POSTER COMMUNICATIONS

Pitfalls in the synthesis and authentication of dopamine-*O*-sulphates

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Several workers have studied the metabolism of dopamine and L-DOPA to dopamine-3- and 4-*O*-sulphates (Jenner & Rose, 1974; Arakawa, Imai & Tamura, 1979) whilst it has also been claimed that these conjugates are further converted directly to noradrenaline by dopamine-β-hydroxylase (Buu & Kuchel, 1979a,b). In all these reports, the sulphoconjugates were prepared from dopamine and concentrated H_2SO_4 according to Jenner & Rose (1973), but in no case was evidence presented allowing assessment of either authenticity or purity of the conjugates.

In this communication, we describe various conditions for the synthesis of dopamine sulphoconjugates and the application of h.p.l.c. and n.m.r. to their separation, isolation and structural determination.

When phenolic compounds are reacted with concentrated H_2SO_4 or other SO_3 donors, the preferred reaction is with the aromatic ring to yield sulphonic acids (Norman & Taylor, 1965). Table 1 gives the yields of the four components found after the reaction of dopamine with concentrated H_2SO_4 and with chlorosulphonic acid, as estimated by h.p.l.c. analysis of the reaction mixture (Hypersil ODS 5 μm, 8 × 250 mm column eluted with 2% methanol in water; detector 284 nm). Peak IV co-chromatographed with dopamine. Compounds I–III were isolated (10–50 mg) by preparative h.p.l.c. for ^1H and ^{13}Cn.m.r. and elemental analysis. Data will be presented which demonstrate that I, II and III are dopamine-6-sulphonic acid [2-(2′-aminoethyl)-4,5-dihydroxyphenylsulphonic acid], dopamine-4-*O*-sulphate and dopamine-3-*O*-sulphate respectively.

Sulphonation of dopamine thus yields 3 isomeric products whose relative proportions depend critically upon H_2SO_4 specific gravity and temperature. In our hands, the method of Jenner & Rose (1973) gave fractions of sulphoconjugates contaminated with either the sulphonic acid or dopamine. We would therefore urge caution in interpretation of certain biological properties attributed to these metabolites.

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Table 1

Reagent	*T (°C)*	*% yield of h.p.l.c. peak*[2] *I (9.0)*[3]	*II (26.2)*	*III (29.2)*	*IV (128.8)*
H_2SO_4 (s.g.[1] 1.92)	0	90	5	5	n.d.[4]
H_2SO_4 (s.g. 1.92)	20	100	n.d.	n.d.	n.d.
H_2SO_4 (s.g. 1.86)	0	30	35	35	n.d.
H_2SO_4 (s.g. 1.86)	20	95	2.5	2.5	n.d.
H_2SO_4 (s.g. 1.86)	38	95	2.5	2.5	n.d.
H_2SO_4 (s.g. 1.84)	0	5	20	20	55
Chlorosulphonic acid	20	n.d.	15	15	70

[1]Specific gravity
[2] I = dopamine-6-sulphonic acid, II = dopamine-4-*O*-sulphate
III = dopamine-3-*O*-sulphate, IV = unreacted dopamine (see text)
[3]Elution volume (ml)
[4]Means not detected

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Reduction of dopamine and octopamine content of an insect salivary gland on incubation in 6-hydroxydopamine

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Evidence from electrophysiological and secretory experiments and from a radiochemical assay has led to the suggestion that dopamine is the neurotransmitter in the salivary gland of the cockroach, *Nauphoeta cinerea*, Olivier (see House, 1980). We report here the measurement of both octopamine (a poor agonist of the receptors mediating hyperpolarization in these glands (Bowser-Riley & House, 1976) and dopamine by mass spectrometry.

Ten paired salivary glands were dissected from adult cockroaches, the reservoirs removed and five were placed in cockroach Ringer containing ascorbic acid (10^{-2} M), and five in Ringer containing ascorbic acid (10^{-2} M) and 6-hydroxydopamine (10^{-3} M) for up to 9 h. The dopamine and octopamine contents of the pooled glands were measured, after extraction, by gas chromatography-mass spectrometry (cf. Wiesel, 1976) using multiple ion detection.

It has been shown that 6-hydroxydopamine causes degeneration of nerve terminals in the salivary glands *in vitro* (Maxwell, 1980). The present experiments show that after 9 h incubation in 6-hydroxydopamine the contents of octopamine and dopamine in the glands were reduced by 50% and 75% respectively.

M.R.M. is a Walter Smith Kay Research Fellow and S.G.P.W. is an M.R.C. scholar.

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Pharmacological evidence against the occurrence of presynaptic dopamine receptors in sympathetic neurons innervating the sino-atrial node of the rat

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Recently, Lefèvre-Borg & Cavero (1980) reported that N,N-di-n-propyl-dopamine (DPDA), a dopamine receptor agonist, inhibited pressor, but not positive chronotropic, responses to electrical stimulation of the spinal cord in the rat. These results led us to postulate that the rat cardiac sympathetic neurons are probably not endowed with presynaptic dopamine receptors. The purpose of this communication is to present additional data obtained with the dopamine receptor agonist, pergolide (Cavero & Lefèvre-Borg, 1981), which appear to support this hypothesis.

Sprague Dawley male rats (220–250 g) were anaesthetized with pentobarbitone and then pithed. The animals were ventilated and treated with atropine (1.0 mg/kg, i.v.) plus (+)-tubocurarine (5.0 mg/kg, i.v.).

Heart rate was measured with a cardiotachometer triggered by the carotid blood pressure pulse and displayed on a polygraph. Drugs were injected via the femoral veins.

Heart rate was elevated (70–90 beats/min) by sustained stimulation of the thoracic spinal cord. Saline (0.26 ml/kg), phentolamine (0.3 and 1.0 mg/kg) or sulpiride (0.3 and 1.0 mg/kg) were administered intravenously and 5 min thereafter cumulative dose-response curves to pergolide were constructed. The doses of pergolide (means ± s.d.) required to produce 50% inhibition (ED_{50}) of experimental tachycardia were calculated.

In the pithed rat the ED_{50} for pergolide administered intravenously was 9.0 ± 0.6 μg/kg ($n = 6$). Sulpiride (0.3 mg/kg) did not change this value. However, 1.0 mg/kg, i.v., sulpiride shifted the dose-response curve to pergolide in a parallel manner to the right resulting in an ED_{50} value of 36.5 ± 2.9 μg/kg ($n = 5$). In rats pretreated with phentolamine (0.3 and 1.0 mg/kg, i.v.), the ED_{50}'s value of pergolide were 58.0 ± 3.0 and 108.6 ± 6.5 μg/kg ($n = 4$/group), respectively.

These results indicate that pergolide decreases the neural tachycardia by activation of cardiac presynaptic α_2-adrenoceptors. The fact that sulpiride (1.0 mg/kg, i.v.) increased the ED_{50} of pergolide is attributed to possible α_2-adrenoceptor blocking properties of this dose of dopamine receptor antagonist. We have reported that sulpiride (0.3 mg/kg, i.v.) is sufficient to block the effects of pergolide produced on heart rate in intact pentobarbitone anaesthetized rats (Cavero & Lefèvre-Borg, 1981a) and on pressor responses to stimulation of spinal cord which are mediated by activation of dopamine receptors located in the central nervous system and on sympathetic neurons innervating the vascular bed respectively (Cavero & Lefèvre-Borg, 1981).

Since in the dog pergolide activates cardiac presynaptic dopamine receptors (Cavero, 1981), it is again suggested that the rat sino-atrial node lacks a population of these receptors which upon pharmacological activation can inhibit cardiac sympathetic tone (Lefèvre-Borg & Cavero, 1980). It is concluded that species differences may exist concerning the presence of presynaptic dopamine receptors in the heart since Rand, McCulloch & Story (1975) have already reported that the guinea pig atria also lack these receptors.

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Effects of diltiazem and verapamil on pressor responses mediated by stimulation of α_1-, α_2-adrenoceptors, angiotensin II and 5-hydroxytryptamine receptors in pithed rats

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Verapamil decreases perfusion pressure responses of the dog hindlimb and hindpaw to stimulation of α- and β-adrenoceptors as well as angiotensin II receptors (Greenberg & Wilson, 1974). This effect was attributed to a nonspecific depression of vascular reactivity.

In pithed rats the pressor response produced by stimulation of α_2-adrenoceptors with B-HT 920 was inhibited by verapamil, nifedipine and D-600. However, these calcium antagonists failed to significantly affect the pressor effects of methoxamine, an α_1-adrenoceptor agonist (Van Meel, De Jonge, Kalkman, Wilffert, Timmermans & Van Zwieten, 1981).

The aim of this communication is to describe the effects of verapamil and diltiazem, two calcium antagonists, on the increases in arterial blood pressure produced by 5-hydroxytryptamine, angiotensin II, cirazoline, a relatively specific α_1-adrenoceptor agonist, and M-7, a relatively specific α_2-

adrenoceptor stimulant in the pithed rat.

Sprague Dawley male rats (220-250 g) were anaesthetized with pentobarbitone (55.0 mg/kg, i.p.), ventilated and prepared for mean carotid artery blood pressure measurements. Studies were carried out in either intact or pithed rats.

Pressor responses (50–65 mmHg: approximately the middle of dose-response curves) to a single dose of the agonists were elicited before and during (10th min) a 15 min infusion of saline (control), diltiazem or verapamil.

In intact rats, diltiazem (12.5, 25 and 50 μg/kg/min, i.v.) produced dose-related falls in arterial blood pressure of 5, 20 and 30% (initial value: 126 ± 2 mmHg, $n = 13$), respectively. This effect attained a steady state between 3 and 7 min after starting the infusion. Similarly, verapamil (12.5, 25 and 50 μg/kg/min, i.v.) reduced pressure by 10, 20 and 30% (initial value: 128 ± 2 mmHg, $n = 10$) respectively.

The studies in pithed rats, reported below, were carried out by using infusion rates of verapamil (25 μg/kg/min, i.v.) and diltiazem (50 μg/kg/min) producing approximately equal falls in pressure (20%).

Verapamil strongly inhibited (67%) the pressor response elicited by 5-hydroxytryptamine (40 μg/kg, i.v.). In contrast, diltiazem was virtually devoid of antagonist activity (10% inhibition) against this indolamine. Pressure increases induced by angiotensin II (0.25 μg/kg, i.v.) and M-7 (20 μg/kg, i.v.) were inhibited by approximately 50% and 75%, respectively, in pithed rats infused with diltiazem or verapamil. However, the pressor responses due to cirazoline were greater reduced by verapamil (35%) than by diltiazem (20%).

These results confirm the observation of Van Meel *et al.* (1981) that slow calcium channel antagonists, like verapamil or diltiazem, can strongly reduce pressor responses produced by M-7, an α_2-adrenoceptor stimulant. In contrast to these investigators, we found that verapamil and to a lesser extent diltiazem, inhibited the increases in blood pressure induced by the α_1-adrenoceptor agonist, cirazoline. This discrepancy may be due to the fact that our dose of agonist was chosen to produce a response located in the middle of the dose-response curve, whilst Van Meel *et al.* (1981) studied a dose of methoxamine which gave 80% of the maximum pressor effect.

Since verapamil, but not diltiazem, depressed the vasopressor effects of 5-hydroxytryptamine, this effect is unlikely to be related to an impairment of the vascular slow calcium channel. In fact, subsequent to this observation, Briley & Langer (personal communication) found that verapamil inhibited the binding of ^{3}H-spiroperidol to 5-hydroxytryptamine receptors in the rat cortex.

The fact that both verapamil and diltiazem reduced the vasoconstrictor effects of angiotensin II may suggest that the extracellular calcium is necessary for the response produced by this octapeptide. From these observations it is proposed that angiotensin II receptors and α_2-adrenoceptors are directly or indirectly coupled to receptor operated calcium channels (Bolton 1979) which might be voltage activated since they are blocked by diltiazem and verapamil.

