THE EFFECT OF RX781094, A SELECTIVE α_2-ADRENOCEPTOR ANTAGONIST ON (^{3}H)-NORADRENALINE RELEASE IN THE MOUSE VAS DEFERENS

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Noradrenaline released from nerve terminals may inhibit its own release via presynaptic α_2-adrenoceptors. In the guinea pig vas deferens preloaded with ^{3}H-noradrenaline the non-selective α-adrenoceptor antagonist phenoxybenzamine increased tritium release following one pulse (Kalsner, 1979). However, modulation of noradrenaline release within 1 pulse (rather than released transmitter regulating only subsequent release) needs confirmation using a more selective drug. No support for Kalsner's results were obtained in the mouse vas deferens using the α_2-adrenoceptor antagonist yohimbine (Baker & Marshall, 1982). The availability of a more selective antagonist, RX781094 (Chapleo et al., 1981) provides a more stringent test of this hypothesis.

Mice isolated vasa deferentia were set up (Marshall et al., 1978) in Krebs solution containing prazosin, 100 nM. Clonidine (1-1000 nM) cumulative inhibition curves were obtained on twitch responses to stimulation (0.2 Hz, 2 ms) before and after 10 min equilibration with one concentration of RX781094 (30, 100, 300 or 1000 nM). At the highest concentration the dose-ratio was 77 ± 15 (mean ± s.e. mean). The pA_2 value for RX781094 was 7.89 with a slope of the Schild plot of 0.96 for this action at presynaptic α_2-adrenoceptors. To assess the activity of the drug at α_1-adrenoceptors isolated vasa (without prazosin in the Krebs solution) were contracted by phenylephrine (1 μM - 3 mM). RX781094 (10, 30 and 100 μM) shifted the dose-response curve to the right (DR 84 ± 11 at 100 μM) with a pA_2 value of 6.08 (slope 0.93).

The uptake of 1-7,8-(^{3}H)-noradrenaline (Sp. Act. 45 Ci/mmol, 59 nM) over 10 min was unaltered by RX781094 (tested up to 1 μM). The tritium overflowing from vasa preloaded with ^{3}H-noradrenaline (0.59 μM) was separated into noradrenaline and its metabolites (Baker & Marshall, 1982). The composition of the basal release of tritium was unaltered by RX781094 (up to 1 μM) suggesting that the drug did not inhibit noradrenaline breakdown. The twitch response and the fractional release of noradrenaline elicited by 1 pulse (2 ms) were not significantly increased ($P > 0.05$) by RX781094 (30, 100 and 300 nM 20 min equilibration).

The release of (^{3}H)-noradrenaline by 10 pulses (1 Hz) was increased ($P < 0.05$, paired t test) by the antagonist (1 μM) after 2 min equilibration but not by lower concentrations (10-300 nM). However after 20 min equilibration the fractional release of noradrenaline was significantly increased above the control ($6.3 \pm 0.5 \times 10^{-5}$) in a concentration dependent manner by RX781094 (10, 30, 100 and 300 nM; 7.2 ± 0.2, 7.8 ± 0.5, 8.7 ± 0.5 and $9.1 \pm 0.6 \times 10^{-5}$ respectively). These 4 concentrations of antagonist increased the peak twitch height to stimulation by 12 ± 5, 11 ± 4, 23 ± 5 and 37 ± 7%.

These results demonstrate that RX781094 is a highly selective α_2-adrenoceptor antagonist in the vas deferens of the mouse. With trains of 10 pulses RX781094 revealed feedback of noradrenaline onto presynaptic α_2-adrenoceptors. The increase in noradrenaline release is unlikely to be due to blockade of uptake or metabolism. Results with RX781094 suggest that following one pulse noradrenaline does not modulate its own release via α_2-adrenoceptors in the mouse vas deferens.

We thank the MRC for support and Reckitt & Colman for RX781094.

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COMPARISON OF THE EFFECTS OF ICI 118551, ATENOLOL, AND PROPRANOLOL AT β-ADRENOCEPTORS IN THE ANAESTHETIZED CAT

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Experiments on in vitro preparations indicate that ICI 118551 is a potent selective antagonist at β_2-adrenoceptors (Bilski et al, 1980; O'Donnell & Wanstall, 1980). We were interested to examine the selectivity of this compound in vivo. Adult cats of either sex were anaesthetised with α-chloralose. Blood pressure, heart rate, isometric contractions of a nictitating membrane in response to preganglionic stimulation (10 Hz for 10 s every 100 s), and incomplete tetanic contractions of a soleus muscle in response to stimulation of its motor nerve (8 Hz for 1 s every 10 s) were recorded simultaneously by conventional methods (e.g. Houston & Rodger, 1974). Reductions in the tension and degree of fusion of the soleus muscle contractions together with increases in heart rate were induced by i.v. infusions of either terbutaline (1.0 $\mu g\,kg^{-1}\,min^{-1}$) or (-)-isoprenaline (0.2 $\mu g\,kg^{-1}\,min^{-1}$). The infusions were administered in the presence of ganglion blockade induced by hexamethonium (5 $mg\,kg^{-1}$ i.v. sufficient to abolish the nictitating membrane responses), in order to minimise the complications arising from reflexly induced changes in heart rate. At the peak of the effects of the infused β-adrenoceptor agonists, increasing doses of one of the antagonists were injected in a cumulative manner until maximal antagonism of soleus muscle depression and of increase in heart rate were achieved. The results are summarised in Table 1.

Table 1. $Emax_{50}$ antagonistic doses ($\mu g\,kg^{-1}$; mean ± s.e. mean; n = 3 - 5)

	(-)-Isoprenaline		Terbutaline	
	Heart Rate	Soleus	Heart Rate	Soleus
Atenolol	115 ± 60	1180 ± 208	300 ± 53	1650 ± 464
ICI 118551	775 ± 25	34 ± 4	25 ± 13	69 ± 23
Propranolol	78 ± 20	70 ± 15	36 ± 5	79 ± 6

All three antagonists produced dose-dependent antagonism of the infused agonists, the effects of ICI 118551 being about three times slower to develop than those of atenolol. ICI 118551 antagonised the effects of both agonists on soleus contractions (β_2-receptor mediated), and the effect of terbutaline on heart rate (also β_2-receptor mediated) at doses 10 - 30 times lower than those required to reverse the effects of (-)-isoprenaline on heart rate (combined β_1 and β_2 effect in the cat). Atenolol was 5 - 10 times more potent in reducing heart rate responses than in blocking soleus muscle effects.

