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PHARMACOLOGICAL DETERMINATION OF THE pA_2 FOR PIRENZEPINE USING A MAMMALIAN IN VITRO BRAIN SLICE PREPARATION

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Antagonist binding to muscarinic acetylcholine receptors (mAChR) appears to be homogenous when ligands such as quinuclinidinylbenzilate (QNB) scopolamine or atropine are used (Hammer et al, 1980). However agonist binding suggests that the mAChR has several affinity states and this is supported by studies using antagonists such as pirenzepine (Pz). Two mAChR subtypes can be distinguished using Pz: a high affinity site found in the CNS, and a low affinity site detected in smooth and cardiac muscle, these sites being termed M1 and M2 (Hammer et al, 1980). Using pharmacological techniques it has been shown that the pA_2 for Pz in ganglia is 23 fold higher than in ilea (Brown et al, 1980). The present study has attempted to extend such observations to a mammalian brain slice preparation.

Surface slices of guinea-pig olfactory cortex (500 μm thick) were hand-cut and incubated in standard Kreb's media (23-25° C) (Williams et al, 1985). Electrical stimulation of the rostral lateral olfactory tract (0.2 Hz) evoked surface negative field potentials that were recorded using extracellular glass microelectrodes filled with 0.9% saline (1-3 MΩ). All drugs were bath-applied. Schild plots were analysed by linear regression.

Agonists such as carbachol (CCh), Ach, or muscarine (10-200 μM) produced reversible dose-dependent depressions of the evoked field potential, by a postulated presynaptic mechanism; these effects were atropine sensitive, but not mimicked by nicotinic agonists or antagonised by d-tubocurarine (Williams et al, 1985). Pz (0.05-1 μM) had no effect on the field potential when applied alone but it drastically reduced the effects of muscarinic agonists. Pz shifted the CCh log dose-response (DR) curve to the right in parallel fashion with no depression of the maximum. Results pooled from 27 slices yielded a linear Schild plot, with a slope of 0.90 ± 0.14 (s.e.m.). The calculated pA_2 was 8.10 ± 0.08, or 7.99 ± 0.12 if the slope was constrained to unity. When Pz and atropine were used in combination the resultant dose ratio was additive, suggesting an action on similar sites.

Antagonist	Dose (M)	mean DR shift (+ s.e.m.)	n
Atropine	10^{-8}	8.9 ± 1.4	5
PZ	10^{-7}	18.6 ± 4.1	10
Atropine + PZ	10^{-8} & 10^{-7}	32.6 ± 9.4	4

Pirenzepine was clearly a potent antagonist at the olfactory cortical mAChR. This action appeared competitive since parallel shifts of DR curves were seen and the Schild plot slope did not differ significantly from unity ($p<0.05$). Moreover combination studies showed that the observed shift was much closer to that expected for similar site competition, 27.5 than for two dissimilar sites, 165.4. The estimated pA_2 value for Pz was similar to that found in ganglia (Brown et al, 1980) and also corresponds to the high affinity brain binding site (Hammer et al, 1980). This suggests that the mAChR receptor in this brain slice preparation is of the M1 subtype, and that this tissue may be a useful pharmacological model for studying central muscarinic receptors.

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NEURONAL RESPONSES TO ACETYLCHOLINE AND CARBACHOL IN THE CEREBRAL CORTEX: INVOLVEMENT OF M_1 MUSCARINIC RECEPTORS

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Acetylcholine (ACh) applied by microelectrophoresis is a potent excitant of cortical neurones; this effect has been attributed to the activation of muscarinic receptors (Krnjević, 1974). Muscarinic receptors, however, do not form a homogeneous population: two subclasses (M_1 and M_2 receptors) have been differentiated using selective agonists and antagonists (see Birdsall et al, 1984). In the present investigation we have used two M_1 selective compounds (Birdsall et al, 1984): McN-A-343 (selective M_1 agonist) and pirenzepine (selective M_1 antagonist) in an attempt to establish the possible involvement of M_1 muscarinic receptors in the neuronal responses to ACh and its synthetic analogue, carbachol (CCh).

Single spontaneously active neurones were studied in the somatosensory cortex of the halothane-anaesthetized rat. Our techniques for the extracellular recording of neuronal activity and for the microelectrophoretic application of drugs are described elsewhere (Bradshaw et al, 1983). All the drugs were applied by microelectrophoresis. The α_1-adrenoceptor agonist, phenylephrine (PhE), was used as the control agonist. Statistical comparisons were made using Student's t-test with a criterion of $P < 0.05$.

We first compared the agonistic actions of McN-A-343, ACh and CCh. While McN-A-343 (74 cells) and ACh (32 cells) excited all the neurones to which they were applied, CCh had multiple effects: out of 40 neurones, 33 were excited, 2 depressed and 5 responded in a biphasic fashion (excitation/depression).

The effects of pirenzepine on excitatory responses to McN-A-343, ACh and PhE were examined on 9 cells. Pirenzepine significantly attenuated the responses to both McN-A-343 (percentage change in the size of the response in the presence of pirenzepine, mean ± s.e.mean: -88.3 ± 3.9) and ACh (-33.7 ± 13.6), although the response to McN-A-343 was significantly more sensitive to pirenzepine than was the response to ACh. The response to PhE was unaffected.

We examined next the effects of pirenzepine on excitatory responses to McN-A-343, CCh and PhE (11 cells). Pirenzepine failed to discriminate between the responses to McN-A-343 (-74.2 ± 5.3) and CCh (-79.0 ± 6.4). Responses to PhE were not diminished. On 4 of these cells pirenzepine not only abolished the excitation to CCh, but also unmasked a depressant response to this agonist.

Finally, we compared the effects of pirenzepine on excitatory responses to CCh and ACh on 8 PhE-sensitive cells. Pirenzepine significantly reduced the size of the responses to both CCh (-85.6 ± 6.6) and ACh (-56.2 ± 4.8), although the response to CCh was significantly more susceptible to antagonism than was the response to ACh. The response to PhE was unaffected. On 3 of these cells a depressant response to CCh was unmasked by pirenzepine.

These results provide evidence for the involvement of M_1 muscarinic receptors in mediating the excitatory responses of cortical neurones to ACh and CCh. The nature of the depressant response to CCh remains to be determined.

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EFFECT OF NICOTINE ON MOTONEURONES IN THE RAT HEMISECTED SPINAL CORD PREPARATION

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Carbachol has been shown in previous experiments to produce depolarizing muscarinic responses in motoneurones as recorded from ventral roots of immature rat hemisected spinal cord preparations. An atropine-resistant component of the effect of carbachol was never observed (Evans, 1978).

In the present experiments the effect of nicotine has been tested on these preparations. Nicotine (5-50μM) ((-)nicotine di(+)tartrate) was found to produce depolarizing responses as recorded from ventral roots. Isolated ventral roots, sectioned at their point of emergence from the cord, failed to respond to nicotine (10-20μM) although they were sensitive to glycine (0.1 - 1mM). Desensitization to the depolarizing effect of nicotine occurred with a half time of approximately 5 min and such desensitization persisted for at least 2 hours following a single 2 min application. Because of desensitization blocking agents were tested on five preparations, for their ability to prevent, at the $P > 0.05$ level (sign test), a depolarizing response to the first application of 10 μM nicotine. The amplitude of depolarizing responses ranged from 0.15 to 0.55 mV in all of 23 naive preparations treated with a first dose of 10 μM nicotine.

Sensitivity to nicotine persisted in the presence of sufficient tetrodotoxin (0.1μM) to block all spontaneous and evoked synaptic activity. Similarly, sensitivity to nicotine persisted in the presence of the amino acid antagonist kynurenic acid (2mM) (Perkins and Stone, 1982) which depressed synaptic activity by more than 90%. However, treatment of preparations with the ganglion blocker hexamethonium (250μM) abolished sensitivity to nicotine.

These observations suggest that motoneurones of the immature rat spinal cord possess nicotinic receptors in addition to the muscarinic receptors previously described.

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ANALYSIS OF THE MUSCARINIC AGONIST ACTION OF ARECAIDINE PROPARGYL ESTER (APE)

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The agonist, arecaidine propargyl ester (APE), has been reported to exhibit 5 fold selectivity for muscarinic receptors (mAChRs) present in the atria in comparison to those present in the ileum (Mutschler & Lambrecht, 1984). These data were considered to be consistent with the concept of different mAChRs present in the ileum and atria as originally proposed by Barlow et al (1976).

The present study has assessed the muscarinic activity of APE relative to carbachol in a number of preparations to investigate further its muscarinic activity.

The potency of APE and carbachol was assessed (Clague et al, 1984) at 30°C in the following tissues from Dunkin-Hartley guinea-pigs: ileum, atria (spontaneously beating), trachea and bladder. The affinity was determined (Furchgott & Bursztyn, 1967) using phenoxybenzamine (3×10^{-6} mol.litre^{-1}) as the irreversible antagonist. The results are shown below:-

Tissue	$-\log EC_{50}$		$-\log K_A$	
	C	A	C	A
Ileum	6.77	7.70	5.09	6.74
Atria	6.72	7.85	4.76	6.71
Trachea	5.62	5.89	4.19	5.32
Bladder	5.89	5.80	5.01	6.00

Table 1 Agonist potency ($-\log EC_{50}$) and affinity ($-\log K_A$) of carbachol (C) and APE (A) in various tissues. Values are mean, sem less than 5% in each case, n = 4.

The results showed that carbachol and APE exhibited similar potencies at mAChRs present in the trachea and bladder, which were lower in comparison to those observed at atrial and ileal mAChRs. The affinities observed for carbachol and APE in the trachea were lower than those in the other tissues. The relative efficacy of APE, in comparison to carbachol, was similar in all four tissues (ileum 0.21, atria 0.17, trachea 0.16 and bladder 0.21).

The bladder and trachea possess a low receptor reserve in comparison to the ileum and atria. APE is an agonist of low efficacy in comparison to carbachol. In this study APE did not exhibit any marked degree of selectivity between atrial and ileal mAChRs either in terms of potency or affinity.

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DIURNAL VARIATION IN 5-HT_1 RECEPTOR FUNCTION

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The diurnal variation in 5-hydroxytryptamine (5HT) metabolism is well-known and there are reports of 24 h variations in receptor binding (Kafka et al, 1983) and function (Moser & Redfern, 1985). Here we present data to suggest that the behavioural response produced by the $5HT_1$ receptor agonist 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)-H-indole (RU 24969), exhibits a variation which may reflect diurnal changes in $5HT_1$ receptor function.

Male BK.TO mice (30-40 g) were housed under a 12:12 h light:dark cycle and allowed free access to food and water. The hyperlocomotion induced by i.p. injection of RU 24969 was quantified as the number of times that an infra-red beam across a black box (20x30x33 cms) was broken in the 30 minutes post-injection. Animals were familiarised in the box for 30 minutes before injection. All drugs were administered in 0.9% w/v saline and control animals received 0.9% saline.

RU 24969 induced a significant dose-dependent increase in locomotion (ANOVA, $P<0.001$, n=6 at each dose), the ED_{50} being 4 mg/kg and R_{max} at 24 mg/kg. A significant ($P<0.05$) variation in the activity counts was observed after administration of saline to groups of 6 animals 2, 5, 8 and 11 h after lights on and 2, 5, 8 and 11 h after lights off. The highest scores occurred during the hours of darkness. RU 24969 (4 mg/kg) significantly increased these counts ($P<0.001$) and to a different extent according to the time of administration (2 way ANOVA $P=0.05$). The maximum increase occurred 8 h after lights on (L8) and the minimum 5 h after lights off (D5). This suggested a difference in receptor function at these two times. In order to test this hypothesis we examined dose-response curves to RU 24969 in the presence of the 5HT receptor antagonist metergoline (2 and 5 mg/kg) given 30 min before RU 24969 and observed a significant dose-dependent (co-variance analysis, $P<0.001$) parallel shift to the right. There was however a clear difference in the dose ratios at the two times (L8-4.6 and 10.9; D5-1.3 and 2.0).

In conclusion, the results demonstrate that $5HT_1$ receptor function varies throughout the normal 24 h light-dark cycle. We have previously reported that $5HT_1$ receptor stimulation in the SCN decreases 5HT release and metabolism (Marsden & Martin, 1985). It is therefore tempting to speculate that the variation in $5HT_1$ receptor function seen here may be related to circadian rhythm genesis.

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PERIPHERAL 5-HT_2 RECEPTOR ACTIVATION FOLLOWING ADMINISTRATION OF TRYPTAMINE DERIVATIVES IN RATS

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There is considerable evidence that there are a number of subtypes of 5-HT receptors. Central 5-HT receptors have been designated 5-HT_1 and 5-HT_2 on the basis of ligand binding studies. The 5-HT_1 binding sites have been further subdivided into 5-HT_{1A} and 5-HT_{1B}. In an attempt to determine a functional role for the different 5-HT receptors, behavioural studies have been previously undertaken. It has been reported that the centrally acting 5-HT receptor agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) induces some behavioural effects by activation of putative 5-HT_{1A} receptors (Tricklebank, 1984). In the rat fundus strip preparation 5-MeODMT has been reported to be an antagonist of 5-HT (Glennon & Gessner, 1979). In view of the reports that 5-MeODMT has both agonist and antagonist activity and that activation of a large number of receptor systems may contribute to its activity, it was decided to investigate the effects of a number of tryptamine derivatives on the blood pressure of rats.

Male hooded Wistar rats weighing 250 - 320 g were used. To prevent centrally-mediated reflex activity contributing to the cardiovascular effects, the rats were pithed following anaesthesia with halothane and were artificially respired. Drugs were injected intravenously (i.v.) and the blood pressure (BP) was recorded continuously.

Injection of 5-HT (3 - 100 µg/kg) produced a dose-dependent increase in BP, the maximum being an increase of 107 ± 9 mmHg (mean ± s.e., n = 12). The other tryptamine derivatives caused a similar dose-dependent increase in BP. The ED_{50} values are presented in Table 1. 5-HT was the most potent agent and N-methyltryptamine (NMT) was the least potent. Ketanserin, at doses of .75 to 75 nmol/kg (0.3 - 30 µg/kg) resulted in non-competitive antagonism of the pressor responses to 5-HT, 5-MeODMT, 5-OHNMT, 5-MeOT and tryptamine. Higher doses of ketanserin were required to inhibit the N-methyl tryptamine derivatives.

Table 1. ED_{50} (± s.e.m.) values of the tryptamine derivatives and the dose of ketanserin required to inhibit the pressor response by 50%.

Compound	*ED_{50} (nmol/kg)*	*n*	*Ketanserin (nmol/kg)*
5-HT	185 ± 21	12	2.5
5-MeODMT	210 ± 119	5	30
5-hydroxy-N-methyltryptamine (5-OHNMT)	313 ± 36	5	50
5-MeOtryptamine (5-MeOT)	500 ± 37	6	7.5
N,N-dimethyltryptamine (DMT)	1660 ± 350	4	315
Tryptamine	2173 ± 285	6	20
N-methyltryptamine (NMT)	3954 ± 920	5	2500

The pressor action of the tryptamine derivatives was not altered by reserpine pretreatment (2 mg/kg, i.p. 18 h).

It is suggested from these results that, except for DMT and NMT, the tryptamine derivatives produced vasoconstriction by activation of 5-HT_2 receptors. DMT and NMT may produce vasoconstriction by an action on a 5-HT receptor less sensitive to ketanserin or another receptor mediating vasoconstriction.

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ADENOSINE ENHANCEMENT OF A23187 INDUCED 5-HYDROXYTRYPTAMINE RELEASE FROM RAT MAST CELLS IS NOT P_1- OR P_2- PURINOCEPTOR MEDIATED

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Adenosine enhances mediator secretion from rat mast cells induced by immunological and non-immunological stimulation (Marquardt et al 1978; Welton & Simko, 1980). We have confirmed these observations and examined the site of action of adenosine of calcium ionophore A23187 induced 5-hydroxytryptamine (5-HT) release from purified rat peritoneal mast cells.

Mast cells were purified to >90% by centrifugation through Percoll (1.09 g_3 ml^{-1}) and incubated for 2h with [^{3}H]-5HT (5 uCi per 10^6 cells). [^{3}H]-5-HT release following incubation with A23187 (0.1 μM) for 15 min was assessed by liquid scintillation spectrometry. Cyclic AMP was measured by radioimmunoassay.

Adenosine (0.1 - 100 μM) enhanced A23187 induced 5-HT release by up to 100%, the effect being most pronounced with simultaneous addition of nucleoside and secretagogue. That the action of adenosine was likely to be at the cell surface rather than intracellular was evidenced by the failure of adenosine uptake blockers dipyridamole (1 μM) or β-nitrobenzylthioguanosine (5 μM) to inhibit enhancement. The relative potencies of adenosine, AMP and ADP (1:0.06:0.002) are not consistent with an effect at ATP-sensitive P_2-purinoceptors. That the effects of AMP and ADP result from their metabolism to adenosine is suggested by the findings that α,β-methylene ATP was inactive and inhibition of 5'-nucleotidase by α,β-methylene ADP abolished the effects of AMP. Adenosine-sensitive P_1-purinoceptors consist of two sub-types both linked to adenylate cyclase, A_1 being inhibitory and A_2 causing stimulation (Londos & Woolf, 1977). The presence of functional A_2-purinoceptors on rat mast cells was demonstrated by a transient rise in cyclic AMP following incubation with adenosine. Although A23187 alone produced 5-HT release without changes in cyclic AMP, it may be postulated that enhancement of A23187-induced mediator release results from a concomitant elevation of cyclic AMP. However, this hypothesis is unlikely to be correct because theophylline (50 μM) and 8-phenyltheophylline (3 μM), despite abolishing the cyclic AMP response, did not inhibit enhancement of 5-HT release. Furthermore, the relative potencies of adenosine (=1) and P_1-purinoceptor agonists, D-N^6-phenylisopropyladenosine (0.61), L-N^6-phenylisopropyladenosine (0.56), 5'-N-ethyl-carboxamideadenosine (0.57) and N^6-cyclohexyladenosine (0.19) are not consistent with an interaction with either A_1 or A_2 subtypes of P_1-purinoceptors.

These results suggest that, like antigen-induced release (Church & Hughes, 1985), enhancement of A23187-induced 5-HT release from rat mast cells by adenosine is independent of its ability to elevate intracellular cyclic AMP. Although the cyclic AMP response is likely to be mediated by an A_2-purinoceptor, that mediating enhancement of 5-HT release appears to be a novel cell-surface purinoceptor.

