NEUROGENIC ANION SECRETION BY GUINEA-PIG ISOLATED DUODENAL MUCOSA

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Experiments on guinea-pig isolated duodenal mucosa revealed spontaneous, rhythmic increases in short-circuit current (SCC) in voltage clamped tissues, and a pharmacological study of the control of these effects was undertaken.

Duodenal mucosae, dissected free of muscle, were mounted in Ussing chambers (Ussing & Zehran, 1951). The mucosae were bathed bilaterally with Krebs-Henseleit solution at 37°C gassed with 95%O_2/5%CO_2; indomethacin (1μM) was added to the serosal bath. The transepithelial potential was maintained at zero using a voltage clamp amplifier and the applied SCC continuously measured. The spontaneous increase in charge transfer across the epithelium above the basal rate was determined from the area under the SCC/time traces, and converted to equivalents of monovalent ion flux using the Faraday relationship. This charge transfer was measured during a 30min control period, the test compound added and charge transfer measured again for a further 30min. All drugs were added to the serosal bath and their effects recorded as percentage inhibition compared with control.

In control experiments the spontaneous increase in charge transfer above basal was 0.12 ± 0.03μEq$(cm^2.30min)^{-1}$ (mean ± s.e. mean, n=4), and there was no difference between two consecutive 30min periods. The loop diuretic piretanide (1mM) inhibited the spontaneous increase in charge transfer by 64.1 ± 8.8% (n=4: $P<0.05$), indicating that the SCC was due to electrogenic anion secretion (Frizzell et al, 1979). Tetrodotoxin (0.3 to 30 nM) produced a concentration-related inhibition of charge transfer, with an ID_{50} of 2.4 (1.1-4.7, 95% confidence limits)nM, indicating inhibition of neuronal transmission (Catterall, 1980). The following antagonists did not affect charge transfer; phentolamine (1μM), sotalol (1μM), mepyramine (0.1μM), 8-phenyl theophylline (adenosine, 10μM) and MDL72222 (5HT, 0.3μM). Hexamethonium inhibited charge transfer by 42.5 ± 4.7% at 100μM, but no inhibition was observed at 1mM. Ketanserin and N-methylscopolamine inhibited charge transfer at concentrations which are selective for $5HT_2$ and muscarinic receptors respectively, the results are shown in Table 1.

Table 1 Effect of antagonists on the spontaneous secretion in duodenal mucosa

Antagonist	Receptor	Concentration(μM)	%inhibition
N-methylscopolamine	Muscarinic	0.001	30.2 ± 10.0
		0.01	58.0 ± 6.3*
		0.1	62.8 ± 5.8*
Ketanserin	Serotonin($5HT_2$)	0.003	46.8 ± 9.1*
		0.03	55.1 ± 7.4*
		0.3	79.9 ± 2.5*

* : significant inhibition ($P<0.05$, paired t-test): Mean ± s.e. mean, n=4-6

These results suggest that enteric neurones in the mucosa and submucosa of the duodenum control mucosal ion transport, and that $5HT_2$ and muscarinic receptors are involved.

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205P

SOME PROPERTIES OF MAST CELLS OBTAINED BY HUMAN BRONCHOALVEOLAR LAVAGE

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Asthma is characterized by widespread and variable intrathoracic airflow obstruction caused, in part, by the release of chemical mediators from mast cells. These cells are widely distributed throughout the human lung but the mastocytes lying immediately adjacent to the airways might be expected to be of major importance in modulating the initial phases of the allergic response. These cells would come into immediate contact with inhaled antigen and release their mediators directly onto the airway surface. For this reason, we have now studied the pharmacological properties of mast cells obtained by human bronchoalveolar lavage (BAL).

BAL was carried out, using conventional techniques, on patients undergoing bronchoscopy for routine diagnostic purposes. All gave informed consent. Cells were recovered by centrifugation and processed for morphological examination and measurement of histamine release as previously reported (Atkinson et al, 1979).

Of the cells obtained by BAL of non-asthmatic subjects, 0.25 ± 0.07% (mean ± s.e. mean, n = 14) were mast cells as identified by staining with alcian blue-safranin after fixation in Carnoys solution (6:3:1; ethanol:chloroform:acetic acid). This number was reduced twenty-fold when formol saline (10% formalin in physiological saline) was used as fixative. This property is characteristic of mucosal mast cells of the gastrointestinal tract (Strobel et al, 1981).

The mast cells released histamine in dose-dependent fashion on challenge with anti-human IgE, showing that they carry this antibody on their surface and possess membrane receptors for the isotype. The release process was rapid, being essentially complete within 2 minutes, and the cells secreted up to 30% of their total histamine. The release was blocked by metabolic inhibitors, showing that it depended on an intact cell metabolism. The cells were unresponsive to the polybasic chemical histamine liberators compound 48/80, polylysine and peptide 401 from bee venom ($\leq$10 µg/ml).

Immunologically induced histamine release was blocked in dose-dependent fashion by disodium cromoglycate. The maximum inhibition approached 50% (IC_{30} ca. 3 µM) and was obtained following a 10 minute period of incubation. The drug was significantly more active against the lavage cells than against those isolated by enzymic dissociation of whole lung. In contrast, salbutamol was equiactive against the two preparations.

Most interestingly, increased numbers of mast cells (1.41 ± 0.3%, n = 10) were recovered by lavage of asthmatic subjects and these cells responded to immunological challenge with an enhanced release of histamine.

In total, the present findings show that human mast cells may be conveniently isolated by BAL and used directly for functional studies. In view of the potential importance of this cell type in immediate hypersensitivity reactions in the lung, their further pharmacological properties should be studied in great detail. Work to this end is currently in progress.

This work was supported by grants from Fisons Pharmaceuticals Ltd, The Jules Thorn Trust, MRC, NATO, SERC and Wellcome Trust.

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ALTERATIONS IN HISTAMINE H_1-RECEPTOR ACTIVITY BY 1,4-DITHIOTHREITOL IN GUINEA-PIG ILEUM

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1,4-Dithiothreitol (DTT), an agent which reduces disulphide linkages to sulphydryl groups, has been reported to potentiate selectively histamine H_1-receptor-mediated contractions in rabbit aorta (Fleisch et al, 1973). A similar selective increase in sensitivity to histamine has been reported in guinea-pig ileum (Glover, 1979), although, other studies have suggested that DTT produces a non-specific potentiation of spasmogenic activity in this tissue (Watson & Iversen, 1982). In order to resolve this difference we have compared the effect of DTT on contractile responses to histamine (in the longitudinal smooth muscle of guinea-pig ileum) with the effect of this reducing agent on the responses to other spasmogens in the same smooth muscle segment.

Strips of the longitudinal muscle layer of guinea-pig (Hartley strain, 300-400g) ileum were suspended in 10 ml of Krebs-Henseleit solution, gassed with O_2/CO_2 (95:5%) at 37°C. Contractions to alternate doses of histamine and a second spasmogen were recorded isotonically. DTT (1mM) was added to the reservoir solution and allowed to equilibrate with the tissue for 30 min prior to the determination of subsequent agonist dose-response curves. Treatment with DTT was found to increase the potency of all histamine H_1-receptor agonists tested, as indicated by the parallel shift to the left of agonist dose-response curves (Table 1). In contrast, the effects on contractions elicited by acetylcholine and KCl were minimal.

Table 1 The effect of DTT on contractile responses in guinea-pig ileum

Agonist	Potentiation (EC_{50} [-DTT]/EC_{50} [+DTT]) of response to: Agonist	Histamine	(n)
Acetylcholine	1.8 ± 0.4 #	14.5 ± 1.1	(6)
KCl	1.5 ± 0.3 #	14.0 ± 2.8	(6)
H_1-agonists			
2-Thiazolylethylamine	6.3 ± 1.3	6.0 ± 1.4	(4)
2-Pyridylethylamine	8.2 ± 3.9	8.8 ± 3.9	(3)
N^{α},N^{α}dimethylhistamine	13.0 ± 2.9	10.7 ± 1.8	(3)

Values are mean ± s.e.mean of the ratio of EC_{50} values obtained in the presence and absence of DTT. # $P < 0.01$ (paired t test) with respect to the increase in sensitivity to histamine measured in the same muscle strip.

The apparently selective enhancement of H_1-mediated responses by DTT did not appear to be due to inhibition of histamine-metabolising enzymes since a similar increase in sensitivity was observed following blockade of both diamine oxidase and histamine N-methyltransferase with aminoguanidine (1μM) and SKF91488 (10μM, Beavan & Shaff, 1979) respectively. The mechanism of action of DTT on H_1-mediated responses is unclear but it is likely to result from the reduction of disulphide bonds since it was readily reversed by the sulphydryl oxidising agent 5,5'dithiobis-(2-nitrobenzoic acid). These results suggest that DTT may be a useful tool with which to investigate histamine H_1-receptor mechanisms in ileal smooth muscle.

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207P

DOSE- AND VEHICLE-RELATED VARIATION IN THE DISPOSITION OF BENZYL ACETATE IN THE RAT

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Benzyl acetate (BA) finds application as a fragrance agent in perfumes and toiletries and as a flavour in foods and non-alcoholic beverages. Due to the widespread exposure of the human population to this compound, there is interest in its safety evaluation. Knowledge of the metabolism and pharmacokinetics of test compounds is important in the design and interpretation of animal toxicity tests, and we now report on the effect of dose size and dose vehicle upon the metabolism and pharmacokinetics of benzyl acetate in the rat.

Male Fischer 344 rats (200g b.w.) received [methylene-^{14}C]-BA by gavage at 5, 250, or 500 mg/kg (5μCi/animal), as the neat substance or in corn oil or propane-1,2-diol (5 ml/kg). Their urine and faeces were collected and urinary metabolites assayed by TLC and HPLC. Other animals were sacrificed at various times, exsanguinated and plasma levels of ^{14}C and metabolites determined.

Peak levels of ^{14}C occurred earliest and were highest when BA was given neat, with lower peak levels and delayed absorption with the propane-1,2-diol vehicle. The use of corn oil as the dose vehicle led at the higher doses to the maintenance of plateau plasma levels, about one-half the peak levels seen with the neat compound, for up to 8h after administration. At the 5 mg/kg dose, the plasma levels of ^{14}C were essentially the same whether the dose was given in corn oil or propane-1,2-diol. At the 250 and 500 mg/kg doses, at all time points the major metabolite in the plasma was benzoic acid, accompanied by smaller amounts of hippuric acid. Benzyl alcohol was also detected in some plasma samples. At the 5 mg/kg dose, the major plasma metabolite was hippuric acid, together with a smaller amount of benzoic acid. When propane-1,2- diol was used as the vehicle, benzyl mercapturic acid was also present. At all dose levels, there were also one or more minor unknown metabolite(s) present in plasma. The urinary elimination of ^{14}C was rapid in all cases, with 70-89% of the dose recovered in 24h. There was no systematic variation in rate or route of excretion of radioactivity with either dose size or vehicle. In each case, the major urinary metabolite was hippuric acid (ca. 66% of dose), with benzoic acid (2%) and benzyl mercapturic acid (1%) also present. The elimination of benzoyl glucuronide increased with increasing dose, from ca. 3% to 11% of dose.

This study has shown the ability of both dose size and dose vehicle to influence the plasma pharmacokinetics, but not urinary metabolic pattern, of benzyl acetate. These data should be borne in mind when attempting to use results of experiments using high doses of benzyl acetate in corn oil, to indicate the potential hazard of the low dose dietary exposure of man to this compound.

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THE INFLUENCE OF THE IMMUNE MODULATOR, POLYrI.rC UPON THE METABOLISM OF ASPIRIN IN MICE

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It is known that perturbation of the immune system can markedly influence the metabolism of xenobiotics, and it appears that increased levels of the interferons mediate most of the metabolic changes seen (Renton, 1984). However, scant attention has been paid to the conjugation reactions in this regard and as part of a systematic evaluation of this problem, we have studied the influence of the interferon inducer polyrI.rC on the metabolism of aspirin in mice. As aspirin is extensively conjugated with glycine and glucuronic acid, it is an appropriate compound for this purpose.

Male DBA/2 mice (25g b.w.) received polyrI.rC (min. m.w. 100kD; Sigma) in sterile saline 10 mg/kg i.p. or an equivalent volume of vehicle 24h prior to use. Groups of mice were then given hexobarbitone (85 mg/kg i.p.) for determination of sleeping time or [carboxyl-^{14}C] aspirin (100 mg/kg p.o.; 5μCi/mouse). Urine and faeces were collected daily for 3 days, and urinary metabolites assayed according to Hutt et al. (1982).

Pretreatment of mice with polyrI.rC resulted in a significant prolongation of hexobarbitone sleeping time, from 42.1±7.3 min (mean ± S.D.) to 70.2±17.2 min ($p<0.01$, $n=12$). Urinary metabolites of aspirin in treated and control animals are summarized in the Table.

Table 1 Influence of polyrI.rC on the metabolism of aspirin in mice

Metabolite	Saline treatment	polyrI.rC	p
Salicylic acid	17.1±5.1	13.7±2.1	n.s.
Salicyluric acid	34.4±2.9	45.4±7.0	<0.01
Gentisic acid	3.2±0.7	0.3±0.2	<0.001
Total glucuronides	44.0± 5.0	39.5±7.3	n.s.
% dose in 0-24h urine	86.3±6.8	77.4±10.8	n.s.

The demonstration of prolonged hexobarbitone sleeping time in poly rI.rC treated mice confirms the depression of oxidative drug metabolism by this agent as reported by others (see Renton, 1984). However, the metabolic conjugation of salicylate appeared to be unaffected by polyrI.rC treatment, apart from an increase in the excretion of salicyluric acid. The significance of this is presently unknown. In contrast, polyrI.rC elicited a 10fold reduction in the excretion of the oxidation product of aspirin, gentisic acid. It will be necessary to examine other substrates and other conjugation pathways, and to study the effects of other immune modulators, before firm conclusions can be drawn as to the effects of altered immune status on drug conjugation. We are presently performing such studies.

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209P

INHIBITION OF GANGLIONIC TRANSMISSION REVEALS A PRESSOR EFFECT OF EXOGENOUS CORTICOSTERONE ACETATE IN CONSCIOUS LONG EVANS RATS

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Inhibition of ganglionic transmission with pentolinium initially causes a profound hypotension in conscious rats, after which BP recovery is dependent on angiotensin II and vasopressin (Bennett & Gardiner, 1983). In the present study we have examined the effects of prior acute administration of corticosterone acetate (7.5 $mgkg^{-1}$; 5 $mgkg^{-1}h^{-1}$ i.v.; n=8) or its vehicle (propylene glycol; n=8) on BP in this model of hypotension.