The results confirm that in vivo, ICI 118551 is a selective antagonist at β_2-adrenoceptors, having a potency similar to that of propranolol.

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HYPOTHERMIA INDUCES SUPERSENSITIVITY AT β_1-ADRENOCEPTORS BUT NOT AT β_2-ADRENOCEPTORS

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Lowering the bath temperature of isolated guinea-pig atria has been shown to produce supersensitivity of the β-adrenoceptor-mediated positive inotropic and chronotropic responses to sympathomimetic amines (Broadley, 1980). This study examines whether hypothermia-induced supersensitivity occurs in other tissues containing β-adrenoceptors. Right and left atria, papillary muscles, ileum, lung parenchymal strips and uterine strips from guinea-pigs (>300g) were mounted in Krebs-bicarbonate solution at either 38 or 30°C, gassed with O_2:CO_2 (95:5) and containing metanephrine (10^{-5}M) to inhibit extraneuronal uptake. Cumulative concentration-response curves to orciprenaline were constructed. Geometric mean EC_{50} values and their 95% confidence limits were compared by Student's t-test.

Left atria and papillary muscles were paced at 2Hz and the EC_{50} values for the increase in developed tension at 38°C (left atria, 1.53μM (0.28-31.7) n=7; papillary muscle, 1.53μM (0.32-7.4) n=9) were significantly ($P<0.05$) greater than the values at 30°C (left atria, 0.17μM (0.06-0.46) n=9; papillary muscle, 0.52μM (0.09-3.1) n=6). The EC_{50} value for right atrial rate increases at 38°C (0.97μM (0.28-3.3) n=7) was also significantly ($P<0.05$) greater than at 30°C (0.33μM (0.05-2.3) n=5). Thus supersensitivity to orciprenaline was obtained at the lower temperature with the cardiac preparations, the responses of which are mediated via β_1-adrenoceptors (Zaagsma, Oudhof, van der Heijden and Plantjé, 1979). Relaxation of the coaxially stimulated (0.1Hz) ileum by orciprenaline in the presence of phentolamine (10^{-6}M) was used as an alternative β_1-adrenoceptor-mediated response (O'Donnell & Wanstall, 1975). Supersensitivity was again evident, the EC_{50} value at 30°C (0.86μM (0.41-1.8) n=10) being significantly ($P<0.001$) lower than at 38°C (5.17μM (1.45-18.4) n=6).

Orciprenaline-induced relaxation of lung strips was recorded after contraction with carbachol (10μM). The EC_{50} values at 38°C (1.18μM (0.38-3.6) n=11) and 30°C (1.02μM (0.34-3.0) n=11) were not significantly different ($P>0.05$). Similarly, when histamine (10μM) was the contracting agent, there was no significant difference ($P>0.05$) between EC_{50} values at 38°C (1.18μM (0.38-3.6) n=11) and 30°C (0.84μM (0.15-4.7) n=6). Uterine strips were contracted by substituting the Na^+ in the bathing solution by K^+. The EC_{50} values for orciprenaline-induced relaxation were not significantly different ($P>0.05$) at 38°C (0.54μM (0.14-1.8) n=9) and 30°C (0.82μM (0.24-2.8) n=6).

In contrast to the heart and intestine, the responses of the lungs (Siegl, Rossi & Orzechowski, 1979) and uterine preparations (O'Donnell, Persson & Wanstall, 1978) are thought to be mediated via β_2-adrenoceptors. This study therefore suggests that hypothermia-induced supersensitivity occurs in guinea-pig tissues only where the responses are mediated via β_1-adrenoceptors.

This work was supported by a grant from the British Heart Foundation.

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EFFECTS OF β-ADRENOCEPTOR BLOCKING AGENTS ON URINE AND ELECTROLYTE EXCRETION IN CONSCIOUS RATS

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Lockett and her group of workers were probably the first to suggest the existence of ß-adrenoceptors in the kidney (Botting & LOckett, 1961; Lees & Lockett, 1963). They showed that, in conscious rats, either isoprenaline, a ß-adrenoceptor agonist, or dichlorisoprenaline (DCI), a ß-adrenoceptor blocker with intrinsic sympathomimetic activity (ISA), causes anti-diuresis coupled with anti-natriuresis. These responses were attributed to an action on ß-adrenoceptors, since the effects of isoprenaline were antagonised by pronethalol. However, in spite of several ß-blockers, with or without ISA becoming available since, little clear evidence exists as to their effects on urine and electrolyte excretion.

Male Charles River U.K. rats (CD strain) were deprived of food 24 h prior to the experiment, but allowed access to water up to 1 h before dosing. 12 rats were used in each experiment. There were 4 or 6 rats per group. Each rat received an oral dose of 2.0 ml/100 g of physiological saline (0.9%). Drugs were incorporated in the saline. Controls received saline only. The rats were placed in individual containers, and the urine output collected for 5 h. The cumulative volume excreted by each animal was noted at hourly intervals after dosing, and the Na^+ and K^+ levels of the 5 h total volume was determined using a Corning 430 flame photometer.

Pindolol, a ß-blocker with marked ISA, caused a dose related inhibition of diuresis ($E_{max}50$ = 0.003 mg/kg). Concurrently, Na^+ and K^+ excretion were also inhibited. Similar effects, albeit to a considerably lesser extent, were obtained with acebutolol ($E_{max}50$ = 24 mg/kg) and diacetolol ($E_{max}50$ = > 90 mg/kg). (-)-acebutolol was more potent than (+)-acebutolol. Salbutamol, a ß-stimulant, also behaved in the same manner ($E_{max}50$ approx. = 0.03 mg/kg). In contrast, the non-selective ß-blocker, propranolol, and a vascular-selective experimental compound, each without ISA caused increased urine and electrolyte excretion and were able to antagonise the antidiuretic effects of pindolol and acebutolol. This antagonism appeared to be dose related. Combination with hydrochlorthiazide easily abolished the anti-diuretic effects of acebutolol. Atenolol and metoprolol, the cardioselective ß-blockers without ISA, had no effect on urine excretion. It is suggested that ISA confers antidiuretic activity to ß-blockers as a positive correlation, and that $ß_2$ blockade may be responsible for the precipitation of diuresis. In man, propranolol has been shown to cause significantly increased diuresis and decreased urinary osmolarity (Imbs et.al., 1976, 1977), and also to reverse the urine and electrolyte retaining properties of isoprenaline (Levi, et.al., 1976). The method provides a simple and sensitive means of evaluating the ISA of ß-adrenoceptor blocking agents in a single dose.