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FURTHER CHARACTERIZATION OF THE PREJUNCTIONAL INHIBITORY 5-HT_1 RECEPTOR IN RAT KIDNEY

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We have recently demonstrated the presence of an inhibitory prejunctional receptor to 5-hydroxytryptamine (5-HT) on the sympathetic nerves to the rat kidney (Charlton et al, 1984). According to current thinking, this receptor appears to conform to criteria defining a 5-HT_1-like recognition site. However, ligand binding studies have demonstrated the existence of multiple sites within the 5-HT_1 grouping (5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C}). Therefore, the present experiments were undertaken to further define the prejunctional receptor. From studies done with 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), (-) propranolol and mesulergine we conclude that the prejunctional receptor does not fit criteria defining the 1A, 1B or 1C binding sites.

Rat kidneys were perfused in vitro with [^{3}H] noradrenaline to label the sympathetic innervation, as described previously (Charlton et al, 1984). The renal periarterial nerves were stimulated at supramaximal voltage with square wave pulses of 1msec at 2Hz for 20 sec. Stimulus-induced release of tritium was measured in the venous effluent.

As reported previously (Charlton et al, 1984), 5-HT (0.01 to 1.0 μM) produced concentration-related decreases in stimulus-induced release of tritium. In this regard, the 5-HT_1 agonists, 5-carboxamido-tryptamine and RU-24969, were 6.0 and 0.25 times respectively as active as 5-HT. However, 8-OH-DPAT (0.1μM), a 5-HT_{1A} agonist (Middlemiss and Fozard, 1983), failed to alter stimulus-induced release, even though the concentration used is sufficient to displace [^{3}H]5-HT entirely from cortical 1A binding sites. The experiments with 5-HT receptor antagonists were done using 5-HT (0.1μM). At this concentration, 5-HT does not increase basal release of tritium and is clearly submaximal at the presynaptic receptor, producing about a 40% decrease in stimulus-induced release. This inhibitory action of 5-HT was antagonized significantly by co-perfusion with methysergide (IC_{50}= 0.1μM) but not by co-perfusion with (-) propranolol (1.0 and 10μM). (-) Propranolol has been shown to displace [^{3}H]5-HT from cortical 1B binding sites with a pIC_{50} of 6.31 (Middlemiss, 1984). Furthermore, the 5-HT_{1C} antagonist, mesulergine (0.1 and 1.0μM), also failed to attenuate the inhibitory action of 5-HT on stimulus-induced release. Mesulergine has been reported to exert a nanomolar affinity for cortical 1C binding sites (Pazos et al, 1985). Thus, while the presynaptic inhibitory action of 5-HT in rat kidney appears to be mediated via a 5-HT_1-like receptor, the subtype involved does not conform to criteria defining the 1A, 1B or 1C binding sites. A major distinction between this receptor and the 5-HT autoreceptor on brain cortical 5-HT neurons is that (-) propranolol is ineffective in the kidney whereas it inhibits the autoreceptor with a pA_2 value of 6.67 (Middlemiss, 1984).

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PLATELET [^{3}H]-IMIPRAMINE AND [^{3}H]-YOHIMBINE BINDING ARE REDUCED IN CUSHING'S DISEASE

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Platelet binding capacity for (^{3}H)-imipramine is reportedly decreased in depressed patients whilst the binding of (^{3}H)-yohimbine is unchanged (Elliott, 1984). In addition, depressed patients have relatively high plasma cortisol levels particularly following the dexamethasone suppression test (Carroll et al, 1981). Since platelet (^{3}H)-imipramine binding is restored to normal values when the illness remits (Suranyi-Cadotte et al, 1982) this phenomenon cannot represent a trait marker and must be mediated by some humoral factor. We have therefore studied platelet (^{3}H)-imipramine and (^{3}H)-yohimbine binding in patients with Cushing's disease in order to investigate the possible role of cortisol as a modulator of platelet receptors.

Patients with Cushing's disease were admitted to the Endocrinology Department after diagnosis of active pituitary or adrenal tumours. Control subjects were matched for age amd sex and had no history of psychiatric illness. Blood samples (40 ml) were anticoagulated with 1% EDTA/saline and the platelets isolated by differential centrifugation and resuspended intact in 0.1% EDTA/150 mM NaCl, pH 7.5. In each case (^{3}H)-imipramine binding was carried out at 2°C for 60 min using 6 concentrations in the range 0.3 - 3 nM. Non-specific binding was defined by 1 µM fluoxetine. (^{3}H)-Yohimbine binding was carried out at 37°C for 30 min in the range 1.5 - 15 nM free concentration using 5 µM phentolamine to define non-specific binding. Specific binding was analysed by iterative non-linear regression analysis to determine binding affinity (Kd, nM) and capacity (Bmax, fmol/10^8 platelet).

Platelet binding characteristics for 5 patients with Cushing's disease and 5 matched controls are shown below as means ± s.e.m.

	(^{3}H)-Imipramine		(^{3}H)-Yohimbine	
	K_d	B_{max}	K_d	B_{max}
Control	0.56 ± 0.05	154 ± 17	4.2 ± 0.3	49 ± 5
Cushing's	0.76 ± 0.16	101 ± 14*	5.4 ± 1.6	30 ± 2*

As indicated above, platelet (^{3}H)-imipramine and (^{3}H)-yohimbine binding capacities were significantly ($p<0.05$, Student's unpaird t-test) lower in patients with Cushing's disease than in matched controls. There were no significant differences in binding affinity for either ligand.

Although cortisol is not the only hormone whose levels are significantly altered in patients with Cushing's disease, it remains the most likely agent responsible for the observed changes. Since cortisol levels tend to be higher than normal in depressed patients, these data suggest that the decrease in platelet (^{3}H)-imipramine binding previously associated with depression may be directly modulated by the increased cortisol concentration.

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SOLUBILIZATION OF THE SEROTONIN TRANSPORTER COMPLEX OF RAT CEREBRAL CORTEX

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Paroxetine is a very specific and potent inhibitor of serotonin (5HT) uptake, with a chemical structure different from that of the tricyclic antidepressants (Buus-Lassen, 1978). Recently, we have described the use of [^{3}H] paroxetine as a ligand to selectively label the 5-HT transporter complex in rat cortical membrane preparations (Habert et al., 1985). Using [^{3}H]paroxetine, we now report an initial pharmacological characterization of the 5-HT transporter complex upon solubilization of the transporter from its membrane environment.

Rat cortical membranes were prepared using the procedure of Sette et al. (1983). Membrane preparations were solubilized for 1 h at 0°C in 1 % (w/v) digitonin in the presence of 100 µM phenylmethylsulphonylfluoride, 16 m Units/ml of aprotinin and 2.5 mM EDTA. The detergent extracts were then centrifuged at 4°C for 1 h at 100,000 g, and the supernatant used as the source of the solubilized transporter. Binding assays with [^{3}H]paroxetine were carried out at 22°C and specific binding of [^{3}H]paroxetine was defined as the difference between the total binding and that remaining in the presence of 10 µM fluoxetine. [^{3}H]Paroxetine subsequently bound to detergent-solubilized transporter was separated from free [^{3}H]paroxetine by a modification of a rapid filtration assay for soluble receptors which utilizes polyethylenimine-treated filters (Bruns et al., 1983).

Specific, saturable binding of [^{3}H]paroxetine to the solubilized preparation was displayed in a concentration-dependent manner. Specific binding represented 84 % of total binding at a [^{3}H]paroxetine concentration of 0.10 nM. Scatchard transformation of the equilibrium saturation isotherm revealed a single class of binding sites, and the mean values from 3 experiments gave an equilibrium dissociation constant (Kd) of 0.12 ± 0.03 nM, and a density of binding sites (Bmax) of 350 ± 43 fmoles/mg protein. This Kd value is similar to the Kd of [^{3}H]paroxetine binding to rat cortical membranes (Kd = 0.15 nM). Also, 63 % of the specific [^{3}H]paroxetine binding sites present in the membrane preparation were solubilized using the detergent digitonin.

The binding of [^{3}H]paroxetine to the solubilized preparation was inhibited by chlorimipramine (Ki = 1.7 nM), fluoxetine (Ki = 8.7 nM) and 5-HT (Ki = 466 nM). This profile of inhibition closely resembles that obtained for the displacement of [^{3}H] paroxetine binding by these drugs to rat cortical membranes.

Thus, the binding properties of [^{3}H]paroxetine to these detergent extracts indicate that the neuronal 5-HT transporter complex can be efficiently solubilized with retention of its pharmacological profile towards 5-HT uptake inhibitors.

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NEUROPHARMACOLOGICAL PROFILE OF SR 95191, A NEW ATYPICAL ANTIDEPRESSANT DRUG WITH MAOI AND DOPAMINOMIMETIC ACTIVITIES

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SR 95191, 3-(2-morpholinoethylamino)4-cyano 6-phenyl pyridazine, was studied in rodent models of antidepressant and dopaminomimetic activities.
Female Swiss CD_1 mice (18-22 g) and male Wistar rats (200-230 g) (Charles River France) were used. The following tests were done : antagonism of reserpine-induced ptosis in mice (2 mg/kg i.v.) or rats (4 mg/kg s.c.) ; potentiation of yohimbine lethality in mice (20 mg/kg s.c.) ; potentiation of L-5-HTP-induced tremor in mice (1 mg/kg p.o.) ; behavioural despair in mice (Porsolt et al, 1977) ; antagonism of haloperidol catalepsy in rats (0.75 mg/kg i.p. ; Worms and Lloyd, 1980) ; induction of stereotyped behaviour in rats (Bizière et al, 1984) ; induction of turning in 6-OHDA lesioned mice (Von Voigtlander and Moore, 1973).

Table 1

	ED50's (mg/kg, p.o.)a							
Test Drug	Reserp. (M)	Ptosis (R)	Despair (M)a	Yohimb. (M)	5-HTP (M)	Tremor (R)	Halop. (R)	Oxot. (M)
SR 95191	5.8	12	40	>100	6.6	10.3	6.9	>100
IMI	2.4	6	20	9	>30 i.p.	-	16 i.p.	6.4
NOM	0.3	2.5	10	7	>100	-	6	5
IND	5.4	>30	>100	37	3.1	24	-	7
MOCL	0.6	2	10	>100	0.4	1.3	5.8	100

(M) = mice ; (R) = rats ; IMI = imipramine ; NOM = nomifensine ; IND = indalpine ; MOCL = moclobemide; a except despair : MED mg/kg, p.o.

As shown in Tab. 1, SR 95191 antagonized reserpine ptosis, potentiated 5-HTP-induced tremor, and decreased immobility in the despair test. However, SR 95191 did not potentiate yohimbine, nor did it antagonized oxotremorine.
As far as dopaminergic behaviours are concerned, SR 95191 antagonized haloperidol catalepsy (tab. 1) and induced stereotypies, mainly gnawing, licking and biting, in rats (mean $\pm$ SEM stereotypy scores after s.c. injection of SR 0.1 mg/kg : 15.4 $\pm$ 1.0 ; SR 0.3 : 17.0 $\pm$ 0.9 ; SR 1 : 15.0 $\pm$ 0.9 ; SR 3 : 12 $\pm$ 1). This effect of SR (1 mg/kg s.c.) was antagonized by both haloperidol (0.1 mg/kg i.p.) and α-methylparatyrosine (250 mg/kg i.p.). In addition, s.c. SR 95191 induced contralateral rotations in 6-OHDA lesioned mice (mean $\pm$ SEM number of turns after SR 0.25 mg/kg : 4.6 $\pm$ 0.8 ; SR 0.5 : 14.8 $\pm$ 1.9 . SR 1 : 19.7 $\pm$ 2.9). Apomorphine (0.12-0.5 mg/kg) also induced contralateral rotations, whereas d-amphetamine (5 mg/kg) and nomifensine (20 mg/kg) induced ipsilateral rotations.
These data indicate that SR 95191 exhibits an atypical profile of antidepressant and dopaminomimetic activities. This profile appears different from that of the reference drugs tested, whether they are tricyclics (IMI), dopamine uptake inhibitors (NOM), 5-HT uptake inhibitors (IND) or type A MAOI (MOCL). However, available neurochemical studies (Bizière et al, this meeting) indicate that SR 95191 specifically and reversibly inhibits MAO-A, increases 5-HT and decreases 5-HIAA brain levels, without affecting, in vitro, monoamine uptake or receptors.

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THE SUSTAINED RELEASE OF PYRIMETHAMINE BASE AND PYRIMETHAMINE PAMOATE FROM A BIODEGRADABLE INJECTABLE DRUG PREPARATION

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Although malarial prophylaxis requires multiple drug dosage, an injectable biodegradeable drug preparation might provide long term protection from a single dose. Pyrimethamine, when combined with a sulphonamide or sulphone, is in continuous use against chloroquine resistant P. falciparum malaria. We have studied the release of pyrimethamine from oil depot preparations containing pyrimethamine base (BASE) and its pamoate salt (PAM).

The plasma disposition of pyrimethamine, administered s.c.as either BASE or PAM was determined in two parallel groups of male TFW mice (20.0 ± 0.5). Group I (n = 20) received 425mg.kg^{-1} PAM s.c. in 50 µl of an oil mixture (peanut oil/benzyl benzoale: 50/50 v/v). Group II (n = 20) received 425mg.kg^{-1} BASE s.c. in the same oil. The plasma concentration time profiles were followed for four months, drug levels being determined by a previously described H.P.L.C. technique (Coleman et al 1984).

The urinary excretion of unchanged pyrimethamine, ^{14}C radioactivity excretion in faeces and urine, and also the mass fate of ^{14}C radioactivity were determined in a second set of two groups. These animals received either 425 mg.kg^{-1} s.c. PAM (Group A, n = 20) in the oil mixture incorporating a tracer dose of ^{14}C PAM (2.5µCi), or 425mg.kg^{-1}s.c. BASE (Group B) containing 1.5µCi of ^{14}C BASE. Urine and faeces were collected for 4 months, and at intervals of 4 weeks, 5 mice were sacrificed from both groups A and B and ^{14}C levels were determined in the various soft tissues. Unchanged pyrimethamine was determined by HPLC and ^{14}C radioactivity by liquid scintillation counting. Statistical evaluation was by one way analysis at variance and modified t-test (Wallenstein et al. 1980) accepting $p < 0.05$ as significant.

Following s.c. administration of BASE, maximum measured pyrimethamine plasma levels (12.75 ± 3.90µg.ml^{-1}) were attained within 24 hr, and were two-fold ($p < 0.05$) higher than those of PAM (6.51 ± 3.00µg.ml^{-1}) and caused severe toxicity. Drug levels then fell rapidly below the range of the minimum inhibitory concentration (M.I.C.) for P. berghei (100-200µg/ml) by 5 weeks. By contrast, no toxicity was observed in mice dosed with PAM, and plasma concentrations were sustained above the M.I.C. for 13 weeks post dose, the drug being detectable in plasma at four months. Overall, there was no significant difference between the $AUC_{(0-z)}$ of pyrimethamine following administration of PAM (AUC_{0-z} 42.11 ± 11.52µg. day.ml^{-1}) and BASE (AUC_{0-z} 40.73 ± 7.00µg. day. ml^{-1}). The rapid decline in drug plasma concentrations after BASE administration was reflected in the initial rapid mean daily rate of ^{14}C radioactivity excretion seen after ^{14}C BASE administration (2.64 ± 0.47% dose. day^{-1} over 4 weeks). Both BASE and [^{14}C] BASE studies suggest preparation exhaustion occurred at 7 weeks. However the combined urinary and faecal excretion of [^{14}C] radioactivity after s.c. [^{14}C] PAM was gradual and sustained, with a low mean rate which was maintained throughout the study. i.e. 1.21 ± 0.17% day^{-1} (4 weeks) 1.05 ± 0.13% day^{-1} (8 weeks), 0.84 ± 0.12% day^{-1} (12 weeks) and 0.64 ± 0.09% day^{-1} (16 weeks). These studies suggest that the PAM preparation is worthy of further long term evaluation.

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THE PHARMACOLOGICAL RESPONSE TO VITAMIN K_1 IN THE ANTICOAGULATED RAT

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Vitamin K_1 is an essential co-factor for the post-ribosomal γ-carboxylation of glutamyl residues in clotting factors II, VII, IX and X during which it is converted to the biologically inactive metabolite vitamin K_1 2,3-epoxide. Under normal circumstances the epoxide is converted back to vitamin K_1 by the enzyme vitamin K_1 epoxide reductase. The enzyme is thought to be inhibited in the presence of 4-hydroxycoumarin anticoagulants and thus clotting factor synthesis is blocked. Patients poisoned with coumarin anticoagulants are thought to require frequent pharmacological doses of vitamin K_1 to restore clotting factor synthesis (Barlow et al., 1982) and high plasma concentrations of vitamin K_1 are required to maintain clotting factor synthesis in anticoagulated rabbits (Park et al., 1984). The normal daily requirement of vitamin K_1 is, ca 1 μg kg^{-1} (Frick et al., 1967; Barkhan and Shearer, 1977). Therefore we have determined the relationship between hepatic and plasma concentrations of vitamin K_1 and the epoxide and the pharmacological response to the vitamin in anticoagulated rats.

Male Wistar rats (210-265g) were pretreated with either warfarin (W) (63 mg kg^{-1}) in 0.9% NaCl solution (1 ml kg^{-1}) or brodifacoum (B) (10 mg kg^{-1}) in polyethylene glycol 200 (1 mg kg^{-1}). 6h later, together with control animals, the rats were anaesthetised with urethane (14% solution : 1 ml $100g^{-1}$). Vitamin K_1 (1 mg kg^{-1}) was administered via the left jugular vein. Serial blood samples were taken via the right carotid artery for determination of prothrombin complex activity (P.C.A.) as a measurement of pharmacological response. Following collection of a blood sample for the determination of vitamin K_1 and the epoxide in plasma, the liver was blanched and removed. Vitamin K_1 and vitamin K_1 2,3-epoxide concentrations in plasma and liver were determined by HPLC.