Finally, the suggestion of Van Meel *et al.* (1981) the α_2-adrenoceptors may be responsible for the antihypertensive effects of calcium antagonists remains to be proven as both verapamil and diltiazem can decrease arterial pressure in pithed rats where no sympathetic vascular tone is present.

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Evaluation of the selectivity of M7 for α_1 and α_2-adrenoceptors *in vitro*

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M7 was originally demonstrated to be a selective presynaptic α_2-adrenoceptor agonist *in vivo* (Hicks & Cannon, 1979). Following the discovery that *in vivo* the postsynaptic alpha-adrenoceptor population consists of both α_1- and α_2-subtypes (Timmermans, Kwa & Van Zwieten, 1979; Langer, Massingham & Shepperson, 1980), Drew (1980) reported that M7 preferentially stimulated the α_2-subtype. We have now investigated the effects of M7 on α-adrenoceptors *in vitro*, presynaptically in the rat vas deferens and postsynaptically in the rabbit pulmonary artery and the dog saphenous vein.

The prostatic half of rat vasa deferentia was removed from Sprague-Dawley rats, cut open, and mounted in a 2 ml organ bath in magnesium free Krebs' solution, maintained at 37°C and bubbled with 95% O_2, 5% CO_2, under a basal tension of 2 g. Prazosin (30 nM) was added to the Krebs' solution to prevent α_1-adrenoceptor mediated contractions, and cocaine (1 μM) was added to inhibit neuronal catecholamine uptake. Twitch responses of the tissue were evoked by electrical stimulation via parallel platinum wire electrodes on either side of the tissue (0.1 Hz, 2 ms and a supramaximal voltage, usually 40–50 V). Main pulmonary arteries were removed from rabbits and cut into 3 mm wide spiral strips. Lengths of 2–3 cm of the tissue were mounted vertically under 2 g resting tension between a tissue holder and a transducer. The strips were superfused at 6 ml/min with normal Krebs' solution, prewarmed to 37°C and bubbled with 95% O_2, 5% CO_2. The strips were allowed to stabilize for 30 min, after which cumulative dose response curves were determined by injecting the agonist into the perfusion stream. Saphenous veins were removed from dogs anaesthetized with pentobarbitone (35 mg/kg), cut into spirals and mounted as described for the rabbit pulmonary artery. Grass FT03 transducers were used to measure the changes in tension of all the preparations and recordings were made on Grass model 7D polygraphs.

M7 inhibited the twitch response of the rat vas deferens to electrical stimulation in a concentration dependent manner with an IC_{50} of 12.0 ± 0.1 nM. This inhibition was identical to that produced by the preferential α_2-agonist clonidine, the ratio of IC_{50}'s, clonidine/M7, compared in the same preparation being 1.02 ± 0.1 ($n = 4$). The inhibition of the twitch response produced by both agonists was completely reversed by yohimbine (1 μM).

The rabbit pulmonary artery was contracted in a concentration dependent manner by M7, producing a maximum tension of 3.2 ± 0.19 g with an EC_{50} of 204.4 ± 27.1 nmoles. This contraction was inhibited by prazosin (3–30 nM), a Schild plot giving a pA_2 of 9.05 ± 0.11, from a line with a slope of -1.16 ± 0.16.

The dog saphenous vein was contracted, in the presence of 30 nM prazosin, by M7 in a concentration dependent manner, producing a maximum tension of 6.23 ± 0.47 g with an EC_{50} 1.70 ± 0.3 nmoles. Phenylephrine also contracted this tissue giving a maximum response of 8.8 ± 0.66 g with an EC_{50} of 18.0 ± 2.2 nmoles. The response to phenylephrine was inhibited by prazosin (pA_2 of 8.0 ± 0.1 from a Schild plot with a slope of -0.94 ± 0.035). In contrast to these results the response to M7 was not affected by prazosin in concentrations up to 100 nM.

In the presence of 30 nM prazosin the contraction produced by M7 was inhibited by yohimbine (3–30 nM) giving a pA_2 value of 8.64 ± 0.3 from a Schild plot with a slope of -1.03 ± 0.16. This is a significantly greater pA_2 than was obtained with yohimbine using phenylephrine as agonist ($pA_2 = 7.6 \pm 0.1$, slope $= -0.92 \pm 0.01$).

In conclusion, *in vitro* M7 stimulates presynaptic α_2-adrenoceptors and postsynaptic α_1– and α_2-adrenoceptors. Comparing the EC_{50}'s obtained in the pulmonary artery and saphenous vein gives a selectivity ratio of 122 between α_1- and α_2-receptors on vascular smooth muscle, in agreement with the selectivity seen *in vivo*. Furthermore these results show that the dog saphenous vein is a good model for demonstrating postsynaptic α_2-adrenoceptors.

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RX 781094, a new potent, selective antagonist of α_2-adrenoceptors

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Langer (1974) proposed that α-adrenoceptors located on presynaptic nerve terminals and on postsynaptic effector organ cells be designated α_2- and α_1-adrenoceptors, respectively. Presently however, α-adrenoceptors are classified not in terms of biological function or anatomical position but rather with reference to their relative affinities to a wide range of agonists and antagonists (Starke & Langer, 1979). Prazosin, a selective antagonist of α_1-adrenoceptors has a widespread clinical usage. In contrast, a clinically acceptable antagonist for α_2-adrenoceptors is not presently available. This communication describes the actions of a new compound, RX 781094 [2-(2-(1,4-benzodioxanyl))-2-imidazoline HCl] which is a potent and highly selective antagonist at α_2-adrenoceptors.

The pre- (α_2) and postsynaptic (α_1) adrenoceptor antagonist activities of RX 781094 and reference compounds (Table 1) were assessed *in vitro* by determining pA_2 values against clonidine and noradrenaline in the rat vas deferens and anococcygeus muscle, respectively (Doxey, Smith & Walker, 1977). *In vivo* presynaptic α_2-adrenoceptor antagonist activity was determined in the pithed rat. Stimulation-evoked contractions of the vas deferens (6.0 Hz, 50 μs, 40 v for 2 s every 30 s) or anococcygeus muscle (1.0 Hz, 500 μs, 40 v for 20 s every 2 min) were respectively inhibited by clonidine (100 μg/kg, i.v.) and guanabenz (30 μg/kg, i.v.). The cumulative i.v. doses of antagonists causing 50% reversals of these inhibitions were used to express α_2-antagonist potency.

Results from *in vitro* experiments demonstrate that RX 781094 was the most potent and selective antagonist of α_2-adrenoceptors; RX 781094 being respectively 3.8 and 6.5 times more potent and selective than yohimbine for α_2-adrenoceptors (Table 1). Under the present experimental test conditions the selectivity of RX 781094 for α_2-adrenoceptors was greater than the selectivity of prazosin for α_1-adrenoceptors (288 fold compared to 174, respectively).

In pithed rats only prazosin failed to reverse the inhibitory (α_2) effects of clonidine in the vas deferens; RX 781094 being the most potent antagonist. In the anococcygeus muscle only RX 781094 and yohimbine were sufficiently selective α_2-adrenoceptor antagonists to reverse guanabenz (Table 1). RX 781094 was more selective than yohimbine in the anococcygeus since noradrenaline-induced contractions (α_1) were little affected by RX 781094 (1.0 mg/kg, i.a.) but were virtually abolished by the same dose of yohimbine.

These results demonstrate that RX 781094 is a potent and selective α_2-adrenoceptor antagonist.

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Table 1 Relative potencies and selectivities for RX 781094 and four standard α-adrenoceptor antagonists in isolated tissue and pithed rat experiments. pA_2 values were calculated according to Arunlakshana & Schild (1959) and are the means ± s.e.means of in each case a minimum of 6 experiments. The α_2/α_1 selectivity ratio is the antilog of the difference between the pA_2 values at α_2- and α_1-adrenoceptors

	in vitro experiments			*in vivo experiments – pithed rat*	
Antagonist	Vas deferens (α_2) pA_2 value against clonidine	Anococcygeus (α_1) pA_2 value against noradrenaline	α_2/α_1 Selectivity ratio	Vas deferens (α_2) Cumulative i.v. dose reversing clonidine by 50% (mg/kg)	Anococcygeus (α_2) Cumulative i.v. dose reversing guanabenz by 50% (mg/kg)
RX 781094	8.56 ± 0.05	6.10 ± 0.05	288.4	0.024 ± 0.001	0.005 ± 0.001
Yohimbine	8.14 ± 0.05	6.49 ± 0.06	44.7	0.67 ± 0.29	0.11 ± 0.03
Piperoxan	7.72 ± 0.03	6.61 ± 0.08	12.9	0.50 ± 0.10	no effect at 14.4
Phentolamine	8.38 ± 0.09	7.70 ± 0.17	4.8	0.12 ± 0.60	no effect at 1.4
Prazosin	5.94 ± 0.10	8.18 ± 0.11	0.0057	no effect at 4.3	no effect at 1.4

Neuropharmacological evaluation of RX 781094, a new selective α_2-adrenoceptor antagonist

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Presynaptic α_2-adrenoceptors have been implicated in the autoregulation of noradrenergic transmission (Langer, 1974). Selective α_2-adrenoceptor agonists induce behavioural depression (Drew, Gower & Marriott, 1979) EEG synchronization (Florio, Bianchi & Longo, 1975) and hypothermia (von Voigtlander, Triezenberg & Losey, 1978). Studies on the neuropharmacological effects of α_2-adrenoceptor antagonists have been limited by the relatively low α_2/α_1-adrenoceptor selectivity and specificity of the antagonists currently available, e.g. yohimbine.

RX 781094 [2-(2-(1,4-benzodioxanyl))-2-imidazoline HCl] is a potent, highly selective α_2-adrenoceptor antagonist (Chapleo, Doxey, Myers & Roach, 1981). The present report describes investigation of (1) the ability of RX 781094 to antagonize the neuropharmacological effects of the α_2-adrenoceptor agonists clonidine and guanoxabenz (*in vivo* α_2/α_1-selectivity ratios of 31 and >237 respectively) (Doxey, Frank and Hersom, 1981) and (2) the effects of RX 781094 on general behaviour and sleep-waking activity. Yohimbine was included for comparison.

Antagonism of behavioural depression induced by clonidine (0.1 mg/kg, i.p.) or guanoxabenz (1 mg/kg, i.p.) was assessed by recording the activity of groups of 10 mice (male, BKW, 26–28 g) in cages designed to monitor locomotor (horizontal movements), exploratory (hole dips) and rearing activity. Antagonism of clonidine (0.1 mg/kg, i.p.)-induced hypothermia was determined in groups of 8 mice (male BKW, 20–24 g) by measuring body temperature (thermistor probe in the oesophagus), prior to and at 15 min intervals following drug treatment. Drugs or drug vehicle were injected i.v. 15 min before the α_2-adrenoceptor agonist.

Both RX 781094 and yohimbine counteracted the behavioural depression induced by clonidine (RX 781094, 0.03–1 mg/kg; yohimbine, 0.1–1 mg/kg) and the more selective agonist guanoxabenz (RX 781094, 0.1–3 mg/kg; yohimbine, 1–3 mg/kg). The hypothermic response to clonidine was blocked by both RX 781094 (0.03–10 mg/kg) and yohimbine (0.03–1 mg/kg).

Effects on EEG activity were measured in rats (Sprague-Dawley, 350–400 g) chronically implanted with electrocortical and electromyographical electrodes. Both clonidine (0.2 mg/kg, i.p.) and guanoxabenz (0.5 mg/kg, i.p.) produced a synchronized EEG pattern and a reduction in muscle tone; these effects parallelled the observed behavioural depression. RX 781094 (0.1–1.0 mg/kg, i.v.), injected 20 and 30 min after clonidine and guanoxabenz respectively, produced an immediate and complete antagonism of the agonist effects. Against guanoxabenz (1 mg/kg), RX 781094 completely blocked the EEG synchronization for 93 ± 10 min ($n = 4$). During the antagonism the cortical EEG was desynchronized, typical of the awake state. Yohimbine (0.5–2.0 mg/kg, i.v.) produced a qualitatively similar antagonism.