The antidiuretic effect of acebutolol is lost when given daily for 5 days, and has not been reported in man.

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PILOCARPINE SELECTIVELY STIMULATES MUSCARINIC RECEPTORS IN RAT SYMPATHETIC GANGLIA

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Brown et al (1980) have compared the potencies of various muscarinic agonists on rat isolated ganglion and ileum and found that all the agonists tested had similar potencies on the two tissues. In our studies of muscarinic effects on ganglion and ileum we have used pilocarpine, muscarine and arecoline as standard muscarinic agonists, but have obtained results with pilocarpine which conflict with those of Brown et al. (1980).

Depolarizations of rat isolated superior cervical ganglion were recorded extracellularly using a modification of the partition bath system described by Brown & Marsh (1978). The ganglion bath was continuously perfused with Krebs' solution prebubbled with 95% O_2/5% CO_2 and maintained at 34°C. Segments of rat ileum were suspended in Krebs' solution at 34°C, at a resting tension of 0.5g and responses were recorded isometrically. The agonist potencies (ED_{50} values) of arecoline, pilocarpine and muscarine on ganglion and ileum are shown in Table 1.

Table 1 Median ED_{50} values for muscarinic agonists on rat ganglion & ileum

	GANGLION		ILEUM	
	ED_{50}	n	ED_{50}	n
Pilocarpine	4.3×10^{-7}M	6	*1.5×10^{-5}M	5
Arecoline	3.7×10^{-7}M	7	9.5×10^{-7}M	5
(±)Muscarine	1.2×10^{-7}M	13	4.1×10^{-7}M	10

*partial agonist

Muscarine and arecoline were about equipotent on the two tissues, whilst pilocarpine was approximately thirty times more potent on the ganglion than on the ileum. Our ED_{50} value for pilocarpine on the ganglion is approximately ten times lower than the value quoted by Brown et al (1980). This could have been due to differences in the temperature at which the experiments were performed: our studies were carried out at 34°C, while those of Brown et al were at 24°C. However, in preliminary experiments at 24°C we have found pilocarpine and muscarine to have ED_{50}s indistinguishable from those at 34°C. The greater potency of pilocarpine on the ganglion cannot be attributed to nicotinic receptor activation, as atropine (10^{-6}M) produced 3000-fold shifts of both pilocarpine and muscarine dose-response curves in this tissue.

The only other appreciable difference in methodology between the two ganglion studies is that Brown et al (1980) used a one minute application period for all agonists. In contrast, in the present study, agonists were applied continuously until the depolarizations reached a plateau. Using this procedure, it was evident that responses produced by pilocarpine were very much slower in onset than those of the other agonists tested, sometimes requiring a ten minute application before responses peaked. In accord with this, we have found that application of pilocarpine for only one minute resulted in an underestimate of its potency on the ganglion.

The present results therefore demonstrate that pilocarpine is a selective stimulant of muscarinic receptors in ganglion.

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The authors are indebted to Carolyn Bula for excellent technical assistance.

SOME PHARMACOLOGICAL EFFECTS OF DELTA-AMINOLAEVULINIC ACID ON THE ISOLATED SMALL INTESTINE OF THE RABBIT

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We have previously reported that delta-aminolaevulinic acid (ALA) exerts pharmacological effects in isolated preparations of rabbit small intestine. It inhibits spontaneous contractile activity at concentrations above 0.2 mM in the bathing fluid and inhibits contractions induced by acetylcholine, ouabain or barium chloride at concentrations above 1.5 mM (Cutler et al, 1980). These findings are relevant to gastrointestinal symptoms observed in acute episodes of intermittent porphyria and in lead poisoning when blood levels of ALA are significantly elevated from a value around 3 μmol/l to values in the range of 20-200 μmol/l in porphyria and 8 μmol/l in lead poisoning (Meredith et al, 1978).

In the present experiments the effects of pretreatment with phentolamine, propranolol, indomethacin and atropine followed by ALA administration have been examined. In addition, inhibitory effects of ALA have been compared with those of gamma-aminobutyric acid (GABA). Preparations of rabbit small intestine were bathed in oxygenated Ringer-Locke solution at 37°C and contractions of the preparation were recorded by isotonic transducer and displayed on a calibrated Washington 400 MD2R oscillograph.

Complete inhibition of spontaneous contractions occurred for 28±8 sec after administering ALA to reach a concentration of 0.8 mM and for 378±80 sec when the concentration of ALA was 7.6 mM (mean ±s.e.mean). The duration of inhibitory effects of ALA at a level of 7.6 mM was significantly reduced to 60±35 sec (mean ±s.e.mean, $P<0.01$) when the preparation was pretreated with phentolamine (2 μM) but was not affected by pretreatment with propranolol (7.7 μM) or by atropine (3.5 mM). Phentolamine (2 μM) completely prevented adrenaline's effects (0.5 μM). It would thus appear that part but not all of ALA's inhibitory action on the small intestine is mediated by interaction with alpha-receptors or by release of noradrenaline.

On return of contractile activity following its inhibition by ALA, the amplitude of contractions progressively increased, occasionally reaching values greater than those seen prior to ALA administration, and the rate of contractions was always slower than that seen before ALA treatment. Pretreatment with indomethacin (56 μM) significantly reduced the amplitude of returning contractions.

Treatment of the preparation with GABA did not bring about effects similar to those of ALA. In fact, treatment with GABA had no detectable effect in 5 out of 13 preparations at concentrations up to 5 mM, and in the other preparations either reduced the amplitude or inhibited contractions for 30 to 500 sec with concentrations ranging from 1 mM to 5 mM. In 2 preparations an enhancement of contractile activity followed the period of inhibition produced by GABA.