Male Long Evans rats were anaesthetized (sodium methohexitone; 60 $mgkg^{-1}$ i.p.) and the jugular vein and abdominal aorta were cannulated. Recordings of systolic and diastolic BP and heart rate were begun 4½-5 h after the rats had regained consciousness. Corticosterone acetate, or its vehicle alone, were without effect on BP although there was a small bradycardia in the steroid-treated group. Administration of pentolinium (5 $mgkg^{-1}$; 5 $mgkg^{-1}h^{-1}$) 45 min after the steroid or vehicle initially caused a hypotension in both groups, but the level to which BP fell remained higher ($P<0.01$) in the steroid-treated group (93±5/57±4 mmHg; systolic/diastolic; mean±s.e.mean) than in the group receiving vehicle (72±4/44±2 mmHg). During the following 45 min of pentolinium infusion, BP showed some recovery in both groups but the levels reached in the steroid-treated animals were always higher than in the vehicle-treated rats. Subsequent treatment with captopril (2 $mgkg^{-1}$; 1 $mgkg^{-1}h^{-1}$) 45 min after the start of the pentolinium administration, caused falls in BP of similar magnitude in both groups (steroid= -28±5/20±3; vehicle= -28±6/21±5 mmHg). In the presence of captopril and pentolinium, BP showed some recovery which was more effective in the steroid-treated group (106±6/64±3 mmHg, 15 min after captopril) than in the vehicle-treated animals (84±4/52±3 mmHg; $P<0.001$). Antagonism of the cardiovascular actions of vasopressin with $d(CH_2)_5$DAVP ([1-(β-mercapto-β, β-cyclopentamethylenepropionic acid), 8-D-arginine] vasopressin; 10 $\mu g.kg^{-1}$; 10 $\mu gkg^{-1}h^{-1}$) abolished the recovery of BP in the vehicle-injected animals but not in the steroid-treated group.

Other workers have reported a beneficial effect of corticosteroids in hypotensive shock induced by endotoxin (Brackett et al 1983) although the mechanism is unclear. The present results show that corticosterone acetate can exert a significant pressor effect following antagonism of ganglionic transmission, angiotensin II production and the vascular actions of vasopressin.

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LACK OF RESPONSE OF GUINEA-PIG ILEUM PREPARATIONS TO METOCLOPRAMIDE FOLLOWING ABLATION OF ENTERIC NEURONES WITH TRITON X-100

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In an attempt to investigate the relative importance of pre- and postjunctional mechanisms in the action of metoclopramide (MTC) upon the gastrointestinal tract (See review by Fernández & Massingham, 1985), we have now examined the effects of this drug in guinea-pig ileum preparations taken from animals treated with Triton X-100 (TX) to produce a selective ablation of the enteric neurones (Fox et al. 1983). Male tricolour guinea-pigs (350±50g) were anaesthetized with pentobarbitone (40 mg/kg ip). Following laparotomy an 8-10 cm segment of ileum, identified using suture tags, was brought outside the peritoneal cavity and the serosal surface bathed with a 1% solution of TX every 5 min for 30 min. The ileum was then rinsed thoroughly with 0.9% saline and returned to the peritoneal cavity. Control animals received local application of saline for a similar time period. 30 days after the operation the animals were killed, the TX or sham-treated ileum segments removed, cleaned and placed in Krebs' solution for histological and mechanical studies. Low power (x32) microscopic examination of TX-treated ileum longitudinal muscle-myenteric plexus (LM-MP) preparations (Paton and Vizi, 1969) showed the absence of a typical network of nerves and a reduced number of ganglia was confirmed by high power (x125) examination of longitudinal sections (5 μM) of whole ileum mounts following haematoxylin-eosin staining. Mechanical studies using LM-MP preparations demonstrated that although tissues treated with TX responded normally to exogenous acetylcholine (ACh, 0.001-1 μM), they exhibited much less spontaneous activity than control strips and were unresponsive to dimethylphenylpiperazinium (10 μM) and MTC (30 μM). In addition concentration-response curves to ACh in TX-treated preparations were not significantly modified by the presence of MTC (10 μM) in the Krebs' solution. In control ileum segments the putative neuronal 5-hydroxytryptamine (5-HT) antagonist cisapride (Neya et al. 1984) caused a concentration-dependent inhibition of 5-HT (3 μM) responses (0.1 μM, 22±7%; 0.3 μM, 29±5%; 1 μM, 72±9% inhibition). Cisapride had little sustained intrinsic activity and did not affect twitch responses to MTC (30 μM) but did inhibit contractions to MTC in unstimulated preparations; the inhibitions being 37±5%, 52±6% and 59±8% for 0.1, 0.3 and 1 μM cisapride. However, at 0.3 and 1 μM, cisapride reduced maximum responses of the tissue to ACh by 14±3% and 28±3% respectively. These results suggest that, as in rat jejunum (Fox et al. 1983), TX can ablate myenteric neurones in the guinea-pig ileum. Furthermore, in agreement with the findings of Massingham et al. (1985), MTC-induced contractions of this tissue would appear to be mediated indirectly and predominantly via a pre- rather than a postjunctional effect of the drug, the involvement of a 5-HT receptor in this response however remains unproven.

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211P

A NON-ADRENERGIC NON-CHOLINERGIC (NANC) EXCITATORY RESPONSE IN FROG ATRIA

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Frog heart receives its innervation only through the vagosympathetic trunks which contain both preganglionic parasympathetic and postganglionic sympathetic fibres. Stimulating these nerves or the intramural nerve endings with a short pulse train produces an initial inhibitory response, followed by an excitatory response mediated through adrenaline, the sympathetic transmitter in the frog (Angelakos et al, 1965). I show here that when the initial inhibitory response is blocked by atropine, a NANC response is revealed.

Male Rana temporaria of about 20 g body weight were decapitated and pithed. The atria were removed and mounted in an organ bath superfused with room-temperature Ringer's solution. The atria beat spontaneously. Isometric tension or shortening was recorded. Square wave pulses of up to 50 V were applied via silver-silver chloride field stimulation (FS) electrodes of 0.5 cm^2 surface area.

In the presence of atropine 10^{-5}M (used throughout the following experiments unless otherwise stated) exogenous acetylcholine in concentrations up to 10^{-3}M evoked only inhibitory responses, though its LDR curve was shifted 2.3 log units to the right. The amplitude of the NANC response to FS was not reduced by the β blockers propranolol, pronethalol or practolol in concentrations up to 10^{-4}M or by the α blockers phentolamine, co-dergocrine or phenoxybenzamine in concentrations up to 10^{-5}M, but was reduced greatly by guanethidine 10^{-5}M. The muscarinic response to FS was not reduced by 10^{-5}M guanethidine. If the vagosympathetic trunks were cut (in the living frog under MS222 anaesthesia), one week later there was no trace of monoamines when the atria were examined by the fluorescence technique of Falck and Hillarp (Gillespie & Kirpekar, 1966). The adrenergic and NANC responses to FS were absent in the denervated atria.

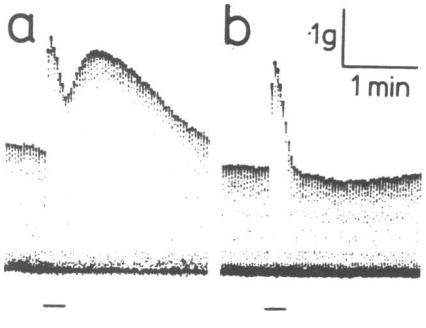

Figure 1. Responses to FS (320 pulses at 20 Hz; pulse width 0.01 ms, pulse height 15 V) in atropinised atria. (a) before and (b) after addition of propranolol 10^{-6}M to the bathing fluid.

I conclude that during FS a mediator that does not interact with α, β, muscarinic or nicotinic receptors is released from a postganglionic neurone that has similar characteristics to the postganglionic adrenergic neurone and, indeed, may be the postganglionic adrenergic neurone.

Some of this work was carried out while FD was in receipt of an MRC scholarship.

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FURTHER ANALYSIS OF ANOMALOUS pK_B VALUES FOR MUSCARINIC ANTAGONISTS ON THE MOUSE STOMACH ASSAY

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Although Angus & Black (1979) found atropine to be a simple competitive antagonist at muscarinic receptors coupled to gastric acid secretion in the isolated, stomach preparation of the mouse, the pK_B ($-\log K_B$) estimates were significantly lower than those found on other muscarinic receptor systems. Rather than proposing receptor subclasses these differences in pK_B's were attributed to a reduction of the steady-state concentration in the receptor compartment due to loss of the antagonist into the gastric secretion. Since then, m_1 and m_2 subclasses of muscarinic receptors have been proposed and the selective inhibition of acid secretion by pirenzepine has been attributed to its high affinity for m_1-receptors (Giachetti et al., 1982). We have re-examined this problem using guinea pig trachea (Emmerson & Mackay, 1979) and an improved isolated mouse stomach assay (Black & Shankley, 1985). Using 5-methylfurmethide as agonist, pK_B's for atropine, pirenzepine and N-methylatropine (a highly polar derivative of atropine) have been measured on both assays.

The pK_B for atropine on the stomach assay (table 1) was significantly different ($P < 0.001$) from that obtained on the trachea. No significant differences in the estimated affinities of either N-methylatropine or pirenzepine were detected between the stomach and trachea assays.

Table 1 pK_B estimates for muscarinic receptor antagonists on the mouse stomach and guinea pig trachea assays.

	pK_B ($\pm$ s.e.mean)			
	guinea pig trachea	mouse stomach	ΔpK_B	log P
Atropine	8.93 $\pm$ 0.16	7.78 $\pm$ 0.11	1.15 $\pm$ 0.19	1.83
N-methylatropine	9.69 $\pm$ 0.13	9.67 $\pm$ 0.11	0.02 $\pm$ 0.17	-0.40
pirenzepine	6.87 $\pm$ 0.09	6.67 $\pm$ 0.09	0.20 $\pm$ 0.13	0.20

We conclude that the oxyntic cell receptors are homogeneous with those in the guinea pig trachea. Apparent differences in the affinity of antagonists (ΔpK_B) may correlate with their lipophilicities (log P). This supports the hypothesis that the underestimation of antagonist affinity is due to loss of the antagonist into the gastric secretion from the receptor compartment. Apparently relatively selective inhibition of acid secretion, compared to atropine, could be explained without the need to postulate heterogeneity of muscarinic receptor populations.

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213P

ACTION OF PHYSOSTIGMINE ON INOTROPIC AND CHRONOTROPIC SELECTIVITY OF MUSCARINIC AGONISTS

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Muscarinic receptors (mAChRs) present in the myocardium may differ from those present in either the CNS or ileum (Mitchelson, 1984). It has also been reported (Barlow et al, 1980; Chaissing et al, 1984) that from agonist potencies mAChRs mediating negative inotropic and chronotropic effects may represent two mAChR subtypes. However, high concentrations of acetylcholinesterase (AChase) in nodal tissue (Webb, 1950) may influence the negative chronotropic effects of such muscarinic agonists. We have investigated the effect of physostigmine on the potency of muscarinic agonists at mAChRs mediating negative chronotropic and inotropic effects.

The agonist potency (pD_2) of muscarinic agonists was determined as described previously (Clague et al, 1984). Chronotropic and inotropic effects were determined simultaneously either in the absence or presence of physostigmine (5×10^{-7} mol.litre^{-1}) from spontaneously beating atria.

Only acetylcholine and AMP exhibited a significant difference between pD_2 values at mAChRs mediating chronotropic and inotropic effects (Table 1). The selectivity of these agents for mAChRs mediating negative inotropic effects was abolished in the presence of physostigmine.

Table 1 Agonist potencies (pD_2) at atrial mAChRs in absence and presence of physostigmine.

			+ Physostigmine	
Agonist	Rate	Force	Rate	Force
Acetylcholine	5.95	6.92*	7.15	7.07
Methacholine	6.42	6.32	6.30	6.31
Carbachol	6.92	7.04	6.51	6.48
Bethanechol	4.62	4.71	4.95	4.95
Oxotremorine	7.65	7.76	7.60	7.65
Muscarine	6.13	6.11	6.23	6.23
Arecoline	6.61	6.79	6.81	6.91
APE#	7.33	7.26	7.58	7.49
AMP##	No effect	2.83*	No effect	No effect

APE - Arecaidine propargyl ester
##AMP - 3 acetoxy N methylpiperidine methiodide
Values are mean, n = 6; SEM is less than 5% in each case. *$P < 0.005$.

We conclude that the majority of muscarinic agonists do not discriminate between mAChRs mediating negative inotropic and chronotropic effects. Also, the selectivity observed with acetylcholine may be due to its susceptibility to AChase degradation. Finally the low potency of AMP makes difficult any definitive conclusion with regard to its selectivity

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THE EFFECT OF MONOCLONAL ANTIBODIES ON THE FUNCTION OF MOUSE ACETYLCHOLINE RECEPTOR

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Monoclonal antibodies against the acetylcholine receptor (AChR) bind acutely to specific determinants on the receptor/channel protein (Whiting, 1984). We have investigated the effect of such specific binding on the functioning of the AChR. In previous work the effect of polyclonal anti-AChR antibodies on AChR function was studied (Dolly et al, 1983) but such preparations have limited specificity.

Monoclonal antibodies were raised in mice against human AChR as previously described (Whiting, 1984). Solutions containing 1-3 mg/ml monoclonal antibody in phosphate buffer were dialysed against Krebs solution. Phosphate buffer alone, treated similarly, was used for controls. Mouse diaphragm muscles were incubated in oxygenated control or antibody-containing solution for 2-3½ hours. Microelectrodes were then used to record miniature endplate potentials (mepps) in the same solution (22-26°C). Muscles were washed with Krebs solution for at least 15 minutes and mepps again recorded. After this, the voltage noise produced by acetylcholine (5μm) was recorded in the presence of eserine (3μm) and tetrodotoxin (250nM) and analysis was carried out as previously described (Wray, 1981).

Of eight monoclonal antibody preparations tested, six produced no significant effect on mepp amplitudes either before or after washing with Krebs solution, indicating that these antibody preparations do not bind at the acetylcholine (ACh) binding site on the receptor.