The effects of RX 781094 on sleep-waking activity in rats, were assessed by EEG and EMG measurement. During a 3 h period after injection of RX 781094 (0.1–1 mg/kg, i.v.) the percentages of time spent in wakefulness, non rapid eye movement (NREM) sleep and REM sleep were not significantly different from those following drug vehicle.

Latency to NREM sleep did not change significantly (15 ± 2 min after RX 781094, 1 mg/kg and 18 ± 4 min after vehicle; $n = 5$ rats). An initial transient (< 15 min) increase in locomotor activity was observed over a wide dose range (0.03–3 mg/kg, i.v.) of RX 781094. This was not dose-related and of much shorter duration than the RX 781094-induced antagonism of the α_2-adrenoceptor agonist effects. (+)-Amphetamine (1 mg/kg, i.v.), in contrast to RX 781094, markedly prolonged latency to NREM sleep onset (133 ± 7 min) and caused a sustained increase in locomotor activity.

The results are consistent with RX 781094 being a potent antagonist at central α_2-adrenoceptor sites.

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α-adrenoceptor blocking properties of R28935 in the rat

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R28935 [erythro-1-{1-{2-(1-4-benzodioxan-2yl)-2-OH-ET)-4-peperidyl}-2-benzimidazolinone] is a hypotensive agent in a number of species and has been reported to have a central mode of action (Finch, 1975; Wellens, De Wilde, Van Bogaert, Van Bogaert, Wouters, Reneman & Janssen, 1975; Taylor & Antonaccio, 1978). Although some reports indicate a lack of involvement of R28935 with α-adrenoceptors (Wellens *et al.*, 1975; Finch, 1975) this compound was able to displace [^{3}H]-prazosin from binding sites in brain slices (Kwa, Timmermans & Van Zwieten, 1980). The *in vivo* evaluation has relied on the failure of R28935 to antagonize vasoconstrictor responses to NA. However, it is now apparent that more than one post synaptic α-adrenoceptor is involved in NA, induced vasoconstriction *in vivo* (see review: Timmermans & Van Zwieten, 1981). Against this background, we chose to examine R28935 against more selective α_1- or α_2-receptor agonists in the pithed rat and in the rat isolated thoracic aorta.

Male normotensive Wistar rats (300–400 g) were used throughout, anaesthetized with pentobarbitone (60 mg/kg i.p.) and pithed. Diastolic pressure changes (DBP) were recorded in response to i.v. injection of agonists. Intracerebroventricular (i.c.v.) injections were made in intact anaesthetized rats (co-ordinates: 1.0 mm lateral; 1.25 mm posterior; 3.5 mm vertical to bregma). Ring segments of rat thoracic aorta were set up under 2 g tension in Krebs solution at 37°C gassed with 95% O_2 and 5% CO_2, and contracted with (−)-noradrenaline added cumulatively.

In pithed rats R28935 was a potent and preferential antagonist of the DBP response induced by a submaximal dose of phenylephrine (PE). Responses to the α_2-receptor agonist TL99 (Hicks & Cannon, 1980) were also blocked by R28935 but at higher doses. The responses to electrical stimulation of the entire sympathetic chain and exogenous NA were less susceptible to blockade by R28935 (Table 1), but the potency of R28935 against these stimuli was increased after treatment with propranolol (1 mg/kg i.v.). The tachycardia induced by exogenous NA was also antagonised by R28935 at doses > 0.25 mg/kg i.v.

The results indicate that R28935 has pronounced α_1-adrenoceptor blocking effects but these effects are not observed using NA *in vivo*. *In vitro* in the rat aorta, the pA_2 value for R28935 against NA was 9.08 (8.43–9.72), with a -slope of 0.88 (9.64–1.12).

Table 1 α-adrenoceptor blocking effects of R28935 in the pithed rat

Agonist	*n*	*R28935 EC_{50}* (mg/kg)*
PE (5 μg/kg)	9	0.02 (0.014–0.028)
T199 (5 μg/kg)	9	0.56 (0.41–0.76)
NA (0.5 μg/kg)	8	2.03 (1.22–3.39)
Stimulation (30 v, 2.5 H_z, 1 ms)	8	2.59 (1.21–5.59)

*EC_{50} = dose of antagonist causing 50% reduction (+95% condifence limits) in diastolic pressor response

In further experiments, the effects of a threshold hypotensive dose of R28935 was studied after i.c.v. administration. PE (5 μg/kg i.v.) or angiotensin II (AII; 0.25 μg/kg i.v.) were administered before or 10 min after saline (10 μl i.c.v.) or R28935 (10 μg i.c.v.). A significant ($P < 0.05$) reduction in the pressor response to PE but not to AII was obtained.

These results indicate that R28935 can also antagonise peripheral α_1-adrenoceptors after central administration. At higher doses R28935 also antagonised post synaptic α_2-adrenoceptors and has significant β-adrenoceptor blocking activity. The results emphasise the need to evaluate α-adrenoceptor antagonists using either selective agonists, or preparations containing a homogenous receptor population.

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Selectivity of RS 21361 for α_2-adrenoceptors as determined by *in vitro, in vivo* and ligand binding studies

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RS 21361 (2-(1- ethyl-2-imidazolyl methyl)-1,4-benzodioxan) has been shown in the rat, isolated, transversely bisected vas deferens to be a selective α_2-adrenoceptor antagonist (Michel & Whiting, 1981a). The selectivity of RS 21361, obtained using the rat vas deferens, has been compared with that determined in ligand binding, *in vivo* and further *invitro* studies.

In vitro, α_2-adrenoceptor antagonist affinity was determined against the inhibitory effect of xylazine, on the response of the rat vas deferens (Michel & Whiting, 1981b) and guinea-pig ileum (Drew, 1978) to transmural nerve stimulation. α_1-Adrenoceptor antagonist affinity was assessed against the contractile effect of amidephrine, in the rat deferens (Michel & Whiting, 1981b) and rat anococcygeus muscle (Doxey, Smith & Walker, 1977).

In vivo, α_2-adrenoceptor antagonism was assessed against the inhibitory effect of clonidine on the tachycardia produced by cardiac sympathetic nerve stimulation (60V, 1msec., 1Hz) in the pithed rat (Drew, 1976). α_1-Adrenoceptor antagonism was determined against the pressor response to phenylephrine in the same preparation.

Binding assays were performed using membranes prepared from rat cerebral cortex by homogenization, centrifugation and resuspension in 50 mM Tris HCl buffer (pH 7.8). [^{3}H]-Yohimbine (Barnett *et al.*, 1981) and [^{3}H]-prazosin (Greengrass & Bremner, 1979) were used to label α_2- and α_1-adrenoceptors, respectively. Noradrenaline (200 μM) was used to define non-specific binding in both studies. The specific binding of [^{3}H]-yohimbine and [^{3}H]-prazosin represented 60–70% and 80% respectively of the total binding. Competition curves for antagonists were performed against these radioligands and the K_i value for the antagonist calculated from the IC_{50}.

At the α_1-adrenoceptor of the rat anococcygeus muscle and rat vas deferens, RS 21361 was without effect at concentrations of up to 10^{-4} mol/l, whereas in the guinea-pig ileum and rat vas deferens RS 21361 was a competitive α_2-adrenoceptor antagonist with pA_2 values of 6.50 ± 0.17 and 6.71 ± 0.06 respectively. When compared with a series of standard compounds RS 21361 was the most selective α_2-adrenoceptor antagonist studied. The order of selectivity of the antagonists for the α_2-adrenoceptor was: RS 21361 $\gg$ rauwolscine $>$ yohimbine $\gg$ phentolamine $\gg$ prazosin.

In the ligand binding studies the affinity of RS 21361 for α_2-adrenoceptors was 375.0 times greater than its affinity for α_1-adrenoceptors ($K_i\alpha_2 = 8 \times 10^{-8}$ mol/l; $K_i\alpha_1 = 3 \times 10^{-5}$ mol/l), Rauwolscine and yohimbine possessed only 24 and 64 times, respectively, higher affinities for the α_2-adrenoceptor, than for the α_1-adrenoceptor. The order of selectivity of the compounds for the α_2-adrenoceptor was: RS 21361 $>$ yohimbine $>$ rauwolscine $>$ phentolamine $\gg$ prazosin.

In the pithed rat RS 21361 was 300 times more

potent as an α_2- than as an α_1-adrenoceptor antagonist (ED_{50} against clonidine = 0.2 mg/kg i.v.; DR_2 against phenylephrine = 30 mg/kg i.v.). For yohimbine a 30 fold separation was obtained. The order of selectivity of the compounds studied for the α_2-adrenoceptor was: RS 21361 > yohimbine > phentolamine ≫ prazosin.

The results of the present study demonstrate that RS 21361 is a selective α_2-adrenoceptor antagonist, as determined by *in vitro, in vivo* and ligand binding studies.

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α_2-Adrenoceptors in the rabbit vasculature

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The concept of two vascular, post-junctional α-adrenoceptors (α_1, α_2) can explain the pressor effects of circulating catecholamines or sympathetic nerve stimulation in rats (Docherty & McGrath, 1980; Flavahan & McGrath, 1980). In conscious rabbits, effects of 'selective' drugs suggest the presence of the same two sub-groups of vascular α-adrenoceptor (Hamilton & Reid, 1980). We now report experiments in pithed rabbits which (1) allow comparison with rats (2) seek the role of α_1- and α_2-adrenoceptors in vascular responses to catecholamines and to sympathetic nerve stimulation.

Male New Zealand white rabbits were pithed (McGrath & MacKenzie, 1977), carotid arterial pressure monitored and drugs injected via a jugular vein. The diastolic pressor effects of agonists or sympathetic nerve stimulation (T8, 20 pulses, 0.1–10 Hz) and the effects on these of 'selective' antagonists (each at 1 mg/kg) were examined.

Rauwolscine antagonized responses to guanabenz (1 μg/kg – 1 mg/kg) but not those to phenylephrine (0.1–30 μg/kg). This confirmed that guanabenz and rauwolscine are an α_2-agonist and an α_2-antagonist, respectively.

Prazosin shifted the dose-response curve to phenylephrine to the right but to a smaller degree than in the rat (Drew & Whiting, 1979). Subsequent addition of rauwolscine produced a further shift to the right. Thus, at low doses, phenylephrine may act predominantly on α_1- but at higher doses activate also α_2-adrenoceptors.

Responses to noradrenaline (0.1–10 μg/kg) were susceptible to either rauwolscine or prazosin. Against responses to low doses of noradrenaline (< 1 μg/kg), rauwolscine produced a greater inhibition than did prazosin. Against responses to higher doses of noradrenaline, prazosin was more effective than rauwolscine. The effects of the two antagonists were additive. Thus, noradrenaline may act at both α_1- and α_2-adrenoceptors, with a balance in favour of α_2 at low doses and α_1 at high doses.

Against nerve stimulation, rauwolscine reduced responses to low frequencies (< 0.5 Hz) but at higher frequencies (> 1 Hz) responses were unchanged or increased. Responses to each frequency were reduced by prazosin. The combination of antagonists produced at least the same amount of inhibition as did prazosin. Thus, at low frequencies (pre-

junctional feedback at a minimum) transmitter noradrenaline acts at both α_1- and α_2-adrenoceptors on vascular smooth muscle. At high frequencies, rauwolscine interrupts feedback, offsetting post-junctional antagonism.