These results suggest that effects of ALA on rabbit small intestine are independent of interaction with GABA receptors unlike actions previously reported when effects on neuronal preparations were studied (Brennan & Cantrill, 1979). Interaction with alpha-receptors or release of noradrenaline appears to be in part responsible for inhibitory effects in the rabbit small intestine.

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INHIBITORY ACTION OF 5HT ON REBOUND CONTRACTIONS OF RAT GASTRIC CORPUS TO NON-ADRENERGIC, NON-CHOLINERGIC NERVE STIMULATION

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Field and vagal stimulation of non-adrenergic, non-cholinergic (NANC) nerve fibres of the rat gastric corpus muscle produces an inhibitory response which is followed after stimulation by a rebound contraction (Hunt et al 1978). During an investigation of the use of 5HT in conjunction with a 5HT uptake blocker (fluoxetine) for raising the tone of the rat gastric corpus muscle we noted that both fluoxetine and 5HT were independently capable of reducing the rebound response to field stimulation. Reported here is an analysis of the effect of 5HT on the rebound contractions suggested by these preliminary observations.

The rat gastric corpus muscle was prepared and field stimulation carried out as described by Hunt et al (1981). Parameters of vagal stimulation were supramaximal voltage, frequency 7Hz, pulse width 0.5ms for 30 - 45 s duration every 500 s.

5HT (10^{-7}-10^{-4}M) induced a contractile response in the rat gastric corpus muscle which could be antagonised completely by a combination of atropine (10^{-6}M) and methysergide (10^{-6}M). 5HT apparently stimulated both cholinergic excitatory nerve fibres and the muscle. Furthermore in the presence of atropine (10^{-6}M) and methysergide (10^{-6}M) there was no 5HT induced relaxation of the tissue even in preparations where the tone had been increased by $BaCl_2$ (10^{-4} - 10^{-3}M). However in the rat gastric corpus muscle 5HT (10^{-6} - 10^{-4}M), in the presence of atropine 10^{-6}M) and methysergide (10^{-6}M), suppressed the rebound contraction to field and vagal stimulation. Repeated application of 5HT (10^{-3}M) every 8 min for 30 min failed to desensitise the mechanism inducing the inhibition of the rebound contraction to field stimulation.

In view of our recent observation (Hunt et al 1981) that low frequency sympathetic nerve stimulation inhibits rebound contractions following field stimulation we decided to investigate the effect of drugs that interfere with sympathetic nerve transmission on the 5HT induced effect. Propranolol (10^{-5}M) and guanethidine (10^{-6}M) abolished 5HT (10^{-4}M) suppression of the rebound contraction to field and vagal stimulation. Sympathectomy produced by 6-hydroxydopamine pretreatment 24 hours before the experiments (250mg kg^{-1} i.p.) prevented 5HT suppression of the rebound contraction to field stimulation. As field but not vagal stimulation would be expected to activate sympathetic neurones in the stomach wall the observation that the noradrenaline uptake blockers cocaine (10^{-6}M) and desipramine (10^{-7}M) could inhibit the rebound response to field but not vagal stimulation was expected. Furthermore this suppression of the rebound response to field stimulation by uptake 1 blockers was antagonised by guanethidine (10^{-6} - 10^{-5}M) and 6-hydroxydopamine pretreatment. Propranolol (10^{-5}M) and guanethidine (10^{-5}M) failed, however, to potentiate the rebound contraction to either stimulation, suggesting that there was negligible sympathetic effect following vagal and field stimulation.

These results suggest that 5HT can suppress field and vagally stimulated rebound contractions by releasing noradrenaline and furthermore that the noradrenaline released by field stimulation in the presence of uptake 1 blockers can also inhibit this response, 5HT may be stimulating sympathetic neurones or displacing noradrenaline from storage sites. The amount of noradrenaline released by 5HT was insufficient to produce a relaxation in preparations with either low or high tone.

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INHIBITION OF 1-O-ALKYL-2-ACETYL-SN-GLYCERYL-3-PHOSPHORYLCHOLINE (PLATELET-ACTIVATING FACTOR)-INDUCED HUMAN PLATELET ACTIVATION

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Platelet-activating factor (PAF-acether), a mediator of allergy and inflammation synthesized and/or released by several cell types, including blood platelets, may be the endogenous mediator of platelet aggregation responses that are independent of ADP and arachidonate metabolites (PGG_2, PGH_2 and TxA_2) (Chignard, Le Couedic, Tence, Vargaftig and Benveniste, 1979). Although the effects of PAF-acether on platelets of animal species are well documented its actions on human platelets have not been studied in detail. We have examined the effects of several anti-platelet agents on human platelet activation induced by purified PAF-acether (1-O-heptadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) dissolved in an albumin buffer as described by McManus, Hanahan and Pinckard (1981). Platelet aggregation, 5HT release and TxB_2 production were monitored in 0.17 ml samples of human citrated platelet-rich plasma (PRP) or gel-filtered platelets (GFP) suspended in modified Tyrodes solution (McMillan, MacIntyre, Booth and Gordon, 1978).

In PRP and GFP, low concentrations of PAF-acether (PRP, 10-100nm; GFP, 1-10nm) induced reversible aggregation in the absence of TxB_2 production or 5HT release (primary aggregation). Responses to higher concentrations of PAF-acether were irreversible and accompanied by TxB_2 formation (<500pmol/10^9 cells) and release of 5HT (<60% of total) (secondary aggregation). Primary and secondary aggregation induced by PAF-acether were inhibited by PGD_2 (10-100nM), PGI_2 (1-10nM), 8-bromo-cyclic AMP (0.5-1mM), 8-bromo-cyclic GMP (0.1-1mM), Verapamil (10-100μM), 8-(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate (TMB-8, 10-100μM), Quinacrine (10-100 μM) and Tosyl-l-arginine methyl ester (TAMe, 0.03-1mM). The ADP antagonist β,γ-methylene-ATP (<100μM) and agents such as aspirin (900mg p.o., 12 hours previously) which block the synthesis, or 13-Azaprostanoic acid (<100μM) and Trimethoquinol (<100μM) which block the actions of PG endoperoxides and TxA_2, only inhibited secondary aggregation. The thrombin-inhibitor, Hirudin (25u/ml) and Acetylcholine, Atropine, Gallamine, Decamethonium, Hexamethonium and Pancuronium (all at <100μM) failed to inhibit PAF-acether-induced platelet aggregation.