At t = 0 those animals treated with W and B had P.C.A. of 20.5 ± 2.7% and 21.2 ± 4.8% respectively. After administration of vitamin K_1, P.C.A. was seen to increase steadily. At t = 3h P.C.A. was 47.9 ± 18.3% and 52.6 ± 17.9% for the W and B treated animals respectively and thereafter declined at a rate which indicated complete inhibition of clotting factor synthesis (Leck and Park 1980). Hepatic vitamin K_1 concentrations were significantly reduced ($p < 0.001$) by both W (2.95 ± 1.70 μg g^{-1}) and B (2.93 ± 1.18 μg g^{-1}) compared with controls (18.47 ± 1.08 μg g^{-1}); there was no difference between W and B treated animals. However vitamin K_1 2,3-epoxide concentrations were significantly ($p < 0.01$) raised by both W (4.43 ± 2.00 μg g^{-1}) and B (4.28 ± 1.75 μg g^{-1}) compared with controls (1.06 ± 0.48 μg g^{-1}); there was no significant difference between W and B treated animals. Similarly plasma concentrations of vitamin K_1 were significantly reduced ($p < 0.02$) by both W (185.3 ± 95.3 ng ml^{-1}) and B (193.7 ± 109.6 ng ml^{-1}) when compared to controls (813.3 ± 485.6 ng ml^{-1}). Vitamin K_1 2,3-epoxide was detected in the plasma of both W (215.3 ± 78.7 ng ml^{-1}) and B (262.8 ± 78.3 ng ml^{-1}) treated animals. In only one of the six control rats could vitamin K_1 2,3-epoxide be detected (122 ng ml^{-1}). Thus, it can be concluded, that the minimum hepatic concentration of vitamin K_1 required for clotting factor synthesis is of the order of 3 μg g^{-1}.

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METHYLTRANSFERASE INHIBITORS MAY INHIBIT HISTAMINE RELEASE BY ELEVATING CYCLIC AMP IN MAST CELLS AND BASOPHILS

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In recent years, evidence has been presented which indicates that phospholipid methylation plays an essential role in IgE-dependent stimulus-secretion coupling in mast cells and basophils (Ishizaka & Ishizaka, 1984; Morita et al, 1981). That the methylation inhibitor 3-deazaadenosine (DZA) in combination with homocysteine thiolactone (Hcy) is able to almost totally inhibit histamine release from these cells lends support to this hypothesis. However, some workers have been unable to detect increased phospholipid methylation in immunologically-activated rat mast cells (Boam et al, 1984; Moore et al, 1984). We have therefore sought an alternative explanation for the inhibitory action of these drugs on histamine release and present evidence that this drug combination elevates cyclic AMP in mast cells and basophils, a process associated with inhibition of histamine release.

In rat peritoneal mast cells purified to >85% by centrifugation through Percoll, incubation for 60 min with the combination of DZA (100 uM) and Hcy (100 μM) inhibited anti-IgE-induced histamine release by 65.2 ± 4.7%. In untreated cells, anti-IgE activation elevated cyclic AMP by 51.0 ± 8.0% above baseline levels of 0.96 ± 0.05 pmole/10^6 cells at 15 seconds after challenge. Incubation of cells for 60 min with the drugs increased basal cyclic AMP to 1.17 ± 0.06 pmole/10^6 cells, an increase of 21.8 ± 1.1%. In these cells, activation with anti-IgE increased cyclic AMP by 60.7 ± 4.5% over this new baseline. Similarly, in preparations of human leukocytes comprising 20% basophils, preincubation for 60 min with DZA (100 μM) and Hcy (100 μM) inhibited anti-IgE-induced histamine release by 41.0 ± 6.5%. Stimulation of untreated cells with anti-IgE elevated cyclic AMP by 77.3 ± 8.9% over basal levels of 6.1 ± 0.1 pmole/10^6 cells at 30 seconds following challenge. Preincubation for 60 min with the drugs increased basal cyclic AMP by 80.3 ± 4.7% to a new level of 11.0 ± 0.6 pmole/10^6 cells. In these cells, activation with anti-IgE increased cyclic AMP by 192 ± 55% over this new baseline. In one experiment with dispersed human lung cells comprising 33% mast cells, preincubation for 60 min with DZA (100uM) and Hcy (100 uM) inhibited anti-IgE-induced histamine release by 65.0%. Anti-IgE activation of untreated cells elevated cyclic AMP by 32.7% above basal levels of 0.41 pmole/10^6 cells. Preincubation for 60 min with the drugs increased basal cyclic AMP by 18.0% to a new level of 0.48 pmole/10^6 cells whilst anti-IgE activation elevated cyclic AMP by 37.3% above this new baseline.

These elevations of cyclic AMP by DZA and Hcy indicate that compounds previously assumed to inhibit histamine release by inhibition of methylation reactions may have an alternative or additional mode of action. Furthermore, our results do not confirm previous observations that phospholipid methylation inhibitors reduce the cyclic AMP response following immunological challenge.

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ON THE TRANSMITTER CHEMISTRY OF THE THALAMO-STRIATAL PATHWAY IN THE RAT

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The caudate-putamen complex (CP) of the rat is partially innervated by the parafascicular (Pf) and intralaminar thalamic nuclei (Veening et al, 1980). Whilst thalamo-striatal fibres are clearly excitatory in nature (Vandermaelen & Kitai, 1980), the identity of their transmitter(s) is unknown. Previously, however, we have shown that acetylcholine is an unlikely candidate (Barrington-Ward et al, 1984). In the present study, we explore the possibility that S(+)-aspartate (ASP) or S(+)-glutamate (GLU) may serve as transmitters in these fibres.

Under halothane anaesthesia (1.5% in O_2) and full asepsis, male Porton rats (180-220g) received bilateral stereotaxic injections of either the excitotoxin, sodium ibotenate (IBO; 2 µg/0.2 µl) or 0.15M saline vehicle (0.2 µl) into the Pf using the co-ordinates: bregma -3.4; L ± 1.0; V 6.0 (from cortex). One week later, animals (including a group of naive controls) were killed by cervical dislocation, the brain rapidly removed onto a chilled Petri-dish containing ice-cold Krebs' solution and the anterior CP rapidly dissected into medial (CP_M) and lateral (CP_L) segments which were then immediately frozen on dry ice. After thawing, blotting and weighing, each piece of tissue was homogenised in borate buffer containing a known amount of RS-homocysteic acid as internal standard. Tissue amino acid content was then estimated by reverse phase HPLC of pre-column amino acid/O-phthalaldehyde/2-mercaptoethanol derivatives. The remaining tissue block was taken for histological verification of the injection and lesion sites.

Table 1 illustrates the tissue content of ASP and GLU, in the various treatment groups. For clarity, data from only one hemisphere is presented and represents the mean and S.E.M. of 4-6 experiments.

Table 1. Selected amino acid content of CP_M and CP_L segments (µmol g^{-1})

	ASP	GLU	p
Naive CP_M	2.60 ± 0.40	10.34 ± 0.67	
Naive CP_L	2.53 ± 0.35	10.03 ± 0.47	
Saline CP_M	2.81 ± 0.42	8.69 ± 0.51	N.S.
Saline CP_L	2.71 ± 0.41	9.95 ± 0.64	N.S.
IBO CP_M	3.56 ± 0.45	9.56 ± 0.60	N.S.
IBO CP_L	2.84 ± 0.38	9.33 ± 1.10	N.S.

No significant differences in the concentrations of ASP, GLU (or any other common amino acid) were noted between any treatment group. Since much of the CP acidic amino acid content will be devoted to metabolism, an unknown dilution factor of the metabolic : transmitter compartments exists. Thus, it is not yet possible to discount the role of ASP and/or GLU in thalamo-striatal transmission. More detailed studies are under way.

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THE ANTINOCICEPTIVE EFFECT OF CHOLECYSTOKININ (26-33) IN THE MOUSE

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The sulphated C-terminal octapeptide of cholecystokinin (CCK 8) has been reported to exert antinociceptive effects in experimental animals (Zetler, 1980). However, a lack of effect of CCK8 in analgesia studies has also been noted (Tang et al, 1984). These conflicting results made further investigation seem worthwhile.

Male mice (CFLP strain) 20-25g were maintained on a 12h light-dark cycle and tested between the hours of 1000 and 1400 in the hot plate test (53-55°C), recording latency to licking of the the forepaws. Compounds were dissolved in artificial cerebrospinal fluid (ACSF) and administered directly into the cerebroventricular system, injections of 10µl being made perpendicularly through the lambda. The site of injection was verified in each animal post mortem and response latencies associated with 'off-target' injections discarded. Results were compared to ACSF injected controls in every experiment and differences analysed statistically using the Mann-Whitney 'U'-test (one tailed), the level of significance being set at $p < 0.05$.

Sulphated CCK 8 was shown to reproducibly increase hot plate latencies as compared to controls. The antinociception was evident at 15' but not 45' after injection and was dose related. In a typical experiment a control hotplate latency of 10.76 $\pm$ 0.66s was increased to 15.49 $\pm$ 2.96s by a 3µg dose of CCK-8 and further increased to 21.43 $\pm$ 2.65s by 30µg of CCK 8 (all values mean of n = 7 $\pm$ SEM). The antinociception produced by a given dose of CCK could be increased further ($p = 0.027$) by coadministration of the peptidase inhibitors bestatin (25µg), thiorphan (25µg) and captopril (25µg) although each inhibitor alone when coinjected with CCK 8 had little effect and the inhibitors themselves did not produce analgesia. A reduction of the analgesia produced by CCK 8 (3µg icv) could not be demonstrated by coadministration of the CCK antagonist proglumide (5µg icv). A similar finding has been reported for the antinociception produced by caerulein (Sheehan and De Belleroche, 1984) although Barbaz et al (1985) do claim to antagonise the actions of CCK 8 with proglumide. The lack of antagonist effect on CCK 8 induced antinociception is not wholly surprising since proglumide does not appear to bind to central CCK receptor sites (Clark et al, 1985)

The present experiments have shown CCK 8 to be antinociceptive in the mouse hot plate test when given by the icv route. The anti-nociception can be enhanced by peptidase inhibitors but is unaffected by CCK antagonists and its mechanism remains to be determined.

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A TACHYKININ ANTAGONIST INHIBITS THE SUBSTANCE P-INDUCED ACCUMULATION OF INOSITOL BISPHOSPHATE IN GUINEA-PIG SMALL INTESTINE

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Breakdown of membrane polyphosphoinositides has been suggested to provide the link between agonist-receptor interactions and the mobilisation of intracellular calcium in some smooth muscle systems (Berridge & Irvine, 1984). Substance P (SP) has been shown to enhance the accumulation of inositol mono-, bis-, and tris-phosphates (InsP, $InsP_2$, and $InsP_3$, respectively) in the guinea-pig small intestine, and this mechanism has been suggested to underly its contractile activity in this tissue (Watson & Downes, 1983; Holzer & Lippe, 1985). We have studied further the concentration-dependence of the effects of SP and the related tachykinin, kassinin, on inositol phosphate levels in this tissue at different time points. The tachykinin antagonist [D-Pro^4,D-Trp^{7,9,10}]-SP_{4-11} was employed also in an attempt to identify the receptor(s) mediating the inositol phosphate response with those responsible for contraction.

Briefly, longitudinal muscle strips (10-15 mg) from the small intestine of the guinea-pig were prelabelled (2 h) with [^{3}H]-inositol. The strips were washed for 30 min, with Li^+ (12 mM) present for the final 10 min and thereafter, to inhibit the dephosphorylation of InsP. Single strips were incubated with SP or kassinin for 30 s or 10 min, following which the inositol phosphates were extracted and separated as described previously (Berridge, 1983).

Ten min incubation with SP or kassinin (20 nM - 20 μM) resulted in concentration-dependent increases in the levels of both InsP and $InsP_2$, the maximum response being an approximate doubling in the levels of either metabolite, as compared with peptide-free controls. EC_{50} values were approximately 1 μM for SP and 100 nM for kassinin. In addition, higher concentrations of kassinin (>600 nM) caused a slight accumulation of $InsP_3$; this was not seen with SP, at concentrations up to 20 μM. After 30 s incubation with SP, however, $InsP_2$ levels were preferentially elevated; the maximum effect was a 50 % increase above basal at a concentration of 20 μM. Slight increases (up to 20 %) in InsP levels were also detected, but $InsP_3$ levels were again unchanged. With an agonist contact time of 30 s, the tachykinin antagonist [D-Pro^4,D-Trp^{7,9,10}]-SP_{4-11} (20 μM) produced a 10- and 100-fold shift of the SP- and kassinin-$InsP_2$ dose-response curve, respectively, consistent with its pA_2 values previously determined against their contractile effects on the ileum (Bailey et al., 1983). However, at longer agonist contact times (10 min), the antagonist was less potent (dose ratio ~5). By itself the antagonist produced no significant changes in the levels of any of the inositol phosphates.

The results suggest that the tachykinin receptors mediating the accumulation of inositol phosphates in the guinea-pig small intestine are identical with those receptors which are responsible for the contraction. The data also provide no further compelling evidence for a heterogeneity of the tachykinin receptors in this tissue.

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THE EFFECTS OF NITROPRUSSIDE ON CIRCULATING MET-ENKEPHALIN LEVELS IN DOGS

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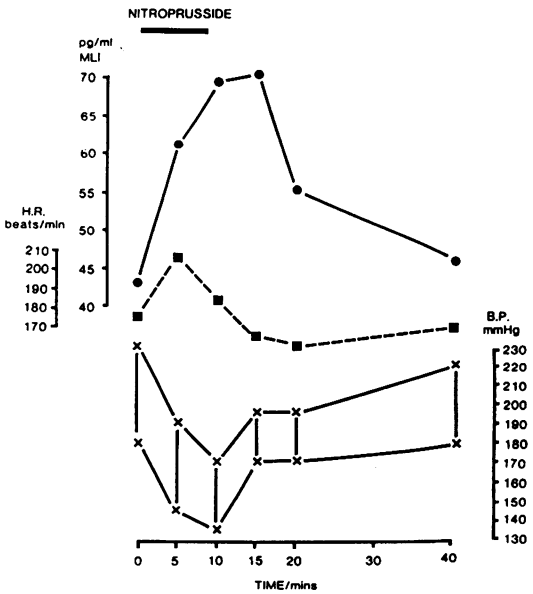
The endogenous opioid peptides may play a role in the control of cardiovascular function. Holaday (1983) and Evans, Hinds et al (1984) have shown that the cardiovascular collapse following endotoxin shock may be reversed by the administration of the opiate antagonist naloxone. Evans, Medbak et al (1984) have assayed the circulating met-enkephalin-like immunoreactivity (MLI) before and after endotoxin shock and have shown that the cardiovascular collapse was accompanied by a rise in MLI. We have examined the effects of hypotension induced by intravenous infusion of sodium nitroprusside on circulating MLI levels in five anaesthetized greyhounds. The MLI were measured using a sensitive radioimmunoassay (Clement Jones et al 1980). Blood pressure and heart rate were recorded. Fig. 1 shows one such experiment.



Infusion of 200 µg/min sodium nitroprusside i.v. for ten minutes in animals with a body weight of 25-35 Kg caused profound falls in blood pressure and raised the circulating MLI from 36 ± 6 pg/ml to 79 ± 11 pg/ml (mean ± S.E.M.; $P < 0.001$). Thus we have been able to show for the first time that a fall in blood pressure will provoke a release of MLI into the blood.

The authors gratefully acknowledge financial support from the MRC and technical help from Mr. R. Croxton.

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THE EFFECTS OF SUBSTANCE P ON ENDOGENOUS DOPAMINE RELEASE FROM RAT CORPUS STRIATUM IN VITRO

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Autoradiographic evidence indicates there are significant concentrations of Substance P (SP) receptors in the rat corpus striatum (Mantyh et al, 1984). SP has also been shown to release Met-enkephalin from striatum (Del Rio et al, 1980) and to alter striatal dopamine (DA) release in vitro (Starr, 1983). The present study further examines the role of SP in the modulation of DA release from rat corpus striatum.

Male C/D albino rats (200-300g) were killed by decapitation, their brains quickly removed and the striata dissected out. Blocks of striatal tissue (1 mm^{-3}) were incubated at 37°C in a modified Tyrodes solution (pH=7.4) which contained Hepes buffer (5 mM), pargyline (350 uM) and 3,4-dihydroxyphenylalanine (l-DOPA; 4 uM) and gassed continually with O_2. Three 15 min periods of incubation in normal media were collected before the tissues were exposed to media containing 20 mM K^+ for a single 15 min sampling period. This was followed by three further periods in normal media, a second high K^+ stimulus and two final periods in normal media. The DA concentrations in the incubation media samples were assayed directly by reverse phase HPLC with electrochemical detection (Mefford, 1980). The results indicate that during an experiment in vitro, there is stable basal secretion of endogenous DA and two repeatable periods of K^+-induced stimulation of release from the same tissue. Both the spontaneous and the K^+-induced DA release are Ca^{2+}-dependent. The effects of SP on DA release are determined by comparing basal and K^+-induced release during the first collection periods (1-5) with the subsequent sampling periods (6-10) following the addition of the peptide.

When SP (1.0-50.0 uM) was added to both the normal and the high K^+-containing media there was a dose-dependent increase in the basal release of DA and a decrease in the K^+-stimulated release. The opiate antagonist naloxone (20 uM), when added to the media had no effect on DA release on its own, but was able to reverse both of the SP-induced effects.

Table 1: Mean (± SEM) Change in DA Release as a Percentage (%) of the Control

		Basal	K^+-stimulated
(i)	Ca^{2+} Free (n=8)	-24.4 ± 5.3*	-110.3 ± 12.3*
(ii)	Naloxone (20 uM) (n=8)	-5.8 ± 7.1 n.s.	-23.6 ± 7.0 n.s.
(iii)	SP (10 uM) (n=8)	+21.0 ± 7.2*	-72.0 ± 10.0*
(iv)	SP (10 uM) + Naloxone (20 uM) (n=8)	+ 0.0 ± 5.0**	-30.5 ± 5.5**

*p<0.05; **p<0.01 (iii) vs. (iv)

These results suggest that SP is able to modulate striatal DA activity by releasing endogenous opiate peptides, possibly the enkephalins and β-endorphin, to act on different receptor types to increase basal secretion and to inhibit K^+-induced release.

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α_2-ADRENOCEPTOR MEDIATED INHIBITION BY β-PHENYLETHYLAMINE OF [^{3}H]-5-HT RELEASE FROM RAT HIPPOCAMPAL SLICES

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The locomotor stimulating properties of phenylethylamine (PEA) have been atributed, in addition to the dopaminergic component, to a noradrenaline (NA) releasing action (Mogilnicka and Braestrup, 1976). Direct stimulation by PEAs of serotonin (5-HT) receptors (Slotiver et al., 1980) or specific receptors for PEAs (Hauger et al., 1982) have also been suggested. The stimulation evoked release of ^{3}H-5-HT from rat hypothalamic or hippocampal slices is modulated by inhibitory α_2-adrenoceptors and 5-HT autoreceptors (Langer and Moret, 1982 ; Frankhuyzen and Mulder, 1982). We have used the electrically-evoked release of ^{3}H-5-HT from rat hippocampal slices as a model to explore the direct or indirect effects of β-PEA. The slices were labelled with ^{3}H-5-HT and perfused with Krebs medium. The release of ^{3}H-5-HT was elicited by two periods of electrical stimulation (S_1 and S_2) at 3 Hz, 24 mA for 2 min and was entirely calcium-dependent.