Thus, in rabbit vasculature, noradrenaline's effects can be explained in terms of two sub-groups of post-junctional α-adrenoceptor as in the rat. However, in rabbit, compared with rat, post-junctional α_2-adrenoceptors play a proportionately greater role and contribute to responses to sympathetic nerve stimulation as well as to circulating noradrenaline.

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Partial agonist effects of medroxalol at β_2 adrenoceptors

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Medroxalol is a new antihypertensive drug with antagonist properties at α_1- and β_1-adrenoceptors (respective ρA_2's 6.09, 7.73; Dage, Cheng & Woodward, 1981) and vasodilator effects, the mechanism of which has not been defined. As partial agonist effects at β_2-adrenoceptors may result in vasodilation, I have assessed medroxalol for such an effect.

Tracheal rings (Coburn & Tomita, 1973), from male guinea-pigs pretreated with reserpine (2 mg/kg, i.p. 24 h previously) and set up in Tyrode solution at 35°C under isotonic conditions (0.2 g load), developed tone over a 60 min incubation period. Medroxalol ($0.1-10\,\mu$M) and pindolol ($0.1-10\,\mu$M), a partial agonist at β_1- and β_2-adrenoceptors (Ozawa, Matsubara & Chen, 1977), caused concentration-dependent relaxations of these preparations, the maximum effects ($10\,\mu$M) being respectively $39\pm4\%$, $n=19$ and $45\pm10\%$, $n=5$ of the maximum relaxation caused by papaverine ($10-30\,\mu$M). The relaxant effects of medroxalol ($0.3-10\,\mu$M) were reduced ($40-65\%$, $n=10$, $P<0.05$) in the presence of (±)-propranolol ($3\,\mu$M). (±)-Propranolol did not affect the tone of the preparations directly, as it is essentially devoid of partial agonist effects at β-adrenoceptors (Barratt, 1972).

Medroxalol ($0.1-10\,\mu$M) did not relax trachea preparations which had been contracted with carbachol ($0.3\,\mu$M). However, preparations contracted by carbachol were less sensitive to the relaxant effects of salbutamol (EC50 196 ± 50 nM, $n=13$) compared with preparations which contracted spontaneously (EC50 42 ± 11 nM, $n=16$, $P<0.001$) and as the antogonist effects of medroxalol ($1\,\mu$M) were similar ($P>0.1$) under both conditions, the failure of medroxalol to relax preparations contracted with carbachol can be ascribed to an adrenoceptor reserve insufficient to allow the partial agonist effects to be manifested.

To investigate the vasodilator effects of medroxalol, male Sprague-Dawley rats (250–320 g) which had been pretreated with reserpine (2 mg/kg, i.p. 24 h previously) were anaesthetized with sodium pentobarbitone (40–50 mg/kg, i.p.) and pithed. The rats were infused with angiotensin II ($0.1-0.2\,\mu$g/kg/min, i.v.) to maintain diastolic blood pressure (BP) between 85 and 105 mm Hg. The pressor effects of angiotensin II were unchanged by (±)-propranolol (1 mg/kg, i.v.) or phentolamine (1 mg/kg, i.v.) and were stable for >1 h. Medroxalol and pindolol ($0.1-10\,\mu$mole/kg, i.v.) caused dose-dependant falls in BP of up to 40 mm Hg. The effects of pindolol, but not medroxalol, were accompanied by an increase in heart rate. The medroxalol-induced fall in BP was significantly attenuated

($P<0.05$) by (±)-propranolol (1 mg/kg, i.v. 15 min previously), but not by phentolamine (1 mg/kg, i.v. 15 min previously).

These findings indicate that medroxalol has partial agonist effects at β_2-, but not β_1-, adrenoceptors which may contribute to its vasodilator effects in some circumstances.

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Antibiotics and autonomic neuroeffector transmission: similarities with neuromuscular junction?

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Timmerman, Long & Pittinger (1959) demonstrated that not all antibiotics possess neuromuscular blocking properties and the effects of those that do are greatly enhanced by the simultaneous administration of (+)-tubocurarine in sub-maximal doses. Recently, we have shown that certain antibiotic compounds are capable of inhibiting evoked and reflex responses in the guinea-pig ileum, and evoked responses in the rabbit colon (Lees & Percy, 1981).

To study further these events, we have used extracellular electrophysiological recording from the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum by the method of Kosterlitz & Lydon (1971), and transmural electrical stimulation of ileal segments (Paton, 1955). Electrical stimuli were produced by a Grass S88 stimulator, mechanical events were recorded on a Linseis LS 24 pen recorder, and electrical events were displayed on a Tektronix 502A oscilloscope via a CFP 8120 preamplifier. Antibiotics were prepared as previously described (Lees & Percy, 1981).

We have found that, following a 15 min exposure to a concentration known to abolish the peristaltic reflex proper of the guinea-pig ileum *in vitro*, clindamycin, lincomycin gentamicin, kanamycin, pivmecillinam and trimethoprim reversibly abolished evoked muscle action potential complexes without affecting evoked nerve action potentials. Of these drugs, lincomycin, gentamicin and kanamycin did not depress spontaneous myoelectric activity in high concentrations.

When a concentration of atropine (0.1–0.5 nM, 5–20 min exposure) or morphine (0.1–5 nM, 1–2 min exposure) producing a maximum of 20% depression of contraction (to transmural electrical stimulation of ileal segments) was followed by administration of a dose of antibiotic known to produce less than a 35% depression, the resulting inhibition was usually greater than would be predicted from the arithmetic sum of the individual events. This potentiation was usually more marked in the presence of atropine and, under these conditions, the blockade produced by gentamicin was not only greater but the spontaneous reversal seen with low concentrations of gentamicin was absent.

From these results it would appear that the ability of the named antibiotic compounds to abolish the peristaltic reflex proper and evoked muscle action potential complexes arises from an effect on interneuronal transmission, since we have previously shown that at these concentrations the antibiotics are virtually without postjunctional activity (Lees & Percy, 1981); their effect is not due to a local anaesthetic action because nerve action potentials are unaffected.

The clinical significance of the interaction between antibiotic compounds and somatic neuromuscular blocking agents is well appreciated and the results of the present study imply that there may be a potentially hazardous interaction between antibiotics and muscarinic antagonists or opiate agonists given for routine surgical procedures.

The effects of these antibiotics on evoked transmitter release from the guinea-pig ileum is currently under study.

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Inhibition by perivascular nerve stimulation of the rebound contraction of the rat gastric corpus muscle to field stimulation

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Field stimulation of the rat gastric corpus muscle in the presence of atropine (10^{-6} M) produces an inhibitory response which is followed, after stimulation, by a rebound contraction (Hunt, Parsons, Wahid & Wilkinson 1978). Furthermore as these responses are unaffected by adrenergic neurone and adrenaline receptor blocking agents, Hunt, *et al.*, (1978) concluded that the responses were due to the stimulation of non-adrenergic, non-cholinergic (NANC) nerve fibres.

In these experiments we were attempting to elucidate the effect of sympathetic nerve stimulation on the NANC responses. A strip of rat gastric corpus was bathed at 36°C in Krebs – Hensleit solution containing atropine sulphate (10^{-6} M). While our observation that perivascular nerve stimulation (1 ms, 30 Hz, supramaximal voltage) had no effect on the inhibitory response to simultaneous field stimulation (2 ms, 7 Hz, supramaximal voltage, duration 30–45 sec) agrees with results reported by Jansson & Martinson (1966) in the cat, we noted that the subsequent rebound contraction was depressed. Field stimulation at 500 s intervals took 10–30 min to restore control rebound contractions.

If perivascular nerve stimulation (30 Hz) was begun immediately after the inhibitory component of the response had ended the subsequent rebound contraction was abolished and instead a sympathetic relaxation induced. Furthermore low frequency (3–8 Hz) stimulation of the perivascular nerve also inhibited the development of the rebound contraction but produced a negligible relaxatory response. This inhibition of the rebound contraction was maintained throughout the duration of perivascular nerve stimulation (2, 4 and 6 min). On termination of the stimulation a contraction occurred.

As perivascular nerve stimulation at low frequencies does not give rise to a rebound contraction it would seem that the contraction observed was the delayed excitatory component of the NANC responses. The size of the rebound contraction was affected by the length of delay and was reduced to $26 \pm 6.8\%$ (mean ± s.e.mean $n = 4$) of control values after 6 min of perivascular nerve stimulation. In control responses the rebound contraction persisted for only 120 ± 10.3 s (mean ± s.e.mean $n = 4$), thus perivascular nerve stimulation apparently allowed this response to appear at a time when the degree of contraction of the tissue should have returned to the base level.

Hexamethonium (5×10^{-5} M) and phentolamine (10^{-5} M) failed to prevent the perivascular nerve inhibition of the rebound contraction, while guanethidine (10^{-5} M), propranolol (10^{-5} M) and section of the perivascular nerve between the electrode and the gastric strip removed the inhibition.

In conclusion, rebound contractions can be inhibited by low frequency adrenergic neurotransmission mediated by perivascular nerve stimulation. Since a contraction is observed after periods of perivascular nerve stimulation in excess of the normal duration of the rebound response, adrenergic mechanisms can apparently prevent the synthesis or the release of spasmogen. Alternatively adrenergic neurotransmission may antagonise the action of the spasmogen and in addition delay its metabolism. It is also possible that the delayed rebound contraction is produced by a different mechanism to that occurring after NANC nerve stimulation.

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An analysis of the receptors involved in 5-hydroxytryptamine-induced smooth muscle relaxation

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5-Hydroxytryptamine (5-HT) produces vasodilatation *in vivo* but the mechanism has frequently been shown to involve inhibition of neurogenic tone (Page & McCubbin, 1953; Feniuk & Humphrey, 1980; Feniuk, Humphrey & Watts, 1981). However evidence is now accumulating that 5-HT can also produce smooth muscle relaxation by a direct action (McKeever, Croft & Coder, 1959; Eyre, 1975) and we are currently attempting to determine the receptor type(s) involved.

Cat lateral saphenous veins were cut into strips and prepared for measurement of isometric contractions as described for the dog saphenous vein (Humphrey, 1978). Segments of guinea-pig ileum were prepared for measurement of isotonic contractions and maintained in a modified Krebs (Apperley, Humphrey & Levy, 1976) containing atropine (1.0×10^{-6} mol/l).

In the cat saphenous vein contracted with potassium chloride (3.0×10^{-2} mol/l), α-methyl-5-HT (1.0×10^{-5} mol/l) or methoxamine (5.0×10^{-6} mol/l), 5-HT (1.0×10^{-7}–1.0×10^{-4} mol/l) added in a cumulative manner caused concentration-dependent relaxation. The maximum effect produced by 5-HT varied according to the spasmogen but when methoxamine was used was equivalent to $69 \pm 3\%$ of the total relaxable tone; the concentration necessary to produce 50% of this maximum effect (EC_{50}) was $2.9 \pm 1.2 \times 10^{-7}$ mol/l (mean ± s.e.mean, $n = 23$). In the guinea-pig ileum contracted with histamine (3.0×10^{-7} mol/l), 5-HT had a similar effect with an EC_{50} of $1.4 \pm 1.1 \times 10^{-6}$ mol/l ($n = 8$). The relaxant effects of 5-HT on the two preparations were not antagonized by propranolol (1.0×10^{-6} mol/l), indomethacin (3×10^{-5} mol/l) or atropine (1.0×10^{-6} mol/l). The effects of the 5-HT-receptor blocking drugs, methysergide and cyproheptadine against the relaxant effects of 5-HT are shown in Table 1.