These results indicate that PAF-acether-induced human platelet aggregation is independent of thrombin generation and of endogenous ADP or arachidonate metabolites. The platelet response is suppressed by the esterase inhibitor, TAMe; by agents which cause or mimic an elevation of the intracellular concentration of cyclic AMP or cyclic GMP; by agents which interfere with intracellular calcium availability (Verapamil, TMB-8), and by Quinacrine acting by an unknown mechanism. Although the configurations of the acetyl and choline groups in PAF-acether and in Acetylcholine are similar (Satouchi, Pinckard, McManus and Hanahan, 1981), the lack of effect of Acetylcholine and its antagonists on PAF-acether-induced platelet aggregation indicates that the putative receptor with which PAF-acether combines to initiate platelet activation is distinct from any of those acted upon by Acetylcholine.

W.K.P. and A.M.S. are MRC scholars. This study was supported by the Medical Research Funds of the University of Glasgow. PAF-acether was a gift from Dr. R.N. Saunders, Sandoz Inc.

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IS THE QUANTITY OF PROSTAGLANDINS REMOVED BY THE LUNGS DEPENDENT UPON THEIR LEVEL OF VENTILATION?

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In a preliminary communication (Francis & Smy, 1978) we reported that i.a. or i.v. infusions of prostaglandins E_1 or E_2 in cats caused a dose dependent decrease in pentagastrin stimulated gastric secretion. It was observed that the dose response lines obtained with i.a. or i.v. infusions of each prostaglandin were not parallel. We presented evidence to suggest that this may be due, in part, to differential changes in gastric mucosal blood flow, estimated by amidopyrine clearance, occurring during i.a. and i.v. infusions.

It has been suggested (Harper *et al*, 1970) that in vagus intact cats the resulting increased response of the stomach to pentagastrin may be due to the increased mucosal blood flow (MBF) observed in these animals compared with that observed in vagotomised animals. A series of experiments was designed to investigate the influence of the vagus on the action of PGE_2 on pentagastrin stimulated secretion. No difference in the mean reduction in acid secretion was observed between vagus intact and vagotomised cats when the PGE_2 was infused i.a. or i.v. (57±11% cf 57±9% i.a.; 47±16% cf 47±6% i.v.; mean ± s.e.m. in vagus intact and vagotomised respectively; n=6 in each group). In the vagus intact group in which PGE_2 was infused i.v. the variance of the mean was greater ($p < 0.05$) than in the corresponding vagotomised group. This increased variance could not be attributed to other recorded parameters, namely gastric MBF or systemic BP. A subsequent series of experiments were undertaken in which the vagus nerve was left intact during an intial infusion of PGE_2 and cut prior to a second infusion of the same dose of PGE_2. The degree of reduction in acid output observed during this second test did not show a consistent change from that observed in the first test. Once again changes in acid secretion appeared to be independent of changes in MBF or BP, but were inversely proportional to, and correlated with the level of ventilation (rate x volume) prevailing at the time of the infusion ($p < 0.001$).

In order to determine the effect of varying levels of ventilation on the PGE_2 induced reduction in gastric acid secretion an artificial ventilator was used to control the level of ventilation in animals with phrenic nerves cut or intact. In four phrenic intact preparations after an initial i.v. infusion of PGE_2, during which the animal was allowed to respire naturally, an articial ventilator was applied and the infusion of PGE_2 was repeated. The mean reduction in acid during the 1st infusion was 46±6% which was greater than during the 2nd infusion 10±9% ($p < 0.05$). In experiments in which the phrenic nerves were crushed, the ventilation level could be changed as required, after reference to a ventilation standards table (Kleinman & Radford, 1964). In one group the ventilation volume was set at a 'normal' level and subsequently increased and in a second group set initially high and reduced. In the 1st group the reduction in acid secretion was 64±10% (n=5) during 'normal' ventilation and 3±27% during the period of increased ventilation ($p < 0.05$). In experiments in which four animals were ventilated at an initially high level which was subsequently reduced the change in pattern of acid inhibition was less clearly defined. During these experiments changes in pH, pCO_2 and pO_2 were monitored, but were not different in any group. In conclusion therefore, we propose that the quantity of prostaglandin surviving passage through the lungs following an i.v. infusion is dependent upon the level of ventilation prevailing at that time.

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INHIBITION OF THROMBOXANE SYNTHESIS WITH UK-37,248 PREVENTS FIBRILLATION FOLLOWING REPERFUSION OF THE ISCHAEMIC MYOCARDIUM

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We have reported recently (Coker, et al., 1981) that thromboxane (Tx) is released into blood draining from the acutely ischaemic myocardium and that this release is related to the occurrence of early post-ligation arrhythmias. The aim of this present study was to determine whether Tx contributes to arrhythmias that arise as a result of reperfusion of a formerly ischaemic area of the myocardium. The arrhythmias induced by reperfusion are particularly severe and frequently lead to ventricular fibrillation (VF). These arrhythmias also appear to be resistant to a number of standard antiarrhythmic agents (Naito, et al., 1981).

Greyhounds were anaesthetised with chloralose and catheters were placed, under fluoroscopic control, in the aorta, pulmonary artery, coronary sinus and in the lumen of the left ventricle. After thoracotomy a catheter was placed in a coronary vein adjacent to the left anterior descending (LAD) coronary artery. This catheter, after LAD occlusion, drains blood predominantly from the ischaemic region (Marshall et al., 1974). Plasma concentrations of TxB_2 (the stable metabolite of TxA_2) and 6-keto $PGF_{1\alpha}$ (a metabolite of prostacyclin) were measured by radioimmunoassay.

After a 40 min LAD occlusion period, the occlusion was released and the subsequent reperfusion of the ischaemic area resulted in ventricular extrasystoles, multifocal in origin, usually progressing to tachycardia and VF. Six out of seven animals in the control group died in VF, four of them within one minute of reperfusion. Since most animals exhibited severe arrhythmias immediately following reperfusion, blood samples were obtained for the analysis of TxB_2 and 6-keto $PGF_{1\alpha}$ in only a few animals.