Table 1 : Effects of β-PEA on the electrically-evoked release of ^{3}H-5-HT

		S_2/S_1		
	μM	–	Idazoxan 1 μM	α-MpT (a)
Control	–	0.83 ± 0.03 (8)	0.99 ± 0.04 (9)	0.91 ± 0.10 (7)
β-PEA (S_2)	3	0.53 ± 0.03* (11)	0.90 ± 0.05 (9)	0.89 ± 0.13 (4)
β-PEA (S_2)	10	0.39 ± 0.06* (6)	0.56 ± 0.07* (7)	1.03 ± 0.15 (8)
6-F-NA (S_2)	0.1	0.34 ± 0.02* ( )	N.T.	0.34 ± 0.07* (4)

Deprenyl 1 μM was present in the medium throughout the experiment. Idazoxan 1 μM was added 20 min before S_1 and kept throughout. β-PEA or 6-F-NA were added 20 min before S_2. (a) α-methyl-p-tyrosine 300 mg/kg i.p., 2 h + 100 μM in the Krebs medium. * $p < 0.005$ vs the corresponding control. Values are mean ± S.E.M. from ( ) experiments per group. N.T. not tested.

When monoamine oxidase B was inhibited with deprenyl 1 μM, β-PEA inhibited the electrically-evoked release of ^{3}H-5-HT from rat hippocampal slices (Table 1). Idazoxan at 1 μM, a concentration which does not modify the release of ^{3}H-5-HT (data not shown), antagonized the inhibitory effect of β-PEA 3 μM (Table 1). Inhibition of tyrosine hydroxilase (TH) activity by α-MpT antagonized the inhibitory effects of β-PEA on ^{3}H-5-HT release (Table 1), but did not affect those elicited by 6-F-NA 0.1 μM (Table 1).

Blockade of α_2-adrenoceptors by idazoxan prevented the inhibitory effects of β-PEA, indicating the involvement of α_2-adrenoceptors in the inhibition by β-PEA of ^{3}H-5-HT release. The lack of effects of β-PEA on the electrically-evoked release of ^{3}H-5-HT after inhibition of TH activity by αMpT indicates that β-PEA acts indirectly on α_2-adrenoceptors through the release of newly synthesized NA.

The present data indicate that in rat hippocampal slices, β-PEA inhibits the electrically-evoked release of ^{3}H-5-HT through the release of NA and subsequent activation of α_2-adrenoceptors. Our results exclude a direct effect of β-PEA on inhibitory 5-HT receptors or on β-PEA recognition sites involved in the regulation of the release of ^{3}H-5-HT.

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NORADRENALINE TURNOVER: DRUG-INDUCED CHANGES EVALUATED USING A NEW HPLC-ECD ASSAY FOR FREE MHPG IN MOUSE BRAIN

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Considerable evidence exists to show that for noradrenaline (NA), reductive metabolism to 3,4-dihydroxyphenylethyleneglycol (DHPG) or, after O-methylation, to 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) are the major catabolic routes. In the rat, MHPG is predominantly conjugated as the O-sulphate and MHPG-SO_4 levels have been proposed as a useful index of cerebral NA turnover (Meek and Neff, 1973), reflecting release from presynaptic sites and thereby providing a measure of functional importance (Bareggi et al, 1978a, b; Glavin et al, 1983). The present work describes a novel reliable assay for free MHPG developed from previous HPLC-ECD methodology (Diggory and Buckett, 1984) by using a silica-gel material containing a phenyl function (Bond-Elut, Analytichem, U.S.A.) for prior sample clean-up. The method was applied to mouse brain tissue since previous work suggested MHPG is essentially unconjugated in this species (Caesar et al, 1974). The effects of various specific inhibitors of monoamine metabolism and of adrenergic agents on the mouse brain free MHPG content were evaluated to assess the use of the method for studying NA turnover. Pargyline (100 mg/kg i.p., 2h) and tranylcypromine (10 mg/kg, i.p., 2h) caused the complete disappearance of MHPG whereas deprenyl (10 mg/kg, i.p., 2h) had no effect. Probenecid (200 mg/kg, i.p.), reserpine (5 mg/kg, i.p.), piperoxan (20 mg/kg. i.p.), phenoxybenzamine (20 mg/kg, i.p.) and yohimbine (5 mg/kg, i.p.) all increased free MHPG by up to 126%, 2h after administration. In addition, a variety of antidepressant and neuroleptic drugs have been investigated acutely for their effects on MHPG levels, as presented in Table 1.

Table 1 Effects of various drugs on free MHPG content (ng/g) in mouse brain, 2h after administration.

Control	44.8 ± 2.1	Mianserin	(50 mg/kg, p.o.)	119.8 ± 14.4***
Control	49.2 ± 3.0	Dothiepin	(100 mg/kg, p.o.)	41.6 ± 0.7*
Control	41.5 ± 2.5	Desipramine	(50 mg/kg, p.o.)	32.9 ± 1.8*
Control	50.4 ± 2.4	Nomifensine	(20 mg/kg, p.o.)	29.2 ± 1.3***
Control	45.5 ± 1.6	Haloperidol	(3 mg/kg, i.p.)	130.8 ± 13.9***
Control	45.5 ± 1.6	Chlorpromazine	(3 mg/kg, i.p.)	97.1 ± 10.2***

*$p<0.05$, ***$p<0.001$ compared to control.

The 5-HT reuptake inhibitors citalopram and panuramine, together with amitriptyline and clomipramine had no effect on free MHPG levels. Clonidine significantly reduced MHPG dose-dependently (e.g. 0.1 mg/kg : 28.9 ± 1.6*** cf. control : 40.5 ± 2.0 ng/g). Prolonged administration of mianserin resulted in an attenuated elevation of MHPG levels observed acutely, indicating adaptive changes at the presynaptic α_2-sites. In summary, these results demonstrate that measurement of free MHPG in the mouse brain by HPLC-ECD provides a sensitive index of noradrenaline turnover reflecting changes in release and reuptake mechanisms observed after various drugs.

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ELECTRICAL STIMULATION OF RAT C1 NEURONES INCREASES BLOOD PRESSURE AND EXTRACELLULAR HYPOTHALAMIC ADRENALINE

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Evidence suggests a role for central monoaminergic systems in the regulation of blood pressure (Dampney et al 1980). The hypothalamus is innervated by adrenergic neurones (C1 group) in the rostral ventrolateral medulla (RVL), an area which also innervates the spinal cord. Using intracerebral dialysis (Sharp et al 1984) we have demonstrated that stimulation of the C1 cell bodies increases hypothalamic extracellular adrenaline and mean arterial pressure (MAP).

Male Wistar rats (270-300g) were anaesthetised with chloral hydrate (600mg/kg i.p.) and MAP was recorded from a cannulated femoral artery. Dialysis loops were implanted stereotaxically into the posterior hypothalamus. The dialysis loops, consisting of a folded dialysis tube (length 2.0mm, diameter 0.25mm) were perfused (0.7µl/min) with physiological saline. Samples were collected at 30 min intervals and the perfusate assayed for noradrenaline (NA), adrenaline (A), 3,4-dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5HIAA) using HPLC-ECD (carbon paste working electrode at +0.65V). Separation was performed on a Spherisorb 50DS2 column using 0.1M NaH_2PO_4 buffer pH3.6 containing 0.1mM EDTA, 1.0mM sodium octyl sulphate and 9% methanol. Modified concentric needle electrodes were implanted into the C1 region of the RVL. For control experiments the electrodes were implanted close to but outside the region. After a 120 min stabilisation period the C1 region was electrically stimulated for 30 mins (2v, 1msec rectangular pulses at 40Hz for 10s every 30s), and the effects monitored for a further 120 mins. Positions of the electrodes were verified histologically.

Table 1 shows estimated extracellular values (corrected for recovery) of A, NA, DOPAC and 5HIAA and the corresponding MAP levels before and during electrical stimulation of the C1 area.

	Extracellular Amine levels (nM) (mean+SEM)		MAP (mmHg) (mean+SEM)	
	Pre-Stim	During stim	Pre-Stim	During Stim
COMPOUND				
A	67±10	112±17*	73±6	121±14*
NA	79±20	80±21		
DOPAC	230±60	230±50		
5HIAA	3400±700	2100±300		

*P<0.05 n=6

Stimulation of the C1 region significantly increased extracellular A compared with pre-stimulation control levels while NA, DOPAC and 5HIAA remained unchanged. A corresponding rise in MAP was observed during C1 electrical stimulation. Stimulation of the control area produced no significant change in MAP or amine and metabolite levels. MAP and A levels returned to basal levels in the post-stimulation collection period. The rise in extracellular A level and MAP following electrical stimulation of the C1 region suggests a role for A in the hypothalamic regulation of blood pressure. Further experiments need to be made to differentiate the effects of A in the hypothalamus and in the spinal cord.

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α_1-ADRENOCEPTOR ANTAGONIST EFFECTS OF ALFUZOSIN IN RABBIT AND DOG LOWER URINARY TRACT

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Urinary retention associated with benign prostatic hypertrophy and other pathological conditions can be relieved by α-adrenoceptor antagonists (Abrahams et al., 1982; Hedland et al., 1983). In the present study, the effects of the selective α_1-adrenoceptor antagonist alfuzosin (Cavero et al., 1984a, b) were studied in rabbit and dog urinary bladder.

Male rabbits (3-4 kg) were sacrificed and strips (5x2 mm) of trigone muscle or rings of urethra (5 mm long), prepared according to the methods of Ueda et al (1984) were set up in Krebs' bicarbonate containing propranolol (1 μM). Concentration-contractile response curves to phenylephrine were determined before and after incubation with alfuzosin (0.3-3.0 μM) or prazosin (0.1-1.0 μM). pA_2 values were obtained by Schild analysis.
Female dogs (14-18 kg) were anaesthetised with pentobarbitone (35 mg/kg and 6.0 mg/kg/h i.v.). A catheter was introduced into the urethra and secured by a ligature around the bladder neck. Increases in intra-urethral pressure (cmH_2O) were evoked by electrical stimulation of the hypogastric nerve fibres (6-10 V, 4 ms, 5 Hz) performed before and after i.v. injection of alfuzosin (0.3 mg/kg).

The potency of phenylephrine as an agonist at α_1-adrenoceptors was similar in rabbit trigone muscle strips (pD_2 = 4.9 $\pm$ 0.07, n=26) and urethral rings (pD_2 = 5.2 $\pm$ 0.07, n=26). Alfuzosin was a competitive antagonist of phenylephrine-induced contractions in both the trigone (pA_2 = 7.05 $\pm$ 0.17; slope = 1.12) and urethra (pA_2 = 7.1 $\pm$ 0.23; slope = 1.15). The corresponding pA_2 values for prazosin in the trigone and urethra were 7.85 $\pm$ 0.24 and 7.96 $\pm$ 0.35, respectively. Stimulation of the postganglionic sympathetic fibres of the hypogastic nerve innervating the urethra evoked reproducible increases in urethral pressure. These effects were not influenced by propranolol (0.75 mg/kg i.v.), atropine (0.5 mg/kg i.v.) or chlorisondamine (0.5 mg/kg i.v.). Alfuzosin (0.3 mg/kg i.v.) caused a significant reduction (-38.6 $\pm$ 6.7%, n=4) in the base-line urethral pressure (13.7 $\pm$ 2 cmH_2O) and abolished the control urethral pressure increases ($\triangle$ 13.9 $\pm$ 3 cmH_2O) evoked by sympathetic nerve stimulation.

The results demonstrate that alfuzosin is a competitive antagonist of α_1-adrenoceptor-mediated contractions in the isolated rabbit urinary bladder and urethra preparations and can antagonise sympathetically mediated urethral tone in the anaesthetised dog. These results suggest that alfuzosin may be of value in the treatment of urinary retention dependent on increased sympathetic tone in the urethra.

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EFFECT OF TWO AMINO-PYRIDAZINE DERIVATIVES ON BOTH FORMS OF MONOAMINE OXIDASE IN RAT BRAIN

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In rat brain, endogenous amines serotonin (5-HT), noradrenaline (NA) and dopamine are preferentially deaminated by MAO-A whereas MAO-B prefers β-phenethylamine as substrate. We have investigated the in vitro and ex vivo effect of two amino-pyridazine derivatives minaprine (3-(2-morpholino-ethylamino) 4-methyl 6-phenyl pyridazine, 2HCl) and SR 95191 (3-(2-morpholino-ethylamino) 4-cyano 6-phenyl pyridazine, citrate) on MAO-A and B activities in rat brain. For comparison, two specific and reversible type A MAOI's moclobemide and cimoxatone were tested.

Male Sprague-Dawley rats (200-220 g, Charles River, France) were used. Drugs were administered i.p. (doses refer to the salt). Rat striatal MAO-A and B activities were estimated using [14C] 5-HT and [14C] PEA as substrate (Kan et al, 1980).

Table 1

Test Drug	MAO inhibition In Vitro (IC50's, M) 5-HT	PEA	Ex Vivo (ED50's, mg/kg, i.p.) 5-HT	PEA
MINA	$9\ 10^{-4}$	$3\ 10^{-3}$	12.8(10.4-15.9)[a]	> 30 (4 %)[b]
SR 95191	$7\ 10^{-6}$	$2.8\ 10^{-4}$	7.5(6.7- 8.5)	> 300 (32 %)
MCCL	$6\ 10^{-4}$	10^{-3}	0.9(0.6- 1.4)	100
CIMO	$2\ 10^{-8}$	$2\ 10^{-7}$	2.8(2.4- 3.3)	54.8(50.7-59.3)

MINA = minaprine ; MCCL = moclobemide ; CIMO = cimoxatone ; a = 95 % confidence limits ; b = % inhibition at this dose.

As shown in Tab. 1, minaprine behaved in vitro as a very weak and non specific inhibitor of MAO-A. However, ex vivo, this drug preferentially inhibited MAO-A with a mild potency. Both in vitro and ex vivo, SR 95191 was much more potent and selective than minaprine towards MAO-A. In comparison moclobemide, ex vivo, strongly inhibited MAO-A despite its lack of activity in vitro. Finally cimoxatone both in vitro and ex vivo, appeared as a potent and specific type A MAOI.

The time course of inhibition of MAO-A induced by equimolar doses of minaprine (20 mg/kg) and SR 95191 (27 mg/kg) indicated that the effect peaked 15 min after drug administration, then decreased until 6 h. For both drugs, MAO-A activity reached control values 24 h after treatment. In this time ranging MAO-B was only slightly inhibited. Pretreatment with minaprine (30 mg/kg) or SR 95191 (41 mg/kg) partially protected against the irreversible inhibition of MAO-A induced at 24 h by clorgyline (1 mg/kg). Such an effect was not observed when MAO-B was irreversibly inhibited by l-deprenil (1 mg/kg). These results suggest that minaprine and SR 95191 are specific and reversible type A MAOI's, minaprine being rather weak in this respect. However the discrepancy between in vitro and ex vivo results suggests that one (or several) metabolite(s) may be responsible for this effect.

Finally, pharmacological data indicate that minaprine and SR 95191 exhibit an atypical profile of antidepressant activity (Bizière et al., 1982 and this congress).

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REPERFUSION ARRHYTHMIAS AND TIME-RESPONSE RELATIONSHIPS: MODIFICATION BY DRUGS IN THE ANAESTHETISED RAT

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The duration of the period of ischaemia prior to reperfusion is one of the most important determinants of the vulnerability of tissue to reperfusion-induced arrhythmias. In animal studies a clear but complex relationship has been demonstrated between the duration of ischaemia and the incidence of reperfusion-induced ventricular fibrillation (RVF), with bell-shaped time-dependancy curves being observed in the dog, isolated rat heart, and in vivo anaesthetised rat (1). Peak vulnerability to RVF within these models occurrs after 25, 15, and 5 min periods of ischaemia, respectively. Various pharmacological interventions have been claimed to reduce the incidence of RVF (1), but it is unclear whether they actually reduce RVF per se, or merely delay the deleterious consequences of ischaemia and thereby alter the relationship between duration (or severity) of ischaemia and the incidence of arrhythmias upon reperfusion.

We have shown previously that slow calcium channel antagonism (2) and beta-blockade (3) may reduce RVF, and in the present study we have attempted to determine whether these interventions bring about an absolute reduction of arrhythmias or merely manipulate the time-response profile. In this study we used the anaesthetised rat with temporary coronary artery occlusion. The drugs were administered i.v. 10 min prior to occlusion.

Table 1. Effects of beta-blockade and calcium-channel antagonism upon the time-response profile of reperfusion-induced ventricular fibrillation.

	Incidence of ventricular fibrillation%				
Duration of Ischaemia prior to reperfusion (min)	3	5	7	10	20
Control	20	75	65	50	17
Nifedipine (5 ug/kg)	0	35*	20*	5**	0
Atenolol (1 mg/kg)	0	10**	33	33	0

n=12-20 in each group; **P≤0.01; *P≤0.05.

Table 1 shows that nifedipine (5 ug/kg) reduced the height of the time-dependancy curve for RVF at each time point; however the peak incidence of RVF still occurred after 5 min occlusion. By, contrast administration of atenolol (1 mg/kg) while also reducing the incidence of RVF after 5 min occlusion, additionally acted to shift the relationship to the right, so that the peak vulnerability to RVF now occurred after 7-10 min occlusion.

These results indicate that in the anaesthetised rat the slow calcium channel antagonist nifedipine can achieve an absolute reduction of RVF, with no effect on the time of peak vulnerability, whereas the beta-blocking agent atenolol both reduces and delays the time of peak vulnerability to RVF. These results suggest that the mechanisms by which these two distinct pharmacological approaches to the control of RVF operate, differ.

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LACK OF EFFECT OF N-2-CHLOROETHYL-N-2-BROMOBENZYLAMINE (DSP4) ON PERFORMANCE IN AN OPERANT DISCRIMINATION PROCEDURE

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The ascending noradrenergic pathway has been implicated in various aspects of operant behaviour, although its precise role remains controversial (see Robbins & Everitt, 1982). We have recently observed that systemic treatment with N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP4), a neurotoxin which produces a selective and irreversible depletion of noradrenaline in the neocortex, hippocampus and cerebellum (Jonsson et al, 1981), failed to affect the operant behaviour of rats in variable-interval schedules of positive reinforcement (Bradshaw et al, 1985). We report here the effect of DSP4 on an operant discrimination.