Table 1 Effects of methysergide and cyproheptadine on the relaxant effects of 5-HT

Antagonist		*Cat saphenous vein*	*Guinea-pig ileum*	*Rabbit aorta (contraction)*	*Dog femoral artery (contraction)*
Methysergide	pA_2	6.75 (6.50 – 7.00)	7.37 (7.02 – 7.73)	8.49 (7.85 – 9.14)	8.52 (8.03 – 9.01)
	slope	1.09 (0.91 – 1.27)	0.88 (0.71 – 1.09)	0.78 (0.51 – 1.05)	0.88 (0.56 – 1.20)
Cyproheptadine	pA_2	<7.0	<7.0	8.73 (8.36 – 9.10)	8.55 (8.39 – 8.71)
	slope	—	—	0.90 (0.71 – 1.09)	1.18 (0.96 – 1.40)

The cat saphenous vein was contracted with methoxamine and the guinea-pig ileum was contracted with histamine such that the contraction was 50–75% of the maximum response obtainable to the given spasmogen.
pA_2 values and slopes were calculated by the method of Arunlakshana & Schild (1959) and values shown are the mean (95% confidence limits) of 4–8 observations.
Results in rabbit aorta and dog femoral artery are taken from, Apperley, Humphrey & Levy (1976) and Apperley, Feniuk, Humphrey & Levy (1980) for comparison.

Our results suggest that 5-HT mediates smooth muscle relaxation in both preparations by stimulation of 5-HT receptors which although weakly blocked by methysergide are not of the classical D-receptor type like those which mediate contraction in rabbit aorta and dog femoral artery.

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The effect of chronic treatment with Cd^{2+} on the noradrenaline and dopamine-β-hydroxylase contents of the tissues of the rat

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Chronic treatment of rats with Cd^{2+} has been found to induce hypertension (Fadloun & Leach, 1980) and to increase the responsiveness of vascular and non-vascular preparations to field stimulation, noradrenaline (NA) and K^+, NA and field stimulation being most affected and the K^+ the least, (Fadloun & Leach, 1981a, b). The present experiments were designed to elucidate whether these changes in the responsiveness and the Cd^{2+}-induced hypertension are a reflection of biochemical changes at the neurotransmitter turnover level.

Neonatal 6-hydroxy dopamine sympathectomy was achieved using the method reported by Fadloun & Leach (1981a). Sympathectomized and non-sympathectomized rats aged 14 weeks were treated with Cd^{2+} (25 ppm) for 4 weeks, Cd^{2+} added to the drinking water. These treatment parameters had previously been found to induce suitable hypertension, (Fadloun & Leach, 1980). At the end of the four week treatment period, NA and dopamine-β-hydroxylase (DBH) concentrations were measured in five different tissues: heart, kidney, portal vein, vas deferens, anococcygeus muscle according to the method of Fadloun & Leach (1978).

The NA content of the tissues removed from non-sympathectomized Cd^{2+} treated rats (NScd) was significantly increased compared to that of the non-sympathectomized untreated rats (NSc). The NA content of the heart, kidney, portal vein, vas deferens and anococcygeus muscle were increased by 53, 16, 99, 57, 38% respectively over the controls. DBH concentration in these same tissues was increased, although the extent of the increase being smaller than that seen with NA. DBH levels in the heart, kidney, portal vein, vas deferens and anococcygeus muscle were increased by 17, 6, 19, 22, 34% respectively over the control values.

Neonatal sympathectomy caused considerable depletion in the NA of the five tissues studied. NA concentrations in the heart, kidney, portal vein, vas deferens, anococcygeus were 3.5, 14, 11, 14, 7% respectively of those of the non-sympathectomized. DBH concentrations in these same tissues were also reduced, but to a lesser extent than that of NA reduction. The DBH content in the homogenates of the heart, kidney, portal vein, vas deferens and anococcygeus were 20, 26, 27, 30 and 25% respectively of that of the non-sympathectomized controls. Cadmium treatment to sympathectomized animals did

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not significantly change the levels of either NA or DBH in the five tissues under investigation.

These results support our previous suggestions that the modification of adrenergic function of the rat is an important part of the action of Cd^{2+} and that the observed hypertension following Cd^{2+} administration is the result of altered sympathetic nervous system activity.

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Differences in the inhibitory potency of opioids on the epididymal and prostatic halves of the mouse vas deferens

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Evidence has accumulated to suggest that the mouse vas deferens contains μ-receptors mediating the actions of morphine-like compounds, as well as the predominant δ-receptors, for which the enkephalins have a high affinity (Wüster, Schulz & Herz, 1980). Since the epididymal and prostatic halves of the vas differ in their contractile response to field stimulation (McGrath, 1978) it was decided to investigate whether inhibitory responses to opioids, designated as μ- or δ-agonists, differ in the two halves of the vas deferens.

Albino mice, BALB/C strain weighing 25–30 g, were used. In all experiments, a single vas deferens was bisected into epididymal and prostatic halves which were mounted in identical tissue baths as previously described (Kitchen & Hart, 1981). Field stimulation between linear platinum electrodes was provided by a Grass S 88 stimulator (0.1 Hz, 1 ms, 390 mA) and isometric contractions were recorded

Table 1 Relative potencies of opioids in the epididymal (EPI) and prostatic (PROST) halves of the mouse vas deferens.

	ID 50 (nM) (mean ± s.e.mean)		*Potency ratio (mean ± s.e.mean)*
	EPI	PROST	$\frac{\text{EPI}}{\text{PROST}}$
Met-enkephalin	13.7 ± 1.6	17.1 ± 1.3	0.86 ± 0.14 (11)*
Leu-enkephalin	7.4 ± 0.9	9.1 ± 0.8	0.90 ± 0.13 (11)*
(D-Ala^2, D-Leu^5)-enkephalin	0.65 ± 0.067	0.68 ± 0.035	0.96 ± 0.09 (11)*
Normorphine	305 ± 35	257 ± 31	1.25 ± 0.13 (7)
Morphine	339 ± 51	243 ± 17	1.43 ± 0.20 (10)

ID_{50} values (concentration producing 50% inhibition of the twitch) were calculated for each compound from linear regression of four points between 20 and 80% maximum. Potency ratios (paired experiments) are expressed as $\frac{ID_{50}\text{ epididymal}}{ID_{50}\text{ prostatic}}$

Number of observations in parentheses, Student's t-test versus morphine (log potency ratios) $^*P<0.05$

using Dynamometer UFI transducers linked to a Devices M4 recorder. Tissues were stimulated at 8 min intervals and drug additions made on a 9 min time cycle. The inhibitory responses of the two tissues to met-enkephalin, leu-enkephalin, (D-Ala^2, D-Leu^5)-enkephalin, normorphine and morphine were investigated in paired experiments.

The enkephalins were more effective at inhibiting the twitch response in the epididymal half, whilst the alkaloids, morphine and normorphine, were more potent in the prostatic half (Table 1). Excluded from the data in Table 1 are two experiments where the epididymal half of the vas was extremely insensitive to met- and leu-enkephalin ($ID_{50} > 67$ nM). It is interesting that in these two experiments the sensitivity to the other opioids was normal.

These results suggest that μ- and δ-receptors are not equally distributed along the whole length of the vas, and that the μ-receptors may predominate in the prostatic end.

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Effects of guanidine, guanethidine and tetrodotoxin on the outflow of tritium from rat vas deferens preloaded with [^{3}H]-noradrenaline.

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Guanethidine and tetrodotoxin (TTX) contain a guanidine group. Guanidine HCl increases the release of noradrenaline (NA) from the electrically stimulated perfused spleen of the cat (Hirsch, Kirpekar & Prat, 1979). It has been suggested that guanidine acts by increasing the availability of calcium ions to intraneuronal sites. Guanethidine decreases the release of NA from sympathetic nerves. Different mechanisms have been suggested as responsible for this effect of guanethidine (for references see Hausler & Haefely, 1979). TTX prevents the entry of Na ions into nerves and thereby prevents the propagation of action potentials (Kao, 1966). We have studied the effect of these agents on the release of tritium from [^{3}H]-NA labelled vasa deferentia. Tyrode solution containing 60 mM KCl was used to stimulate NA release. Under these conditions, the release is proportional to the concentration of Ca in the medium, and agents modifying the entry of Ca into nerves will alter the amount of release. In normal Tyrode solution (containing 1.8 mM Ca), these agents did not alter the release of tritium. When the

Table 1 Release of tritium from [^{3}H]-NA labelled rat vasa deferentia

	Ca^{2+} concentration (mM)		
	1.8	0.67	0.25
Control	1.34 ± 0.09	0.59 ± 0.15	0.24 ± 0.07
Guanethidine (2 μg/ml)	1.44 ± 0.11	0.08 ± 0.02	−0.26 ± 0.01
Guanethidine (20 μg/ml)	0.99 ± 0.03	−0.11 ± 0.03	−0.08 ± 0.03
TTX (0.1 μg/ml)			0.32 ± 0.03
TTX (1 μg/ml)	1.55 ± 0.10		0.51 ± 0.06
Guanidine (0.5 mM)	1.28 ± 0.12		0.21 ± 0.05

Release was induced by exposure of the tissue to a Tyrode solution containing 60 mM KCl. Figures are increase of tritium outflow during stimulation period as percent of tissue radioactivity. Mean ± s.e.mean of at least 3 experiments are given.

concentration of Ca was reduced to 0.67 or 0.25 mM, only guanethidine decreased significantly the release of radioactivity. Guanidine did not increase tritium release induced by KCl (Table 1). It is concluded that guanethidine probably decreases the entry of Ca ions into sympathetic nerve endings. Present results are not in accord with the suggestion (Hirsch *et al.*, 1979) that guanidine increases the availability of Ca ions to sympathetic nerve endings.

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The extraneuronal 0-methylation of [^{3}H]-(±)-isoprenaline by guinea-pig tracheal rings *in vitro*

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The extraneuronal disposition of catecholamines in guinea-pig trachea has previously been studied by measuring their accumulation in the tissue (Foster, 1969; Bryan, Cole & O'Donnell, 1980). However, this site of loss involves not only the uptake but also the subsequent metabolism of catecholamines (Trendelenburg, 1980). Thus, we have investigated the 0-methylation of [^{3}H]-(±)-isoprenaline by guinea-pig tracheal rings *in vitro*.

Tracheal rings, each comprising two cartilage bands, were pooled from several male guinea-pigs (400–450 g) and aliquots of 3 to 10 rings were incubated at 37°C in Krebs solution (pH 7.4) with 7-[^{3}H]-(±)-isoprenaline ([^{3}H]-ISO; 0.1 to 200 μmole/l). After appropriate times, rings were separated from respective supernatants before tritiated catecholamine and metabolite was extracted by the method of Head, De La Lande, Irvine & Johnson (1980). The unchanged substrate, in both supernatants and ring extracts, was separated from 0-methyl [^{3}H]-(±)-isoprenaline ([^{3}H]-OMI) by the method of Graefe, Stefano & Langer (1973). When inhibitors were studied, rings were incubated with them at 37°C for 20 min prior to and during subsequent incubation with [^{3}H]-ISO.

At a [^{3}H]-ISO concentration of 1 μmole/l, [^{3}H]-OMI appeared in the tissue with a half-time to steady-state of about 10 min. This approximates the half-time for efflux of [^{3}H]-OMI from the tissue since total [^{3}H]-OMI formation (in tissue and supernatant) attained steady-state rates after a lag of no more than 3 min. When rings were incubated with [^{3}H]-ISO at concentrations of 0.1 to 200 μmole/l total [^{3}H]-OMI formation proceeded at steady-state rates for up to 40 min and could be described by Michaelis-Menten kinetics ($K_M = 6.3$ μmole/1; $V_{max} = 0.31$ nmole g^{-1} min^{-1}); there was also preliminary evidence for a lower affinity 0-methylating system. In a muscle-rich section of the trachea, the rate of 0-methylation was approximately three times greater than in whole rings. However, considerable 0-methylating activity remained in the cartilage-rich section and this was not abolished by removing the endothelium.