The administration of UK-37,248, a thromboxane synthetase inhibitor (Tyler et al. 1981) at a dose of 2 mg/kg i.v. 15 min prior to coronary artery ligation plus 1 mg/kg i.v. 5 min prior to reperfusion, resulted in a dramatic decrease in the incidence of VF following reperfusion. Seven out of eight animals survived ($P<0.01$ compared with controls, chi-squared test). UK-37,248 itself had no effects on heart rate, arterial blood pressure, pulmonary artery pressure, LVEDP, LVdP/dt max or cardiac output or on the circulating TxB_2 concentrations in the aorta; 45±13 pg/ml pre-drug, 50±10 pg/ml post-drug. There was, however, a significant decrease in coronary sinus TxB_2 concentrations; 77±21 pg/ml pre-drug to 52±12 pg/ml ($P<0.05$, t-test). Aortic 6-keto $PGF_{1\alpha}$ concentrations were not significantly altered (354±48 pg/ml to 486±106 pg/ml) although coronary sinus concentrations increased from 397±75 pg/ml to 542±101 pg/ml after the drug ($P<0.05$)

In most surviving animals arrhythmias ceased to occur by 15 min post-reperfusion at which time the various cardiovascular parameters were not significantly different from values prior to reperfusion. The area of occlusion was similar in both the control and the drug treated groups; 35.4±1.3% and 35.6±0.9% of the free left ventricular wall respectively. Thus inhibition of thromboxane synthesis appears to be a successful intervention for preventing VF following reperfusion of the acutely ischaemic myocardium. These results suggest that thromboxane may be an important contributory factor in post-reperfusion VF.

We thank SHERT and Pfizer for financial support.

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THE RELATIONSHIP BETWEEN PGI_2 INFUSION RATE, BLOOD PRESSURE, RENAL FUNCTION AND URINARY 6-KETO-$PGF_{1\alpha}$ EXCRETION IN CONSCIOUS DOGS

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PGI_2 which is synthesised in the renal cortex and by vascular endothelial cells is a vasodilator, stimulates renin release and causes natriuresis (see Jones & Watson, 1980). PGI_2 is unstable at physiological pH and is rapidly hydrolysed to 6-keto-$PGF_{1\alpha}$. The latter is frequently measured as an index of PGI_2 production. 6-keto-$PGF_{1\alpha}$ is thought to be freely filtered and is excreted in the urine. (Rosenkranz et al, 1981). In this study, the excretion rate of 6-keto-$PGF_{1\alpha}$ in conscious dogs has been determined after intravenous infusion of 3 dose levels of PGI_2. Systemic blood pressure (S.B.P.), renal function and plasma renin activity (P.R.A.) have also been determined.

Eight male dogs were surgically prepared with carotid arterial loops and indwelling venous catheters. 5% Dextrose solution containing inulin and P.A.H. was infused intravenously (4 ml/min) to establish a modest water diuresis and permit measurement of glomerular filtration rate (G.F.R.) and renal plasma flow (R.P.F.). A catheter was inserted into the bladder. PGI_2 was infused intravenously at 7.5, 15 and 30 ng kg^{-1} min^{-1} for 4 hours and control values were obtained from infusion of buffer alone. Urinary excretion of 6-keto-$PGF_{1\alpha}$ was estimated by gas-chromatography mass-spectrometry using a multiple ion detection technique.

S.B.P. is decreased within the first 60 mins of the two higher infusion rates of PGI_2 after which compensatory mechanisms return the pressure towards control values despite continued PGI_2 infusion. R.P.F. tended to be higher during the PGI_2 infusions but this effect was not dose-related and G.F.R. and urine flow showed no significant change from control during any PGI_2 infusion. There was a gradual increase in sodium excretion during all infusion rates of PGI_2 although the lowest infusion rate had the greatest effect despite there being no change in G.F.R. Only the two higher infusion rates of PGI_2 increased P.R.A. and it is possible that the angiotensin II consequently formed attenuated the natriuretic action of the higher concentrations of PGI_2. Urinary 6-keto-$PGF_{1\alpha}$ was higher during all PGI_2 infusions than during the control infusions and the total amount excreted during the 1.5-4 h period was 1.73±0.32 ng, 4.46±0.96 ng, 5.86±1.19 ng and 6.75±1.76 ng for control, 7.5, 15 and 30 ng kg^{-1} min^{-1} infusions respectively (n = 5).

It is known that PGI_2 is enzymatically metabolised in the liver (Wong et al, 1980), kidney (Wong et al, 1979) and vessel wall (Wong et al, 1978) and that these metabolites, as with 6-keto-$PGF_{1\alpha}$, are excreted in the urine and can originate from circulating PGI_2 as well as from renal PGI_2. Obviously it would be advantageous to measure more than one metabolite as an index of total body PGI_2 synthesis, however the results from this study show that, over a 4 fold infusion range of PGI_2, there is linear recovery of urinary 6-keto-$PGF_{1\alpha}$ and, presuming that endogenous PGI_2 is metabolised in the same way as exogenous, this would indicate that urinary 6-keto-$PGF_{1\alpha}$ is a reliable indicator of PGI_2 production.

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This work was supported by a grant from the British Heart Foundation.