The remaining two antibody preparations reduced mepp amplitudes significantly to 57 and 71% of control ($P<0.001$). The antibody preparation which produced the most significant reduction in mepp amplitude (from 1.03 + 0.06 mV, n=34 end plates to 0.59 ± 0.02 mV, n=49) was selected for further study. Afer washing a significant reduction ($P<0.001$) in mepp amplitude continued to be observed, indicating irreversible antibody binding. This antibody preparation also caused a significant ($P<0.001$) reduction in the depolarisation caused by ACh (5μm) from a control value of 18.0 ± 1.1 (n=5) to 12.1 ± 1.4 mV (n=9). Noise analysis showed that the depolarisation produced by a single channel opening was not significantly changed by the antibody (control 0.257 ± 0.015 μV) indicating a lack of action of the antibody on the open channel itself. The frequency of channel opening by ACh (5 μm) was decreased ($P<0.05$) by this antibody to 65% of control.

In conclusion, of the eight monoclonal antibodies investigated here, one type appeared to have no effect on receptor/channel protein function and the other type reduced post-synaptic sensitivity whilst having no effect on single open channel properties.

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215P

REDUCED Bmax OF [^{3}H]-IMIPRAMINE BINDING TO PLATELETS OF DEPRESSED PATIENTS FREE OF PREVIOUS MEDICATION WITH 5-HT UPTAKE INHIBITORS

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The high affinity binding sites for [^{3}H]imipramine (IMI) present in brain and platelets (Langer et al., 1981) are associated with the neuronal uptake for serotonin (5HT) and may be a biological marker in depression. Comparisons between Bmax of [^{3}H]IMI binding in platelets of untreated severely depressed patients and control volunteers in different laboratories showed some discrepancy, partly due to methodological problems, and possibly related to the suggestion of a circannual rhythm of the density of platelet [^{3}H]IMI binding (Egrise et al., 1983 ; Whitaker et al., 1984). Another important factor is the time of washout from antidepressant medication. We reported recently that one week administration of chlorimipramine (50 mg) in control volunteers is sufficient to induce decreases in Bmax of [^{3}H]IMI binding even after three weeks of washout (Poirier et al., 1984). Therefore, it was thought of interest to reexamine the KD and Bmax of [^{3}H]IMI binding in platelets from depressed patients with no previous antidepressant therapy with drugs that inhibit 5HT uptake for at least one month. In order to explore the question of seasonal variations of [^{3}H]IMI binding, a preliminary study was conducted for one year on a control population.

Blood samples from normal volunteers (6 male and 5 female, 28 to 60 years old ; mean age : 36.6 $\pm$ 3, in good health and free of drugs, were collected once a month from December 1983 to November 1984. [^{3}H]IMI binding assays were performed according to Langer et al. (1981) each month throughout the study. Data were subjected to an analysis of variance (ANOVA) (one way, 11 subjects, 12 treatments). The results indicate that there is no annual rhythm in the Bmax or in the KD values of [^{3}H]IMI binding to platelets from normal volunteers. Mean values were calculated for the control population : Bmax : 1210.8 $\pm$ 36.0 fmoles/mg prot ; KD : 0.61 $\pm$ 0.01 nM (n = 11).

We studied a group of untreated depressed patients (6 male and 10 female, 19 to 56 years old ; mean age : 39.8 $\pm$ 3) who were suffering from either mono- or bipolar endogenous depression of sufficient severity to require hospitalisation (mean Hamilton rating : 28.7 $\pm$ 2.0 ; NIMH 1967). Patients were chosen to fulfil the criteria of washout described previously (Poirier et al., 1984). Six depressed patients had no previous antidepressant therapy, while ten patients had a washout from antidepressant drugs affecting 5HT uptake of at least 1 month. Two patients had a washout of 2 weeks from maprotiline. This group of severely depressed patients had a significantly lower maximal binding (Bmax : 641.3 $\pm$ 59.9 fmoles/mg prot, $n = 16$, $p < 0.001$), than the control group. The mean KD value 1.04 $\pm$ 0.21 did not differ significantly from that of the control.

These results confirm that there is a significant decrease in Bmax values of [^{3}H]IMI binding in platelets of severely depressed patients when compared with controls. This decrease appears to represent a genuine difference, probably linked to the involvement of 5HT uptake mechanisms in depression. Our present study confirms the importance of previous antidepressant therapy on the variation of the parameters of [^{3}H]IMI binding in depressed patients. Our results failed to demonstrate a circannual rhythm in platelet ^{3}H-IMI, and confirmed the decrease in Bmax of platelet ^{3}H-IMI binding in severely depressed patients with a sufficiently long washout period to exclude artefactual changes in density of ^{3}H IMI binding.

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[^{3}H]-IMIPRAMINE AND [^{3}H]-PAROXETINE BINDING TO HUMAN PLATELETS: CLUES TO A SELECTIVITY PARADOX FROM TEMPERATURE STUDIES

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Considerable evidence has been gathered in recent years favouring a selective association of ^{3}H-imipramine (IMI) binding in various tissues with the 5-HT transporter. The specificity of ^{3}H-IMI binding is surprising in view of, amongst others, the lack of IMI selectivity towards inhibition of 5-HT uptake in comparison with noradrenaline uptake. Some of these arguments, most recently reiterated by Laduron (1984), may easily be countered (Langer, 1984), whereas others remain open to discussion. An important unknown is the contribution of temperature to uptake inhibition (37°C) and ^{3}H-IMI binding studies (0°C). This question was adressed using binding studies with ^{3}H-IMI and ^{3}H-paroxetine (PAR), a newly available selective ligand for the 5-HT transporter.

Platelet membranes prepared from frozen platelet rich plasma were incubated with 0.6 nM ^{3}H-IMI (Amersham, 20 Ci/mmol) at 0°C as described by Segonzac et al. (1984). For assays at 20°C, the incubation volumes were increased pro rata to 1 ml. ^{3}H-PAR binding (New England Nuclear, 20.3 Ci/mmol) was performed in a volume of 2 ml for 180 min at 20 or 37°C. Uptake of ^{3}H-5HT (Amersham, 13-17 Ci/mmol) into fresh human platelets was performed as described by Segonzac et al. (1984).

At 0°C, ^{3}H-IMI binding to human platelet membranes is of high affinity, (Kd : 0.6 nM). The IC_{50} of unlabelled IMI for inhibition of ^{3}H-IMI binding is 5.3 nM (Ki : 2.7 nM). In contrast, the IC_{50} of IMI for inhibition of ^{3}H-5HT uptake in human platelets is 44 nM. An explanation for this apparent discrepancy in IMI affinity may be found in the assay temperature. When ^{3}H-IMI binding is done at 20°C, its affinity decreases (Kd : 2.5 nM ; Ki : 4.1 nM). This affinity agrees well with the affinity of IMI on ^{3}H-PAR binding at 20°C (Ki : 4.6 nM). As ^{3}H-IMI binding cannot reliably be studied at 37°C, ^{3}H-PAR was used as a radiolabel at this temperature. At 37°C, the IC_{50} of IMI on ^{3}H-PAR binding (41 nM) equals that for ^{3}H-5HT uptake inhibition. Thus, the relatively high affinity measured for IMI in ligand binding studies aimed at the 5-HT transporter, can be fully explained by an effect of the incubation temperature (0°C). The selectivity of ^{3}H-IMI for the 5-HT transporter may also arise from this phenomenon, since the IC_{50} of IMI for ^{3}H-desipramine binding to the noradrenergic transporter (220 nM at 0°C) equals its IC_{50} for noradrenaline uptake at 37°C (200 nM) (Raisman et al., 1982). Temperature effects are much less pronounced for chlorimipramine with IC_{50} values of 6.2 nM at 0°C (^{3}H-IMI), and 3.6 nM (^{3}H-PAR) and 4.4 nM (^{3}H 5-HT uptake) at 37°C. In contrast, 5-methoxytryptoline, a putative endogenous ligand for the IMI receptor (Langer et al., 1984), shows a pronounced temperature sensitivity with IC_{50} values of 44 nM at 0°C (^{3}H-IMI), and 1000 nM (^{3}H-PAR) at 37°C, and 630 nM (^{3}H-5-HT uptake) at 37°C.

The present results indicate that the binding of IMI to its 5HT-uptake modulatory receptor in human platelets is temperature-sensitive. This temperature sensitivity most likely explains the selectivity for the 5-HT transporter ; the affinity of IMI for the 5-HT transporter increases with decreasing temperature, whereas its affinity for the noradrenergic transporter is apparently temperature-insensitive. Since the temperature-sensitivity varies with the compound under study, it follows that drug affinities for the IMI receptor, measured at 0°C, may not always be representative for their activity as 5HT uptake inhibitors at physiological temperature.

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217P

CHANGES IN [^{3}H]-IMIPRAMINE BINDING IN RABBITS ASSOCIATED WITH PREGNANCY AND OESTROGEN/PROGESTERONE TREATMENT

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[^{3}H]-Imipramine binding capacity is reportedly decreased in both the brain and platelets of depressed patients (Elliott, 1984). Platelet [^{3}H]-imipramine binding may therefore act as a marker for central binding sites. [^{3}H]-Imipramine binding is also reduced in maternal platelets shortly after parturition (Best et al, 1985). This study was established, therefore, to investigate (i) whether similar changes occurred at parturition in the rabbit, (ii) whether such changes could be related to oestrogenic/progestagenic status, and (iii) whether binding changes in the platelet reflected similar changes in the brain.

Female New Zealand white rabbits (weight 3-5 kg) were used throughout. Oestrogen treatment comprised 150 µg/day ß-oestradiol benzoate s.c. for 7 days whilst animals treated with both oestrogen and progestagen received an additional 10 mg/day progesterone s.c. Control animals received vehicle only. Pregnant animals were tested either ante-partum (27-29 days pregnant; gestation period 31 days) or post-partum (7-8 days after parturition). After treatment animals were anaesthetised and bled out by cardiac puncture and the brain then isolated and dissected. Following homogenisation the brain samples were centrifuged at 1000 g for 10min and the supernatant removed, centrifuged at 30,000 g for 15min and the resulting membrane preparation finally resuspended in incubation buffer (150 mM NaCl, 2.7 mM EDTA, pH 7.5). Platelets were separated from whole blood by differential centrifugation, broken by sonication and membranes prepared in identical incubation buffer. [^{3}H]-Imipramine binding (0.5-5 nM) was carried out at 2°C for 60min using 1 µM fluoxetine to define non-specific binding.

Binding capacities (fmol/mg protein) for platelet and hypothalamic membrane preparations are shown below. Each figure represents the mean ± s.e.m. for 4-8 individual animals. There were no significant differences in binding affinity.

	Control	Oestrogen	Oestrogen + progesterone	Ante-partum	Post-partum
Hypothalamus	1227 ± 70	1101 ± 137	826 ± 127*	1625 ± 137	1515 ± 215
Platelet	13042 ± 567	7538 ± 773**	6378 ± 1787**	19601 ± 1825	8687 ± 2177††

*$p < 0.05$ **$p < 0.01$ vs control ††$p < 0.01$ vs ante-partum. Unpaired t-test.

As indicated above, platelet binding is substantially reduced following parturition but this is not accompanied by an equivalent fall in hypothalamic binding. Following treatment with oestrogen and progesterone, however, a significant decrease in binding is observed in both platelet and hypothalamus. These results suggest therefore that, in the rabbit, platelet [^{3}H]-imipramine binding is more sensitive to changes in hormonal balance than the hypothalamus. Furthermore, the changes associated with pregnancy may not be attributable solely to changes in oestrogen/progestagen status.

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MECHANISM OF TACHYCARDIA ELICITED BY 5-HYDROXYTRYPTAMINE IN THE SPINAL CAT

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5-hydroxytryptamine (5-HT) causes tachycardia in the cat isolated heart directly via 5-HT receptors susceptible to d-lysergic acid diethylamide (LSD) (Trendelenburg, 1960). Since LSD has similar affinities for both 5-HT_1 and 5-HT_2 binding sites (Peroutka & Snyder, 1979), the exact identity of these receptors is uncertain. We have analysed the mechanism of 5-HT-induced tachycardia in vagotomized and spinal cats under chloralose-urethane anaesthesia.

In the initial 15 cats 5-HT (3, 10 and 30 µg/kg) caused a moderate pressor response, and tachycardia of 19±2, 41±4 and 57±4 beats/min, respectively. These responses were little changed by bilateral adrenalectomy, guanethidine (1 mg/kg), propranolol (1 mg/kg), burimamide (10 mg/kg) or by MDL 72222 (0.5 mg/kg). At a dose of 0.5 mg/kg, the 5-HT_2 receptor antagonists (all of which blocked pressor effects of 5-HT) either did not modify (ketanserin, ritanserin and cyproheptadine), or reduced (pizotifen and mianserin) or even completely eliminated (methysergide) the 5-HT-induced tachycardia. In the second group of 15 cats, compared to saline (n=5), the tachycardia caused by 5-HT (3, 10 and 30 µg/kg) was significantly affected by ketanserin (n=5) only at the highest dose, but by methysergide (n=5) at all doses used (fig. 1). Responses to isoprenaline (1 µg/kg) were not modified. Injection of 5-carboxamido-tryptamine (5-CT)

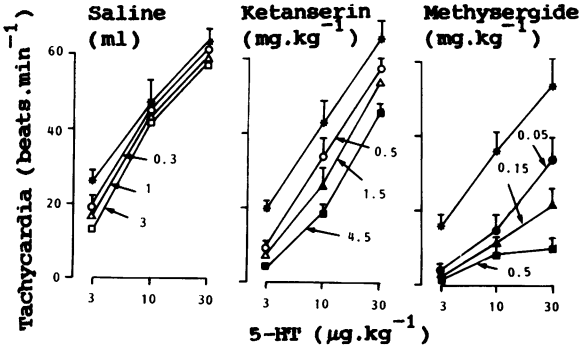

Fig. 1 5-HT-induced tachycardia in spinal cats before (*) and after saline, ketanserin or methysergide (doses indicated by arrows). Filled symbols mean that the effect was significantly (P<0.05; Duncan's test) more than that caused by saline

(0.1-10 µg/kg) caused similar increases in heart rate in cats treated with saline or ketanserin (4.5 mg/kg), but the effect was significantly less in methysergide (0.5 mg/kg)-treated animals. In subsequent 10 cats, we found that methiothepin was more potent, and metergoline less potent than methysergide in suppressing tachycardic effects of both 5-HT and 5-CT. In two other cats, 8-hydroxy-2-(di-N-proylamino)-tetralin (8-OH-DPAT), a putative 5-HT_{1A} agonist (Middlemiss & Fozard, 1983), did not cause tachycardia in a dose of upto 0.5 mg/kg.

Since 5-CT, methysergide, methiothepin and metergoline have high to appreciable affinities for 5-HT_1 binding sites (Peroutka & Snyder, 1979, Engel *et al.*, 1983) our results indicate that 5-HT_1-like receptors, though not of the 5-HT_{1A}-type, mainly mediate tachycardic response to 5-HT in the spinal cat.

