic anticonvulsant drug. There is evidence that in animals and man CBD inhibits cytochrome P-450 (P-450)-mediated drug metabolism both *in vivo* and *in vitro* (Benowitz et al, 1980). We have investigated whether CBD inhibits constitutive (basal), phenobarbitone (PB)-induced and 3-methylcholanthrene (3MC)-induced isozymes of P-450 equally.

The effects of CBD on two rat hepatic microsomal P-450-dependent O-dealkylation reactions were measured, comparing microsomes from untreated, PB-induced and 3MC-induced rats. Ethoxyphenoxazone (ethoxyresorufin) O-deethylase (EPOD) and pentoxyphenoxazone O-depentylase (PPOD) are highly characteristic reactions of the 3MC-induced and PB-induced isozymes of P-450 respectively (Burke and Mayer, 1983). Inducer pretreatments, liver microsome preparation and EPOD and PPOD determinations were carried out as described elsewhere (Burke and Mayer, 1983). CBD (0.01-10μM final reaction concentration) was added to reaction mixtures in DMSO solution (10μl per 2ml reaction). DMSO alone had no effect on EPOD or PPOD.

CBD (10μM) totally inhibited both EPOD and PPOD reactions with control and PB-induced liver microsomes and EPOD with 3MC-induced microsomes. In contrast, 10μM CBD stimulated PPOD with 3MC-induced microsomes by 36%. Lower CBD concentrations caused either weak inhibition (23% at 0.1μM) or were without effect (0.01μM and 1.0μM). IC_{50} values (CBD concentrations causing 50% inhibition compared to reactions run in the presence of DMSO) are shown in Table 1.

Table 1 CBD concentrations causing 50% inhibition of O-dealkylation

Microsome Type	Substrate	CBD-IC_{50} (μM)*
control	ethoxyphenoxazone	0.40 ± 0.11
	pentoxyphenoxazone	0.70 ± 0.19
PB-induced	ethoxyphenoxazone	0.31 ± 0.03
	pentoxyphenoxazone	0.10 ± 0.00
3MC-induced	ethoxyphenoxazone	0.49 ± 0.05
	pentoxyphenoxazone	**

* means ± s.e. mean (n=3) ** reaction stimulated by CBD

These results show that CBD is a potent inhibitor of P-450 O-dealkylases. The sensitivity to CBD varies, however, depending on some microsomal membrane components (possibly certain P-450 isozymes) which change as a result of 3MC treatment. CBD may be a useful inhibitory probe for studies of P-450 isozymes, whilst clinically the inhibition could result in interactions with co-administered drugs.

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N-METHYL-D-ASPARTIC ACID INHIBITS SPONTANEOUS FIRING OF CEREBELLAR PURKINJE CELLS IN VITRO

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The excitatory amino acid. N-methyl-dl-aspartic acid (NMDLA), when applied microiontophoretically to the dendritic fields of rat cerebellar Purkinje cells in vitro only weakly excited cells at low currents but, with larger ejecting current, inhibition of firing was seen (Crepel et al, 1982). This, they suggested, could indicate that the inhibitory interneurones in the cerebellum were more sensitive to NMDLA than were the Purkinje cells. Excitation and inhibition were also seen after both aspartate and glutamate application, the inhibition being attributed to excitation of basket/stellate cells. To further examine this finding we have investigated the effect of bath application of the active isomer, N-methyl-d-aspartic acid (NMDA), on the spontaneous firing of Purkinje cells recorded extracellularly in the in vitro cerebellar slice preparation (Crepel et al, 1981 and Yamamoto, 1973). In an attempt to further characterise the inhibitory response pharmacologically we have used the GABA antagonist, picrotoxin, and the NMDA antagonist, (±)-2-amino-5-phosphonovaleric acid (APV).

Adult male Wistar rats (200-250g) were stunned, decapitated, the cerebella removed and whole sagittal slices (350μm thick) of the vermis prepared using a Vibroslice (Campden Instruments Ltd.). The tissue slice was then transferred to a recording chamber, supported on a nylon mesh, and superfused at a rate of 0.5ml.min^{-1} with continuously gassed (O_2, 95%:CO_2, 5%) medium (composition, mM: NaCl, 124; KCl 5; KH_2PO_4 1.2; $MgSO_4 7H_2O$, 1.2; $NaHCO_3$, 26; glucose, 10 and $CaCl_2$, 2). Warmed, humidified O_2/CO_2 was directed over the tissue which was left to equilibrate for at least 1 hour before recording.

The spontaneous action potentials of Purkinje cells were recorded using glass microelectrodes (tip diameter 4-6μm) filled with 4M NaCl and the signals amplified and displayed using conventional techniques. All compounds were dissolved in the medium, pH 7.4, and continuously gassed with the O_2/CO_2 mixture.