THE EFFECT OF EVENING PRIMROSE OIL ON BODY WEIGHT, FOOD INTAKE AND DIET SELECTION OF CAFETERIA-FED RATS

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Prostaglandins reduce food intake and have been proposed to contribute in the regulation of energy balance (Baile et al, 1973). Vaddadi and Horrobin (1979) reported that evening primrose oil (EPO), a dietary source of the prostaglandin precursor γ-linolenic acid, reduced body weight in normal and schizophrenic individuals. This effect was most effective in those individuals who were overweight by more than 10% of their ideal body weight. This effect of EPO was therefore examined in normal weight rats fed standard laboratory chow and in rats made overweight by palatable food stuffs presented in a cafeteria diet (eg Sclafani and Springer, 1976). 32 female rats were used. 16 rats received ad lib water and lab chow, 16 rats received, in addition, white bread and digestive biscuits. They were allowed to feed undisturbed for 57 days, thereafter they were weighed, handled and sham-injected daily for 7 days. The rats were further divided. 8 rats of each diet group received a dose of 500 mg EPO/ml (i.p.) while the other 16 rats received 1 ml of liquid paraffin daily for 21 days. This dose was then doubled and administered for an additional 14 days. 24-h food intake measures were taken at days 7, 14 and 21 (500 mg EPO) and at days 7 and 14 (1000 mg EPO). Intake measures were corrected for spillage and the results were converted into energy values (Paul and Southgate, 1979). The results were analysed by analyses of variance. At the end of the 64 adaptation days, cafeteria-fed rats weighed more (277.7 vs 252.5 g; $F(1,28) = 7.82$; $p<0.05$) than their chow-fed controls and had a significantly higher energy intake (244.5 vs 168.1 KJ; $F(1,28) = 55.91$; $p<0.01$). Cafeteria-fed rats were selecting approximately 10, 50 and 40% of their total daily energy intake from chow, bread and biscuits respectively. Neither 500 nor 1000 mg of EPO had any effect on body weight on either cafeteria or chow-fed rats. 500 mg of EPO had no effect on energy intake. Chow-fed rats continued to consume as much as during baseline. Cafeteria-fed rats, regardless of treatment reduced energy intake slightly. 1000 mg EPO inhibited an increase in energy intake in both paraffin-treated control groups (Paraffin-treated vs EPO:243.4 vs 201.7 KJ; $F(1,28) = 7.74$; $p<0.01$). 500 mg EPO failed to alter the proportion of total energy selected from chow, bread and biscuits. 1000 mg and 2 ml of liquid paraffin both significantly increased the proportion selected from chow from 9.6 to 20.6% while reducing the proportion selected from biscuits from 43.2 to 23.1%. These results indicate: (1) EPO, at these doses, has very little effect on energy intake, and it is possible that longer administration of this dose could have an effect on body weight; (3) Cafeteria-fed rats are sensitive to paraffin and EPO administration effects on energy intake and diet selection. The reasons for these effects remain to be elucidated.

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METABOLISM AND SERUM PROTEIN BINDING OF PROPRANOLOL IN RATS WITH RAISED ERYTHROCYTE SEDIMENTATION RATES

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Arthritic subjects with a raised erythrocyte sedimentation rate (ESR) have 5-10 fold higher blood concentrations of propranolol than control subjects (Schneider et. al., 1979). This effect can also be observed in rats with adjuvant-induced arthritis (Bishop et.al., 1980). Increased total drug concentrations after oral administration of propranolol could be due to either decreased intrinsic clearance by the liver or to increased serum protein binding (Wilkinson and Shand, 1975).

The mechanisms responsible for the altered total propranolol concentrations reported above have been investigated by measuring both the in vitro metabolism of propranolol by liver microsomes and the in vitro binding of propranolol to serum proteins. Male Sprague-Dawley rats (180-200g) were injected with 0.1ml M.tuberculosis (heat-killed strains PN, DT and C) in paraffin oil (3mg/ml) into the right hind foot pad. The rats were killed approximately 15 days later and the ESR's were measured. Hepatic microsomes were prepared and the metabolism of {^{3}H} propranolol was measured by the disappearance of substrate in an incubation medium containing microsomes, NADPH generating system and propranolol (1-20μM) in 0.1M Tris buffer, pH 7.4. Serum was obtained from a second similarly treated group of rats with raised ESR's and the binding of propranolol (18-354μM) to serum proteins was measured by equilibrium dialysis.

The results are shown in Table 1. Although there was a significant decrease in cytochrome P_{450} concentrations, the in vitro metabolism of propranolol was not significantly different between the two groups. In contrast the "hybrid capacity factor" (n{P}) and the affinity constant for binding (K) were both significantly increased in the treated rats.

Table 1. In vitro Michaelis Menten & protein binding constants for propranolol

	Control rats	Treated rats
ESR	<2	14 ± 2
Cytochrome P_{450} (nmol. mg protein^{-1})	1.01 ± 0.07	0.56 ± 0.06*
Propranolol metabolism		
Km (μM)	3.5 ± 0.8	2.8 ± 1.0
Vmax (nmol. mg protein $^{-1}h^{-1}$)	47.2 ± 8.4	42.5 ± 12.8
Binding constants		
n{P} (μM)	390 ± 43	664 ± 84*
Kx10^{2} (μM^{-1})	0.7 ± 0.1	1.6 ± 0.2*

Results are mean ± s.e. mean, n = 4. * P <0.005 by Student's t test.

The results suggest that increased total drug concentrations of propranolol in rats with elevated ESR's are due to changes in serum protein binding, possibly due to an increased concentration of α -1-acid glycoprotein.

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GROWTH HORMONE - THE PITUITARY "FEMINIZING FACTOR" IN THE RAT?

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Sex differences exist in hepatic drug metabolism in the rat. These differences are controlled by androgens in the male and a pituitary "feminizing factor" (FF) in the female (Gustafsson et al, 1980; Skett & Gustafsson, 1980). The nature of FF is uncertain but has been proposed to be growth hormone (GH) (Kramer & Colby, 1976).

In this study the effects of rat GH (NIAMDD-rat GH-B6) on rat hepatic drug metabolism have been studied using continuous infusion of GH by osmotic mini-pump into castrated male animals and analysis of diazepam metabolism. GH was dissolved in two different ways: 1) distilled water containing 1% albumin (GH1) and 2) 0,01M sodium hydroxide followed by neutralisation and addition of 1% albumin (GH2).

Table 1 Effect of rat GH (10µg/hr) on rat hepatic metabolism of diazepam

	N-demethylation	3-Hydroxylation
control ♂	100 ± 19	100 ± 40
control ♂ + GH1	99 ± 25	89 ± 17
control ♂ + GH2	72 ± 15*	64 ± 12*
control ♀	85 ± 6*	71 ± 3*

Results expressed as % of control ♂, * = $p < 0,05$

It is seen that a sex difference exists in the metabolism of diazepam and that without sodium hydroxide treatment GH has little effect on this difference. A marked change in metabolism occurs, however, following treatment with GH pretreated with sodium hydroxide giving metabolism indistinguishable from the female. A study of the effect of the two pretreatments on GH has been performed using SDS-gel electrophoresis and shows that sodium hydroxide causes breakdown of the hormone to smaller fragments whereas distilled water has little effect.

It appears, therefore that GH is not the pituitary FF but that sodium hydroxide pretreatment can alter the GH to yield a product giving feminization of hepatic drug metabolism. FF may, thus, be a fragment of the rat GH molecule.