The 0-methylation of [^{3}H]-ISO (0.1 μmole/l) by tracheal rings was completely inhibited by the COMT inhibitor U-0521 (100% at 100 μmole/l) whereas corticosterone, an inhibitor of $Uptake_2$, only partially inhibited 0-methylation (a maximum of about 40% at 30 μmole/l and above). Histamine, which is often used to induce tone in the isolated trachea, inhibited 0-methylation approaching a maximum of 75% at 1 mmole/l. This might be due either to competition between histamine and [^{3}H]-ISO for the extraneuronal transport mechanism (Kalsner, 1975) or to competition between COMT and histamine N-methyl transferase for the mthylating co-factor, S-adenosylmethionine. Inhibition of 0-methylation was not associated with tissue contraction since two other contractile agonists, bethanechol and 5-hydroxytryptamine, did not strongly inhibit [^{3}H]-OMI formation.

In summary, the 0-methylation of [^{3}H]-ISO by guinea-pig tracheal rings is a high affinity, saturable process, that seems to involve two mechanisms only

one of which is linked with a steroid-sensitive transport mechanism. It remains to be seen whether these 0-methylating systems are functionally-distinct, one being associated with smooth muscle and the other associated with cartilage.

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Modulation of bronchoconstrictor responses to histamine in pithed guinea-pigs by sympathetic nerve stimulation

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The low noradrenaline content of the lungs when compared with other organs, e.g., the heart (Holtzbauer & Sharman, 1972) is reflected by the generally sparse adrenergic innervation of bronchial smooth muscle (Richardson, 1979). Whilst sympathetic nerve stimulation has been shown to inhibit bronchoconstrictor responses in pithed guinea-pigs (Burden, Parkes & Gardiner, 1971), the bronchodilator contribution of neuronal noradrenaline may have been obscured by the concomitant release of adrenal catecholamines as the technique employed involved electrical stimulation of the complete thoracic spinal outflow. The functional role of sympathetic innervation to the lungs has been re-investigated in the present study by a more precise method of spinal nerve stimulation.

Airway responses in pithed pump-ventilated guinea-pigs were measured by changes in pulmonary inflation pressure, an adaptation of the method originally described by Dixon & Brodie (1903). Bronchoconstriction induced by histamine (2 to 20 μg/kg i.v.) was antagonized by electrical stimulation (40 V, pulse width 0.5 ms, 0.125 to 8 Hz for 10 to 120 s) of thoracic sympathetic nerve trunks via a miniature electrode assembly similar to that described by Armstrong & Boura (1973), incorporated within the pithing rod. Skeletal muscle twitches were prevented by tubocurarine (1 mg/kg i.v.). Inhibition of bronchoconstriction varied with the position of the stimulating electrode in the spinal canal. Using a single stimulus frequency (1 Hz) for 120 s, two maxima were identified (Figure 1a). One was at the level of the 9th and 10th thoracic vertebrae, where it was clear that the adrenal glands were being stimulated. The other was at the level of the 3rd and 4th thoracic vertebrae which was present in adrenalectomized animals and accompanied by tachycardia, not seen at the adrenal position (Figure 1b). This second bronchodilator effect was frequency related over the range 0.125–8Hz and was virtually abolished by pretreatment (48 and 24 h earlier) with reserpine, (5 mg/kg i.p.) as was the accompanying tachycardia. Propranolol (0.01–1 mg/kg i.v.) antagonized the bronchodilator effect of a 10 s stimulus at the level of the 3rd/4th thoracic vertebrae; higher concentrations (1–3 mg/kg i.v.) were required to block the effect of supramaximal (120 s) stimulation. These observations are consistent with bronchodilator tone being derived from neuronally-released noradrenaline within the lungs, probably overflowing from well-innervated vasculature adjacent to sparsely-innervated airways.

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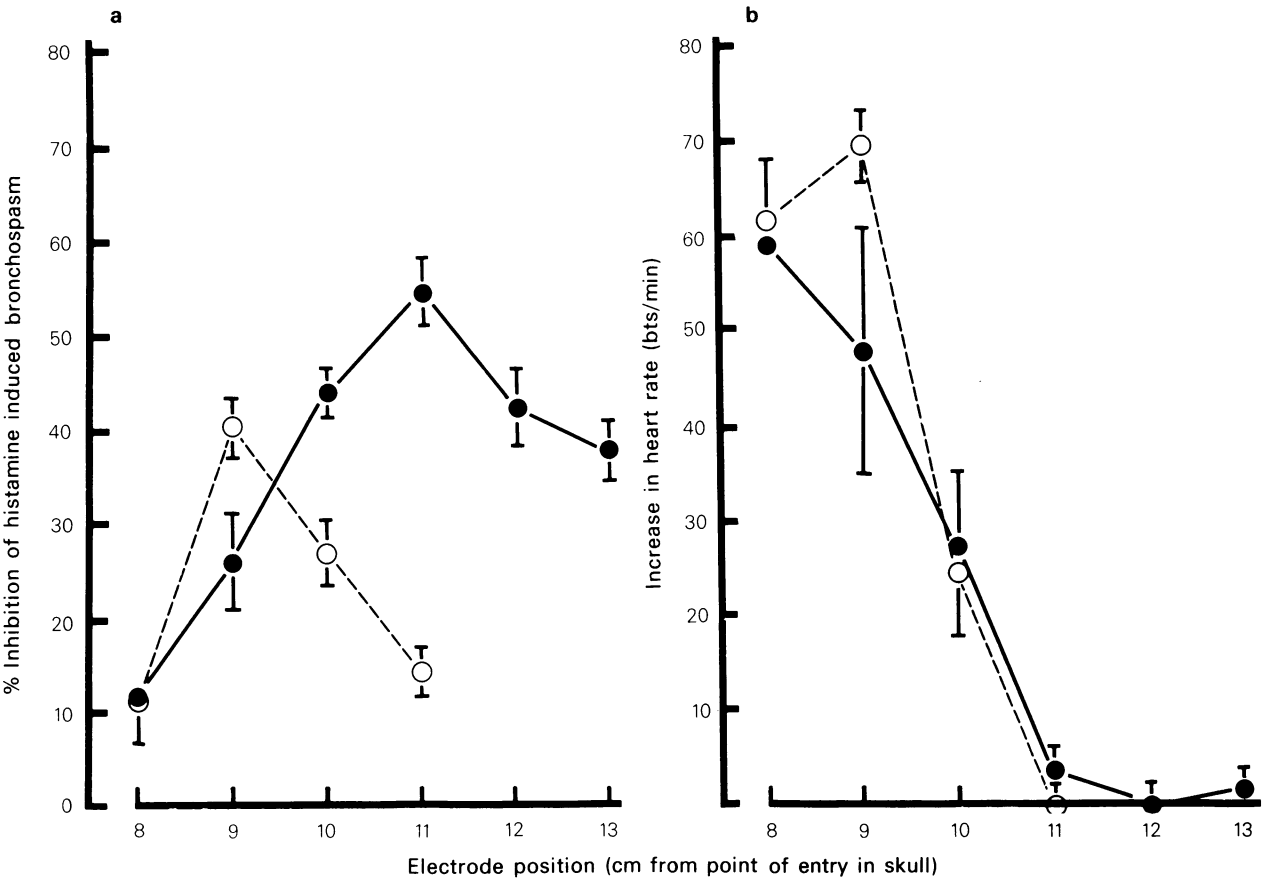

Figure 1 (a) inhibition of histamine-induced bronchoconstriction together with (b) concomitant changes in basal heart rate following electrical stimulation at a frequency of 1 Hz of the spinal nerve trunks of pithed guinea-pigs with the stimulating electrode at various positions. (●—●) Intact animals, $n = 3-7$ (○---○), adrenalectomized animals, $n = 5$. Vertical bars, indicating s.e.mean, are shown where $n = > 3$. The 9 cm and 11 cm positions correspond to thoracic vertebrae 3/4 and 9/10 respectively.

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Antagonism of calcitonin induced analgesia by ionophore A23187

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Calcitonin (CT), by intracerebroventricular (i.c.v.) injection, induces analgesia in several species (Pecile, Ferri, Braga & Olgiati, 1975; Bates, Buckley, Eglen & Strettle, 1981a). The analgesic action of CT can be antagonized in mice by the s.c. injection of naloxone (Bates *et al.*, 1981b) and by the i.c.v. injection of small doses of calcium ions (Bates *et al.*, 1980). The ionophore, A23187, has been shown to facilitate the movement of calcium and magnesium ions across membranes (Reed & Lardy, 1972). We have investigated the effects of A23187, calcium and magnesium on the analgesia induced by i.c.v. injection of CT.

Groups of 6–10 mice (♂,♀CFLP, 30–40 g) were given unilateral i.c.v. injections (volume = 10 μl) of calcium ($CaCl_2$), magnesium ($MgCl_2$), ionophore A23187 or salmon calcitonin (SCT 2 Iu/kg = 1.18×10^{-10} moles/kg). These agents were dissolved in a solution of 50% dimethyl sulphoxide in tris-saline pH 7.4 (Bates *et al.*, 1981a, DMSO vehicle). Control animals received vehicle alone. Analgesia was assessed 10 min later by the acetic acid – abdominal constriction test (Bates *et al.*, 1981a). Statistical analyses were performed with the Mann-Whitney *U* test.

The sensitivity of the control animals receiving DMSO vehicle to peritoneal irritation by acetic acid (3.05 ± 0.11 constrictions/min, mean ± s.e.mean), was not significantly different from that of animals receiving tris-saline vehicle (Bates *et al.*, 1981a).

SCT (2 Iu/kg) produced a significant ($P < 0.001$) decrease of 30% in the frequency of abdominal constriction. However, 3.3 μmoles/kg calcium ions significantly ($P < 0.001$) increased the frequency of abdominal constriction by 36%. Magnesium ions (3.3 μmoles/kg) had no effect on the frequency of abdominal constriction. A23187 (117 nmoles/kg) caused a significant ($P < 0.05$) increase of 26% in the frequency of constrictions. Doses of 11.7 nmoles/kg and 1.17 nmoles/kg had no effect. The hyperalgesic effects of A23187 (117 nmoles/kg) and calcium ions (3.3 μmoles/kg) were not additive. Administration of magnesium ions (3.3 μmoles/kg) did not affect the response to either ionophore or SCT. In contrast, magnesium ions have been shown to be equipotent with calcium ions in antagonizing morphine-induced analgesia (Harris, Loh & Way, 1975).

SCT analgesia was antagonized by A23187 (117 nmoles/kg and 11.7 nmoles/kg). The analgesic potency of SCT was reduced approximately 100 fold by the simultaneous injection of 11.7 nmoles/kg A23187. This dose of A23187 alone was not hyperalgesic and did not affect neuromuscular co-ordination or exploratory behaviour as assessed by standard rotating drum and head-dipping tests respectively.

We conclude that the analgesic effect of SCT can be antagonized by A23187. This antagonism is possibly produced by alteration of the fluxes of calcium ion, rather than magnesium ion, within the brain.

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Autoradiographical localization of cholecystokinin-receptor binding in rat brain and pancreas in ivtro using ^{3}H-CCK$_8$ as radioligand

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Cholecystokinin (CCK) is a peptide hormone produced by the small intestine in response to the movement of food from stomach into the intestine. It causes contraction of the gallbladder and activation of the pancreas. Immunohistochemical studies have shown that CCK is also present in the CNS, where, in part, it coexists with dopamine in a sub-population (40%) of meso-limbic neurons (Hökfelt, Skirboll, Rehfeld, Goldstein, Markey & Dann, 1980). Cell firing is potently stimulated by CCK in cerebral cortex, hippocampus and in the A9, A10 regions. Although CCK$_8$ is the main fragment released from nerve endings, most laboratories have used CCK$_{33}$ modified by [^{125}I]-Bolton-Hunter reagent as radioligand for receptor binding studies (Saito, Sankaran, Goldfine & Williams, 1980). We therefore synthesized [^{3}H]-CCK$_8$ in order to characterize and localize CCK receptors by autoradiography.