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219P

RU 24969 DECREASES 5-HT RELEASE IN THE SCN BY ACTING ON 5-HT RECEPTORS IN THE SCN BUT NOT THE DORSAL RAPHE

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Neuronal release of 5-hydroxytryptamine (5HT) is modulated by an autoreceptor, thought to be of the $5HT_1$ sub-type (Middlemiss, 1982). The $5HT_1$ agonist 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)-H-indole (RU 24969) decreases 5HT release in the frontal cortex both in vitro (Middlemiss, 1984) and in vivo (Brazell et al, 1984). We now present in vivo data suggesting that RU 24969 decreases 5HT release and metabolism in the suprachiasmatic nucleus (SCN) via $5HT_1$ receptors located on the nerve terminals rather than the cell bodies.

Differential pulse voltammetry was performed using carbon fibre working electrodes implanted into the SCN of chloral hydrate (600 mg/kg) anaesthetised male Wistar rats (290-310 g). Some animals also had injection cannulae implanted in the contralateral SCN or the nucleus raphe dorsalis. The height of the oxidation peak recorded at +300 mV (peak 3) was used as an indication of the extracellular concentration of the 5HT metabolite 5-hydroxyindole acetic acid (5HIAA) (Crespi et al 1983).

Saline (2 mls/kg i.p.) did not affect the extracellular level of 5HIAA in the SCN. However the size of peak 3 fell by 37±3% (n=5) 2 hours after administration of RU 24969 (10 mg/kg i.p.). Conversely, administration of the $5HT_1$ receptor antagonist methiothepin (10 mg/kg i.p.) was associated with an increase in the height of peak 3 of 23±7% (n=4). The decrease observed 90 min after RU 24969 (28±2%, n=5) was markedly attenuated when methiothepin was given 30 min before RU 24969, there being only a 6±9% (n=4) difference from pre-injection control.

In an attempt to localise the site of action of RU 24969 we administered saline or RU 24969 into the contralateral SCN or the nucleus raphe dorsalis. Saline (1 µl) was without effect in the SCN and the nucleus raphe dorsalis. Administration of up to 10 µg of RU 24969 into the nucleus raphe dorsalis had no effect on the extracellular level of 5HIAA in the SCN. However, a dose-dependent decrease in the size of peak 3 was observed following the injection of RU 24969 into the SCN contralateral to the one in which the working electrode was implanted. The peak had decreased by 26±7% 1 hr after 5 µg and by 82±7% 1 hr after a further 5 µg (n=4).

These data suggest that the release and metabolism of 5HT in the SCN is under the control of 5HT receptors located on the nerve terminals and not the cell bodies. These receptors are probably of the $5HT_1$ sub-type.

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ALLOSTERISM IS NOT NEEDED TO EXPLAIN COMPLEX BINDING OF [^{3}H]-IMIPRAMINE IN RAT HYPOTHALAMUS

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In rat brain cortex, 5HT and non-tricyclic inhibitors of 5HT uptake have been shown to inhibit ^{3}H-imipramine (^{3}H-IMI) binding in a complex manner (Sette et al., 1983) whereas tricyclic antidepressants (TCA) showed classical competitive inhibition. The authors suggested that an allosteric modulation could exist between the IMI binding site and the 5HT uptake site. We report here, that, in the rat hypothalamus at least, the complex inhibition of ^{3}H-IMI binding is more easily explained by the existence of two independent ^{3}H-IMI binding sites.

^{3}H-IMI binding was measured essentially according to Raisman et al. (1980) using rat hypothalamic membranes. Experiments were carried out in the absence or in the presence of 120 mM sodium chloride. ^{3}H-IMI binding to rat hypothalamic membranes in the presence of sodium was inhibited by citalopram, paroxetine, F 2207, a new uptake inhibitor, and 5HT in a complex manner, with biphasic curves and Hill coefficients less than 1.0 (Table 1). In contrast to the cortex (Sette et al., 1983), TCA such as IMI, chlorimipramine and amitriptyline also inhibited ^{3}H-IMI binding with biphasic curves and Hill coefficients less than 1.0 (Table 1). In addition, in the biphasic inhibition curve, the percentage ^{3}H-IMI binding inhibited by 5HT with high affinity is decreased as the ^{3}H-IMI concentration is increased.

In the absence of sodium, IC_{50} values for the inhibition by TCA and non TCA were increased by at least 1000 fold (Table 1). In addition, the inhibition curves became classically monophasic with Hill coefficients close to 1.0 (Table 1).

Table 1 ^{3}H-IMI (3 nM) binding in the rat hypothalamus

	+ Na^+		- Na^+	
	IC_{50} (nM)	n Hill	IC_{50} (μM)	n Hill
Imipramine	39 ± 5 (n=4)	0.58	20 ± 9 (n=3)	1.12
Chlorimipramine	14 ± 2 (n=3)	0.52	34 ± 5 (n=4)	1.08
Amitriptyline	54 ± 8 (n=3)	0.76	15 ± 6 (n=4)	1.18
Citalopram	18 ± 5 (n=3)	0.33	107 ± 61 (n=3)	0.90
Paroxetine	11 ± 5 (n=4)	0.22	31 ± 6 (n=3)	0.82
F 2207	90 ± 55 (n=4)	0.29	900 (n=2)	0.90
5HT	6600 ± 4400 (n=5)	0.22	> 1000 (n=3)	-

These data are most simply explained by the existence of two independent binding sites for ^{3}H-IMI in the hypothalamus. One site is sodium dependent with a high affinity for the drugs tested, the other is sodium independent and has a low affinity for these drugs. From various recent data the sodium dependent site seems to be identical to, or associated with, the 5HT uptake site whereas the role, if any, of the low affinity site remains to be elucidated.

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EFFECT OF PAROXETINE AND OTHER 5-HYDROXYTRYPTAMINE UPTAKE INHIBITORS ON CENTRAL MONOAMINE UPTAKE IN VITRO AND EX VIVO

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Buus Lassen (1978a,b) provided evidence that paroxetine is a potent and selective inhibitor of central 5HT uptake in pharmacological tests in rats and mice and subsequently Magnussen et al (1982) confirmed this selectivity using mouse forebrain synaptosomes. Furthermore, Nelson et al (1984) showed that the compound possesses only weak affinity for rat cortical catecholamine, 5HT and histamine receptors in vitro. In the present study, the kinetics of inhibition by paroxetine of (^{3}H)-5HT uptake into rat hypothalamic synaptosomes have been investigated. In addition, its selectivity for (^{3}H)-5HT versus (^{3}H)-noradrenaline (NA) uptake has been compared with other 5HT uptake inhibitors in vitro and ex vivo.

Monoamine uptake inhibition was measured by incubating rat hypothalamic crude synaptosomes for 10 minutes at 30°C in the presence of (^{3}H)-5HT (5-250nM) or (^{3}H)-ℓ-NA (20-500nM) with or without test drug. For the experiments ex vivo, male CFY rats (5 per group) were administered orally with test drug and synaptosomes prepared 2 hours later as for the experiments in vitro.

Lineweaver-Burk analysis showed paroxetine to be a competitive inhibitor of (^{3}H)-5HT uptake in vitro with an inhibition constant (Ki) of 1.1 ± 0.1nM (Table 1). Paroxetine showed marked selectivity for 5HT compared to NA uptake in vitro with only citalopram showing greater specificity for the 5HT uptake system. Paroxetine was also a potent and selective inhibitor of (^{3}H)-5HT uptake ex vivo following acute dosing (ED_{50} 1.9mg/kg p.o.). This potency and selectivity were maintained after 14 days repeated dosing, since paroxetine (5mg/kg/day p.o.) inhibited 5HT uptake by 81 ± 3% (n=3) without affecting NA uptake under these conditions.

Table 1: Effect of 5HT uptake inhibitors on (^{3}H)-monoamine uptake into rat hypothalamic synaptosomes (a) in vitro and (b) ex vivo (acute).

Compound	(a) Ki (nM) ± s.e. mean (n≥3)		(b) ED50mg/kg p.o.	
	(^{3}H)-5HT	(^{3}H)-NA	(^{3}H)-5HT	(^{3}H)-NA
Paroxetine	1.1±0.1	352±6	1.9	>30
Citalopram	2.6±0.4	3850±664	5.9	>30
Fluvoxamine	6.2±0.1	1130±42	23	>30
Fluoxetine	25±1	504±180	7	>30
Femoxetine	36±4	706±57	28	>30
Zimelidine	171±14	8560±112	2.3	>30
Imipramine	101±11	65±4	>30	10
Desipramine	1400±200	12±5	>30	7
Kinetic parameters of control uptake : Km(nM), Vmax (pmoles/h/mg tissue)				
Km	48.2±4.1	254±27		
Vmax	10.4±0.5	12.5±0.9		

In conclusion, we have confirmed that paroxetine potently and selectively inhibits central 5HT uptake both in vitro and ex vivo and this selectivity is maintained after repeated dosing. The inhibition is competitive in nature implying a direct interaction with the 5HT uptake site. Therefore, paroxetine is particularly useful for investigating the effect of inhibition of the 5HT uptake system pharmacologically and in the treatment of depressive illness.

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5-CARBOXAMIDO-TRYPTAMINE IS A SELECTIVE AGONIST AT 5-HT RECEPTORS MEDIATING VASODILATION AND TACHYCARDIA IN THE ANAESTHETISED CAT

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5-Hydroxytryptamine (5-HT) has phasic and complex effects on blood pressure mediated via a number of different mechanisms involving more than one type of 5-HT-receptor (see Humphrey, 1983). We have therefore examined the cardiovascular effects of 5-carboxamido-tryptamine (5-CT), a selective agonist at 5-HT receptors in vitro (Feniuk, Humphrey & Watts, 1981; 1984).

Cats of either sex were anaesthetised with pentobarbitone (36-42 mg/kg i.p.) and blood pressure, heart rate, common carotid vascular resistance and tracheal inflation pressure recorded using standard techniques. Drugs were administered intravenously (i.v.) into a left femoral vein. Prior to each experiment the vagus and cervical sympathetic nerves were sectioned bilaterally and propranolol (1 mg/kg i.v.) administered. Dose-response curves to 5-HT or 5-CT were constructed and repeated after increasing doses of antagonist. In some specificity studies, isoprenaline was used as an agonist and in these experiments the cats were not pretreated with propranolol.

5-Carboxamido-tryptamine (0.01-1 µg/kg i.v.) caused dose-related decreases in diastolic blood pressure, common carotid arterial resistance and increases in heart rate which were unaffected by low doses of methysergide (10-100 µg/kg i.v.) or a high dose of ketanserin (1 mg/kg i.v.). These effects were however potently and dose-dependently antagonised by methiothepin (10-100 µg/kg i.v.) and higher doses of methysergide (300 µg/kg - 1 mg/kg i.v.). Unlike 5-CT, 5-HT (1-100 µg/kg i.v.) caused dose-related bronchoconstriction and tachycardia and complex and variable effects on diastolic blood pressure and carotid vascular resistance. The 5-HT-induced bronchoconstriction was potently and dose-dependently antagonised by all three 5-HT antagonists studied (10-100 µg/kg i.v.) whilst the 5-HT-induced tachycardia, like that induced by 5-CT, was antagonised by methiothepin (10-100 µg/kg i.v.), and high doses of methysergide (300-1000 µg/kg i.v.) but not by ketanserin (1000 µg/kg i.v.). The highest doses of methiothepin, methysergide and ketanserin studied did not modify the cardiovascular effects of isoprenaline or the bronchoconstriction induced by prostaglandin $F_{2\alpha}$.

Our findings indicate that 5-CT has a more selective action than 5-HT in vivo and suggest that its ability to produce vasodilation, hypotension and tachycardia in the cat appears to be mediated via a specific 5-HT-receptor type which is different to the 5-HT_2-receptor mediating bronchoconstriction. Thus the 5-HT_2-receptor is characterised by the high antagonistic potencies of ketanserin, methysergide and methiothepin. In contrast the 5-HT receptor mediating vasodilation, hypotension and tachycardia is characterised by the high agonistic potency of 5-CT and a rank order of antagonist potency of methiothepin > methysergide >> ketanserin (inactive). This receptor appears similar to that mediating relaxation in some isolated vascular preparations (Feniuk, Humphrey & Watts 1983, 1984; Cohen, Shepherd & Vanhoutte, 1983).

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THE MECHANISM OF INDOMETHACIN-INDUCED AIRWAY HYPER-REACTIVITY

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Histamine-induced contractions of the guinea-pig isolated trachea are augmented in the presence of arachidonate cyclo-oxygenase inhibitors (Orehek et al., 1975) and this effect is reversed by inhibitors of arachidonate lipoxygenase (Adcock & Garland, 1980). It has been proposed that selective inhibition of cyclo-oxygenase allows more arachidonic acid to be converted to a lipoxygenase product which is then responsible for the increased reactivity of the tissue.

In this study, contractions of guinea-pig tracheal strips were recorded either with auxotonic levers in a superfusion system or with isometric transducers in an organ bath. Tissues were bathed in Tyrode solution and dose-response curves to histamine were obtained in the presence and absence of the selective cyclo-oxygenase inhibitor indomethacin, the dual cyclo-oxygenase and lipoxygenase inhibitor BW755C and the cyclo-oxygenase product prostaglandin E_2 (PGE_2).

Bolus injections of histamine (10^{-9} to 10^{-5} moles) into the Tyrode superfusing tracheal strips caused dose-related contractions. In the presence of indomethacin (2.8 µM) the dose-response curves were steeper and the size of the maximum contraction was increased by 80-100% of control values. The augmentation of maximum was reversed by addition of BW755C (150 µM). At lower concentrations, however, BW755C (5-20 µM) had a similar effect to indomethacin, increasing the steepness of the histamine dose-response curve and enhancing the maximum response by up to 100%.

Tracheal strips suspended in organ baths at a tension of 1g gained tone which could be partially reduced by washing. After washing, the tissues slowly regained tone to reach equilibrium after about 10 min. Cumulative dose-response curves to histamine (1-50 µM) were obtained with equilibrated tissues and repeated in the presence of indomethacin (5 µM). Indomethacin caused a similar reduction in resting tone to that seen after washing except that the tissues did not regain tone. In the presence of indomethacin, histamine-induced contractions were augmented and the maximum was increased by approximately 100%. The increased size of the maximum response could not be accounted for by the reduced tone of the tissues. Reduction of tone caused by indomethacin was reversed by the addition of PGE_2 (0.03 µM) to the organ bath. In the presence of both indomethacin and PGE_2, histamine responses resembled those obtained before the addition of indomethacin.