Of all the cells studied (>50) the spontaneous firing rate was inhibited by the application of both GABA (50μM to 1mM) and NMDA (10 to 50μM). In the presence of the NMDA antagonist, APV (10 to 100μM) the response to NMDA was blocked (7 cells of 7 tested) but not that to GABA (0/7) whereas, the GABA antagonist, picrotoxin (5 to 50μM), was shown to reduce the inhibitions produced by both GABA (7/7) and NMDA (6/6). Aspartate (100 μM to 1mM) and glutamate (50 μM to 500 μM), 6 and 5 cells respectively, produced excitation.

The block of NMDA's inhibitory action by both APV and picrotoxin is consistent with the suggestion that NMDLA's indirect inhibitory effect was mediated through an activation of the basket cells to release GABA onto the Purkinje cells (Crepel et al) and also with the current understanding of cerebellar neuronal circuitry (Eccles, 1973). Our findings suggest that the neurotransmitter(s) released onto the basket cells from the parallel fibres act on NMDA sensitive receptors. Aspartate/glutamate have been proposed as the neurotransmitters at this site (see Crepel et al, 1982) but in our studies both these amino acids failed to produce NMDA-like inhibition.

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CORTICAL AMINO ACID CHANGES AFTER "LONG TERM" ELECTRICAL KINDLING IN RATS

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Previous studies have suggested that electrical kindling may be accompanied by changes in the concentrations of glutamate (Peterson et al, 1983) and glutamine (Leach et al, 1983) in the brain. Kindling is a chronic animal model of epilepsy characterised by a progressive increase in response to the same regularly applied stimulus. We now report the effect of 'long term' (3 month) electrical kindling on the concentrations and release of certain cortical amino acids.

Male Wistar rats (Charles River 250-300g) were implanted with epidural screw electrodes over the frontal and parietal cortex and kindled electrically by stimulation of the frontal cortex as described previously (Leach et al, 1983). Control rats were implanted but not shocked. When three kindled responses had been obtained, the rats were shocked once weekly for approximately 3 months and sacrificed one week after the last shock. The brain was rapidly removed, cerebral cortex dissected and hemisected, one section being frozen on dry ice for amino acid analysis after prechlorate extraction, and the other section processed for endogenous release studies. Prewashed cortical slices (0.1 x 0.1 mm prisms) were incubated with or without veratrine (2 µg/ml, 10 min incubation) in gassed (95% O_2/5% CO_2 pH 7.4) Tyrode medium, recovered by centrifugation for protein determination and the supernatant analysed for amino acid content using DL-homocysteic acid as internal standard. Amino acids were determined by separation on a Locarte amino acid analyser and detected fluorometrically as the o-phthaldialdehyde derivatives.

All rats electrically stimulated showed kindled responses (i.e. after-discharge duration were significantly greater than threshold values) which occurred after 16 ± 2 stimulations (n = 10/group). In these 'long term' kindled rats, the first kindled mean after-discharge duration was 82 ± 11s, which was not significantly different from the last kindled response some 3 months later of 77 ± 7s. There were no significant changes in cortical concentrations of taurine, aspartate, glutamate, glutamine or GABA. Veratrine stimulation of cerebral cortex slices prepared from kindled rats evoked a greater release of both glutamate and aspartate than from slices prepared from control (non-shocked) rats: the respective values for glutamate were 405% vs 316% ($p < 0.05$, n=10) and for aspartate were 294% vs 216% ($p = 0.07$, n=10).

After correction for basal release of glutamate (6.09 ± 0.89 nmol/mg protein in controls; 6.79 ± 0.59 nmol/mg protein in kindled rats) and for aspartate (4.78 ± 0.62 nmol/mg protein in controls; 5.46 ± 0.58 nmol/mg protein in kindled rats, mean ± s.e.m., n=10 per group) this represented an increased stimulated release of 43% for both amino acids in kindled rat cortex.

The excitatory amino acids glutamate and aspartate are considered to be involved in the generation and spread of abnormal epileptic activity (Bradford and Dodd, 1976). The present results indicate that the cortex of kindled rats has an increased capacity to release glutamate and aspartate which may be a permanent effect. However it remains to be determined if this increased release, results from or causes kindling.

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EXAMINATION OF THE INTERACTION OF α_2-ANTAGONISTS WITH GABA-RECOGNITION SITES IN RAT BRAIN MEMBRANES

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Recent work has shown that selective α_2-antagonists can lower seizure threshold in rodents at a supraspinal site (Fletcher and Forster, 1984). Although evidence indicates that central catecholamines modulate seizure threshold, an action of these compounds on other systems must be considered. In particular, an interaction with GABA, a major inhibitory transmitter in the CNS may be relevant. We describe in this report the interaction of the selective α_2-antagonists Wy 25309, Wy 26392 and Wy 26703 (Lattimer et al., 1982) and RX 781094 (Doxey et al., 1983) with GABA-recognition and benzodiazepine binding sites.