In cerebral cortex, high affinity binding sites for [^{3}H]-CCK$_8$ were found. The radioligand could be displaced by CCK$_8$ (sulfated IC_{50} = 2.8 nmol/l, non-sulfated IC_{50} = 20), CCK$_4$ (IC_{50} = 31), [Mox^3]-CCK$_8$ (IC_{50} = 22), [Mox^3 – Mox^6]-CCK$_8$ (IC_{50} = 47), Gastrin I (IC_{50} = 21), but not by CCK$_{26-29}$ tetrapeptide (sulfated IC_{50} > 1000 nmol/l).

In order to localize these binding sites in the CNS, we have used a recently developed dry-mount technique for the autoradiographical localization of drug and neurotransmitter receptors (Kuhar, 1981). Slide-mounted cryostat sections were incubated with [^{3}H]-CCK$_8$ (s.a. 33 Ci/mmol/l; 1 nmol/l). Alternating sections were incubated with the radiolabel in the presence of nonradioactive CCK$_4$ or CCK$_8$ (1 and 0.5 μmol/ respectively). The sections were then processed as described by Kuhar (1981).

A high density of [^{3}H]-CCK$_8$ binding sites was observed in the olfactory bulb (plexiform layer), in limbic structures (n. accumbens, tuberculum olfactorium, cortex cinguli, n. amygdaloideus medialis), ventral neostriatum and occipital cortex as well as pancreas whereas in septum, substantia nigra, cerebellum and white matter only background amounts of radioactivity were present. When adjacent sections were incubated in the presence of an excess amount of nonradioactive CCK$_8$ (CNS and pancreas) or CCK$_4$ (CNS), the intensity of labelling correspond to background levels in grey as well as in white matter.

These findings demonstrate that CCK receptors are present in brain regions (limbic areas and striatum) where CCK-immunoreactive nerve terminals are found but not in A9, A10 cell body regions. Moreover, they lend further support to the possibility of co-transmission of CCK and dopamine in limbic cortex.

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Depression of glucose utilization in primary visual areas by quipazine

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Central 5HT systems have been reported to interact with the primary visual areas with regard to both perception (Williams, 1979) and sleep (Jouvet, 1969). Quipazine (2- (1-piperazinyl) quinoline maleate) has been shown to affect sleep mechanisms (Rodriguez, Rojas-Ramirez & Drucker-Colin, 1973) in a manner compatible with an interaction with central 5HT receptors (Green, Youdim &

Table 1 Glucose utilization in primary visual areas (μmols $100\,g^{-1}\,min^{-1}$)

Structure	*Saline*	*Quipazine maleate (mg/kg i.v.)* 1	3	10
Occipital Cortex	104±4	94±4	84±3*	63±3**
Lateral Geniculate	93±4	86±4	74±4*	67±3**
Superior Colliculus (Superficial Layer)	83±2	80±4	64±4**	60±3**
Superior Colliculus (Deep Layer)	82±2	83±3	79±4	73±3*

Data is derived from groups of five rats and are presented as mean ± s.e.mean. Statistical analysis by ANOVA and Scheffe Test. *P0.05, **P0.01.

Grahame-Smith, 1976). Therefore, in this study, we have measured the effect of quipazine administration on glucose utilization in the primary visual areas of the conscious rat (occipital cortex, superior colliculus and lateral geniculate body).

Male Sprague-Dawley rats had femoral catheters inserted under light halothane anaesthesia. A loose plaster cast was fitted around the lower body and the rats were allowed to recover consciousness. Quipazine (1.3 or 10 mg/kg i.v.) or the vehicle (0.4 mls saline) were given 10 min before the bolus injection of [^{14}C] 2-deoxyglucose (50 μCi i.v.). The quantitative autoradiographic measurement of local glucose utilization was subsequently carried out as described by Sokoloff, Reivich, Kennedy *et al.*, (1977).

Glucose utilization in the occipital cortex fell by 39% ($P<0.01$) and the lateral geniculate body was reduced metabolically by 28% ($P<0.01$). There was a differential effect by quipazine on the superficial and deep layers of the superior colliculus: with decreases in glucose utilization of 28% ($P<0.01$) and 11% ($P<0.05$) respectively (see Table 1). These changes were clearly visible on inspection of the autoradiographs.

Since glucose catabolism provides the major energy requirements for the brain, regional glucose utilization affords a direct measure of the level of function in these brain areas. This data, therefore, suggests an inhibitory role for central 5HT systems in modulating the functional activity of visually related areas in the rat brain.

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Lisuride binding to human brain

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The ergot derivative lisuride is a potentially useful drug in the treatment of Parkinson's disease (Schachter, Marsden, George, Dick, Parkes, Smith, Wilson, Horowski & Dorow, 1981), and its pharmacological effects suggest that it acts directly on dopamine receptors as an agonist.

We have investigated (^{3}H)-lisuride binding, and the effect of lisuride on (^{3}H)-spiperone binding, using partially purified membranes from human putamen taken post-mortem. Scatchard analysis was used to assess ligand affinity and the density of bind-

ing sites (B_{max}), defining saturable (^{3}H)-spiperone binding as that displaced by (+)-butaclamol (0.1 μM) (Owen, Crow, Poulter, Cross, Longden & Riley, 1978). A (^{3}H)-lisuride binding assay was developed from the spiperone method with the same conditions, again using (+)-butaclamol to define specific binding.

(^{3}H)-Lisuride binding to human putamen was found to be saturable with a B_{max} of 22.0 ± 1.7 pmol/g tissue (± s.e.mean, $n = 5$) and a typical normal K_D of 1.1 nM. Lisuride inhibition of (^{3}H)-spiperone binding showed a single sigmoidal inhibition curve yielding of K_I of 1.2 nM. The B_{max} for (^{3}H)-spiperone binding to the brains investigated above was found to be 22.7 ± 2.1 pmol/g tissue.

These data suggest that lisuride and spiperone bind to the same saturable sites in the human putamen and that lisuride inhibition of antagonist binding is similar to lisuride binding itself. This suggests that in this system lisuride exhibits binding of an antagonist type, supporting a preliminary report from Fujita, Saito, Yonehara & Yoshida (1978). Further evidence for the similarity in binding of the two ligands is found in our preliminary observations of the similar effects of ADTN which has K_I values of 1.9 μM and 2.3 μM against (^{3}H)-spiperone and (^{3}H)-lisuride respectively, and of the close correlation ($P < 0.05$) between K_D values for the two ligands in brain samples from different individuals (Reynolds & Riederer, 1981).

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Comparative studies on dihydrofolate reductase in rabbit, rat and human brain

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Dihydrofolate reductase (DHFR; tetrahydrofolate dehydrogenase E.C.1.5.1.3) catalyses the conversion of folic acid and dihydrofolate to tetrahydrofolate. Brain DHFR activity was initially reported absent from several animal species, but was later found in rabbit, calf and rat brain (see Colman & Herbert, 1980). In view of the importance of folates in brain development and their possible role in epilepsy (see Colman & Herbert, 1980); the recent proposal that 5-methyltetrahydrofolate may be an endogenous excitatory neuromodulator of central nervous transmission (Ruck, Kramer, Metz & Brennan, 1980), and the fact that DHFR reduces 7,8,dihydrobiopterin to tetrahydrobiopterin, the cofactor for tyrosine and tryptophan hydroxylases (Spector, Fosburg, Levy & Abelson, 1978), we decided to investigate DHFR activity in human brain in comparison with rabbit and rat.

Post mortem samples of human temporal cortex (grey matter) previously shown to be high in folate (Yoshino, Koike, Wakabayashi & Sawaguchi, 1979) were assayed for DHFR activity using a [^{3}H]-methotrexate (MTX) binding assay essentially as described by Kamen, Takach, Vatev & Caston (1976). This technique is based upon measurement of the stable complex formed between DHFR and methotrexate in the presence of $NADPH_2$. The age, sex, cause of death and approximate time elapsed between death and freezing of the brain after post mortem of the four cases studied were respectively: 63 year old female, renal failure, 24 h; 66 year old male, bronchopneumonia, 25 h; 67 year old male, heart failure, 5 h (but this brain was removed to cold Krebs within 30 min); 83 year old female, bronchopneumonia, 7 h. To the best of our knowledge these were essentially normal brains and none of the patients had received drugs known to inhibit dihydrofolate reductase prior to death.

DHFR was estimated in human brain samples using a crude preparation. Tissue was homogenized in 3 volumes (w/v) of 0.01M KPO_4 buffer pH 7.0 containing 0.15M KCl. the homogenate centrifuged at 12,000 × g for 15 min at + 4°C and the supernatant used in the assay. Endogenous folate competes with [^{3}H]-MTX so as to underestimate the activities found in crude liver, but not crude brain preparations (Table 1). Human brain DHFR activity was approximately 100 × less active than rabbit brain and 9 × less

Table 1 Dihydrofolate reductase activities** in rabbit, rat and human tissues

	Rabbit (New Zealand Whites; ♂ ≃ 1.5 kg)		*Rat (Wistars; ♂ ≃ 150g)*		*Human*
Enzyme preparation	Brain‡‡	Liver	Brain	Liver	Brain†
Crude	588 ± 56(4)	3141 ± 483(4)	66 ± 3(12)	2084 ± 197(6)	5.57 ±1.29
Purified*	393 ± 118(4)	9988 ± 1447(4) $P<0.005$	80 ± 14(8)	4027 ± 625(6) $P<0.020$	—

** Units are pmol ^{3}H-MTX bound/g protein (mean ± s.e.mean no. of animals in parenthesis). Activities were determined from protein saturation curves using 1 pmol of ^{3}H-MTX in an incubation volume of 1.1 ml 0.05M KPO_4 buffer for 10 min at +4°C.

* Purified DHFR was prepared through the second ammonium sulphate step described by Kamen *et al.* (1976).

† Brain samples were temporal cortex (grey matter). Individual activities were: Case 1 = 4.79; Case 2 = 4.87; Case 3 = 3.32; Case 4 = 9.29 pmol/g protein.

‡‡ Whole brain estimates; however DHFR in temporal and parietal cortex > caudate nucleus = globus pallidus = frontal cortex = cerebellum > hippocampus.

active than rat brain DHFR (Table 1). Post mortem degradation of DHFR is unlikely since there was no significant decrease in DHFR activity in rat brains left for 1 h at room temperature and then for 24 h at + 2°C before freezing compared with brains frozen immediately (109 ± 18(6) and 69 ± 6(5) pmol [^{3}H]-MTX bound/g protein respectively).

DHFR was found to be present only in low activity in these samples of human temporal cortex examined. These preliminary results may indicate that man is more dependent upon transport of reduced folates into CSF to supply the brain with metabolically active folate coenzymes. The effect of age on human DHFR activity remains to be determined.

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Enhancement of the α-receptor stimulated growth hormone release after 7 allylglycine primed seizures in the photosensitive baboon

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The stimulation of plasma growth hormone (GH) in mice to a combined infusion of apomorphine and clonidine, is markedly potentiated by pretreatment with a course of 7 electroconvulsive shocks (ECS) (Eden & Modigh, 1977). In rodents, it has been shown that the method of seizure induction is less important than the number and frequency of seizures, for producing enhanced behavioural activity to monoamine receptor stimulation (Green, 1978).

In baboons (*Papio papio*) where photically induced seizures are a naturally occurring syndrome (Meldrum, 1975), an adrenergic mechanism for the stimulation of growth hormone (GH) has been assessed.

Using chair-restrained baboons (5–6 kg), all drugs were infused intravenously through a femoral cannula, over 15 min and blood samples withdrawn at 15 min intervals post infusion, for the radioimmunoassay of GH.