These results confirm that BW755C reverses indomethacin-induced augmentation of histamine responses but the concentration of BW755C required is 30 times greater than that which inhibits cyclo-oxygenase and lipoxygenase in other tissues. At lower concentrations BW755C has the same efect as indomethacin. This suggests that hyper-reactivity to histamine is related to inhibition of cyclo-oxygenase rather than potentiation of lipoxygenase. This is supported by the observation that PGE_2 reverses the augmentation. These experiments also indicate that isolated tracheal strips produce an endogenous PGE_2-like substance which may modulate the contractions caused by exogenous histamine.

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PHOSPHODIESTERASE IN THE GUINEA-PIG CARDIAC VENTRICLE: SPECIFIC INHIBITION OF TYPE III ACTIVITY BY SK&F 94120

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SK&F 94120 is a novel and potent positive inotropic agent which also possesses vasodilator activity (Coates et al, 1985). Evidence has suggested that SK&F 94120 acts as a positive inotrope via a cyclic AMP dependent mechanism. Thus i) SK&F 94120 potentiated response to isoprenaline ii) the positive inotropic responses of SK&F 94120 were abolished by carbachol and iii) SK&F 94120 caused significant increases in intracellular cyclic AMP (Cameron et al, 1985).

Other positive inotropic agents (e.g. MDL 17,043 and amrinone) have been reported to inhibit a specific form of phosphodiesterase (PDE) isolated from dog cardiac tissue (Takashi Kariya et al, 1982). Inhibition of this specific 'low Km' form of PDE was suggested to be the mechanism by which such agents act as positive inotropic agents.

Three major forms of guinea-pig ventricular PDE could be separated by DEAE-cellulose ion-exchange chromatography and were labelled fraction I, II and III in order of their elution from the ion-exchange column. Fraction I could use both cyclic AMP and cyclic GMP as substrates and was stimulated by Ca^{2+}/calmodulin complex. Fraction II an enzyme which demonstrated a low affinity for cyclic AMP could also hydrolyse both cyclic nucleotides. Low concentrations (μM) of cyclic GMP were also shown to stimulate cyclic AMP hydrolysis by this fraction. Fraction III the smallest activity detected was cyclic AMP specific and demonstrated a high affinity for this substrate.

SK&F 94120 at a threshold concentration of 1 μM and at concentrations up to 100 μM inhibited specifically fraction III activity. This concentration range correlated well with that which caused positive inotropic effects as measured using guinea-pig ventricle strips (see fig below). A K_i could not be calculated because of the complicated kinetics demonstrated by fraction III activity for hydrolysis of cyclic AMP. Isobutylmethyl-xanthine (IBMX) was a potent and non-specific inhibitor of all fractions of PDE activity.

These data indicate that a specific inhibition of PDE fraction III is the primary mechanism of action of SK&F 94120 as a positive inotropic agent.

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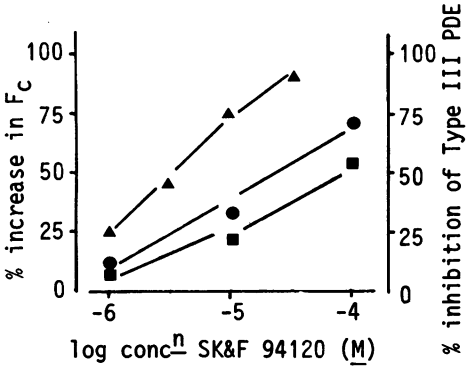

Comparison of the effects of SK&F 94120 on force of contraction (▲—▲) and inhibition of Type III PDE at 0.25 μM cAMP (●—●) and 1 μM cAMP (■—■).

225P

α,β-METHYLENE ATP INHIBITS THE VASOCONSTRICTION TO PERIARTERIAL FIELD STIMULATION IN SHR, BUT NOT WKY TAIL ARTERIES IN VITRO

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ATP released as a cotransmitter from sympathetic nerves is thought to mediate the electrical activity (e.j.p's) recorded in vas deferens and blood vessels (Meldrum and Burnstock, 1983 ; Sneddon and Burnstock, 1984). The stable ATP analogue α,β-methylene ATP (α,β-MATP) blocks these fast electrical events, but not the slow depolarisation in smooth muscle. The present paper compares the effects of α,β-MATP on responses to periarterial nerve stimulation and exogenous agonists in the tail artery preparations of SHR or WKY.

Tail artery preparations were obtained from age matched SHR or WKY (12-15 weeks), and were perfused and superfused with Krebs' bicarbonate, according to the methods of Hicks et al. (1984). Vasoconstrictor responses (Δ perfusion pressure) were evoked by either periarterial field stimulation (60 V, 0.6 ms, 0.1 - 30 Hz), NA (0.01 - 1 μM), 5HT (0.01 - 1 μM), KCl (40 mM) or the stable ATP analogue β,γ-methylene ATP (3 - 30 μM), before or after exposure to α,β-MATP (0.1 or 1 μM, 20 min). In SHR-arteries, the effects of α,β-MATP were examined on ^{3}H-release after labelling of the tissues with ^{3}H-NA.

The responses to periarterial field stimulation in SHR-arteries were significantly greater than in WKY arteries. These vasoconstrictor responses were virtually abolished in the presence of prazosin (100 nM). In both SHR and WKY arteries, α,β-MATP (1 μM) caused a short lasting contraction. In SHR arteries α,β-MATP (0.1 μM) significantly inhibited the responses to periarterial field stimulation at all frequencies, but these inhibitory effects were not further increased at 1 μM. In WKY, α,β-MATP (0.1 or 1 μM) failed to inhibit the responses to periarterial field stimulation. α,β-MATP (1 μM) did not modify the stimulation (1 Hz, 2 min), evoked release of ^{3}H in SHR-arteries. End organ responses to exogenous NA were only slightly inhibited in SHR-arteries by α,β-MATP (1 μM), and the responses to 5HT were not modified. The rapid spike response evoked by KCl (40 mM) was however significantly antagonised by α,β-MATP (1 μM), in SHR tail arteries. Vasoconstrictor responses to β,γ-MATP were not modified by α,β-MATP (0.1 μM), but were antagonised by α,β-MATP (1 μM) in a non-competitive manner.

The ATP-receptor desensitising agent α,β-MATP at concentrations which block the e.j.p.'s in the rat tail artery (Sneddon and Burnstock, 1984), failed to antagonise contractions to field stimulation in normotensive rat tail arteries. In contrast, this compound caused a highly significant inhibition of vasoconstrictor responses evoked by periarterial field stimulation in SHR tail arteries, without modifying ^{3}H-NA release. It is possible that cotransmitter release of ATP has a more important role in SHR than in normotensive rat tail arteries, and may contribute to the lower membrane potential (Cheung, 1984), increased sensitivity and contractile properties of these vessels.

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POSITIVE INOTROPIC ACTIONS OF SK&F 94120 ON HUMAN ISOLATED VENTRICULAR MYOCARDIUM

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SK&F 94120 is a novel agent possessing both positive inotropic and vasodilator activity (Coates et al, 1984) and has recently been shown to be a very selective inhibitor of cardiac type III phosphodiesterase (PDE III) activity (Gristwood et al 1985).

We have now compared the effects of SK&F 94120 and isoprenaline on human isolated ventricular preparations obtained from cardiac transplant recipients with ischaemic heart disease (n=9) or congestive cardiomyopathy (n=4), and from patients with mitral valve disease (n=5).

On electrically driven (at 1 Hz) preparations (incubated at 37°C in Krebs solution) SK&F 94120 (threshold ca 1×10^{-6}M) and isoprenaline (threshold ca 1×10^{-8}M) caused concentration dependent increases in force of contraction (F_C). Tissue responsiveness to either SK&F 94120 or isoprenaline appeared to be unrelated to the cardiac disease aetiology. Mean % increases in F_C (± s.e. mean) caused by SK&F 94120 at 1×10^{-6}M, 1×10^{-5}M and 1×10^{-4}M were 9 ± 3, 45 ± 11 and 119 ± 31 respectively. The response to SK&F 94120 at 1×10^{-4}M represented 50% (±9%) of the isoprenaline maximum response (at 1×10^{-5}M).

In preparations in which intracellular action potentials were recorded (these driven at 0.2 Hz) both SK&F 94120 and isoprenaline were found to increase the plateau height of normal action potentials ($[K^+]_o$ 5.9 mM) and also to markedly enhance slow response action potentials ($[K^+]_o$ 27 mM), indicating the ability to increase the second, Ca^{++} carried, inward current (Isi).

Evidence was obtained that Isi enhancement by SK&F 94120 in human ventricle was mediated via cyclic AMP and as a consequence of PDE inhibition. Thus, SK&F 94120 potentiated responses to isoprenaline (an effect consistent with PDE inhibition); and the positive inotropic actions of SK&F 94120 (1×10^{-4}M) were inhibited by carbachol 1×10^{-7}M - 1×10^{-6}M (indirectly implying a cyclic AMP involvement) and were associated with a significant (64% ± 18%, n=5) increase in intracellular cyclic AMP content (measured by radioimmunoassay).

The results indicate that SK&F 94120 is a positive inotrope in human ventricle and further that inhibition of PDE III in human ventricle is an effective mechanism to increase F_C.

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227P

COMPARISON OF THE EFFECTS OF ADENOSINE ON ATRIAL AND VENTRICULAR CARDIAC PREPARATIONS

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Adenosine is well known to have negative inotropic and chronotropic effects on isolated atria (Evans et al. 1982; Collis,1983). It has also been shown to exert inhibitory effects upon stimulation of the whole heart by catecholamines (Schrader et al. 1977). In the present study we have compared the effects of adenosine on isolated left and right atria with ventricular muscle.

Isolated left and right atria, papillary muscles and ventricular strips of guinea-pigs were set up in Krebs-bicarbonate solution at 38°C gassed with 5% CO_2 in oxygen. Tension responses of left atria, papillary muscle and ventricular strips paced at 2Hz (5ms, threshold voltage + 50%) and rate responses of spontaneously beating right atria were recorded.

Adenosine produced concentration-dependent inhibitions of left atrial tension and right atrial rate. The tension IC50 value was 82.5(41.3-167.3)μM and the inhibitions of tension and rate at the maximum adenosine concentration (2.25mM) were 80.6±1.1 and 44.5±13.6% (n=6). Cumulative concentration-response curves for isoprenaline were determined in left and right atria before and in the presence of adenosine (75μM). Adenosine displaced the curves to the right with significant depression ($P<0.05$) of the maximum developed tension from 0.95±0.2 to 0.61±0.12g (n=4) and the maximum developed rate from 355±2 to 339±1 beats min^{-1} (n=5). Pre-adenosine curves were corrected from controls. Adenosine was then added cumulatively to atria already stimulated by submaximal concentrations of isoprenaline (right 9.47nM; left 18.9nM). There were concentration-dependent inhibitions of rate and tension. The tension IC50 value was 35.7(16.0-80.0)μM with a 90.0±2.4% inhibition at the maximum concentration (2.25mM). The inhibition of rate was 53.7±6.7% at this maximum concentration.

Adenosine failed to exert negative inotropic effects on papillary muscles and ventricular strips, but instead produced small non-significant increases in tension. These reached 6.0±2.8 and 9.8±3.7% (n=4) respectively at the maximum concentration (2.25mM). Concentration-response curves for the positive inotropic responses of papillary muscles to isoprenaline obtained before and in the presence of adenosine (0.75mM) were superimposed at the lower part. However, in contrast to atria, the maximum developed tension was significantly increased ($P<0.05$) from 0.39±0.05 to 0.45±0.05g (n=5). Finally, when adenosine was added cumulatively to papillary muscle pre-stimulated by isoprenaline (18.9nM), there were concentration-dependent inhibitions reaching a maximum of 21.4±1.9% at 0.75mM (n=8).

Thus, in contrast to the atria, adenosine failed to exhibit negative inotropy of ventricular muscle or to antagonize isoprenaline concentration-response curves. When pre-stimulated with isoprenaline, however, there was inhibition. The high concentration required for this effect throws doubt upon the importance of its possible physiological cardioprotective role by this mechanism.

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THE EFFECTS OF ADENOSINE ON ARRHYTHMIAS INDUCED BY MYOCARDIAL ISCHAEMIA AND REPERFUSION IN DOGS

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Accumulation of myocardial adenosine and subsequent leakage occurs with seconds of an abrupt reduction in coronary flow (Foley et al., 1979). A recent study in rats has hown that exogenously administered adenosine exerts antiarrhythmic activity following coronary artery ligation (Fagbemi & Parratt, 1984). The aim of the present study was to investigate the effects of an intra-ventricular infusion of adenosine on the arrhythmias that occur in a canine model of ischaemia and reperfusion.

Greyhounds, anaesthetised with chloralase, were prepared for occlusion of the left anterior descending coronary artery (LAD). Catheters were placed in the coronary sinus (draining the essentially normal myocardium) and in a local coronary vein (draining the area rendered ischaemic by the occlusion). Adenosine (10 μg kg^{-1} min^{-1}) was infused directly into the left ventricle via a catheter advanced from the left common carotid artery. The infusion was commenced fifteen minutes prior to the occlusion of the LAD and maintained for the duration of the experiment.

Adenosine markedly reduced the number of ventricular ectopic beats (VEB) during ischaemia from 836 ± 256 in control dogs to 54 ± 38 ($P < 0.05$). Further, 4 of the 6 control dogs surviving the ischaemic period experienced bursts of ventricular tachycardia (VT), defined as 7 or more consecutive ectopic beats, whereas none of the five surviving adenosine dogs had VT. All 5 dogs that fibrillated during the ischaemic period (3 adenosine and 2 control) exhibited rapid multifocal VT in the few seconds prior to the onset of VF. Following the release of a 40 minute coronary artery occlusion all 6 remaining control dogs fibrillated within 1 minute of reperfusion while the incidence of VF was 2 out of 5 in the adenosine group.

Administration of adenosine caused a significant fall in mean arterial blood pressure of 19 ± 3 mmHg ($P < 0.01$) within 15 minutes of the start of the infusion and this was sustained for the duration of the experiment. There were no significant effects on heart rate. Blood gas levels in the coronary sinus and local coronary vein were similar in both groups during the ischaemic period. On reperfusion, however, the PO_2 in local coronary venous blood in dogs receiving adenosine was increased from 24 ± 1 to 41 ± 7 mmHg. The latter figure is significantly greater than the corresponding value in the control groups ($P < 0.05$).