Eight female Wistar rats were randomly assigned to two groups. Group 1 (n=4) received intraperitoneal injections of DSP4 (two doses of 50 mg/kg, separated by an interval of seven days); Group 2 (n=4) received injections of distilled water. The rats were maintained at 80% of their free-feeding body weights, and were trained to press a lever in an operant conditioning chamber using 0.05 ml of 0.6 M sucrose as the reinforcer. In Phase I of the experiment (35 sessions), they were trained under a multiple variable-interval 60-sec variable-interval 60-sec schedule, the two components being associated with the presence and absence of three lights (6 W) on the front panel of the chamber. In Phase II (30 sessions), the component associated with the presence of the lights was changed to extinction (multiple variable-interval 60-sec extinction). In Phase III (35 sessions), the original schedule was reinstated. After the completion of Phase III, the rats were sacrificed and their brains assayed for concentrations of catecholamines using high-performance liquid chromatography with electrochemical detection.

During Phase I, there was no significant difference between the DSP4-treated and the untreated rats in terms of the steady-state response rates obtained in the two components (Student's t-test, $P > 0.1$ in each case). The acquisition of discrimination during Phase II was assessed by computing a 'discrimination ratio' (response rate during the extinction component divided by the overall response rate in both components) for each session. A two-factor analysis of variance (sessions, treatment group) showed that there was a progressive decrease in the discrimination ratio across sessions ($F(29,174) = 51.39$; $P < 0.001$), but no difference between the treated and untreated groups ($F(1,6) = 0.44$; $P > 0.1$); the interaction was not significant ($F(29,174) = 1.38$; $P > 0.1$). During Phase III there was no significant difference between the groups with respect to the rate at which the discrimination reversed ($F(1,6) = 0.43$; $P > 0.1$). The concentrations of noradrenaline in the parietal cortex, hippocampus and cerebellum in the DSP4-treated group were less than 10% of those of the untreated group (t-test, $P < 0.001$ in each case).

These results fail to provide support for a role of the ascending (caeruleo-cortical) noradrenergic pathway in operant discrimination.

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420P

EVIDENCE FOR A LACK OF MEMORY IMPAIRMENT IN SOCIALLY ISOLATED RATS

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Short term isolation of rats immediately after weaning results in a retardation of habituation of object contact in the open-field (Einon & Morgan, 1976; 1977). As habituation of exploration in a novel environment has been shown to be a valid test of memory in the rat (Lilley & Morinan, 1984), the effect of social isolation on this behaviour was used as an index of memory impairment.

Male Sprague Dawley rats (50-55g) were randomly assigned to either individual (ISOL) or grouped housing conditions (SOC) for 21 days (Morinan & Leonard, 1980). On day 22, each animal was given an acquisition trial (T1) in an elevated X-maze, followed 72 hours later by a retention trial (T2). The number of rears, arm entries and locomotor activity counts were recorded for the two 5 min trials (Lilley & Morinan, 1984).

Results for these behaviours and their product score (rears x entries x activity) were analyzed by a two-way ANOVA (mixed design), with any significant interactions between the main effects evaluated by the Tukey test for unconfounded means (Table 1).

Table 1 Effect of Social Isolation on Habituation of Exploration

	Rears		Arm Entries		Activity		Product Score ($\times 10^{-4}$)	
	T1	T2	T1	T2	T1	T2	T1	T2
SOC	12.3±1.4	11.6±1.2	8.3±0.6	5.9±0.6	589±34	487±45	6.4±1.2	4.3±1.1
ISOL	9.3±1.0	6.9±1.0	5.2±0.6	3.8±0.5	446±34	310±50	2.5±0.5	1.4±0.5

Each value represents the mean ±S.E.M. for 16 rats.

The ANOVA revealed a significant effect ($P<0.01$) of both isolation and trials on exploration. ISOL rats explored less than SOC controls, while exploration in both groups decreased from T1 to T2. The only significant ($P<0.01$) interaction occurred for the product score. This was due to a significant difference ($P<0.05$) between SOC and ISOL rats on T1 but not T2. As SOC animals initially showed a higher level of exploration, it would appear that habituation was faster than for ISOL rats.

In conclusion, evidence of memory impairment in ISOL rats is lacking, since there was no difference in the rate of habituation for three of the four parameters measured. The inhibited behaviour of ISOL rats in this novel environment provides further support for the concept of isolation-induced neophobia that has also been reported at this meeting (Morinan & Parker, 1985).

VP wishes to thank the BPS for a grant from the Bain Memorial Fund to attend this meeting.

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THE EFFECT OF THE PROSTAGLANDIN ENDOPEROXIDE ANALOGUE, U46619, ON GASTRIC MUCOSAL ULCERATION AND NON-PARIETAL SECRETION IN THE RAT

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The effects of the stable thromboxane A_2-mimetic, U46619 (11α, 9α - epoxymethano - prostaglandin H_2; Coleman et al, 1981), on ethanol-induced gastric mucosal ulceration and on gastric non-parietal secretion, and the way in which indomethacin treatment modifies the actions of U46619, have been examined in the rat.

In the conscious rat gastric mucosal ulceration was produced by oral dosing with 1ml of absolute ethanol; the gastric mucosa was examined and scored for lesion formation by planimetry after 1h. Dosing the rats orally with U46619 30min before the ethanol challenge inhibited lesion formation with an ED_{50} value of 0.24 (0.14-0.39, 95% confidence limits) μmol/kg, and a mean inhibition of 80% at the highest dose of U46619 used (2.8μmol/kg = 1mg/kg p.o.). This effect of U46619 was markedly diminished by pretreatment with indomethacin (14μmol/kg s.c.) such that U46619 (2.8μmol/kg p.o.) only inhibited lesion formation by 41%. For comparison, the ED_{50} values for PGE_2 against ethanol-induced ulceration in the absence and presence of indomethacin pretreatment were 0.06 (0.04-0.08) and 0.04 (0.02-0.06)μmol/kg p.o. respectively; these values were not significantly different.

Gastric non-parietal secretion was studied in pentobarbitone anaesthetised rats treated with atropine (3μmol/kg i.v.) to inhibit gastric acid secretion. The prostaglandins were applied intraluminally and secretion collected over a 1h period; results are expressed as mean ± s.e. mean, n = 5-7 throughout. In preliminary experiments a dose of PGE_2 of 0.3μmol/kg, the largest dose used, stimulated a secretory rate of 1.1 ± 0.2 ml/h with net ion outputs of Na^+ 152.1 ± 10.4, Cl^- 126.2 ± 8.7, K^+ 3.5 ± 0.6 and HCO_3^- 19.4 ± 4.5μEq/h. Since Na^+ is a major constituent of non-parietal juice but only a minor constituent of parietal juice this cation was used as the index of non-parietal secretion. Gastric Na^+ output under control conditions was 59.4 ± 3.8μEq/h, and pretreatment with indomethacin (14μmol/kg s.c.) inhibited this to 27.4 ± 6.1μEq/h ($P<0.05$). Since indomethacin inhibited basal Na^+ secretion, the responses to the prostaglandins were expressed as change in secretion above the corresponding mean basal value. In the absence of indomethacin, doses of U46619 of 0.3 and 3μmol/kg stimulated mean increases in sodium output of 54.8 ± 17.9 and 99.2 ± 21.1μEq/h respectively, but in indomethacin treated animals the corresponding increases in Na^+ output were 7.6 ± 3.8 and 5.6 ± 7.0μEq/h (in each case $P<0.05$). For comparison, doses of PGE_2 of 0.03, 0.1 and 0.3μmol/kg stimulated mean increases in Na^+ output of 27.9 ± 6.3, 53.1 ± 7.5 and 92.7 ± 10.4μEq/h respectively, and pretreatment with indomethacin did not significantly affect these responses.

In conclusion, we have confirmed the observation (Tao & Wilson, 1984) that U46619, a thromboxane A_2-mimetic, ameliorates gastric mucosal ulceration and stimulates non-parietal secretion in the rat, and have further shown that since these effects of U46619 were reduced by indomethacin pretreatment they may be indirect and mediated by endogenous prostaglandins. Thus, any release of endogenous thromboxane A_2 that may occur in the gastric mucosa will not necessarily predispose the tissue to ulceration as suggested by Whittle et al (1981).

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THE LEVELS OF PAF AND LYSO-PAF IN THE JOINT FLUIDS OF RABBITS WITH ANTIGEN-INDUCED ARTHRITIS

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Platelet activating factor (PAF) has been proposed as an important inflammatory mediator because it stimulates neutrophil chemotaxis and degranulation (Roubin et al., 1983). PAF also increases vascular permeability directly. The activation of phospholipase A_2 results in the formation of 1-O-alkyl-SN-glyceryl-3-phosphorylcholine (lyso-PAF) which can be enzymatically acetylated to 1-O-alkyl-2-O-acetyl-SN-glyceryl-3-phosphorylcholine (PAF) which in turn can be metabolised to lyso-PAF by an acetyl hydrolase (Roubin et al., 1983). In this study, we have measured the concentration of PAF and lyso-PAF in inflammatory exudates induced in an experimental model of chronic arthritis.

New Zealand white rabbits were sensitized to ovalbumin and challenged by injection of antigen into one knee joint (Dumonde & Glynn, 1962). The contralateral joint received the same volume of saline. The animals were killed 4h - 35 days after challenge and joint fluids were collected from both knees by washing the joint space with 1 ml saline containing 0.25% bovine serum albumin (BSA). The joint wash was centrifuged and the supernatant was precipitated with cold acetone (-20°C) and extracted with chloroform (Parente & Flower, 1985). At this stage, one half of the extract was dried down and assayed for PAF activity and the other half dried down, redissolved in 0.1 ml dry pyridine, acetylated with 0.1 ml acetic anhydride and then assayed for PAF activity. This gives a measure of PAF and lysoPAF activity, respectively. PAF activity was assayed by measuring the effect of extracts on aggregation of rabbit platelets treated with indomethacin and ADP scavengers (Vargaftig et al., 1981). In some experiments extracts were also subjected to thin layer chromatography using the solvent system, chloroform/methanol/water. Material which co-migrated with synthetic PAF was assayed for platelet aggregating activity.

The efficiency of extraction of synthetic PAF from saline containing 0.25% BSA was 92.8% ± 3.9 (mean ± S.E. mean; n=5). Extracts of arthritic or control joint washes collected 4h - 35 days after challenge did not contain any detectable PAF (< 0.10 ng/ml). However, an extract of pooled washes from 3 arthritic joints one day after challenge contained 0.34 ng PAF-like activity (equivalent to 112.5 pg/ml of joint wash). There was no activity in the pooled washes from control joints. All joint washes contained lyso-PAF. The mean concentration of lyso-PAF in control joint washes did not exceed 3.3 ng/ml at any time, whereas lyso-PAF in arthritic joint washes increased from 8.5 ng/ml ± 2.8 (n=3) at 4h to 17.9 ng/ml ± 1.7 (n=3) at 1 day. After 7 days lyso-PAF had not significantly decreased (16.8 ng/ml ± 2.0; n=6) but from days 14-35 concentrations fell to less than 5 ng/ml.

The low but biologically active level of PAF at day 1 supports the view that PAF is a mediator of acute inflammation. It remains to be determined what proportion of the lyso-PAF present is a precursor or a breakdown product.

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OVALBUMIN-INDUCED EICOSANOID RELEASE IN GUINEA-PIG ISOLATED LUNGS PERFUSED VIA THE PULMONARY ARTERY AND VIA THE TRACHEA

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The profile of eicosanoid release from the guinea-pig isolated lung is dependent on the stimulus employed. This could be due to the different sites of action of the various stimuli or to a different mechanism of release (Bakhle et al., 1985). We have now investigated whether the same stimulus (ovalbumin) releases a different profile of eicosanoids when given to guinea-pig isolated lung perfused via the pulmonary artery or via the trachea.

Male guinea-pigs (250-300g) were sensitised with ovalbumin (50mg i.p. and 50mg s.c.) and used 17-21 days later. The guinea-pigs were anaesthetised (Sagatal 60mg/kg), the thorax opened and the heart injected with 1000 U of heparin. The pulmonary artery was cannulated and the lungs perfused for 5 min with 25ml of heparinised Krebs' solution (10 U/ml). The trachea was cannulated and the lungs removed and placed in a heated chamber. The lungs were inflated with 20ml of air and the pulmonary artery perfused with oxygenated (95%O_2-5%CO_2) and warmed (37°C) Krebs' solution at a flow rate of 5ml/min. The lungs were left to stabilise for 30 min and then challenged with an infusion of ovalbumin (100ng/ml) for 5 min. The lung effluent was collected and analysed by radioimmunoassay (without prior extraction or purification) for TXB_2, 6-oxo-$PGF_{1\alpha}$, PGE_2, LTB_4 and LTC_4.

For perfusion via the trachea, the lungs were prepared as described above and were left to stabilise for 10 min. The pulmonary artery cannula was removed and the cannula in the trachea was connected to the Krebs' flow. The lungs were then allowed to stabilise for a further 20 min and challenged with ovalbumin (100ng/ml) infused in the Krebs' flow.The experiments lasted no more than 40 min. The challenge with ovalbumin was always performed via the perfusion system and each pair of lungs was used for one challenge with ovalbumin only. In the lungs challenged via the pulmonary artery the levels of eicosanoids after challenge were as follows: TXB_2 - 78.1±20.2 ng/ml, PGE_2 - 1.8±0.4 ng/ml, 6-oxo-PGF_1 -10.5±2.2 ng/ml, LTB_4 - 0.2±0.1 ng/ml and LTC_4 - 1.2±0.2ng/ml, (n=5). In the lungs perfused via the trachea the levels were TXB_2 - 201.8±18.1 ng/ml, PGE_2 -7.5 ±1.2 ng/ml, 6-oxo-$PGF_{1\alpha}$ -45.3±6.5 ng/ml, LTB_4 - 2.2±0.4 ng/ml and LTC_4 -9.7±1.5 ng/ml (n=5).

Although the release of immunoreactive eicosanoids was always larger when the challenge was applied through the trachea, our results show that in the lungs perfused via the trachea there was a greater increase in the levels of leukotrienes (10-fold) than in the levels of cyclooxygenase products measured (3-fold). This preferential increase in leukotriene release suggests that ovalbumin when infused via the trachea has an easier access to the population of cells responsible for leukotriene synthesis. The cyclooxygenase inhibitor indomethacin (5.6 µM) was infused into the Krebs' stream for 20 min before challenging the lungs with ovalbumin. In the lungs perfused via the trachea indomethacin blocked TXB_2 release completely and enhanced leukotriene release (1.5±0.3 and 11.1±2.5 ng/ml compared to 7.3±1.3 and 24.8±3.6 ng/ml after indomethacin treatment, for LTB_4 and LTC_4 respectively, n=5). Indomethacin when infused via the pulmonary artery also blocked TXB_2 and enhanced LTB_4 and LTC_4 release.

Our present results suggest that perfusing the guinea-pig isolated lung via the trachea is a better model for analysing lipoxygenase activity in anaphylaxis, since this route of challenge leads to a a greater release of leukotrienes, allowing a better assesment of drug actions.

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RAT ACTIVE PERITONEAL ANAPHYLAXIS

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An immediate hypersensitivity reaction in an asthmatic can result in an early phase of respiratory embarrasment which is thought to be due to the release of mediators such as histamine and the leukotrienes. Four to six hours later there is a delayed response in some patients and the mediators involved in this response have yet to be identified. The reaction has been shown to be associated with a cellular infiltration but there is no evidence that this is causal.

Rat peritoneal anaphylaxis provides a model in which it is possible to measure the mediators released and the pathology following an antibody-antigen reaction. At the same time it is possible to measure changes in the number of leucocytes in the peritoneal cavity and in the blood (Sharpe et al, 1979). The rats used had a blood eosinophilia induced by sephadex (Lemanske and Kaliner, 1982). The effect of drugs administered prior to antigen were determined on the various stages of the reaction.

Intraperitoneal antigen challenge of actively sensitised rats produced an increase in the concentration of histamine, slow-reacting substance of anaphylaxis (SRS-A) and dye labelled plasma proteins in the peritoneal washings 5 minutes after antigen challenge, (Sharpe et al, 1979). At this time there was a decrease in cells in the peritoneal washings, (with mean values, from 5.5 to 2.7 x 10^6ml $p<0.01$), with no change in volumes collected. After 4 hours there was an increase in neutrophils both in the blood (from 4.1 to 7.7 x 10^6/ml $p<0.01$) and peritoneal washings (from 0.006 to 5.5 x 10^6/ml $p<0.01$) and a fall in blood eosinophils (from 0.43 to 0.16 x 10^6/ml $p<0.001$). After 24 hours there was an increase in the peritoneal washings of numbers of eosinophils (from 1.7 to 3.3 x 10^6 /ml $p<0.001$ with no change in volume), and mononuclear cells (from 4.7 to 7.8 x 10^6/ml $p<0.001$).

The 5'-lipoxygenase inhibitor Phenidone, at 100mg/kg p.o., inhibited SRS-A release to control levels but had no effect on subsequent cellular events. Dexamethasone, at doses of 0.1 and 1mg/kg p.o., produced little inhibition of SRS-A release but inhibited neutrophil infiltration (72.8% and 69.1% $p<0.05$ and <0.01) with complete inhibition of eosinophil and mononuclear infiltration. These results suggest that lipoxygenase products are not the prime mediators for the cellular events which occur in this reaction.

Isoprenaline at 0.05 and 0.2mg/kg s.c. inhibited extravasation to control levels with no effect on histamine release but only the highest dose inhibited neutrophil infiltration (78.1% $p<0.001$) with complete inhibition of eosinophil infiltration.

Aminophylline at 25 and 50mg/kg had no effect on the immediate reaction but the higher dose inhibited neutrophil infiltration at 4 hours (50.3% $p<0.05$). Disodium cromoglycate at 100mg/kg inhibited histamine release and extravasation (to within 20% of negative control levels $p<0.01$), with a partial non-significant inhibition of SRS-A release 1mg/kg s.c., but with no significant effects on cellular infiltration after 5 minutes. Cyproheptadine at 1mg/kg s.c. inhibited extravasation ($>50\%$ $p<0.05$) but had no effect on the cellular events. It appears therefore that, in this system, factors other than those derived from the mast cell are responsible for the subsequent cellular changes occurring after the antibody-antigen reaction.

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EFFECT OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID) ON THE ADHESION OF HUMAN LEUCOCYTES TO CULTURED ENDOTHELIUM

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Endothelial cells have a unique position in being the ultimate barrier between blood and tissue. They are thus ideally situated to control the migration of leucocytes from the vasculature into an inflammatory site. Since leucocyte adhesion to endothelium (margination) is an essential cellular component of inflammation we have investigated the action of five NSAID (diclofenac, indomethacin, piroxicam, BW755C & benoxaprofen) on the adhesion of human polymorphonuclear cells (PMN) and monocytes to cultured endothelium using a modified quantitative monolayer adhesion assay (de Bono 1974).