Cortical membranes were prepared and benzodiazepine and GABA binding measured according to the methods of Horton et al (1982) and Bowery et al (1982). The following ligand concentrations were employed: [^{3}H]-flunitrazepam (0.5nM) for benzodiazepine binding, [^{3}H]-GABA (5nM) for high affinity $GABA_A$ binding and [^{3}H]-GABA (100nM) for low affinity $GABA_A$ binding. A concentration of 10nM [^{3}H]-GABA, under appropriate incubation conditions, was used for $GABA_B$ binding. Inhibitory potency at α_1-adrenoreceptors was assessed from the displacement of [^{3}H]-prazosin (0.15-0.25nM) in Hepes buffer 20mM (pH 7.4 at 25°C). The activity at α_2-adrenoreceptors was assessed from the displacement of [^{3}H]-rauwolscine (1.5-2.5nM) in an identical buffer.

All four compounds were potent inhibitors of [^{3}H]-rauwolscine binding (Table 1). In addition, all compounds resulted in complete (ie 100%) displacement of [^{3}H]-prazosin from its binding site. In marked contrast, at concentrations up to 10^{-4}M, none of the compounds displaced the GABA related ligands by > 20%. It is unlikely, therefore, that brain levels achieved on systemic administration of these drugs would result in significant interactions with GABA recognition or benzodiazepine binding sites. The most likely explanation of the proconvulsant action of these compounds is due to an interaction at adrenoceptors, although not necessarily at pre-junctional α_2-adrenoreceptors (Fletcher & Forster, 1984).

Table 1. Interaction of compounds with adrenoreceptors, GABA and benzodiazepine binding sites.

	Potency (K_I,nM)					
Compound	α_2	α_1	$GABA_A$		$GABA_B$	BZ
			HA*	LA*		
Wy 25309	9.4±0.8(4)	2200±220(3)	ND	ND	ND	ND
Wy 26392	3.5±0.6(3)	2200±370(3)	ND	ND	ND	ND
Wy 26703	6.2±1.5(4)	2200±470(3)	ND	ND	ND	ND
RX 781094	5.6±0.8(4)	1000±330(5)	ND	ND	ND	ND

* HA = High affinity binding site, LA = Low affinity binding site, BZ = benzodiazepine binding site, ND = $K_I > 10^{-4}$M.
Means ± S.E.M. for the number of observations in parenthesis.

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BRAIN DOPAMINE LEVELS FOLLOWING EXPERIMENTAL CEREBRAL ISCHAEMIA

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The finding that the blood supply to the brain of the Mongolian gerbil (Meriones unquiculatus) is unique in lacking connecting arteries between the basilar and internal carotid circulations has made it a suitable model for the study of stroke (Kahn, 1972). As only 30-50% of gerbils suffer a stroke following ligation of the common carotid artery, an assessment of animals affected must be undertaken. This study describes a simple subjective system for assessing the possibility of stroke in an animal by analysis of dopamine changes in the striatum and by anatomical investigation of the circulus arteriosus.

Male gerbils (60 - 80 g) were anaesthetised with 6 mg pentobarbital (i.p.) and the left common carotid artery exposed in the paratracheal region. The artery was dissected free and doubly ligated. In sham operated controls the carotid artery was exposed and ligatures put in place but not tied. After 3 h the animals were decapitated, the corpus striatum separated into left and right hemispheres and stored under liquid nitrogen until analysed for catecholamine content by high performance liquid chromatography coupled to electrochemical detection.

Table 1 shows the percentage change in the dopamine levels in the left relative to the right hemispheres of the striatum for each group.

Group	Type 1 Complete Circulus Arteriosus	Type 2 Incomplete Circulus Arteriosus
Sham operated	103.9 ± 8.4 (8)	84.0 ± 9.1 (5)
Ligated	116.1 ± 15.9 (9)	45.9 ± 4.1 (7)*

*$p < 0.001$ for type 2 ligated compared to type 1 ligated.
Each value represents the mean ± SE for the number of determinations in brackets.

The results of this study show an ipsilateral decrease in striatal dopamine levels following cerebral ischaemia which confirms the observations of Zervas et al (1974). Anatomical separation of stroked and non-stroked animals by examining the circulus arteriosus has increased the reliability of using the change in dopamine level as an indication of stroke.