These results suggest that adenosine can suppress ischaemic arrhythmias and raises the possibility that it can act as an 'endogenous antiarrhythmic agent'. This may be due to an improved oxygen supply as a result of coronary vasodilation.

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AN AGONISTIC EFFECT OF A SUBSTANCE P ANTAGONIST

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(D-Pro^4, Trp^{7,9}) SP4-11 (DPT2) has been reported to block the action of substance P in cardiac, vascular, intestinal and tracheal preparations (Mizrahi et al., 1982) but in experiments in which both DPT2 and substance P were applied iontophoretically to neurones in the C.N.S. no such antagonism was observed (Jones et al., 1983). This suggests that the receptors involved in the peripheral and central actions of substance P may differ, but it may also be possible that the amount of DPT2 released iontophoretically may have been insufficient to cause antagonism. Substance P injected intrathecally inhibits reflex milk-ejection in the lactating rat (Wright, 1984) and in the present study this has been used as a model to determine whether higher doses of DPT2 antagonise substance P action in the central nervous system.

Lactating Wistar rats (7-10 days post partum), separated from all but one of their pups overnight, were anaesthetised with urethane (1.0g/kg, i.p.) and intrathecal and intramammary cannulations were made under halothane (0.5-1.5% in O_2/NO_2). Three hours after induction of anaesthesia 10 hungry pups were placed on the nipples. The reflex ejection of milk was detected by both increased intramammary pressure and the characteristic behaviour of the pups (Lincoln et al., 1973). This usually commenced within 30 min and continued regularly (mean interval = 5.8 ± 0.3 min) for a period of several hours. The intrathecal injection (i.t., 5µl + 15µl saline flush) was made after 5 or 6 responses and the interval until the subsequent response was measured and expressed as a percentage of the mean basal interval.

Whereas intrathecal injection of 0.9% saline did not significantly alter the pattern of this reflex (mean percentage increase = 70.2 ± 20.7, n=9) the interval was significantly increased by substance P (5µg i.t.); the mean percentage increase was 397.4 ± 75.6 (n=5, $P<0.01$). Intrathecal administration of DPT2 at a dose of 0.1µg did not inhibit reflex milk-ejection (mean percentage increase = 79.5 ± 10.5, n=5, $P>0.05$) nor did it antagonise the effect of substance P (5µg i.t.) when the two peptides were injected together; the mean percentage increase after injection of DPT2 and substance P was 333.2 ± 64.1 (n=8, $P>0.05$). At higher doses DPT2 itself significantly inhibited reflex milk-ejection in a dose-related manner. The mean percentage increase after DPT2 at doses of 0.5, 5, and 10µg were, respectively 249.8 ± 57.1 (n=8, $P<0.02$), 1066.9 ± 330.0 (n=5, $P<0.02$) and 1040.6 ± 312.2 (n=5, $P<0.01$).

These results are consistent with those of the iontophoretic study of DPT2 action in the C.N.S. (Jones et al., 1983) and provide further evidence to suggest that central receptors affecting substance P action differ from those in peripheral systems. The mechanism underlying the inhibition of the milk-ejection reflex by DPT2 is unknown and is under investigation.

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A COMPARISON OF THE RESPONSES OF CINGULATE CORTICAL NEURONES TO SUBSTANCE P AND NEUROKININ A

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Recent autoradiographic studies (Mantyh, Maggio and Hunt, 1984, Rothman, Danks, Herkenham, Cascieri, Chicchi, Liang and Pert, 1984) have shown that binding sites for substance P (SP) in the rat neocortex are found mainly in the superficial layers (I-III) whereas those for the related tachykinin, neurokinin A (NKA, also known as substance K) are localised in the deeper layers (IV-VI). SP, applied by iontophoresis consistently excites single neurones in the deeper layers of the cingulate and parietal cortices (Jones and Olpe, 1982, Lamour, Dutar and Jobert, 1983). In my studies in the cingulate cortex I have occasionally noted cells in the more superficial layers which exhibit repeatable depressant responses to SP (Jones, unpublished observations). In view of the differential laminar distribution of binding sites for the tachykinins it was of interest to compare responses of neurones at different levels in the cortex to SP and NKA.

Conventional techniques were used to record action potentials extracellularly from neurones in the cingulate cortex of rats anaesthetised with chloral hydrate. SP and NKA were applied by iontophoresis (10-200 nA, 30-60s) from similar solutions (0.001M in 0.165M NaCl). The depth of each cell from the pial surface was noted from the micromanipulator.

Of 36 cells tested, 21 were excited by both peptides. 17 of these cells were located below a depth of 650μ from the pia. However, 9 cells were depressed by SP, all of which were located superficially, within 600μ of the pia. Of these 9 cells, 7 were unaffected by NKA and two were weakly excited. SP was without effect on 5 other superficial cells, one of which was excited by NKA and 4 unaffected. Two further cells located superficially were excited by both SP and NKA.

Thus, generally, on the more superficial cells SP was depressant and NKA without much effect whereas both peptides were strongly excitatory in the deeper layers. In view of the laminar difference in binding sites for the peptides these results could indicate that the responses to the tachykinins may be partly mediated by separate populations of receptors in the cortex.

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COMPARISON OF THE HAEMODYNAMIC ACTIONS OF NEUROPEPTIDE Y, ANGIOTENSIN II AND NORADRENALINE

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Neuropeptide Y (NPY) is a 36 amino acid peptide originally isolated from porcine brain (Tatemoto 1982), and subsequently purified from extracts of human phaeochromocytoma tissue (Corder et al 1984). Immunocytochemical methods have been used to demonstrate its abundant presence in sympathetic neurones, where it is thought to be co-stored with noradrenaline (Lundberg et al 1983). Systemic administration of NPY has been shown to produce a rise in blood pressure and to evoke a vasoconstrictor response which is not antagonized by α-adrenoceptor blockade (Lundberg & Tatemoto 1982). This study was performed to investigate further the pressor action of NPY and to compare its haemodynamic properties with two other endogenous vasoconstrictor substances, angiotensin II (AII) and noradrenaline (NA).

Experiments were carried out in five open-chest cats, anaesthetised with a mixture of α-chloralose (80mg kg^{-1}) and pentobarbitone sodium (12mg). All animals were pretreated with 1mg atropine sulphate after surgical preparation and artificially ventilated. Arterial blood gases and pH were maintained within the physiological range. The blood pressure was measured from the femoral artery from which heart rate was derived electronically. Regional blood flows were recorded using electro-magnetic flow probes placed on the left renal artery, left femoral artery and aortic root. These measurements were used to derive renal resistance (RR), femoral resistance (FR), cardiac output (CO), and total peripheral resistance (TPR). All drugs were given by i.v. injection in the jugular vein.

Dose (n mol kg^{-1}) response curves were obtained for NPY (0.1-3), AII (0.001-0.04) and NA (0.06-1.8). The effect of the dose producing a 50mmHg rise in mean BP on changes (Δ) in RR, FR, CO and TPR is shown in Table 1.

Table 1: Comparison of the haemodynamic action of NPY, AII and NA producing a 50mmHg rise in mean BP

	Dose nmol.kg^{-1}	ΔCO ml min^{-1} kg^{-1}	ΔRR	Δ FR mmHg min ml^{-1}	ΔTPR
AII	0.02	11.5±2.2	14.5±3.5	-3.6±0.9	0.108±0.016
NA	0.29	21.3±12	2.3±0.7	2.0±0.6	0.054±0.008
NPY	2.20	0.2±5	4.6±0.6	4.8±1.6	0.140±0.018

Results are mean (n=10) ± s.e.m.

The actions of NPY were found to be resistant to blockade by phenoxybenzamine (5mg kg^{-1}) and propranolol (1mg kg^{-1}). These data indicate that the pressor action of NPY is 100 and 7 times less potent than AII and NA respectively. However, it was found that NPY had a similar potency to NA in increasing RR, FR and TPR. Therefore NPY may play a role in the maintenance of peripheral vascular resistance.

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DISTRIBUTION OF TRITIATED SOMATOSTATIN BINDING SITES IN THE HUMAN BRAIN

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In a previous communication (Brundish et al., 1984) we reported the distribution of somatostatin binding sites in the rat brain using [4-^{3}H-(Phe^{6})]-somatostatin-14 as the radioligand. We now wish to report the distribution of somatostatin binding sites in the human brain using this ligand.

Tissue was dissected before freezing from the brains of 10 patients (7 males, ages 45-75 years, post mortem delay 12-29h; 3 females, ages 58-79 years, post mortem delay 12-24h) who had no clinical history of neurological or mental illness. In the binding assay, 0.7nM ^{3}H-somatostatin-14 was incubated with 1mg of washed membrane protein in the presence and absence of 1μM somatostatin-14 for 30min at 30°C (equilibrium was reached after 20min and remained stable for at least 60min). The reaction was terminated by centrifugation. Specifically bound ^{3}H-somatostatin accounted for up to 50% of total binding, depending on the region assayed. 1μM Tyr^{11}-somatostatin displaced 92 ± 6% of the ligand bound, while displacement was <12% with 1μM TRH, met-enkephalin, morphine, CCK-8, bombesin, substance P and β-endorphin. The distribution of binding sites in a number of brain regions is shown in Table 1.

Table 1. The regional distribution of somatostatin binding in human brain

Region	Specific Binding (fmol/mg protein)	Region	Specific Binding (fmol/mg protein)
Frontal Cortex	62.0 ± 4.2	Putamen	31.6 ± 3.4
Temporal Cortex	69.7 ± 5.2	Amygdala	30.5 ± 5.3
Parietal Cortex	52.5 ± 3.2	Hypothalamus	13.9 ± 5.8
Occipital Cortex	33.2 ± 4.0	Thalamus	4.2 ± 1.8
Hippocampus	48.1 ± 4.3	Substantia nigra	7.1 ± 2.4
Claustrum	46.0 ± 4.6	Substantia innominata	5.07(1)
Caudate	41.7 ± 7.8	Cerebellum	74.3 ± 3.5(5)

(n=4 except where indicated).

The distribution in the human brain is similar to that previously reported in the rat brain (Brundish et al., 1984), with the highest densities of binding sites present in the cerebral cortex and hippocampus. Surprisingly, there is also a very high density of binding sites in the human cerebellum, which contrasts with the low density present in the rat cerebellum. Preliminary localization of the binding sites within the human cerebellum using tritium film autoradiography has shown that the highest density of binding sites is associated with the granule cell layer.

These results show that high affinity somatostatin binding sites are present in the human brain. Their gross distribution is similar to that found in the rat brain, with the exception of the cerebellum.

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233P

LOCAL OEDEMA INDUCED BY SYNERGISM BETWEEN CALCITONIN GENE-RELATED PEPTIDE AND MEDIATORS OF INCREASED VASCULAR PERMEABILITY

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Calcitonin gene-related peptide (CGRP) is a novel 37 amino acid putative neuropeptide (Rosenfeld et al, 1983). Immunoreactive CGRP has been detected in nerves associated with blood vessels (Rosenfeld et al, 1983) and local injection of CGRP produces intense vasodilatation (Brain et al, 1985). As vasodilators, such as PGE_2 , have a striking enhancing effect on oedema induced by other mediators, eg. histamine and bradykinin (Williams & Morley, 1973), we have investigated the possibility that CGRP, if released from local nerve endings, could similarly modulate oedema formation. Local oedema was measured in NZW rabbit skin using the 30 minute accumulation of intravenous ^{125}I-albumin in response to intradermally-injected agents, in replicates of 6 per rabbit. Responses were expressed in terms of volumes of plasma per 16 mm diameter skin sample. Results using human synthetic CGRP (Bachem) are shown in the table.

AGENTS INJECTED INTRADERMALLY (mol/0.1 ml injection)	OEDEMA RESPONSE (µl plasma equivalent/skin sample)
Saline control	7.1 ± 0.9 (n=4 rabbits)
Histamine (1.6×10^{-8})	25.9 ± 3.6 (n=4)
CGRP (10^{-11})	8.9 ± 1.0 (n=4)
Histamine + CGRP	62.8 ± 10.8 (n=4)
	TIME FOR HALF ACTIVITY LOSS IN VIVO
CGRP (10^{-11}) injected 0-80 min before Bradykinin (8×10^{-11})	$t_{½}$ = 40.1 ± 7.5 min (n=4)
PGE_2 (10^{-10}) injected 0-80 min before Bradykinin (8×10^{-11})	$t_{½}$ = 18.0 ± 1.0 min (n=4)
	% OF INITIAL ACTIVITY AFTER INCUBATION IN PLASMA IN VITRO
CGRP (10^{-10}) incubated in 1 ml rabbit plasma (1h,37°C) diluted 10 fold before injection with Histamine (1.6×10^{-8})	102.0 ± 6.3 (n=3)
PGE_2 (10^{-9}) incubated in 1 ml rabbit plasma (1h,37°C) diluted 10 fold before injection with Histamine (1.6×10^{-8})	102.6 ± 16.8 (n=4)

Thus CGRP potentiates histamine and bradykinin-induced oedema. Synergism was also observed between CGRP and the complement peptide C5a. CGRP has a longer duration of action in rabbit skin when compared with PGE_2. The activity of CGRP appears stable when incubated in plasma. These results suggest that CGRP could be involved in neurogenic inflammation. In the rabbit, the local release of CGRP from nerve endings would not itself cause oedema. However the potent vasodilator activity of CGRP (Brain et al, 1985) could have a marked enhancing effect on oedema induced by other mediators released concomitantly. The mechanisms involved in the metabolism of CGRP are unknown although its vasodilator activity appears to be stable in blood plasma.

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COMPARATIVE AUTORADIOGRAPHIC STUDIES WITH [^{3}H]-GABA AND [^{3}H]-(-)-BACLOFEN IN RAT BRAIN IN VITRO

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GABA receptor binding sites in the mammalian central nervous system can be readily demonstrated by in vitro autoradiography (Palacios et al 1981; Wilkins et al 1981). Moreover the distribution of each of the GABA receptor subtypes, $GABA_A$ and $GABA_B$, can easily be distinguished (Bowery et al 1984a). Although both sites can be labelled with ^{3}H GABA it has yet to be established that radiolabelled baclofen, the selective ligand for $GABA_B$ sites in synaptic membrane homogenates, labels the same population of $GABA_B$ sites as ^{3}H GABA in brain sections. Previous autoradiographical studies have employed racemic ^{3}H-baclofen but the resolution has been limited by the low specificity of the ligand (Bowery et al 1984b). Recently the radiolabelled active isomer ^{3}H(-) baclofen has become available and this binds with greater specificity to synaptic membranes (Bowery et al 1984c). The present study compares autoradiographically the pattern of binding sites labelled by ^{3}H(-)-baclofen and ^{3}H GABA in rat brain sections.