The assay consisted of coculturing one million PMN or monocytes with confluent layers of porcine aortic endothelial cells grown on glass coverslips. After 60 mins incubation at 37°C, each coverslip was removed and washed five times with phosphate-buffered saline to remove the non-adherent cells, fixed in propan-2-ol and stained with haematoxylin. Leucocyte adhesion was expressed as the number of cells/9 high power fields. All experiments were performed in triplicate.

Pretreatment of either PMN or cultured endothelium with diclofenac, piroxicam, indomethacin & BW755C did not modify the number of adherent PMN. In 4/6 experiments 5 & 50 μg/ml benoxaprofen produced a mean 33% inhibition of PMN adhesion, when the endothelial cells had been preincubated with the drug for at least 6 hrs. Both indomethacin and benoxaprofen produced a dose-related inhibition of monocyte adhesion, following either a 2 or 24 hr incubation of the monocytes with the drugs. At 0.05 & 50 μg/ml benoxaprofen induced a mean 33% & 83% inhibition of adhesion respectively (n = 6), whilst indomethacin at 0.1 & 10 μg/ml induced a 24% & 61% inhibition respectively (n = 5). Diclofenac, piroxicam and BW755C had no significant effect on monocyte-endothelial cell adherence. The inhibition of monocyte adhesion to endothelial cells by benoxaprofen and indomethacin was due to the direct action of these drugs on the monocyte. This is in contrast to the inhibition of PMN endothelial cell adherence by benoxaprofen, where abrogation was only manifest when the endothelium had been preincubated with the drug.

The failure of most of the NSAID tested to abrogate the adhesion of PMN or monocytes to cultured endothelium, probably reflects their limited clinical effectiveness. As leucocyte margination is a necessary prerequisite to the diapedesis of these cells into an inflammatory lesion, we believe that the leucocyte-endothelial cell adherence assay will prove to be a valuable screening test for agents active in the pharmacological regulation of inflammation.

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EFFECTS OF FORSKOLIN, AN ADENYLATE CYCLASE ACTIVATOR, ON GASTRIC ACID SECRETION IN THE RAT, IN VIVO AND IN VITRO

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The role of cAMP in the mechanism of stimulus-secretion coupling in the parietal cell is still the subject of debate. A new tool which could serve to elucidate further the importance of cAMP is forskolin. This is a plant derived diterpene which is a potent activator of membrane bound adenylate cyclase. In addition to many other actions, forskolin will stimulate gastric acid secretion in vitro (Hersey et al 1983). The aim of this study was to characterise the acid secretory effects of forskolin in the rat, both in vivo and in vitro.

Acid secretion was studied in vivo in the lumen-perfused, anaesthetised rat (Main and Whittle, 1973). In addition ^{14}C-aniline clearance was measured as an indication of gastric mucosal blood flow. Forskolin has a profound hypotensive effect and the influence of this on the acid secretory response was of interest. Bolus doses of forskolin (80-330 μg/kg iv) elicited acid secretory responses, but failed to show dose-dependency. The duration of the fall in blood pressure did show a dose-dependency. IV infusions of 3.8 and 7.7 μg/kg/min (each for a period of 75 minutes, with a 75 minute recovery period in between) increased acid secretion from 3.1 $\pm$ 0.57 to 5.2 $\pm$ 0.88 μequiv/hr (n = 7) for the low dose, and 2.7 $\pm$ 0.65 to 19.2 $\pm$ 7.36 μequiv/hr for the high dose, indicating a steep dose-response relationship. With the lower dose of forskolin, arterial blood pressure was reduced but there was no change in clearance. With the high dose, clearance increased to 270% of basal, although the ratio of clearance to acid secretion decreased.

In vitro studies were carried out on the rat isolated mucosal preparation of Main and Pearce (1978). Forskolin added to the serosal bathing solution stimulated responses which were, like those to dibutyryl cyclic AMP (dbcAMP), well maintained and readily reversible, allowing sequential or cumulative dose-response curves to be constructed. An EC_{50} of approximately 2 μM was obtained similar to values of 0.4-1 μM for aminopyrine accumulation in rabbit gastric glands (Chew 1983) and parietal cells (Takahashi et al 1983). Forskolin was 20 and 100 times more potent than histamine and dbcAMP respectively.

We conclude that forskolin is a potent stimulator of acid secretion in the rat, both in vivo and in vitro. The direct effect of forskolin on secretory cells may be modified in vivo by its other actions, including those demonstrated on the cardiovascular system.

G.W. is an M.R.C. scholar.

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INHIBITION OF LEUKOTRIENE C_4 AND B_4 GENERATION BY HUMAN EOSINOPHILS AND NEUTROPHILS

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The biological activities of LTC_4 in man include bronchoconstriction, with specific effects on peripheral airways, promotion of changes in vascular permeability and tone and enhancement of mucus secretion. LTB_4 is a potent chemotactic agent for both neutrophils and eosinophils.

We have recently identified eosinophils as a major source of LTC_4 (Shaw et al, 1984). The quantities of leukotriene produced are potentially physiologically active, pointing to possible modes of action for this cell type in the pathogenesis of allergic disease and other conditions characterised by an eosinophilia. We have used eosinophil/neutrophil cell mixtures, in which eosinophils predominate, to study the pharmacological modulation of both LTC_4 and LTB_4 production via the 5-lipoxygenase pathway.

Eosinophils from patients with an eosinophilia of greater than 10% were purified on metrizamide gradients ($\geq$75% purity, 10^6 cells). The balance of the cell population was comprised of neutrophils. When stimulated by the calcium ionophore A23187 (5×10^{-6} M) eosinophils produced the equivalent of 55.5 ± 4.4 pmol LTC_4 per 10^6 cells, and neutrophils 117 ± 19 pmol LTB_4. Eosinophils also produced LTC_4 when incubated with immunoglobulin G (IgG) covalently coupled to agarose beads, but in significantly smaller amounts (c. 5 ng/10^6 cells). Both LTC_4 and LTB_4 were assayed in a double antibody radioimmunoassay.

The prostacyclin analogue, 6,9-deepoxy-6,9-phenylimino-$\Delta^{6,8}$-prostaglandin I (U-60,257), and 3-amino-1-(3-trifluoromethyl phenyl)-2-pyrazole (BW755C) inhibited both A23187 and IgG-agarose bead stimulated production of LTC_4 and LTB_4 by eosinophil/neutrophil mixtures containing 84 ± 2.4% (n = 3) eosinophils. The ID_{50} values for U-60,257 and BW755C were 2×10^{-6} and 5×10^{-6} M respectively.

Lipoxygenase inhibitors may be useful in influencing the pathophysiological events associated with the asthmatic response, particularly through their effects on eosinophils which are frequently associated with disease.

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DIETARY OIL MODIFIES DUODENAL AND GASTRIC PROSTAGLANDIN SYNTHESIS IN THE RAT

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Dietary polyunsaturated fatty acid (PUFA) profiles are reflected in many body tissues, thereby affecting prostaglandin (PG) synthesis (Galli, 1980). The effects of such dietary manipulation, in the gastrointestinal tract, where PGs have important actions (Wilson, 1981), are unknown.

The aims of this study were to examine the effects of dietary supplementation with coconut oil (CNO), corn oil (CO), fish oil (FO), and evening primrose oil (EPO) on PUFA composition and PG synthesis, in rat stomach and duodenum.

Male Wistar rats were fed either a standard diet (SD) or the same diet supplemented with one of the oils (80g oil/kg diet). After 14 days the rats were sacrificed, and specimens of duodenal mucosa, stomach and liver taken for PUFA and PG assay. Fatty acids were extracted with chloroform/methanol and quantified by gas liquid chromatography. After incubation of tissue for one hour, at 37°C, synthesis of PGE2, PGF2a, 6oxoPGF1a and TXB2 was determined by radioimmunoassay.

	PGE2	PGF2a	6oxoPGF1a	TXB2
DUODENUM				
SD (n=7)	12.6 ± 7.8	25.7 ± 15.0	12.5 ± 7.5	11.3 ± 5.9
CNO (n=6)	10.9 ± 3.4	25.1 ± 10.3	11.1 ± 2.0	9.8 ± 5.5
FO (n=6)	2.5 ± 1.6	6.3 ± 2.6	3.3 ± 1.6	2.5 ± 1.5
CO (n=6)	7.8 ± 2.1	17.4 ± 6.4	7.9 ± 3.1	5.9 ± 3.3
EPO (n=6)	8.5 ± 1.7	18.8 ± 3.7	8.9 ± 4.5	5.4 ± 3.6
STOMACH				
SD (n=5)	2.8 ± 0.6	8.6 ± 4.3	25.5 ± 11.0	1.6 ± 0.9
CNO (n=6)	3.8 ± 2.4	6.7 ± 2.1	21.9 ± 3.8	1.4 ± 0.7
FO (n=6)	1.3 ± 0.8	3.5 ± 1.4	17.5 ± 8.8	0.7 ± 0.2
CO (n=6)	4.9 ± 3.1	9.8 ± 4.3	29.8 ± 5.6	1.7 ± 1.1
EPO (n=6)	5.4 ± 3.9	10.3 ± 3.2	35.1 ± 10.4	2.1 ± 0.9

all given in ng/mg protein as mean ± standard deviation

In FO rats there was a significant reduction of C20:4 with increased C20:5, and significant inhibition of synthesis of all measured PGs, in both stomach and duodenum ($p<0.05$, Student's t-Test). EPO increased tissue C18:2 and C20:4, but only synthesis of 6oxoPGF1a in stomach was significantly increased ($p<0.05$). Neither tissue C20:4 nor prostaglandin synthesis were signicantly altered after the CO diet. In duodenum more PGF2a than other PGs was synthesised, but in stomach, 6oxoPGF1a appeared to be dominant.

Dietary PUFA does modify fatty acid composition in the mucosa of the upper gastrointestinal tract, with associated effects on PG synthesis. The possible correlation of these changes with disease requires further evaluation.

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DO ENDOGENOUS PROSTAGLANDIN E_2 AND THROMBOXANE A_2 MODULATE THE MITOGEN-INDUCED PROLIFERATION OF PERIPHERAL BLOOD LYMPHOCYTES?

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Mitogens such as phytohaemagglutinin (PHA) stimulate human peripheral blood lymphocytes to proliferate. The role of exogenous and endogenous eicosanoids in modulating this response to mitogens has been investigated by using the relevant compound or adding selective blocking drugs; however, using the following methodology highly variable and conflicting results have been published.

The majority of investigators have cultured peripheral blood mononuclear cells (PBMNC) for 48h in the presence or absence of a mitogen with or without the addition of a test substance. Cell proliferation was measured by adding ^{3}H-thymidine from 48-72h and assessing its cellular uptake (Akbar & Jones, 1984). We additionally measured PGE_2 and TxA_2 synthesis (Hillier et al, 1985) with an added chromatography step.

PGE_2, when added at the same time as maximal stimulating concentrations of PHA (5μg/ml), concentration-dependently inhibited proliferation; IC_{50} approx. 50nM. However, if addition of PGE_2 was delayed it was much less effective. A 50% inhibitory concentration of PGE_2 caused only 20% inhibition when added 4h and less than 10% inhibition when added 16h after PHA. We reasoned that endogenously synthesised PGE_2 would also need to be present in effective concentrations in the early part of the incubation period if it was to affect proliferation substantially. We examined this in a time course study measuring PGE_2 and TxA_2 synthesis in the presence of PHA and PHA plus 1μM UK37248 (a TxA_2 synthesis inhibitor which stimulates PGE_2 synthesis). PGE_2 concentrations were (with PHA alone in brackets) 0h, 0.3 ± 0.3 (0); 4h, 9.5 ± 2.5 (2.0 ± 0.8); 16h, 55.7 ± 20.4 (7.5 ± 3.6); 30h 99.7 ± 18.8 (21.2 ± 5.5); 48h, 113.1 ± 26.1 (34.4 ± 10.7). Values are nM ± SE (n = 4-8). TxA_2 synthesis was abolished with UK37248. The accumulation of PGE_2 is relatively low in the first 4h. The high levels of endogenously produced PGE_2 present at 16h but not at 4h following UK37248 administration cannot, therefore, be expected to influence proliferation, and this was confirmed with concentrations up to 100μM. U46619 (a TxA_2 receptor stimulant) inhibited proliferation (IC_{50} approx. 10μg/ml). EP045 (a TxA_2 receptor antagonist) was without effect on PHA-induced proliferation at concentrations of 100nM-10μM.

We conclude that the period of sensitivity to inhibition by PGE_2 is limited following proliferative stimuli, yet substantial endogenous accumulation does not occur until after this period of maximum sensitivity has passed. Drugs added to influence endogenous PG production are unlikely to affect proliferation unless the time course of PGE_2 synthesis is altered to effect substantial changes within the early phase of the culture period. The time course of synthesis and sensitivity needs to be evaluated for all eicosanoids.

We appreciate gifts of U46619, UK37248 and EP045 from Upjohn, Pfizer and Dr. R. Jones, University of Edinburgh respectively.

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2-AMINOTHIAZOLINE-4-ACETIC ACID - A NOVEL, SPECIFIC $GABA_A$ RECEPTOR AGONIST

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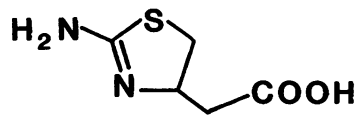

2-aminothiazoline-4-acetic acid

Because of its structural similarity to the inhibitory neurotransmitter γ-aminobutyric acid (GABA), 2-aminothiazoline-4-acetic acid (ATAA)[2] has been examined in several tests of central GABA-ergic activity.

The effects of ATAA on the binding of [^{3}H]-GABA to either $GABA_A$ or $GABA_B$ receptors were investigated in homogenates of rat cerebral cortex according to the method of Bowery et al (1983). ATAA induced a dose-dependent inhibition of [^{3}H]-GABA binding to $GABA_A$ receptors, possessing an IC_{50} value (concentration producing 50% inhibition of specific binding) of 0.5 ± 0.15 μM (n=6). For comparison, IC_{50} values for GABA and the selective $GABA_A$ agonists THIP (4,5,6,7-tetrahydroisoxazolo 5,4-c pyridin-3-ol) and isoguvacine were 0.1 μM, 1 μM and 0.49 μM, respectively. In contrast, ATAA was essentially inactive at displacing [^{3}H]-GABA binding to $GABA_B$ sites (IC_{50} value 700 μM). Likewise, ATAA was only a very weak inhibitor of [^{3}H]-GABA uptake into rat cortical synaptosomes (IC_{50} value 680 μM). In keeping with the ability of $GABA_A$ receptor agonists to enhance [^{3}H]-benzodiazepine binding (Wong and Iversen, 1985), 100 μM ATAA induced a maximal 142 ± 20% (n=3) stimulation of [^{3}H]-diazepam binding to rat cortical membranes at 23°c, exhibiting an EC_{50} value (concentration inducing 50% of maximum enhancement) of 3.4 ± 0.82 μM (n=3). These data compare with a maximal stimulation of binding of 156 ± 15% (n=4) and an EC_{50} of 3.1 ± 0.65 μM (n=4) for the $GABA_A$ agonist isoguvacine.

In electrophysiological experiments, ATAA depressed the CA1 population spike in the rat hippocampal slice (Kemp et al, 1984) in a dose-related manner with a mean EC_{50} value of 28.3 ± 4.1 μM (n=11) compared to EC_{50} values for isoguvacine and THIP of 13 ± 1 μM (n=26) and 55 ± 10 μM (n=7), respectively. The specific $GABA_A$ receptor antagonist bicuculline methochloride (10-100 μM) induced parallel shifts to the right of the ATAA dose-response curve, giving rise to a Schild plot with a slope of 0.96 and pA_2 value of 6.19. Similar values have been obtained previously using the $GABA_A$ agonists muscimol and isoguvacine (Kemp et al, 1984).

In summary, these results indicate that 2-aminothiazoline-4-acetic acid is a new and selective $GABA_A$ receptor agonist of comparable potency to THIP and isoguvacine.

[2]Synthesized by MMC while on sabbatical at the laboratories of Pfizer Inc., Groton, Connecticut, USA.

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INTERACTIONS OF DIHYDROAVERMECTIN B_{1A}, GABA AND IBOTENIC ACID ON LOCUST (SCHISTOCERCA GREGARIA) MUSCLE

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Dihydroavermectin B_{1a} (DHAVM) was found to induce dose dependant reversible and irreversible increases in Cl^- conductance when microperfused onto GABA sensitive bundles of locust (Schistocerca gregaria) extensor tibiae muscle. In muscle bundles where increases in input conductance were irreversibly induced by DHAVM, subsequent GABA induced changes in conductance were potentiated (Duce and Scott 1983). This and the finding that DHAVM induced irreversible responses in muscle fibres which were insensitive to GABA suggested that DHAVM may be acting by activating Cl^- channels not associated with GABA receptors (Duce and Scott 1983).

Ibotenic acid increases Cl^- permeability in locust muscle by activating extrajunctional glutamate H receptors (Lea and Usherwood 1973). In the present study ibotenic acid (10^{-6}M - 10^{-3}M) induced dose dependent increases in conductance. A mean change in input conductance of $62.1\pm8.8\times10^{-7}$S (mean ± S.E.M., n=22) was induced by microperfusion of ibotenic acid (10^{-4}M) from a micropipette; which was reduced to $9.3\pm1.1\times10^{-7}$S (mean ± S.E.M., n=9) in 90% Cl^- free saline. These changes in input conductance induced by ibotenic acid were reduced by DHAVM, (Table 1).

Table 1

Reduction of ibotenic acid responses by DHAVM. Expressed as % reduction of the input conductance induced by 10^{-4}M ibotenic acid.

DHAVM µg/ml	Mean % reduction of control ibotenic acid response ± S.E.M. (n).	
	GABA sensitive fibres	GABA insensitive fibres
1.0	95.0 ± 1.7 (9)	100 (1)
0.1	92.0 ± 0.9 (16)	100 (1)
0.01	86.0 ± 1.3 (7)	100 ± 0 (3)
0.001		59 ± 4.1 (13)
0.0005		51 ± 7.8 (4)

DHAVM was still able to induce irreversible responses even after extrajunctional H receptors were desensitised by ibotenic acid. One possible interpretation of this observation is that DHAVM can directly activate the Cl^- channels associated with glutamate H receptors despite receptor desensitisation.

Lower doses of DHAVM (0.001-0.0001µg/ml) caused reversible increases in conductance in the presence of ibotenic acid. However removal of DHAVM resulted in transient conductance increases, implying an interaction at the glutamate H receptor.

It is concluded that DHAVM has a number of sites of action on the locust extensor tibiae muscle, which include the glutamate H receptor Cl^- ion channel complex.