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EFFECT OF THYROID STATUS ON THE DIFFERENT FORMS OF Na^+, K^+-ATPase IN IMMATURE AND MATURE RAT BRAIN

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Na^+,K^+-ATPase is sensitive to changes in thyroid status in many target tissues. Hormone deficiency during brain maturation results in a retarded development of enzyme activity and also appears to be important for enzyme development in brain reaggregate cultures (Valcana & Timiras, 1969; Atterwill et al 1983). Since it has been established that the different ouabain-sensitive components of brain enzyme activity represent different molecular forms of this enzyme (see Atterwill et al 1984, Sweadner, 1979), we have examined these forms in the brain of developing and mature rats with altered thyroid status.

Rats were rendered hypo-(PTU 50mg/day p.o.) or hyperthyroid (T_4 0.3µg/g B.W.) from birth and at 11, 22 and 30 days Na^+,K^+-ATPase activity was measured by a radiometric method using [^{32}P]ATP (Atterwill et al 1984). Kinetic equations (Marks & Seeds, 1982) were used to resolve the two ouabain-sensitive components of enzyme activity(α^+ form, high ouabain affinity; α form, low ouabain affinity). At 11 days postnatally changes in thyroid status did not significantly alter the Na^+,K^+- or Mg^{++}-ATPase activities of either cerebellum (CBL) or forebrain, although PTU treatment tended to reduce activity in the CBL. However, at 22 and 30 days, although hyperthyroidism had no effect on enzyme activity, there were reductions in Na^+,K^+-ATPase activity in brains from the hypothyroid animals, especially in the CBL (50% reduction approx.). This reduction in CBL Na^+,K^+-ATPase activity appeared to show a differential effect on the two enzyme forms with a greater reduction occurring in the specific activity of the form with low ouabain affinity. Interestingly, more immature cultured neural cells from brain appear to possess higher proportions of this enzyme form than mature cells (as do certain peripheral tissues such as the kidney) and it is possible, therefore, that hypothyroidism may be affecting the development of the form of Na^+,K^+-ATPase in a specific cerebellar cell type or types.

Mature rats made either hyperthyroid (T_3 100µg/kg for 14 days) or hypothyroid (PTU, 50mg/day p.o. for 14 days) showed no changes in cerebellar Na^+,K^+-ATPase activity. We are currently examining the adult rat striatum for ATPase changes, an area where there is known to be a marked perturbation of neurotransmitter function during hyperthyroidism (see Atterwill, 1981).

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THE EFFECT OF VALPROIC ACID AND DIAZEPAM ON CATECHOL-INDUCED SPONTANEOUS CONVULSIONS

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Administration of catechol (1,2 dihydroxybenzene) to urethane anaesthetised mice (2g/kg i.p.) produces a central excitatory state during which the animal convulses spontaneously. Convulsions occur within 1 min of i.p. injection, reach a peak after 3 mins and last 15-20 mins. This study tests the potential of using catechol convulsions as a suitable model for evaluating anticonvulsants, by investigating the effects of diazepam and valproic acid.

The total body activity of anaesthetised mice (female albino, 16-25 g) was measured by suspending groups of three animals in a plastic container from a strain gauge which formed part of a bridge circuit. The bridge output was amplified, half-wave rectified and integrated over 30 s intervals (Angel, 1970). Two successive injections of catechol (80 mg/kg i.p.) were given exactly 60 mins apart, and the ratio of the convulsive response to the first injection (C1) to that of the second (C2), calculated. The test drugs were then administered i.p. 30 mins before the second injection of catechol and their effects on the C2:C1 ratio determined. Diazepam was administered in saline in a volume of 10 ml/kg and valproic acid was given in a volume of 200 or 400 µl/kg. Control animals received only saline. Results are presented below, each being the mean of 10 groups of three mice.

Drug	Dose mg/kg	Mean C2:C1 ± s.e.	P (paired t-test)
Control (saline)		1.44 ± 0.08	
Valproic acid	200	1.21 ± 0.03	< 0.01
	400	0.65 ± 0.09	< 0.001
Diazepam	5	0.80 ± 0.18	< 0.001
	10	0.78 ± 0.10	< 0.001

The results indicate that anticonvulsants which enhance GABA-mediated inhibition reduce catechol-induced convulsions, since both diazepam and valproate have been shown to potentiate the inhibitory effects of GABA (McDonald and Bergey, 1979; Costa et al, 1975). However, although catechol convulsions have been shown to be decreased by cholinoceptor blocking drugs and intensified by anticholinesterases, there is little evidence of a direct effect of catechol on GABA-ergic transmission (Angel et al, 1977; Minchin and Pearson, 1981). The results do suggest that catechol convulsions are a sensitive chemical model for evaluating anticonvulsant drugs.