The α-agonist clonidine (0.02 mg/kg; $n=6$) rapidly stimulates GH release; this response can be abolished by the prior infusion of yohimbine (0.2 mg/kg; $n=4$) or piperoxan (1.0 mg/kg; $n=4$), but not by prazosin (2.0 mg/kg; $n=4$).

Due to variability in natural seizure responsiveness between baboons, a subconvulsive dose of DL-allylglycine (180 mg/kg) was administered to ensure full tonic-clonic convulsions after stroboscopic stimulation. These allylglycine-primed seizures were induced in 6 animals 3 times per week, until a course of 7 was completed. 24 h, 7 days and 15 days after the course of 7 seizures, clonidine (0.02 mg/kg) was infused and the GH response measured. Significant elevations ($P<0.01$) occurred at 24 h, the GH response was still enhanced 7 and 15 days later.

The course of allylglycine alone did not alter the GH response to clonidine at these time intervals.

Stimulation of GH by clonidine appears to be mediated via an α_2-receptor mechanism and alteration in the GH response post seizure may involve receptor changes.

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Histaminergic and serotonergic interaction in the Δ^9-THC-induced hypothermia of the rat

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Δ^9-Tetrahydrocannabinol (Δ^9-THC) administered i.v. to rats in a dose which produces hypothermia (2 mg/kg) reduces histamine (HA) and elevates 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the whole brain (Taylor, Lewis & Fennessy, 1978; Taylor & Fennessy, 1977). To characterize further these Δ^9-THC-induced biochemical modifications, we have determined the effects of Δ^9-THC (2 mg/kg) on regional brain concentrations of HA, serotonin (5-HT) and 5-HIAA. The possible involvement of brain HA and 5-HT in the hypothermic effects of Δ^9-THC was examined further by pretreating rats with drugs which specifically interact with these aminergic systems.

Groups of 5 male Wistar rats, with permanently indwelling i.v. catheters were injected with Δ^9-THC (2 mg/kg) and the concentrations of HA, 5-HT and 5-HIAA were determined spectrophotofluorometrically (Lewis, Fennessy, Laska & Taylor, 1980; Taylor & Fennessy, 1978) at various times after injection in the hypothalamus, medulla oblongata/pons, cortex, cerebellum and mid-brain. In other groups of similarly prepared rats, body temperatures were recorded intracolonically after the animals were injected with Δ^9-THC (2 mg/kg, i.v.) following a 30 min pretreatment time with i.p. doses of atropine sulphate (1.2 mg/kg), a muscarinic receptor antagonist, mepyramine maleate (10 mg/kg), an H_1-receptor antagonist, iprindole HCl (5 mg/kg), an H_2-receptor antagonist (Kanof & Greengard, 1978) or methysergide hydrogen maleate (5 mg/kg), a 5-HT-receptor antagonist. In another experiment, L-histidine (500 mg/kg, i.p.) was given to rats 30 min before receiving a dose of Δ^9-THC which did not affect body temperature (2 mg/kg, i.p.).

After 30 min, Δ^9-THC (2 mg/kg, i.v.) decreased the brain concentrations of HA in the hypothalamus, mid-brain and cortex by 46%, 61% and 72%, respectively ($P<0.05$). HA concentrations were not significantly ($P>0.05$) altered in the medulla oblongata/pons or cerebellum. In addition, the 5-HIAA concentration in the hypothalamus was increased ($P<0.05$), the peak effect (25%) occurring at 60 min after Δ^9-THC.

Neither atropine nor mepyramine modified the hypothermic response to Δ^9-THC. However, L-histidine pretreatment resulted in a significant

hypothermia in rats treated with a dose of Δ^9-THC not affecting body temperature (2 mg/kg, i.p.). On the other hand, iprindole significantly ($P < 0.05$) attenuated the hypothermic response to Δ^9-THC while methysergide significantly ($P < 0.05$) potentiated Δ^9-THC-induced hypothermia.

These results suggest that the Δ^9-THC-induced hypothermia may be mediated by a facilitation of the release of HA within the hypothalamus, an effect possibly manifested through an H_2-receptor. Furthermore, the observations with methysergide suggest that the Δ^9-THC-induced hypothermia may initiate a reflex activation of tryptaminergic heat gain mechanisms. As such, Δ^9-THC may activate both tryptaminergic heat gain mechanisms and histaminergic heat loss mechanisms, with heat loss being the overall response.

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The effects of phospholipids and phospholipases on brain microtubule assembly *in vitro*

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Microtubules isolated *in vitro* are associated with a number of phospholipids (Daleo, Piras & Piras, 1976) and the presence of phospholipids persists in highly purified microtubule preparations (Hargreaves & McLean, unpublished observation). The aim of the present work was to investigate the effect of enzymic modification of microtubule-associated phospho-lipids and the addition of exogenous phospholipids on the rate and extent of microtubule formation.

Pig brain microtubule proteins were prepared by one cycle of temperature-reversible assembly and disassembly (Larsson, Edström & Wallin, 1977). Microtubule formation was initiated in buffered solution of 2–3 mg/ml microtubule proteins by the addition of GTP (0.5 mM) and was monitored spectrophotometrically at 37°C as an increase in turbidity at 350 nm (Gaskin, Cantor & Shelanski, 1974).

Commercially prepared phospholipases A_2 (V.russelli) or C (C.welchii) were pre-incubated for 15 min with the microtubule protein solution at 37°C prior to the addition of GTP. Commercial preparations of the phospholipids or lysophospholipids of ethanolomine and choline were evaporated to dryness under vacuum and resuspended by sonication in the microtubule assembly buffer. These were added to the microtubule proteins immediately before the GTP.

The rate of microtubule assembly was reduced to $54.5 \pm 7.8\%$ (mean ± s.e.mean; $n = 7$) of control value in the presence of phospholipase C (0.5 units/ml) and to $80.3 \pm 4.1\%$ ($n = 5$) in the presence of phospholipase A_2 (0.22 units/ml). The maximal extent of assembly after 20 min was reduced to $69.2 \pm 6.0\%$ ($n = 7$) by phospholipase C and to $78.0 \pm 1.9\%$ ($n = 6$) by phospholipase A_2 at those concentrations. Inhibition by phospholipase A_2 was partially reversed in the presence of the phosphatidyl choline analogue dimethyl-DL-2,3-distearoyloxypropyl-2-hydroxyethyl ammonium acetate (20 μM). The effect of phosphilipase C was not reversed by soya bean trypsin inhibitor, but electrophoretic analysis of the microtubule proteins showed that phospholipase C treatment reduced the amount of high molecular weight microtubule proteins.

Phosphatidyl ethanolamine (30 μM) increased the extent of microtubule assembly at a number of microtuble protein concentrations (for example by $31.0 \pm 5.6\%$; $n = 3$, at 1.6 mg/ml) and significantly reduced the critical concentration (i.e. the minimum concentration of microtubule proteins necessary for assembly to be detected). Phosphatidylcholine (30 μM) had a very slight qualitatively similar effect. The lysophospholipids (30 μM) produced a slight re-

duction in the extent of microtubule assembly and a slight increase in critical concentration.

The results indicate that at least one of the phospholipids associated with microtubule proteins may have a role to play in microtubule formation.

AJH was an SRC scholar.

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Hyperactivity induced by ergot alkaloid derivatives following injection into the nucleus accumbens

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The bilateral injection of ergometrine into the nucleus accumbens of conscious rats causes a strong and long-lasting stimulation of locomotor activity. This drug does not however cause locomotor stimulation following intraperitoneal injection (Pijnenburg, Woodruff and Van Rossum, 1973). In the present study, we have further characterized the locomotor stimulant action of ergometrine and have additionally investigated the actions of some other ergot alkaloid derivatives. The techniques of implantation of cannulae and microinjections were as described by Pijnenburg *et al.*, 1973. Locomotor activity was measured in conventional activity cages. As previously reported, the bilateral injection of ergometrine into the nucleus accumbens caused an initial period of hypoactivity, which lasted for about 35 min followed by a long-lasting (7 h) stimulation of locomotor activity. The threshold dose for ergometrine was about 11 nmol and the maximum response was produced by ergometrine (45 nmol). The locomotor stimulant action of ergometrine was unaffected by α-methyl-*p*-tyrosine (250 mg/kg) but was blocked by the bilateral intraaccumbens injections of the following neuroleptics; (±)-sulpiride (1.5 nmol, each side), spiperone (11 nmol), fluphenazine (15 nmol), cis-flupenthixol (30 nmol). Other ergot derivatives were similarly active in producing locomotor stimulation following bilateral injections into the nucleus accumbens. Pergolide (90 nmol) and bromocriptine (105 nmol) caused strong locomotor stimulation which lasted for 20 h and 18 h respectively.

The intraperitoneal injection of ergometrine (10 mg/kg or 20 mg/kg) in rats had little effect on locomotor activity. However, rats which had been injected i.p. with haloperidol for 28 days (days 1–7, 0.35 mg/kg twice daily; days 8–14, 0.65 mg/kg twice daily; days 15–21, 1.5 mg/kg twice daily and thereafter 2.5 mg/kg twice daily), showed a strong stimulation of locomotion (duration 4 h) when injected with ergometrine (10 mg/kg, i.p.) 2 days following cessation of haloperidol treatment. Thus, it seems that rats with supersensitive central dopamine receptors respond to i.p. injections of ergometrine.

Our results provide further evidence that the locomotor stimulant action of ergometrine is mediated via dopamine receptors and additionally show that pergolide and bromocriptine have a similar action to ergometrine following injection into the nucleus accumbens.

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Intracerebroventricular hemicholinium-3 (HC-3) impairs learning of a passive avoidance task in mice

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In senile dementia of the Alzheimer type (SDAT), one of the most marked features is a disturbance of memory. Biochemical studies using brain tissue obtained at autopsy have consistently demonstrated a severe loss of choline acetyltransferase and acetylcholinesterase in various brain regions in SDAT (see, for example Perry, Perry, Blessed & Tomlinson, 1977). However, muscarinic receptor levels in SDAT are indistinguishable from those in age matched controls (Davies & Verth, 1977). In view of this apparent association between abnormal presynaptic cholinergic function and memory loss in SDAT, the present study was carried out to determine whether disruption of presynaptic cholinergic function by pharmacological intervention affects memory processes in animals.

We have examined the effects of intracerebroventricular (icv) administration of hemicholinium (HC-3), on the learning of a one-trial passive avoidance task (after Jarvik & Kopp, 1967). Training consisted of administering a 1 s footshock (0.5 mA) on entry to the dark compartment of a two-compartment box. Control animals retested after 24 h showed a median latency of entry into this compartment greater than 120 s. Drugs were dissolved in Krebs' solution and injected, in a volume of 5 μl, directly into the third ventricle of conscious mice (Haley & McCormick, 1957).

Direct icv injection of HC-3 (0.31–5 μg) 1 h prior to training produced a significant and dose-related reduction in latency of entry on retesting compared to vehicle treated controls. Thus, median entry latencies were typically reduced to less than 30 s by HC-3 treatment ($P<0.05$, Mann-Whitney U-test). The learning deficit induced by HC-3 (1.25 μg) was apparent when injected 0.5, 1 h or 2 h pretraining, but not 4 h pretraining, or at any time post-training. It is unlikely that changes in sensory perception or motor performance contributed to the effects of HC-3 since there were no changes in either nociceptive footshock threshold, spontaneous locomotor activity, or coordination (rotarod) in drug treated animals.

The present results using HC-3, and those of Glick, Crane, Barker & Mittag (1975) using pyrrolcholine, provide general support for the concept that cholinergic mechanisms are involved in the acquisition and consolidation of learning tasks in animals (see Hunter, Zornetzer, Jarvik & McGaugh, 1977). Moreover, it is suggested that disruption of central cholinergic neurotransmission by drugs acting presynaptically may provide a useful animal model of the memory disturbance of SDAT.

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