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PHARMACOLOGICAL PROFILE OF ZOLPIDEM: A NOVEL HYPNOTIC AGENT

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Displacement of high affinity binding to benzodiazepine (BZD) receptors can be achieved by compounds non chemically-related to BZD, indicating that the chemical requirements of these compounds for the interaction with these receptors is not restricted to the BZD structure. We describe here the properties, at central and peripheral BZD receptors and on the electricorticogram patterns, of zolpidem (SL 80.0750-23N : N,N,-6-trimethyl-2-(4-methylphenyl)imidazo-[1,2-a]pyridine-3-acetamide hemitartrate), a novel hypnotic with affinity for BZD receptors which is not chemically related to BZDs.

^{3}H-Diazepam (DIAZ) binding to cerebellum and hippocampus (Briley and Langer, 1978), ^{3}H-Ro 5-4864 binding to kidney (Schoemaker et al., 1983), and ^{3}H-Ro 15-1788 binding to cereberal cortex as well as the GABA shift (Möhler and Richards, 1981) were measured in the rat. Electrocorticographic analyses in rats and cats were carried out according to Depoortere (1985).

Table 1 : ^{3}H-benzodiazepine receptor binding profile of zolpidem

	IC_{50} (nM)			GABA ratio
	^{3}H-DIAZ cerebellum	^{3}H-DIAZ hippocampus	^{3}H-Ro 5-4864 kidney	^{3}H-Ro 15-1788 cerebral cortex
Zolpidem	27	109	1900	3.35
Flunitrazepam	1.9	2.2	430	2.78
Clonazepam	0.7	1.2	42000	2.62
Ro 14-7437	4.1	5.4	> 100000	1.15

Shown are mean values from at least 3 experiments.

Zolpidem inhibits ^{3}H-DIAZ binding in the nanomolar range, and it is 4 times more potent in cerebellar than in hippocampal membranes (Table 1). In contrast, zolpidem has low affinity for the peripheral BZD receptor labelled with ^{3}H-Ro 5-4864 in the rat kidney. The affinity of zolpidem for central BZD receptors labelled with the BZD antagonist ^{3}H-Ro 15-1788 is enhanced by a factor of 3 in the presence of 100 µM GABA (Table 1). After oral or i.p. administration to the rat, zolpidem exhibits rapid onset short acting hypnotic properties. In freely moving cats and rats chronically implanted with electrodes, zolpidem, in contrast to BZDs, does not affect qualitatively slow-wave sleep or paradoxical sleep. In cats, over 6 h recording, zolpidem 1 mg/kg p.o. does not change the total duration of the different sleep phases. These hypnotic effects of zolpidem are antagonized by Ro 15-1788. In addition, no rebound effects occur on withdrawal.

Zolpidem is a short acting hypnotic with an atypical binding profile, which induces physiological sleep. As recently confirmed in man by Nicholson and Pascoe (1985), zolpidem represents a novel chemical class of BZD agonists, with pharmacological advantages over classical BZDs.

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CHARACTERISATION OF 5-HT SENSITIVE NEURONES IN THE RAT CNS USING IONOPHORESED 8-OH-DPAT AND KETANSERIN

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Radioligand binding studies using tritiated 5-HT and spiperone revealed two serotonin recognition sites in the rat CNS designated 5-HT_1 and 5-HT_2 respectively (Peroutka & Synder, 1979). The 5-HT_1 sites may be differentiated by their preferential binding with agonist (5-HT_{1A} sites: 8OH-DPAT; 5-HT_{1B} sites: RU 24969), while 5-HT_2 sites bind preferentially with antagonists (e.g. spiperone; keranserin). Autoradiographic studies have demonstrated a heterogenous distribution of 5-HT binding sites within rat CNS (Biegon et al, 1982; Cortes et al, 1984; Kohler, 1984). The present study was undertaken to compare the effects of ionophoresed 8OH-DPAT (8-hydroxy-2-(di-n-propylamine) tetralin) and ketanserin on central 5-HT sensitive neurones.

Male Wistar rats were anaesthetised with urethane (1.3-1.5 g/kg^{-1}, i.p., n=15). Five- or 7-barrel micropipettes were used for extracellular recording and ionophoretic application of drugs: 5-HT creatinine sulphate (20 mM, pH 4.0), 8OH-DPAT (20 mM, pH 5.0), ketanserin HCl (20 mM, pH 4.0), imipramine HCl (10 mM, pH 5.0), fluoxetine HCl (10 mM, pH 5.0) or Na glutamate (100 mM, pH 8.5). The time for a neurone to recover by 50% (RT50) following application of 5-HT or 8OH-DPAT was used as an index of uptake.

Ionophoresis of 8OH-DPAT mimicked that of 5-HT in suppressing the spontaneous or glutamate-evoked discharge of the majority of neurones recorded in the hippocampus (27 of 31 neurones tested), superior colliculus (13/15), dorsal raphe nucleus (12/13) and suprachiasmatic nucleus (11/11). 8OH-DPAT was less effective in suppressing the discharge of cells in the frontal cortex (19/33) than in the parietal cortex (15/23). The degree of firing rate suppression induced by 8OH-DPAT was proportional to the ejecting current (0-100 nA) and had little affect on spike amplitude. Recovery from 8OH-DPAT-induced suppressions (RT50 hippocampus: 9.1±2.4 s, n=20 neurones, mean ±s.e. mean) was similar to that for 5-HT-induced suppression (RT50 hippocampus: 8.0±2.3 s, n=20). Simultaneous ejection of the uptake blockers imipramine or fluoxetine (0-10 nA) prolonged ($P<0.001$) the recovery from the suppression induced by 5-HT (RT50 hippocampus: 47.0±8.4 s, n=22) and 8OH-DPAT (RT50 hippocampus: 51.0±7.3 s, n=14), suggesting that 8OH-DPAT may be a substrate for the 5-HT uptake system.

The effect of ionophoresed ketanserin was more complex. Ejection of ketanserin (10-50 nA) enhanced 5-HT inhibitory responses in some frontal (15/29) and parietal (10/21) cortical cells and occasionally elevated the firing rate. Some cells (n=7) were suppressed by ketanserin application. Only rarely, with high ejection currents (40-100 nA), were 5-HT or 8OH-DPAT-induced suppressions of cortical, hippocampal, suprachiasmatic, superior collicular or dorsal raphe neurones blocked by ketanserin.

The predominance of 8OH-DPAT-sensitive (5-HT_{1A}) neurones recorded in the hippocampus, superior colliculus and dorsal raphe nucleus is in agreement with the differential distribution of 5-HT_{1A} and 5-HT_2 recognition sites in the CNS found autoradiographically (Cortes et al, 1984).

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APOMORPHINE INDUCES GROOMING BEHAVIOUR IN RATS PRETREATED WITH SULPIRIDE

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Dopamine agonists initiate stereotyped behaviour via brain D-2 dopamine receptors (Leysen et al 1978) but selective D-1 receptor activation increases general activity and in particular grooming (Molloy et al 1984). We have investigated the effect of the D-2 antagonist, sulpiride, and D-1 antagonist, SCH 23390, on the behavioural effects of apomorphine in the rat and compared these with the effects produced by the D-1 agonist, SKF 38393.

Female Wistar rats (200-250 g) were observed for a 10 sec. period at 2 min. intervals during a 1 h period following dopamine agonist or vehicle administration. Grooming was assessed as present (score 1) or absent (score 0) and sniffing rated on a scale of 0-3 as absent, occasional, periodic or continuous. An overall behaviour index was determined by summation of individual scores.

Administration of apomorphine hydrochloride (0.25 mg/kg s.c.) induced continuous stereotyped sniffing, but did not alter grooming. Pretreatment with (±)-sulpiride (25-80 mg/kg i.p; 3 h previously) caused a dose-dependent inhibition of apomorphine-induced sniffing, but increased grooming (Table 1). In contrast, pretreatment with SCH 23390 (0.0125-4.0 mg/kg i.p; 1 h previously) dose-dependently inhibited both apomorphine-induced sniffing and grooming. Administration of SKF 38393 (0.5-8.0 mg/kg s.c.) induced a dose-related increase in grooming but only weak sniffing. Pretreatment with (±)-sulpiride (40 mg/kg i.p.) did not alter the dose response curve for grooming (0.5-8.0 mg/kg s.c.) but antagonised sniffing. In contrast administration of apomorphine (0.06-1.0 mg/kg s.c.) induced dose-related sniffing but no alteration in grooming was observed. Following (±)-sulpiride (40 mg/kg i.p.) pretreatment, apomorphine (0.06-2.0 mg/kg) produced a dose-related increase in grooming but sniffing was reduced.

Table 1: Alteration of apomorphine and SKF 38393 induced grooming and sniffing

		Behavioural Index			
		Apomorphine (0.25 mg/kg)		SKF 38393 (1.5 mg/kg)	
		Sulpiride (40 mg/kg)	SCH 23390 (0.2 mg/kg)	Sulpiride (40 mg/kg)	SCH 23390 (0.2 mg/kg)
Grooming	Control	2.1	3.5	7.8	9.0
	Antagonist	8.3*	0.9*	6.3	5.2*
Sniffing	Control	54.1	47.4	19.9	12.6
	Antagonist	11.9*	8.9*	5.4*	3.0*

$p < 0.05$

Sulpiride inhibits apomorphine-induced stereotyped sniffing but exaggerates grooming behaviour. Pharmacological manipulation would support the contention of Molloy et al, 1984, that grooming behaviour is mediated via D-1 receptors.

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BINDING OF [^{3}H]-SCH 23390, A SELECTIVE D-1 DOPAMINE RECEPTOR ANTAGONIST, IN HUMAN BRAIN

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The 1-phenyl-3H-benzazepine SCH 23390 has been identified as a potent and selective dopamine D-1 receptor antagonist (Iorio et al, 1983; Hyttel, 1983; O'Boyle & Waddington, 1984a). Recently, the binding of ^{3}H-radiolabelled SCH 23390 to rat striatal membranes has been described (Billard et al, 1984) and this ligand may be superior to ^{3}H-piflutixol as a selective D-1 receptor ligand. We have investigated ^{3}H-SCH 23390 binding in human putamen and report preliminary results on its similarity to the D-1 receptor.

A crude membrane preparation was prepared from human post-mortem putamen tissue, and aliquots were stored at -20°C until use. Radioligand binding assays were carried out essentially as described previously for ^{3}H-piflutixol (O'Boyle & Waddington, 1984a) using 8 mg wet weight tissue in a 1 ml assay volume. Specific binding was defined as that displaced by 100 nM piflutixol. The results of saturation studies were consistent with binding to a single population of sites with a B_{max} of 14.2 pmol/g wet weight and a K_d of 1.3 nM (mean of 3 estimations on the same tissue pool). The number of D-2 receptors was measured twice on this same tissue pool using ^{3}H-spiperone and amounted to 8.1 pmol/g wet weight. Relative potencies of dopamine and reference dopaminergic antagonists to displace the binding of 0.8 nM ^{3}H-SCH 23390 are shown in the Table. Non-dopaminergic antagonists such as prazosin (α_1) and ketanserin (5-HT_2) had IC_{50} values >25 μM.

Displacing Agent	IC_{50} (nM)
SCH 23390	2.1
R-SK&F 83566	4.2
S-SK&F 83566	>1,000
Fluphenazine	106
Domperidone	4,910
Sulpiride	>100,000
Dopamine	2,216

means of 2-5 separate estimations

In this study, ^{3}H-SCH 23390 showed a typical D-1 receptor profile, being displaced potently by SCH 23390 and stereospecifically by the R- but not the S-enantiomer of the selective antagonist SK&F 83566 (O'Boyle & Waddington, 1984b). The non-selective agents fluphenazine and dopamine itself were also active. Among the selective D-2 antagonists domperidone was 2000-fold less active than SCH 23390 and sulpiride was inactive. While the density of D-1 binding sites measured under the present assay conditions in this preparation of human putamen was less than that reported using ^{3}H-piflutixol as radioligand, the ratio of D-1:D-2 sites was similar at approximately 2:1 (Cross & Rosser, 1983). The binding dissociation constant and drug displacement potencies are similar to those reported by Billard et al (1984) in rat striatum, consistent with selective labelling of D-1 receptors in human brain.

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STUDIES ON THE TRANSIENT HYPOTENSIVE EFFECTS OF FENOLDOPAM, A DA_1 DOPAMINE RECEPTOR AGONIST, IN RATS

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Fenoldopam (SK&F 82526J) is a DA_1 dopamine receptor agonist which has clinical potential as an antihypertensive and renal vasodilator agent (Ackerman et al., 1983). In this presentation, we report studies on the blood pressure lowering effects of fenoldopam in rats.

Male Sprague-Dawley rats (220-250 g) were anaesthetised with pentobarbitone (55 mg/kg i.p.), placed under artificial ventilation and prepared for measurement of blood pressure and heart rate. The effects of fenoldopam (5.0, 10.0, 20.0, 40.0 and 80.0 µg/kg/min) were studied during 15 min i.v. infusion and for 15 min, after cessation of the infusion. Fenoldopam (20 µg/kg/min, i.v.) was further evaluated in rats bilaterally vagotomised or in rats pretreated with S-sulpiride (0.3 mg/kg), diclofenac (5.0 mg/kg), chlorisondamine (0.5 mg/kg) or SCH 23390 (5 µg/kg/min, 10 min before and throughout the infusion of fenoldopam). In pithed rats, vasoconstrictor responses were evoked by angiotensin II (0.5 µg/kg i.v.), cirazoline (1.0 µg/kg i.v.) or spinal cord stimulation (1 Hz, 1 ms, 60 V, 30 sec duration) before and 1 min during infusion of saline or fenoldopam in rats pretreated with either saline or SCH 23390 (15 min). The hypotensive effects of fenoldopam are expressed as the maximum response and as the area under the response-(fenoldopam infusion) time curve ($AUC_{0-->15\ min}$).

In anaesthetised saline-pretreated rats, fenoldopam at 5, 10, 20, 40 and 80 µg/kg/min produced a maximal change in blood pressure of -15 ± 2, -20 ± 2, -26 ± 3, -31 ± 4 and 33 ± 2 mmHg (n=5/group), respectively, this response occurred 2 min after starting the infusion and was associated with a sustained increase in heart rate only at the higher doses. At the end of the administration period, the blood pressure effects were 0 ± 3, -4 ± 2, -8 ± 1, -6 ± 2 and -5 ± 1 mmHg, respectively. The respective $AUC_{0-->15\ min}$ for each dose of drug was 82 ± 12, 141 ± 23, 199 ± 30, 196 ± 22 and 218 ± 20 mmHg. Bilateral vagotomy, pretreatment with diclofenac or S-sulpiride did not significantly modify the effects of fenoldopam (20 µg/kg/min). The hypotensive response produced by fenoldopam was, however, reduced (90%) by chlorisondamine and abolished by SCH 23390. The control pressor responses to stimulation of the spinal cord, cirazoline or angiotensin II were significantly inhibited (32, 32 and 26% respectively) 1 min after starting the infusion of fenoldopam. These inhibitory effects of fenoldopam were not observed in rats pretreated with SCH 23390.

These results indicate that fenoldopam infusion evoked rapid decreases in blood pressure, however, this effect was not sustained throughout the infusion period. Moreover, the intensity of the hypotension (AUC) was not dose-related. Although several mechanisms can account for this phenomenon of tachyphylaxis, it is possible that fenoldopam behaves as a partial DA_1 dopamine receptor agonist. Finally, the hypotensive effects of fenoldopam are due to the stimulation of DA_1 dopamine receptors as they are blocked by SCH 23390.

Ackerman et al. (1983) Fed. Proc. 42, 186-190.

DOES CHRONIC RESERPINE PRETREATMENT INDUCE SUPERSENSITIVITY TO CATECHOLAMINES IN GUINEA-PIG LEFT ATRIA?

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It is generally believed, based on the work of Antonaccio & Smith (1974), that chronic reserpine pretreatment increases sensitivity of the right atria, but not the left atria to the effects of catecholamines. However, recently Broadley & Lumley (1977) and Hawthorn & Broadley (1984) have demonstrated sensitivity changes in this tissue. This study was therefore undertaken to elucidate this discrepancy.

Tension increases of either guinea-pig whole left atria or left atrial strips and papillary muscles paced at 2Hz with threshold + 50% voltage and 5ms pulse width were recorded. Tissues were suspended in Krebs-bicarbonate solution at 38°C, gassed with 5% CO_2 in O_2, and taken from either control animals or those that had received 0.1mgkg^{-1} reserpine daily i.p. for 7 days. Cumulative concentration-response curves were constructed to noradrenaline and isoprenaline.

After reserpine pretreatment, the sensitivity to the left atrial inotropic response to isoprenaline was increased with the geometric mean EC50 value falling from 15.17 x 10^{-9}M in controls (n=5) to 2.28 x 10^{-9}M in tissue, from treated animals giving a dose-ratio of 6.75. An increased sensitivity, although smaller than that for isoprenaline, was observed to noradrenaline with EC50 values falling from 10.31 x 10^{-7}M (n=5) to 3.28 x 10^{-7}M (n=5), giving a dose-ratio of 2.71. As in left atria, reserpine pretreatment increased the sensitivity of papillary muscles to isoprenaline with EC50 values of 9.51 x 10^{-9}M (n=5) and 2.78 x 10^{-9}M (n=5) in control and pretreated preparations respectively, giving a dose-ratio of 3.43. However, there was no increase in the sensitivity of the tissue to noradrenaline, where the EC50 values were 2.63 x 10^{-6}M (n=5) in untreated and 1.67 x 10^{-6}M (n=5) in treated tissues.

When left atrial strips were used in place of whole atria, reserpine pretreatment failed to increase the sensitivity of the tissue significantly to either isoprenaline or noradrenaline, where the EC50 values were 12.42 x 10^{-9}M (n=6) in untreated and 6.94 x 10^{-9}M (n=6) in reserpine treated animals for isoprenaline and 12.74 x 10^{-7}M (n=6) and 8.62 x 10^{-7}M in control and reserpine treated animals respectively for noradrenaline. In papillary muscles taken from the same animals, there was again an increase in sensitivity to isoprenaline with EC50 values falling from 12.21 x 10^{-9}M (n=5) in control animals to 4.72 x 10^{-9}M (n=5) after reserpine pretreatment.

It therefore appears that chronic reserpine pretreatment increases the sensitivity of left atria to catecholamines but only in the whole tissue, not in atrial strips which were used by Antonaccio & Smith (1974) in their original study. The reason for this is as yet unclear, but may be caused by changes in the ion balance produced by the trauma of dissection (Fleming et al., 1973).