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TRH INCREASES THE RELEASE OF DOPAMINE AND SEROTONIN IN THE NUCLEUS ACCUMBENS AND STRIATUM IN VIVO

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Thyrotropin releasing hormone (TRH) produces marked stimulatory effects on behaviour (Metcalf & Dettmar, 1981), and this has been attributed by some authors to the release of dopamine from the nucleus accumbens (Miyamoto & Nagawa, 1977 ; Heal & Green, 1979). Studies in vitro have indicated that high concentrations of TRH can release dopamine from nerve endings in this brain area, but not in the striatum (Kerwin & Pycock, 1979). However, the relevance of this phenomenon to the behavioural effects of TRH in vivo has been questioned (Metcalf, 1982). We have now used differential pulse voltammetry in order to determine the effects of pharmacologically active doses of TRH on dopaminergic and serotonergic synaptic activity in the nucleus accumbens and caudate nucleus of the rat.

Male Sprague-Dawley rats (250-300 g) were anaesthetised with nembutal (5 %, i.p. 1 ml/kg), held in a stereotaxic frame, and implanted with one working electrode in the striatum and another in the nucleus accumbens. The electrodes used allowed the simultaneous analysis in vivo of ascorbic acid, the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) and the serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA) (Crespi et al, submitted for publication).

TRH rapidly increased extracellular DOPAC, reaching a maximum after 60 min in the nucleus accumbens, and 40 min in the striatum. One hour after 10 mg/kg i.p. TRH, DOPAC values were 135 % of controls in both nuclei, and after 20 mg/kg reached 205 % in the nucleus accumbens, and 175 % in the striatum. There was also a slower increase in extracellular 5-HIAA content in both areas, reaching a plateau after 100 min, at 170 % of control values in the nucleus accumbens, and 150 % in the striatum. The delayed time course of the increase in 5-HIAA, and other data from our laboratory (Crespi et al, submitted for publication) suggested that the increase in 5-HIAA content might be secondary to the increase in dopamine turnover produced by TRH. The effect of dopamine receptor blockade on the TRH-induced increased in extracellular 5-HIAA was thus tested. In both brain areas, haloperidol (1 mg/kg, i.p.) increased even further the extracellular DOPAC content, but reduced that of 5-HIAA.

These results indicate that doses of TRH which produce behavioural stimulation in vivo increase the release of dopamine and serotonin in the nucleus accumbens and striatum of the rat. The increased turnover of serotonin may be secondary to the increased release of dopamine.

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A SIMPLE OBSERVATIONAL TEST FOR DRUG EFFECT ON SLEEP LATENCY IN THE RAT

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One of the commonest forms of insomnia is difficulty in falling asleep or increased sleep latency but tests to investigate the effects of drugs on this parameter in laboratory animals rely on expensive and time consuming EEG techniques. Here we describe a simple observational approach.

Groups of 8 male S-D rats (115-200g) were housed individually in cylindrical metal cages placed on the floor of a quiet, isolated laboratory. The animals, 4 of which received drug and 4 vehicle, were observed from above for behavioural signs of sleep onset (immobility, eye closure, the assumption of a characteristic sleep posture). Provided the other criteria were met, the moment of sleep onset was taken as the time of eye closure. All experiments were carried out at the same time of day to a standard protocol using an accommodation period of 30 min and the observer was unaware of the treatment received by each rat. Animals were used once only and had no prior experience of the test environment.

The use of these behavioural criteria to determine sleep latency was validated by comparison with simultaneous electrographic (EEG and EMG) recordings (Holmes & Sugden, 1982) in the same animals. By simple regression analysis a high level of correlation was found ($r=0.966$, $n=30$, $p<0.001$).

Using this observational technique a range of drugs claimed to promote sleep in man have been examined. All these drugs shortened sleep latency at relatively low doses. However with the majority (phenobarbitone, flurazepam, nitrazepam, methaqualone, perlapine, methyprylone, chloral hydrate and L-tryptophan) decreased sleep latency was detected only at doses approaching those which caused overt behavioural depression and/or muscle relaxation. Chlorpromazine and 5-hydroxytryptophan had biphasic effects, reducing sleep latency at a low dose but having the reverse effect at slightly higher doses. Thalidomide and melatonin were unusual in that they were the only compounds tested which shortened sleep latency at doses well removed from those causing gross behavioural depression (minimum effective doses 5 and 2.5 $mg.kg^{-1}$ i.p. respectively).

This inexpensive method requiring no special equipment provides a reliable and quick means of studying the effects of drugs on sleep latency.