$GABA_B$ receptors in 10um sections of rat brain were labelled as described previously (Wilkin et al 1981) using ^{3}H GABA or ^{3}H(-)-baclofen (50nM). Isoguvacine (40um) was present in the ^{3}H GABA containing solutions to prevent labelling of $GABA_A$ sites. After incubation the dried sections were placed in contact with LKB Ultrofilm for 28 (^{3}H GABA) or 42 (^{3}H(-)baclofen) days at 4^{o}C. Background binding was determined by the addition of 100uM (+)-baclofen. ^{3}H(-)baclofen appears to bind with the same pattern of distribution as ^{3}H GABA at $GABA_B$ sites. Thus the highest concentrations were detected in the interpeduncular nucleus (IPN), superior colliculus, cerebellar molecular layer, thalamic nuclei with somewhat less in the hippocampus and cerebral cortex. This is in marked contrast to the distribution pattern of $GABA_A$ binding sites (Bowery et al 1984a).

To make further comparison we have examined the binding of ^{3}H(-)baclofen in the IPN after injection of kainic acid or electrolytic ablation of the habenula. The habenula is the source of the major efferent to the IPN and we have previously shown that chronic lesions of this region abolished $GABA_B$ binding sites in the IPN whilst kainic acid injected directly into the IPN had no effect (Price et al 1984). The binding of ^{3}H(-)baclofen in the IPN was similarly influenced by electrolytic ablation of the habenula and not affected by kainic acid. We conclude that ^{3}H(-)baclofen only labels $GABA_B$ sites in higher centres of the rat brain. The spinal cord remains to be studied.

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235P

ANTICONVULSANT PROFILE IN MICE OF LAMOTRIGINE, A NOVEL ANTICONVULSANT

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Lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] is a novel anticonvulsant chemically unrelated to current anti-epileptic drugs. The anticonvulsant profile of lamotrigine (LTG) in mice has been determined in two standard anticonvulsant tests (maximum electroshock, MES, and leptazol-induced seizures) by comparison with the effects of the following known anticonvulsants: phenytoin sodium (PH), phenobarbitone sodium (PB), carbamazepine (CBZ), valproate sodium (VAL), diazepam (DIAZ), ethosuximide (ETH) and troxidone (TROX).

Test drugs, each at a range of doses ($n \geq 3$), or control fluid (Celacol 0.25% or saline 0.85%) were administered p.o. at 10ml kg^{-1} to groups of mice (CFLP or CDI, male, 20 to 25g, n=4 or 6 per group) at 2h before the test challenge. MES stimulation was applied via corneal electrodes from an electroshock apparatus (Hugo Sachs) at an intensity (25mA, 250ms duration) sufficient to elicit hind-limb extension (HLE) in almost 100% of control animals (n>200). In the leptazol (LEPT) test the convulsant (10mg ml^{-1} in 0.85% saline) was infused at 0.3ml min^{-1} through a tail vein until HLE was induced or up to a maximum duration of 2 min.: HLE occurred in all control animals (n=250). The latency to clonus (facial and forelimbs) was recorded. ED_{50} values for the abolition of HLE were determined.

TABLE 1. ED_{50} values (mg kg^{-1} p.o.) and 95% limits, for HLE abolition in the MES and LEPT tests and effect on clonus latency in the LEPT test.

TEST	LTG	PH	PB	CBZ	VAL	DIAZ	ETH	TROX
MES(HLE)	3.8 (2.9-4.8)	6.6 (5.1- 8.6)	11.4 (8.4-15.6)	15.8 (12.2-20.4)	461 (361-596)	5.5 (2.3-9.5)	>640	>960
LEPT(HLE)	6.6 (3.4-13.0)	8.0 (4.1-15.4)	5.0 (2.3-10.5)	23.3 (10.3-50.0)	ca640	3.6 (2.6-5.1)	>320	>320
LEPT CLONUS LATENCY*	NE (320)	NE (640)	≥5 (19.7%)	NE (160)	NE (640)	≥1.25 (26.7%)	≥160 (18%)	≥320 (175%)

*Lowest doses which significantly ($p \leq 0.02$) increased latency (% increase at stated dose) over control values (mean ± s.e.m. = 21.2 ± 0.37s).
NE = No effect (highest dose tested).

LTG resembled PH and CBZ in its ability to block HLE in both tests and in failing to increase clonus latency in the LEPT test up to high multiples of the MES ED_{50} values. In contrast, ETH and TROX failed to block HLE but significantly increased clonus latency. PB and DIAZ were fully effective in both tests. VAL was considerably less potent than the other drugs in blocking HLE.

These studies have revealed that the anticonvulsant profile of LTG in mice is very similar to that of PH and CBZ. In rats, although the profile was similar in the MES test, it differed in the visually-evoked after-discharge test, considered predictive for absence seizures (King et al, 1980): LTG (similar ED_{50} to MES), ETH and DIAZ blocked the response but PH and CBZ were ineffective (Miller et al, 1984). In epileptic patients, LTG, given acutely, reduced or abolished the response to photic stimulation (Binnie et al., 1984). The animal studies suggest usefulness for generalised seizures with possible wider profile than PH or CBZ.

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INTERACTION OF BENZODIAZEPINE RECEPTOR LIGANDS WITH ETHANOL IN RODENTS

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Like benzodiazepines (BDZ's), the pyrazoloquinolinones, CGS 9896, CGS 9895 and CGS 8216, bind with high affinity to the BDZ receptor. However, they are reported to vary in their pharmacological profiles at this site, possessing agonist, partial agonist and antagonist actions, respectively (Yokoyama et al, 1982). CGS 9896, in contrast to chlordiazepoxide (CDP), is not sedative per se and has been reported to be inactive (Goldberg et al. 1983), or less potent than diazepam (Petrack et al, 1984), in potentiating the sedative effects of ethanol in rats. As the interaction of BDZ's with alcohol is a particular problem in the clinic, we have now investigated further this aspect of the pharmacology of CDP and the pyrazoloquinolinones in rodents.

When administered orally 1h before ethanol (1.8 g/kg i.p.), CDP (5 mg/kg) and CGS 9896 (40 mg/kg p.o.) significantly ($P<0.05$, Fisher's exact test) increased by 44 and 40%, respectively, the number of rats (male, CFY, 180-220g, n=16) losing their righting reflex (RR) compared to ethanol treated controls. In mice (male, CD-1, 20-25g, $n \geq 10$), CDP (5 mg/kg), CGS 9896 and CGS 9895 (10-40 mg/kg), given orally 1h before ethanol (4g/kg i.p.), significantly ($P<0.05$, Student's t-test) potentiated the duration of the loss of RR induced by ethanol by 184, 137-172 and 119-193%, respectively. CGS 8216 (10-40 mg/kg) was without significant effect.

As the potentiation of ethanol in mice appeared to be associated with BDZ receptor agonist or partial agonist properties, the influence of the BDZ antagonist, Ro 15-1788 (Ro), and of CGS 8216 on the interaction of CGS 9896 and CDP with ethanol was investigated. The results are shown in Table 1.

Table 1. Effect of Ro and CGS 8216 on CDP- and CGS 9896-mediated potentiation of ethanol-induced loss of RR in the mouse.

Treatment	Mean duration(min) of loss of RR ± s.e.m.	% change compared to vehicle	% change compared to CDP or CGS 9896 alone
Vehicle	34.6±1.7		
CDP	97.7±3.9	+182*	
CGS 9896	84.0±4.9	+143*	
Ro	40.0±3.1	+16	
CGS 8216	29.3±2.6	-15	
CDP + Ro	59.1±6.1	+71*	-39*
CGS 9896 + Ro	64.6±4.3	+87*	-39*
CDP + CGS 8216	30.9±4.7	-11	-106*
CGS 9896+CGS 8216	30.6±3.6	-12	-108*

Doses (mg/kg) and treatment time before ethanol: CDP 5 p.o. and CGS 9896 20 p.o., 1h : Ro 10 i.p. and CGS 8216 10 i.p. 0.5h; *$P<0.05$. Student's t-test).

The increase in duration of ethanol-induced loss of RR caused by CGS 9896 or CDP was significantly reversed by both Ro and CGS 8216.

In summary, we have confirmed that, like CDP, CGS 9896 does potentiate the sedative action of ethanol in rats. Furthermore, the effect of CBS 9896 and CDP on ethanol-induced sedation in mice appears to involve the BDZ receptor.

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CDP and Rol5-1788 were gifts from Roche.

237P

PHARMACOLOGICAL STUDIES ON PITRAZEPIN, A $GABA_A$ RECEPTOR ANTAGONIST

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Pitrazepin is a novel $GABA_A$ receptor antagonist which displaces [^{3}H]-muscimol binding with greater than 10 times the potency of bicuculline (Gahwiler, Maurer & Wuthrich, 1984). However, no quantitative estimates of its potency as an antagonist of $GABA_A$-mediated responses have previously been made. We have investigated its action as an antagonist of the ability of isoguvacine to inhibit the CA1 population spike in the rat hippocampal slice. In addition, we have compared the ability of pitrazepin to displace [^{3}H]-bicuculline methochloride binding to that of bicuculline methobromide in rat cerebral cortical membranes.

Electrophysiological experiments were carried out as previously described (Kemp et al, 1984) and the binding experiments performed on an extensively washed, frozen-thawed rat cortical membrane preparation (Bowery et al, 1983).

Pitrazepin ($1 \times 10^{-7} - 3 \times 10^{-6}$M) produced parallel displacements to the right of the isoguvacine dose response curve, with no depression of the maximum response. Dose ratios were calculated from the EC_{50} values (concentration producing 50% inhibition of the population spike) and Schild plot analysis of these data gave a pA_2 of 6.70 $\pm$ 0.14 (mean $\pm$ S.E.M., n = 5) with a slope of 1.08 $\pm$ 0.06. This latter value was not significantly different from unity. Thus, in this preparation, pitrazepin was 3.3 times more potent, as an antagonist of isoguvacine, than bicuculline methochloride (pA_2 = 6.18, Kemp et al, 1984).

Pitrazepin displaced [^{3}H]-bicuculline methochloride binding with an IC_{50} of 24 $\pm$ 3 nM (mean $\pm$ S.E.M., n = 3), compared to an IC_{50} of 115 $\pm$ 13 nM (n = 10) for bicuculline methobromide. The potency of pitrazepin to displace ^{3}H-bicuculline methochloride binding was lowered by the presence of 1 mM pentobarbitone (IC_{50}'s; pitrazepin 57 $\pm$ 4 nM (n = 3), bicuculline methobromide 234 $\pm$ 17 nM (n = 6)). This is a property shared by other $GABA_A$ receptor antagonists (Wong,1984).

These results suggest that pitrazepin and bicuculline methochloride have similar relative potencies as $GABA_A$ receptor antagonists and displacers of [^{3}H]-bicuculline methochloride binding; with pitrazepin being 3-5 times more potent than bicuculline methochloride. This difference is less than would be predicted from their abilities to displace [^{3}H]-muscimol binding, where pitrazepin is 10-50 times more potent than bicuculline methochloride (Gahwiler et al, 1984).

We wish to thank Sandoz Ltd for their gift of pitrazepin.

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THE LOCATION OF $GABA_B$ BINDING SITES IN THE RAT HIPPOCAMPUS

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The presence of $GABA_B$ receptors in the rat hippocampus has been demonstrated by Ault & Nadler (1982) and Newberry & Nicoll (1984). Both of these studies provide electrophysiological evidence for $GABA_B$ sites in the CA1 region of the hippocampus. The former study indicated a presynaptic location on terminals of Schaffer collaterals arising from the CA3 pyramidal cells whilst the latter study described the postsynaptic location of $GABA_B$ sites on dendrites of CA1 pyramidal cells. The present study was undertaken to compare the autoradiographical distribution of $GABA_B$ binding sites in the hippocampus with the electrophysiological data.

Hippocampal $GABA_B$ binding sites were labelled with ^{3}H-GABA by the technique described previously (Wilkin et al 1981; Bowery et al 1984) or with 50nM ^{3}H(-)baclofen. 10um transverse sections of rat brain were incubated for 20 min in tris-HCl buffer containing 190mM sucrose and 2.5mm $CaCl_2$ together with the radiolabelled ligand. Background binding was determined by the addition of unlabelled (+) baclofen (100uM). After rinsing and drying, the sections were placed in contact with LKB Ultrofilm for 22-40 days at 4°C.

$GABA_B$ sites were distributed throughout the CA1, CA2 & CA3 dendritic regions. Only the dendrites were labelled and little or no binding could be detected in the layer containing the pyramidal cell bodies. This concurs with the observation of Newberry & Nicoll (1984) that $GABA_B$-mediated responses could only be detected when baclofen or GABA was applied to the apical or basal dendrites and not when applied to the pyramidal cell bodies. The pattern of binding also fits with the observations of Ault & Nadler (1982), since the Schaffer collaterals primarily innervate the dendrites rather than the cell bodies.

The highest concentration of $GABA_B$ binding sites within the hippocampus occured in the dentate gyrus where distinct bands could be readily observed. The outer layer which corresponds to the dendrites of the granule cells contained the greatest amount with less in the region of the basket cells. To determine whether the binding sites in the outer layer were on presynaptic terminals of the perforant path we have examined the binding of ^{3}H GABA after electrolytic ablation of this afferent pathway. Rats (n=12) were lesioned unilaterally under equithesin 3.75ml/kg anaesthesia (co-ordinates H-4.0, A-8.1, L4.2, V2.5-3.0) and allowed to recover. The binding of ^{3}H GABA was examined on brain sections prepared 5, 7, 10, 27 days post lesion. No significant change in the concentration or pattern of binding occured at any time interval. We conclude, therefore, that $GABA_B$ sites are unlikely to be on presynaptic terminals but may exist on the granule cell dendrites in the dentate gyrus. This is supported by the lack of effect of baclofen on transmission in the perforant path (Ault & Nadler 1982)

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239P

CENTRAL DOPAMINE METABOLISM MONITORED BY IN VIVO VOLTAMMETRY: EFFECTS OF ACUTE AND CHRONIC NEUROLEPTIC ADMINISTRATION

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Acutely, typical neuroleptics induce an increase in dopamine (DA) neuronal firing in the nigrostriatal and mesolimbic systems. However, DA cell firing is reduced in these regions following chronic administration. This is believed to be due to depolarisation inactivation of the DA cells since it can be reversed with hyperpolarising agents such as apomorphine (White & Wang, 1983). Atypical neuroleptics have selective effects on the mesolimbic system both in the acute (White & Wang, 1983) and chronic situation (Chiodo & Bunney, 1983). The present study was carried out to investigate whether that selectivity is associated with similar differential effects on dopamine metabolism using in vivo voltammetry to monitor dihydroxyphenylacetic acid (DOPAC) levels simultaneously in the nucleus accumbens (n.acc.) and striatum of anaesthetised rats.