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STUDIES ON THE ADENOSINE RECEPTORS IN DOG CORONARY ARTERY

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Adenosine and its synthetic analogues are potent coronary vasodilators (Olsson, 1983). Adenosine receptors have been classified into two types, A_1/Ri and A_2/Ra, on the basis of the potencies of 5'N-ethylcarboxamide adenosine (NECA) and the L- and D-stereoisomers of N^6-phenylisopropyl adenosine (PIA) (See Collis and Brown, 1983). From this classification, A_1/Ri receptors are characterised by the rank order L-PIA≥NECA >>D-PIA, whilst for A_2/Ra receptors the rank order is NECA>>L-PIA ≥D-PIA. The aim of the present study was to characterise the adenosine receptors mediating relaxation of dog isolated coronary arteries using these three agonists.

Hearts taken from anaesthetised dogs (barbitone 300mg/kg i.p.) were stored overnight at 4°C. Rings (approx. 0.5-0.75 mm internal diameter) cut from the left anterior descending coronary artery were suspended in organ baths containing modified Krebs solution with indomethacin (2.8μM) at 37°C and gassed with 5% CO_2 in oxygen. Tone was induced with the TxA_2-mimetic U-46619, the concentration used (10-20nM) giving approximately 50% maximal contraction. Concentration-effect curves to adenosine agonists were constructed cumulatively and all responses were expressed as a percentage of the relaxation produced by isoprenaline (30μM). Only one concentration-effect curve was obtained on each preparation.

All three adenosine analogues caused concentration-related relaxations of the coronary artery. NECA (EC_{50} = 30nM, 95% C.L. 15-58nM, n=15) was the most potent, being 6.1 (3.1-11.9, n=8) times more potent than L-PIA and 46.9 (19.7-111.8, n=8) times more potent than D-PIA. 8-phenyltheophylline (10μM) antagonised responses to all three agonists to a similar extent, producing agonist concentration ratios ranging from 11 to 58. Responses to isoprenaline were unaffected by this concentration of 8-phenyltheophylline.

These results are difficult to reconcile with the presence of either A_1/Ri or A_2/Ra receptors. The relatively small difference in potency between L- and D-PIA might point to A_2/Ra receptors, however, if this were the case, then a much larger difference between NECA and L-PIA would have been anticipated (See Collis and Brown, 1983). Recently Mustafa and Askar (1985) have obtained similar results with NECA and L-PIA on bovine coronary artery. However these workers reported a much larger difference (30 fold) between L- and D-PIA, which could be more consistent with A_1/Ri receptors. Very similar results were obtained by Edvinsson and Fredholm (1983) on cat cerebral arteries. Such findings, together with our own, illustrate the problems associated with the use of NECA and PIA for the classification of adenosine receptors. The definitive classification of these receptors must await the development of selective antagonists.

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PROTECTIVE ACTION OF ALLOPURINOL IN AN EXPERIMENTAL MODEL OF HAEMORRHAGIC SHOCK WITH REPERFUSION

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Inhibition of xanthine oxidase by allopurinol has been demonstrated to be a useful intervention to prevent tissue damage in various models of experimental ischaemia where free-radical induced reperfusion injury has been implicated (McCord, 1985). We would now like to report the effect of allopurinol in an experimental model of haemorrhagic shock where reperfusion injury has been produced by reinfusion of shed blood.

Twelve age and sex matched, anaesthetised, open chest, beagle dogs (6 pretreated with allopurinol, 50 $mgkg^{-1}$, i.v., 60 minutes prior to shock) were bled to a mean arterial pressure of 40 mmHg for 30 minutes. At the end of the shock period, the shed blood was reinfused intravenously and the animals monitored for a further 120 minutes. Haemodynamic changes were measured continuously. Myocardial biopsy samples were also taken at frequent intervals and immediately frozen in liquid nitrogen, for subsequent assay of AMP, ADP, ATP and inosine content by the HPLC method of Ingebretsen, Bakken, Segadal and Farstad (1982).

During haemorrhagic shock, cardiac output (CO), left ventricular stroke work index (LVSWI) and the rate of rise of left ventricular pressure (LVP dP/dt) were significantly depressed in both untreated and allopurinol-treated animals. The extent of the myocardial depression during haemorrhagic shock was not significantly modified by the allopurinol treatment. In both groups of animals, reinfusion of shed blood resulted in an immediate improvement in haemodynamic performance which further deteriorated throughout the remaining experimental period. At 60 minutes following reinfusion of shed blood there was a significantly greater deterioration in myocardial function in untreated animals when compared with treated animals (see Table 1). Between 60 and 120 minutes after reinfusion of shed blood 3/6 untreated and 0/6 allopurinol treated animals died. Myocardial adenine nucleotides were not significantly altered during haemorrhagic shock or with reinfusion of shed blood in either group of animals.

Table 1: Myocardial function in control and allopurinol treated animals (a) preshock and (b) 60 minutes after reinfusion of shed blood.

		CO $ml\ min^{-1}$	LVSWI $gm\ bt^{-1}m^{-1}$	LVP dP/dt $mmHg\ sec^{-1}$
Control	(a)	1226 ± 57	14.2 ± 1.5	2708 ± 264
	(b)	503 ± 69	3.1 ± 0.7	2233 ± 390
Allopurinol	(a)	1421 ± 197	11.4 ± 1.8	3679 ± 551
	(b)	*1148 ± 226	*7.5 ± 1.5	*3346 ± 385

* Significant difference ($p < 0.05$) between control and treated animals.

In conclusion, our studies demonstrate that allopurinol maintains myocardial function and prevents mortality in an experimental model of systemic ischaemia with reperfusion in the absence of changes in myocardial adenine nucleotides, and may therefore before acting by preventing free-radical formation.

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A ROLE FOR PERIPHERAL-TYPE BENZODIAZEPINE BINDING SITES IN REGULATING K^+-EVOKED $^{45}Ca^{++}$ UPTAKE IN A RAT PITUITARY CELL LINE

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Benzodiazepines interact with two sites in mammalian tissues: the central-type site (CS), coupled to GABA receptors, and the peripheral-type site (PS), whose function has not been characterized clearly. Recent studies have demonstrated that RO5-4864, a benzodiazepine selective for the PS, can depress calcium action potentials in heart (Mestre et al., 1984) and in spinal neurones (Skerritt et al., 1984), and it appears, at least in the former case, that these effects are mediated by the PS, as they are reversed by the selective PS antagonist PK 11195. The current study examines the action of these compounds on K^+-evoked calcium fluxes in clonal pituitary cells.

The clonal rat pituitary cell line, GH_3, was grown as a suspension culture. For the uptake experiments, cells were incubated in Krebs-HCO_3 buffer at various [K^+] with 0.5 µCi $^{45}Ca^{++}$. After incubation, the cells were recovered by filtration and the radioactivity accumulated therein determined.

Under these conditions, an accumulation of $^{45}Ca^{++}$ inside the cells could be observed which saturated after 10 min. This accumulation could be increased by replacing Na^+ by K^+ in the uptake buffer. Preliminary experiments indicated that a 1 min incubation period gave the optimal "window" between basal and K^+-stimulated uptake (1.39 ± 0.22 and 3.56 ± 0.28 nCi/10^6 cells respectively at [K^+] = 50 mM). The uptake of $^{45}Ca^{++}$ was proportional to [K^+] between 5 and 50 mM.

The effects of various agents on K^+-stimulated $^{45}Ca^{++}$ uptake are listed in the table. Several classical calcium channel blocking agents (nitrendipine, diltiazem, PN 200,110, but not verapamil) could block this uptake, whilst it was enhanced by the novel calcium channel facilitating agent, BAY K-8644. None of these agents had any effect on basal (i.e. 5 mM K^+) uptake.

		% uptake			% uptake
BAY-K-8644	10^{-7} M	138.6 ± 12.1*	RO5-4864	3×10^{-6} M	50.9 ± 6.5*
Nitrendipine	10^{-7} M	50.8 ± 9.9*	Clonazepam	3×10^{-6} M	112.0 ± 20.6
PN 200,110	10^{-7} M	52.5 ± 21.1*	PK 11195	10^{-6} M	111.7 ± 15.6
Diltiazem	10^{-6} M	60.0 ± 7.9*	RO5-4864	3×10^{-6} M	
Verapamil	10^{-6} M	84.8 ± 26.1	+ PK 11195	10^{-6} M	92.4 ± 22.9

Effects of various compounds on $^{45}Ca^{++}$ uptake into GH_3 cells. Data are expressed as the percentage of that uptake in 50 mM K^+ alone and represent means ± sem (n = 5-9). Values significantly ($p < 0.05$) different from their controls according to the Wilcoxon test are indicated by asterisks)

The benzodiazepine RO5-4864 also inhibited $^{45}Ca^{++}$ uptake into GH_3 cells, whilst equivalent concentrations of the CS selective benzodiazepine, clonazepan, were inactive. The PS antagonist PK 11195 was itself without effect on $^{45}Ca^{++}$ uptake, but could reverse the inhibition of uptake by RO5-4864.

These results give additional support to the hypothesis that PS can be coupled to voltage-dependent calcium channels.

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EFFECTS OF DIPYRIDAMOLE AND DEOXYCOFORMYCIN ON THE ACTION OF ADENOSINE IN GUINEA-PIG CARDIAC TISSUES

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Since the negative chronotropic and inotropic actions of adenosine on the heart appear to be exerted via specific extracellular receptors (Schrader et al. 1977), processes such as uptake or deamination should result in a change in potency (Kurahashi et al. 1983). In this study the adenosine uptake blocker dipyridamole and deaminase inhibitor deoxycoformycin were examined to determine the roles of these processes in guinea-pig isolated cardiac preparations.

Isolated left and right atria and papillary muscles were set up in Krebs-bicarbonate solution at 38°C gassed with 5% CO_2 in O_2. Rate and tension responses were obtained from spontaneously beating right atria and inotropic responses from paced left atria and left ventricular papillary muscles (2Hz, 5ms, threshold voltage + 50%). Left and right atria were exposed to dipyridamole (1.98 & 5.94x10^{-6}M) or deoxycoformycin (1.50x10^{-7}M) for 30 minutes before cumulative dose-response curves to adenosine were obtained. Adenosine was also added 4 min after raising the baselines with isoprenaline (left atria and papillary muscles 18.9x10^{-9}M; right atria 9.47x10^{-9}M). Appropriate controls were performed and all experiments involving isoprenaline were carried out in the presence of metanephrine (10^{-5}M).

The concentration of adenosine for 50% (IC50) or 25% inhibition (IC25) was determined and for papillary muscles, the EC50. (n=5-8).

Treatment before adding adenosine	Left atria tension IC50(x10^{-6}M)	Right atria Rate IC25(x10^{-6}M)	Right atria Tension IC50(x10^{-6}M)	Papillary muscle EC50(x10^{-6}M)
Control	36.0	319	113	-
Deoxycoformycin(1.5x10^{-7}M)	29.0(1.24)	408(0.78)	85(1.3)	-
Dipyridamole(1.98x10^{-6}M)	2.93*(12.3)	2.88*(111)	2.18*(51.8)	-
Dipyridamole(5.94x10^{-6}M)	2.33*(15.5)	0.57*(560)	1.15*(98.3)	-
Control + isoprenaline	35.7	539	46.8	24.3
Iso+Deoxycoformycin(1.5x10^{-7}M)	-	-	-	40.4(0.60)
Iso+Dipyridamole(5.94x10^{-6}M)	†0.45*(79.3)	1.05*(513)	0.68*(68.8)	4.2*(5.8)

Significant difference ($P<0.05$) between treated and control*, or between unstimulated and isoprenaline prestimulated†. Control:treated ratio in brackets.

Deoxycoformycin (1.5x10^{-7}M) abolished the destruction of added adenosine in left atria by exogenous adenosine deaminase (0.3Uml^{-1}). However, it had no effect on adenosine alone or after isoprenaline prestimulation. Both concentrations of dipyridamole significantly potentiated the direct effects of adenosine in left atria and to a greater extent in right atria. The inhibition of isoprenaline prestimulated preparations was also significantly potentiated by dipyridamole, significantly more so in left atria than for the direct effect of adenosine.

In conclusion it would appear that tissue uptake is the main mechanism for terminating the action of adenosine in these isolated tissues, and that the right atria, particularly the S.A. node, has a more active uptake system than the left atria followed by ventricular muscle (papillaries).

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THE CARDIAC ELECTROPHYSIOLOGICAL EFFECTS OF METHIMAZOLE PRE-TREATMENT IN THE RAT

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The literature suggests a relationship between thyroid status and susceptibility to cardiac dysrythmia. It has been suggested (1) that the increased susceptibility to arrhythmia in hyperthyroid animals may be explained by a shortening of cardiac action potentials and conversely the relative resistance to arrhythmia observed in hypothyroid animals may be explained by a prolongation. The aim of the present study was to consider the ex-vivo effects of the goitrogen methimazole on atrial and ventricular action potentials in the rat at a range of pacing frequencies and to consider the effects of the extra-cellular Ca concentration on action potential characteristics.

Male rats were pre-treated with methimazole (300 mg/l) in drinking water (group M) and these animals were weight matched by the euthyroid group (E). Right atrial and papillary muscle preparations were superfused (6 cm^3 / min) at 37°C with Krebs solution. Action potential measurements were made using standard microelectrode techniques. Measurements were made of, resting membrane potential (RMP); action potential amplitude (APA); maximal rate of depolarisation (MRD); action potential duration at 50% (APD50) and 90% (APD90) repolarisation. Papillary muscles were stimulated at 0.2 and 1 Hz. Right atria measured at their spontaneous frequency then paced at 5.5 Hz. In some experiments external Ca was reduced from 2.55 to 1.28 mM. The spontaneous atrial rate of E was significantly higher than M (269.8 ± 8.4 and 167.9 ± 21 BPM respectively n=8).

For atria RMP, APA and MRD were not significantly effected by methimazole. There were no differences in APD between E and M at their spontaneous frequencies. When paced at 5.5 Hz, the APD of E was significantly reduced compared to M (APD50 16.5 ± 1.3 versus 9.3 ± 1.1 respectively $p<0.01$, APD90 52.9 ± 3.6 versus 37.9 ± 8 ms respectively $p<0.05$). However, after exposure to 1.28 mM Ca APD90 of M was greater than E (82 ± 8.4 vs 57.6 ± 8 ms respectively).

In papillary muscles, no difference was detected between the groups for RMP or MRD. However, APA of M was reduced compared with E: for example at 1.28 mM Ca 1 Hz M = 80 ± 4.9 mV vs E = 90.1 ± 2.3 mV ($p<0.05$ n=4). There was no difference in APD between groups at either pacing frequency. However, on exposure to 1.28 mM, Ca, APD was prolonged in M (APD50 33.9 ± 6.1 vs 18.8 ± 0.4 ms, APD90 112.2 ± 10.5 vs 86.5 ± 4.2 ms $p<0.05$).

The difference in APD may be assessed by subtraction of the mean APD at 2.55 from 1.28 mM Ca for each experiment. For atria the resulting Δ APD50 were 4 ± 1.8 and 19.3 ± 4.1 ms and Δ APD90 of 10.8 ± 4.6 and 45.7 ± 9.4 ms for E and M respectively. For papillary muscles Δ APD50 were 3.8 ± 1.1 and 16.6 ± 3.3 ($p<0.05$) and APD90 of 16.9 ± 12.4 vs 28.6 ± 11.5 (N.S) for E and M respectively.

In conclusion, significant differences in APD were detected following methimazole pre-treatment. The Δ APD on transition from 2.55 to 1.28 mM calcium indicates a difference between groups and this difference in Ca "reactivity" may provide a method of discrinimating between animals of different thyroid status.

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COMPARISON OF THE EFFECTS OF BRL34915 AND VERAPAMIL ON RAT PORTAL VEIN

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BRL 34915, (±)6-cyano-3,4-dihydro-2,2-dimethyl-trans-4-(2-oxo-1-pyrrolidyl)-2H-benzo[b]pyran-3-ol, is one of a series of benzopyran derivatives with anti-hypertensive properties (Ashwood et al, 1984). In vivo, the effects of BRL34915 show some similarity to those of calcium channel inhibitors, although the pharmacological profiles are not identical (Buckingham et al,1984). In the present study the effects of BRL34915 and verapamil have been compared.

Portal veins were removed from male Wistar rats (300-400g) and incubated in a MOPS-buffered physiological salt solution (PSS) (Jetley and Weston, 1980). Under isometric conditions, BRL34915 (0.1-5μM) produced a rapid, concentration-dependent reduction in spontaneous mechanical activity. Intracellular recording (Small and Weston, 1980) showed that this was accompanied by a reduction in the duration of multi-spike complexes and by membrane hyperpolarisation. At a concentration of 5μM, BRL34915 produced a hyperpolarisation of 28 ± 1mV (mean ± SE, n=5). In contrast, verapamil (0.01-1μM) produced a slowly-developing reduction in spontaneous mechanical activity. At 1μM, verapamil virtually abolished spontaneous electrical and mechanical activity with a membrane depolarisation of 4 ± 1mV (mean ± SE, n=4).

BRL34915 (0.1-5μM) and verapamil (0.01-1μM) each produced a concentration-dependent reduction in mechanical responses to noradrenaline (NA, 0.1-100μM) and BRL-34915 delayed the appearance of NA-induced contractions. Using microelectrodes, the delay was associated with the time taken for the cell membrane to depolarise from its hyperpolarised state to its firing threshold. Once this had been reached, the degree of NA-induced depolarisation and spike generation was reduced, although spike dV/dt was unaffected. In the presence of verapamil (1μM) NA failed to induce an increase in spike firing although the degree of NA-induced membrane depolarisation was little affected.

Responses to K^+ (5-80mM, added to the PSS) were examined in the presence of either BRL34915 or verapamil. BRL34915 (0.5-5μM) was only able to inhibit responses to K^+ (5-20mM); responses to K^+ (40-80mM) were little affected. In contrast, verapamil (0.1-1μM) inhibited responses to all concentrations of added K^+. When portal veins were loaded with (^{86}Rb) for 2h and the Rb allowed to efflux into Rb-free PSS, the efflux rate coefficient became constant after approximately 10min. Tissues were then challenged with BRL34915 (5μM) and a marked increase in the Rb efflux rate coefficient was detected.

These results reveal that BRL34915 has a different mechanism of action from calcium channel inhibitors like verapamil. They suggest that BRL 34915 acts, in a novel manner for an antihypertensive agent, by opening potassium channels thereby allowing the cell membrane to approach the potassium equilibrium potential. In this way, BRL34915 delays and reduces responses to vasoconstrictors resulting in its observed anti-hypertensive activity.

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