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PREPARATION OF PRIMATE TISSUE FOR RECEPTOR BINDING STUDIES FROM [^{3}H]-2-DEOXYGLUCOSE AUTORADIOGRAPHY SECTIONS

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The introduction of high-resolution autoradiographic techniques has proved to be a useful tool for localising the anatomical distribution of central neurotransmitter receptor sites (Young and Kuhar, 1979). The neural tissue needed for such studies should ideally be removed from the animal as quickly as possible, frozen, and sectioned without strong fixation in order to preserve the binding characteristics of the receptor sites. The routine use of the (^{3}H)-2-deoxyglucose (2-DG) uptake technique, in the monkey, in this laboratory has generated a large reserve of such tissue. This report provides evidence that all but trace amounts of the (^{3}H)-2-DG can be removed from these sections leaving them perfectly suited to autoradiographic receptor binding studies.

Representative cryostat-cut (^{3}H)-2-DG sections, which had been stored for several months, were scraped into scintillation vials and assayed using standard scintillation techniques. Each section was found to contain approximately 122,000 counts per minute (c.p.m.). A similar set of sections were given 15 x 10 minute washes in 50 mM Tris-HCL buffer (pH 7.4) at 4°C, then dried and assayed or exposed to LKB Ultrofilm. This procedure was found to reduce the radioactivity in the sections to 570 c.p.m. ($\pm$ 35, N = 9), which is approximately 0.46% of the original level. Such sections failed to produce an autoradiographic image after 42 days exposure.

The validity of using this procedure for preparing sections for receptor binding studies from tissue which has been used in 2-DG autoradiography was tested by studying the distribution of $GABA_A$ and $GABA_B$ binding sites as has been previously described (Crossman et al, 1983). Using scintillation analysis the specificity of (^{3}H)-GABA binding at A and B sites was estimated in the presence of non-specific ligands. Specific binding of (^{3}H)-GABA to A and B sites was 92% and 62.7% respectively. Following exposure of bound sections for 42 days the anatomical distribution of $GABA_A$ and $GABA_B$ sites as shown in the resultant autoradiographs correlated well with descriptions from studies using fresh rat brain (Crossman et al, 1983).

We conclude that high-resolution autoradiographic studies of exceptionally high specificity can be conducted on primate tissue which has been originally prepared for (^{3}H)-2-DG experiments.

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EFFECTS OF SUBSTANCE P ON RESPONSES OF CAT CAROTID BODY CHEMORECEPTORS TO DOPAMINE, NORADRENALINE AND 5-HYDROXYTRYPTAMINE

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The cat carotid body contains substance P (SP)-like material (Cuello & McQueen, 1980) as well as the putative transmitters dopamine (DA), noradrenaline (NA), and 5-hydroxytryptamine (5-HT). Preliminary investigations showed that SP can modify spontaneous discharge and some drug-induced responses of the carotid chemoreceptors (McQueen, 1980), and we have now studied the influence of SP on responses of cat carotid chemoreceptors to DA, NA and 5-HT. Experiments were performed on pentobarbitone-anaesthetized cats (42 mg kg^{-1} i.p., supplemented as necessary), artificially ventilated and paralysed with gallamine (3 mg kg^{-1} i.v.). Chemosensory discharge was recorded from the peripheral end of a cut sinus nerve, and drugs were injected or infused into the common carotid artery as previously described (McQueen, 1980). Infusions of SP (10 µg min^{-1}) or drug vehicle (Locke solution) were made at a rate of 0.1 ml min^{-1} for 2 min and injections of the amines made 90 s after starting the infusion. This protocol was chosen to minimise the tachyphylaxis caused by SP.

Within 15 s of onset of SP infusion there was a small but significant decrease ($P < 0.05$) in spontaneous discharge from the pre-infusion control frequency; Locke solution had no marked effect on discharge.

NA (0.1 - 50 µg i.c.) caused chemodepression followed by chemoexcitation; chemodepression evoked by the higher, but not the lower doses of NA was potentiated during SP infusion, whereas the delayed chemoexcitation was reduced. 5-HT (1 - 25 µg i.c.) caused dose-related chemodepression that was potentiated during SP infusion. Secondary excitation following chemodepression was smaller in magnitude than that associated with NA, but, in contrast, was potentiated during SP infusion. DA (0.1 - 10 µg i.c.) induced dose-related chemodepression which was greater than that evoked by NA or 5-HT, but the effect was largely unaltered during SP infusion.

Our results indicate that SP-monoamine interactions are rather complex, and there is evidence for a differential effect of the peptide on the responsiveness of carotid chemoreceptors to the amines studied. Thus, SP potentiated NA and 5-HT-induced chemodepression, but had no effect on the depressant action of DA. Delayed chemoexcitation caused by NA was reduced during SP infusion, but that evoked by 5-HT was potentiated. It remains to be established whether SP modifies chemoreceptor activity by acting directly on some element of the chemosensory complex within the carotid body rather than indirectly by a non-specific action (e.g. vascular effects which in turn influence chemoreceptor discharge). Further studies are needed to determine whether the peptide-amine interactions described have any physiological significance, and also to characterize the type(s) of SP receptor involved in modifying chemoreceptor activity.