Male Sprague Dawley rats were anaesthetised with halothane/N_2O and stereotaxically implanted with electrically pretreated carbon fibre microelectrodes (Sharp et al, 1984). Differential pulse voltammograms were recorded from the n.acc. and striatum every 5 min and drugs administered after a stabilisation period of approx. 1 h. In the chronic experiments, rats were injected once a day for 21 days with drug or saline (s.c.) and subsequently tested on day 22.

Acute administration of thioridazine (20 mg/kg s.c.) produced maximal increases in the DOPAC peaks in the n.acc. and striatum of 44%±7 (s.e. mean) and 55%±5 respectively 75 min after injection (n=5). Similar results were obtained with clozapine (50 mg/kg s.c.), DOPAC levels increasing by 60%±17 and 86%±15 (n=4). Rats injected with saline for 21 days and subsequently administered thioridazine (20 mg/kg s.c.) showed increases in DOPAC in both regions similar to the acute experiment (n=5). No such increase was observed in the n.acc. of rats treated chronically with thioridazine (20 mg/kg s.c.) (n=5). An increase in DOPAC (32%±13, n=5) was observed in the striatum but that response was greatly reduced in comparison with animals chronically treated with saline (95%±17, n=5). Although this suggests a lack of correlation between DA cell firing and DA turnover it is possible to explain the attenuated response in the striatum in terms of elevated DOPAC levels prior to drug challenge as a result of chronic thioridazine administration. This is suggested by a comparison of basal DOPAC peak heights in animals treated chronically with saline and those treated with thioridazine although this fails to reach significance. However, further evidence for a dissociation of DA metabolism and apparent depolarisation inactivation was gained from the failure of apomorphine to produce an increase in DOPAC levels in animals chronically treated with thioridazine. Administration of increasing doses of this drug (0.05-0.25 mg/kg s.c.) 1 h following thioridazine (20 mg/kg s.c.) produced a decrease in the DOPAC signal in the n.acc. and striatum similar to that produced by low doses in control animals.

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MODULATION OF DOPAMINE RELEASE AT DIFFERENT FREQUENCIES OF STIMULATION FROM RABBIT RETINA BY A D_2 DOPAMINE AGONIST AND MELATONIN

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Dopamine agonists inhibit the calcium-dependent release of dopamine from rabbit retina through activation of D-2 dopamine autoreceptors involved in a negative feedback mechanism (Dubocovich & Weiner, 1981; Dubocovich, 1984). The hormone melatonin inhibits the release of dopamine from rabbit retina by activation of a site pharmacologically different from monoamine receptors (Dubocovich, 1983). In the rabbit striatum the potency and efficacy of agonists to inhibit and antagonists to enhance transmitter release via activation of autoreceptors depends on the frequency and duration of stimulation (Cubeddu & Hoffman, 1983). The aim of the present study was to investigate the modulation of [^{3}H]-dopamine release from rabbit retina through the D-2 dopamine autoreceptor and the putative melatonin receptor site, at different frequencies of field stimulation.

Rabbits were killed during the light cycle and retinas labelled in vitro with [7,8-^{3}H]-dopamine (SA.: 41 Ci/mmol) as previously described (Dubocovich & Weiner, 1981). The release of tritium was elicited by field stimulation at either 1 Hz, 3 Hz or 6 Hz (360 pulses, 20 mA, 2 msec) twice within each experiment. The percent of total tissue radioactivity released above spontaneous levels during the first period of stimulation (S_1) was: 2.2 $\pm$ 0.2% (n=16) at 1 Hz, 2.6 $\pm$ 0.2% (n=14) at 3 Hz, and 3.3 $\pm$ 0.2% (n=15) at 6 Hz ($p < 0.05$ when compared with 1 Hz or 3 Hz). The ratio obtained between two consecutive periods of stimulation (S_2/S_1) at each frequency was not significantly different from unity: 0.91 $\pm$ 0.09 (n=3) at 1 Hz, 0.93 $\pm$ 0.05 (n=4) at 3 Hz, and 0.87 $\pm$ 0.09 (n=4) at 6 Hz.

The D-2 dopamine receptor agonist LY 171555 (0.01 -1 uM) inhibited the evoked release of [^{3}H]-dopamine in a concentration-dependent manner at 1 Hz (IC_{50}= 50 nM), at 3 Hz (IC_{50}= 250 nM) and at 6 Hz (IC_{50}= 355 nM). The dopamine receptor antagonist S-sulpiride (0.01 - 1 uM) was more potent to increase the release of [^{3}H]-dopamine at the higher than at the lower frequency of field stimulation (EC_{150}= 2 nM, 6Hz; EC_{150}= 300 nM, 1 Hz). Melatonin (1pM-1nM) inhibited [^{3}H]-dopamine release at picomolar concentrations with an IC_{50} of 45pM, both at 1 Hz and 3 Hz. Melatonin was at least 1000 times more potent than the dopamine agonist in inhibiting [^{3}H]-dopamine release from retina. It is concluded that in the retina the modulation of [^{3}H]-dopamine release by dopamine agonists and antagonists, but not by melatonin , depends on the frequency of stimulation and therefore on the synaptic concentration of endogenous dopamine when the number of pulses is mantained constant.

In the rabbit retina, immunoreactive melatonin exhibits a diurnal rhythm, with higher levels at midnight and lower levels at noon (Dubocovich, Lucas & Takahashi, 1985). The difference in potency of dopamine agonists and exogenous melatonin to inhibit dopamine release in vitro would suggest that in vivo during the dark period endogenous melatonin may be the more important modulator of dopamine release. Conversely, during the light period, modulation of dopamine release in retina may be primarily mediated through activation of D-2 autoreceptors.

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241P

THERMODYNAMICS OF LIGAND INTERACTION WITH THE DOPAMINE D2 RECEPTOR IN NATIVE MEMBRANE AND SOLUBILISED ENVIRONMENTS

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The interaction of dopamine antagonist drugs with striatal D-2 receptors may cause changes in both entropy and enthalpy (Zahniser & Molinoff,1983). We report on thermodynamic changes induced by the interaction of dopamine agonist and antagonist drugs with the rat striatal dopamine D-2 receptor identified by ^{3}H-spiperone in both membrane and CHAPS solubilised preparations at 4,15,26 and 37°C.

The dopamine agonists 6,7-ADTN and dopamine were > 5 times more effective in displacing the specific binding of ^{3}H-spiperone to membrane preparations at 4°C than 37°C. The ability of spiperone and cis-flupenthixol to displace specific ^{3}H-spiperone binding was little affected by temperature while (+)-butaclamol was more effective at higher temperatures. In contrast, the substituted benzamide drugs sulpiride, clebopride and metoclopramide were > 10 times more potent at 4°C than 37°C. The interaction of dopamine agonists with the membrane D-2 receptor labelled by ^{3}H-spiperone was associated with a decrease in enthalpy. The interaction of spiperone, cis-flupenthixol and (+)-butaclamol was mainly associated with alterations in entropy. However, the interaction of sulpiride, clebopride and metoclopramide was accompanied by a decrease in enthalpy so allowing an energetically unfavourable decrease in entropy. In the CHAPS solubilised striatal preparations the changes in entropy and enthalpy observed for the interaction of dopamine agonist and antagonist drugs with the binding site identified by ^{3}H-spiperone closely resembled those occurring in membrane preparations.

Table 1. Thermodynamic parameters of drug interaction with D-2 dopamine receptors

Drug	ΔG^{o}(Kcal mol^{-1})	ΔH^{o}(Kcal mol^{-1})	ΔS^{o}(Kcal/mol-deg)
Spiperone	- 14.06	5.17	62.0
cis-Flupenthixol	- 11.37	3.52	48.0
(+)-Butaclamol	- 12.63	13.27	83.5
Sulpiride	- 10.02	- 20.95	- 35.3
Clebopride	- 12.31	- 13.00	- 2.2
Metoclopramide	- 9.92	- 13.10	- 10.2
6,7-ADTN	- 8.78	- 16.24	- 24.1
Dopamine	- 8.09	- 8.65	- 1.8

ΔG^{o} = Gibbs free energy change; ΔH^{o} = Enthalpy change; ΔS^{o} = Entropy change; all calculated for 37°C. Calculated as described by Weiland & Molinoff (1981).

The results presented suggest that binding of dopamine agonists and substituted benzamide drugs, but not typical antagonist drugs, may cause a conformational change of the D-2 receptor identified by ^{3}H-spiperone. The effects observed were retained following CHAPS solubilisation so they may represent alterations in the nature of the receptor rather than its lipid environment.

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MECHANISM OF PARADOXICAL BLOCKADE OF D_2 AGONIST-INDUCED STEREOTYPY BY THE ENANTIOMERS OF THE D_1 ANTAGONIST SK&F 83566

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The introduction of the selective D-1 dopamine antagonist SCH 23390 (Iorio et al, 1983) has aided investigation of any behavioural role for D-1 receptors, but has produced unexpected results. Thus, SCH 23390 blocks stereotypy induced both by apomorphine and by the selective D-2 agonist RU 24213 (Molloy & Waddington, 1984, 1985; O'Boyle et al, 1984). SK&F 83566 is a potent D-1 antagonist, this action residing in its R- but not S-enantiomer; residual D-2 antagonist activity is weak and shows negligible enantioselectivity (O'Boyle & Waddington, 1984). Studies of the effects of these enantiomers on stereotypy induced by RU 24213 would provide clues as to the relative roles of D-1 and D-2 blockade.

Male Sprague-Dawley rats were pretreated s.c. with R- or S-SK&F 83566, or with vehicle alone, and were challenged 30 min later with 15 mg/kg s.c. RU 24213. Immediately before and at 10 min intervals after challenge they were assessed using a conventional stereotypy rating scale and a behavioural check list to resolve the individual behaviours contributing to the stereotypy syndrome (Molloy & Waddington, 1984).

RU 24213 induced typical stereotyped behaviour characterised by sniffing and locomotion. The syndrome was unaltered by pretreatment with 200 µg/kg S-SK&F 83566, but was dose-dependently antagonised ($p < 0.01$) by 40-200 µg/kg R-SK&F 83566. Both the sniffing and locomotor components of the syndrome were significantly antagonised (e.g. prevalences of locomotion at 50 min: RU24213 + vehicle, 100%; RU 24213 + 200 µg/kg S-SK&F 83566, 87%; RU 24213 + 200 µg R-SK&F 83566, 0%, $p < 0.01$; $n = 7 - 10$).

The mode of antagonism by SK&F 83566 of stereotypy induced by RU 24213 can distinguish between two possible explanations for this effect. Its blockade of D-1 receptors is potent and enantioselective, while that of D-2 receptors is weak and negligibly enantioselective (O'Boyle & Waddington, 1984). As stereotypy was potently antagonised with complete enantioselectivity, this effect appears to have its basis in D-1 receptor blockade rather than residual activity at D-2 receptors. Thus, D-1 receptor blockade appears able to influence processes initiated by D-2 receptor stimulation.

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243P

PRELIMINARY OBSERVATIONS ON THE EXCITATORY TRANSMISSION TO THE RETRACTOR PENIS OF THE GIANT SNAIL (ARCHACHATINA MARGINATA)

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The entire length (30-40 mm) of the giant African Snail's retractor penis (SRP) was excised (Segun, 1975) and set up as described by Adebanjo and Ononiwu (1985). Electrical field stimulation (EFS) of the muscle strip was achieved via built-in vertical platinum-iridium electrodes (supramaximal voltage, 5Hz, 1ms pulse-width, for 2 seconds at regular intervals of 60s). EFS elicited pure, powerful, and motor responses which were promptly abolished by tetrodotoxin (TTX), (3×10^{-7}M). These electrically-mediated contractions (EMCs) were completely refractory to: ganglion-blocking doses of pentolinium (10^{-8}-10^{-5}M) or hexamethonium (10^{-8}-10^{-4}M); muscarinic antagonist, atropine (10^{-7} - 10^{-5}M); agents that inhibit responses to sympathetic adrenergic nerves activity such as guanethidine (2×10^{-9} - 2×10^{-5}M) or prolonged exposures to bretylium (10^{-7} - 10^{-5}M); α or β -adrenoceptor blocking drugs such as phentolamine (2×10^{-9} - 2×10^{-5}M) or Idazoxan (10^{-8} - 10^{-5}M): dopaminoceptor blockers such as pimozide (10^{-7} - 10^{-5}M) or haloperidol (10^{-8} - 10^{-5}M). Noradrenaline (10^{-8} - 10^{-5}M), adrenaline (10^{-8} - 10^{-6}M), dopamine (10^{-9} - 10^{-5}M), nicotine (10^{-7} - 10^{-5}M), histamine (10^{-8} - 10^{-5}M) or mepyramine (10^{-8} - 10^{-5}M) displayed no effect on the amplitude of the EMCs. However moderate concentrations ACh (10^{-6} - 10^{-5}M) inhibited the EMCs. The two isomers of 5-Hydroxytryptophan (5HTP) 10^{-9} - 10^{-5}M augmented the EMCs profoundly although the L-5HTP was about 100 times more potent than the DL-5HTP. Similarly, 5-hydroxytryptamine (5HT) (10^{-10}M - 10^{-6}M) did enhance the EMCs. 5-HT (10^{-6}M) produced contractions that were susceptible to cyproheptadine (10^{-6} - 10^{-5}M).

TTX (10^{-8}M), cyproheptadine (10^{-8} - 10^{-7}M) and dibenamine (10^{-9} - 10^{-6}M) decreased and abolished the EMCs or augmenting-actions of either 5HTP or 5HT. Morphine (10^{-8} - 10^{-5}M) had no effect on these EMCs. ACh reversed incompletely the EMCs-augmenting actions of 5HT, while only ATP (10^{-6} - 10^{-5}M) enhanced the EMCs by about 10-15%.

It is concluded that there are no autonomic contributions to this excitatory transmission; that the excitatory transmission is sensitive to cyproheptadine and dibenamine; that there is an indication of the presence of 5HT-D receptors (Gaddum & Picarelli, 1957) and that 5HT may be a candidate for the neurotransmitter system to this molluscan site.

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