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THE EFFECTS OF d-AMPHETAMINE ON BRAIN 5-HYDROXYTRYPTAMINE AND PLASMA CORTICOSTERONE IN UNSTRESSED RATS

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Previous studies in this laboratory have shown that the chronic administration of nicotine to unstressed rats causes a regionally-selective reduction in the concentration and biosynthesis of 5-hydroxytryptamine (5-HT) in rat hippocampus (Benwell & Balfour 1979, 1982). In addition it has been shown that plasma corticosterone levels are raised by acute nicotine administration and by its withdrawal following a period of chronic treatment whereas no increase is observed in rats in which chronic treatment is maintained (Benwell & Balfour 1979). d-Amphetamine is a psychomotor stimulant with many behavioural properties which are similar to those of nicotine (Izquierdo & Izquierdo, 1971). The purpose of the present study was to examine the possibility that d-amphetamine might also cause the changes in brain 5-HT and plasma corticosterone observed in response to nicotine.

Male Wistar rats were given daily subcutaneous injections of d-amphetamine sulphate (0.5 mg free base/kg) or saline for 39 days. On day 40, half the saline-treated group were given saline as usual, the remainder being given an acute injection of d-amphetamine. Half the amphetamine-treated animals were given their usual injection of drug whereas in the remaining half d-amphetamine was withdrawn and replaced by an injection of saline. Thirty minutes after this final injection the animals were killed by cervical dislocation, the brains rapidly removed for dissection and blood samples taken from the trunks for the analysis plasma corticosterone (Mattingly 1962). The brains were dissected into hippocampus, hypothalamus and cerebral cortex following the procedure of Glowinski and Iversen (1966) and analysed for 5-HT and 5-hydroxyindole acetic (5-HIAA) acid (Curzon & Green 1970) or homogenised in 0.32M sucrose and the homogenate used for the estimation of 5-HT biosynthesis (Benwell & Balfour 1982).

Preliminary studies showed that the dose of d-amphetamine selected for the study caused a consistent and marked increase ($p < 0.001$) in psychomotor activity measured using an activity monitor (Animex) and a y-maze. Neither the acute nor chronic administration of d-amphetamine nor its withdrawal had any significant effects on the plasma corticosterone concentration. The concentrations of 5-HT and 5-HIAA in the brain regions studied were also not significantly affected by the acute or chronic administration of d-amphetamine. Acute d-amphetamine did, however, cause a significant reduction ($p < 0.05$) in 5-HT biosynthesis in synaptosomes prepared from hippocampal tissue (control 0.22 $\pm$ 0.03 pmoles/mg protein/min); d-amphetamine 0.12 $\pm$ 0,03 pmoles/mg protein/min, $n = 8$), whereas biosynthesis in hippocampal tissue from chronically-treated or withdrawn rats was not significantly affected when compared with saline-treated controls. 5-HT biosynthesis in hypothalamic and cerebrocortical synaptosomes were unaffected by any of the treatments.

The study has shown that d-amphetamine does not elicit the same changes in plasma corticosterone and hippocampal 5-HT as those previously reported for nicotine (Benwell & Balfour 1979, 1982).

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STUDIES ON THE EFFECTS OF d-AMPHETAMINE ON BRAIN 5-HT AND PLASMA CORTICOSTERONE IN STRESSED RATS

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Previous studies in this laboratory have shown that the psychomotor stimulant, nicotine, attenuates adaptation of the adrenocortical response observed in rats repeatedly exposed to the same psychological stress and that this effect of nicotine may be related to regionally-specific changes in the concentration of 5-hydroxytryptamine (5-HT) in the hippocampus (Benwell & Balfour, 1982). The present investigation seeks to examine the possibility that d-amphetamine exerts a similar effect on this adaptive process.

Male Wistar rats were given subcutaneous injections of d-amphetamine sulphate (0.5 mg free base/kg) or saline and were then stressed acutely by being placed on an elevated platform for 30 minutes (Benwell & Balfour, 1982) or returned to their home cages for 30 minutes (unstressed groups). The rats were then killed by cervical dislocation, the brains rapidly removed and dissected for the analysis of hippocampal, hypothalamic and cerebrocortical 5-HT and 5-hydroxyindole acetic acid (5-HIAA) by the method of Reinhard et al (1980) and blood samples taken from the trunks for the estimation of plasma corticosterone (PC) using the method of Mattingly (1962). The effects of chronic d-amphetamine and its withdrawal on the changes in hippocampal 5-HT and PC associated with habituation to psychological stress were examined by injecting d-amphetamine (0.5 mg/kg) in place of nicotine in the treatment protocol described by Benwell and Balfour (1982).

d-Amphetamine had no effects on the raised PC levels found in acutely stressed rats and its chronic administration or withdrawal also failed to change the levels in the stress-adapted rats. Acute stress had no significant effects on brain 5-HT and 5-HIAA. d-Amphetamine administration to acutely stressed rats caused a significant reduction ($p < 0.05$) in cerebrocortical 5-HT (0.15 ± 0.03 $\mu g/g$; $n = 6$) when compared with the other groups tested (sal/no stress 0.27 ± 0.04 $\mu g/g$; amphet/no stress 0.26 ± 0.03 $\mu g/g$; sal/stress 0.26 ± 0.04 $\mu g/g$; $n = 6$ for each gp) and an increase ($p < 0.05$) in hypothalamic 5-HT (0.91 ± 0.22 $\mu g/g$; $n = 6$) when compared with unstressed saline controls (0.37 ± 0.06 $\mu g/g$; $n = 6$). No significant differences in 5-HIAA or hippocampal 5-HT were observed. Adaptation to the stress was associated with a significant reduction ($p < 0.05$) in hippocampal 5-HT from 0.46 ± 0.10 $\mu g/g$ ($n = 8$) to 0.22 ± 0.04 $\mu g/g$ ($n = 10$) which was further decreased ($p < 0.05$) to 0.15 ± 0.03 $\mu g/g$ ($n = 10$) by chronic d-amphetamine but not its withdrawal (0.21 ± 0.05 $\mu g/g$; $n = 10$). No changes in the 5-HT concentration in cerebral cortex or hypothalamus or in 5-HIAA levels in any of the regions investigated were observed.

The study provides evidence for an interaction between d-amphetamine and the effects of both acute and chronic stress on brain 5-HT. These effects do not seem to be related to changes in the adrenocortical response to stress.

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ADRENAL CORTICOSTEROID-INDUCED ALTERATIONS IN OPIOID RECEPTOR SENSITIVITY

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Morphine produces, in addition to antinociception, several behavioural and endocrine effects in rodents (Babbini & Davis, 1972; Van Vugt & Meites, 1980). As the opiate-sensitive systems are differentially affected by glucocorticoids, antinociception being relatively unaffected (Fekete et al, 1984), the possibility of an interaction at one or more opioid receptor subtype must be considered. We describe the effects of adrenalectomy and high corticosteroid replacement on opioid receptor sensitivity in vitro and in vivo.

Three groups of male Sprague-Dawley rats (approx. 300g) were used. In one group, all animals underwent bilateral adrenalectomy. Of the two remaining sham-operated groups, one group received corticosterone acetate 10mg/day or hydrocortisone (cortisol) 25mg/kg/day for four days. The experiments were performed 24h after the last hormone injection. Resting oxygen consumption was measured by closed-circuit respirometry. Opioid receptor numbers were assessed in vivo (Perry et al, 1982) or determined from Scatchard analysis in vitro.

Morphine (1mg/kg, s.c.) elevated resting oxygen consumption in sham-operated animals (Table 1). This effect was greater in adrenalectomised animals and there was no effect of morphine on cortisol or corticosterone-replaced animals. Parallel changes in in vivo cortical ^{3}H-etorphine binding were observed. Adrenalectomy increased opioid binding, whereas replacement therapy produced a substantial reduction in opioid binding. Brain levels of ^{3}H-etorphine were unaffected by treatment. The binding characteristics of ^{3}H-naloxone in vitro were, however, unaffected by the adrenocortical status (Table 1).

The results of in vivo receptor binding are consistent with the modulation of opioid receptor number or senstivity by corticosteroid status. One explanation of the discrepancy with the in vivo data would be the removal of some endogenous factor(s) during the isolaution procedure. As 10^{-5}M hydrocortisone was found to be without effect on ^{3}H-naloxone binding in vitro, it is unlikely that this factor is an adrenal corticosteroid. Over the concentration range of naloxone employed, an interaction with μ-receptors would be predominant. Etorphine is considered to be a 'universal' opioid ligand and it is possible therefore that changes in the binding characteristics of this ligand may reflect changes other than those at μ-receptors.

Table 1 The effect of adrenalectomy and corticosteroid replacement on opioid sensitivity

	VO_2 % increase	^{3}H-etorphine dpm/mg cortex	K_D (nM)	B_{max} (fmol/18mg tissue)
Sham	7.9±1.0 (7)	725±78 (7)	2.0±0.2 (9)	305±22 (9)
ADX	20.9±1.7 (7)	1163±146 (4)	2.3±0.3 (8)	284±17 (8)
Replacement	3.4±1.2 (7)	302±26 (2)	2.2±0.2 (8)	287±21 (8)

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