The valuable assistance of Professor J-Å. Gustafsson and Dr. Agneta Mode is gratefully acknowledged. GH was a kind gift of the NIAMDD. This work was supported by the University of Glasgow Medical Research Funds.

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EFFECTS OF PHENOBARBITONE ON DEVELOPMENT AND SOCIAL BEHAVIOUR IN THE OFFSPRING OF MICE

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Exposure to many drugs and chemical agents during the prenatal and early postnatal period is known to influence subsequent behaviour and phenobarbitone has been found to be both a physical and behavioural teratogen (Diaz & Schain, 1978; Fedrik, 1973). Effects on brain development, subsequent learning ability and levels of activity in the open-field have been reported (Diaz & Schain, 1978; Middaugh et al, 1981) but the way in which such exposure influences social interactions between animals has still to be studied.

We have used ethological procedures as described by Mackintosh et al (1977) to obtain an objective record of acts and postures shown by animals during social encounters and thus examine behavioural changes in juvenile and adult animals exposed in utero and throughout postnatal life to phenobarbitone. Phenobarbitone was given in drinking fluid (0.8 mM) throughout pregnancy and lactation to outbred breeding mice and to their offspring after weaning, while a corresponding group of controls received tap water to drink. This treatment had no effect on breeding performance or development of pups up to the time of weaning and produced only minor effects on maternal behaviour. Weight gain of the pups receiving phenobarbitone was slower than that of controls from 6-14 weeks of age but ceased to be significantly different thereafter. The average daily intake of phenobarbitone had ranged from 35 to 52 mg/kg body weight, depending on age and sex.

Initially, mice were housed in groups of 5-7 animals of the same sex prior to observations of their behaviour. Behaviour was examined for a 10 min period when mice had been placed in a neutral cage during the early phase of the dark period of their 24 hr light-dark cycle. Encounters were always between mice of the same sex and same treatment group that were unfamiliar to each other. At 5 weeks of age treated males showed an increased mean frequency of Explore and Scan, (Treated 177 ± 42; Control 152 ± 33; $P<0.02$), coupled with a decrease in the duration of Immobility, (Treated 35 ± 21; Control 63.9 ± 42; $P<0.01$). At 15 weeks the same categories of behaviour were effected but now both sexes were involved and the effect was more pronounced, in part because controls showed less immobility and explored less as they matured. Phenobarbitone treatment was without any effect on the other forms of behaviour. At 29 weeks of age, male mice were caged in pairs for a 10 day period to stimulate aggressive behaviour. Behaviour of these mice was then re-examined using the same ethological procedures. It was found that phenobarbitone had no effect on agonistic behaviour and under these circumstances no longer stimulated exploration to a significant extent.

It can thus be seen that exploratory behaviour was the only type of behaviour stimulated by phenobarbitone under the circumstances of this experiment. The observed effects might be attributable to delayed development of inhibitory control areas in the brain (Shaywitz & Pearson, 1978). More recent experiments have revealed that exposure to this level of phenobarbitone for one week after weaning does not produce this increase in exploratory activity.

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CHARACTERISTICS OF THE NICOTINE CUE IN RATS

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Tests of nicotine as a discriminative stimulus or cue can provide a behavioural assay of its central actions (Rosecrans & Chance, 1977). We now report further studies of the specificity of the nicotine cue and of its relationship with putative nicotinic receptors. Rats were trained to discriminate the effects of nicotine (0.4 mg/kg sc) from saline in a standard, two-bar, operant conditioning procedure (Stolerman & D'Mello, 1981). In 15-min sessions beginning 15 min after administration of nicotine, the animals were reinforced with food for pressing one of two bars, whereas pressing the other bar was reinforced in sessions after saline injections. Presses on the wrong bar were counted but were neither reinforced nor punished. After 40 training sessions, discriminative effects of nicotine and other drugs were tested in 5-min sessions during which no responses were reinforced. Groups of 6-8 rats were used for these generalisation tests which were interspersed with training sessions.

It was confirmed that the discriminative effect of nicotine (0.025-0.4 mg/kg) was strongly related to dose and the ED_{50} was 0.14 mg/kg. The number of responses on the bar appropriate for nicotine, expressed as a percentage of the total number of responses on both bars, was 88.1 $\pm$ 4.1% after nicotine (0.4 mg/kg), as compared with 2.8 $\pm$ 0.7% after saline (means $\pm$ SEM; $P < 0.001$). The mean plasma concentration of nicotine increased almost linearly from 8.9 $\pm$ 0.3 ng/ml after nicotine (0.025 mg/kg) to 146 $\pm$ 6 ng/ml after nicotine (0.4 mg/kg), and it was estimated as 48 ng/ml at the ED_{50} dose. These values are of the same order of magnitude as those reported previously for cigarette smokers. Nicotine-appropriate responding was detected as early as 2.5 min after injection, it was maximal at 5-20 min, and disappeared after 80-160 min.

The central stimulants amphetamine (0.075-1.2 mg/kg) and cocaine (1-8 mg/kg), the dopaminergic agonist apomorphine (0.019-0.3 mg/kg), and the muscarinic agonist oxotremorine (0.0063-0.1 mg/kg) all failed to significantly increase nicotine-appropriate responding. Cytisine (0.4-3.2 mg/kg) increased nicotine-appropriate responding (59.0 $\pm$ 17.2% at a dose of 1.6 mg/kg; $P < 0.01$) and this effect was confirmed (73.7 $\pm$ 10.0%) in a second group of rats. All drugs were tested up to doses which were behaviourally active as shown by marked reductions in overall numbers of responses. The rats continued to discriminate nicotine reliably throughout the period of testing other drugs, as shown by repeated tests in which mean scores of 4.1% and 94.9% were obtained after saline and nicotine (0.4 mg/kg) respectively. Thus, of the drugs tested only cytisine, a high-affinity ligand for putative nicotinic-cholinergic receptors in rat brain (Romano & Goldstein, 1980), produced any significant degree of nicotine-like cueing effect.

We thank Mr. C. Feyerabend for determining plasma nicotine concentrations and the Medical Research Council for financial support.

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RESPONDING FOR ELECTRICAL STIMULATION OF THE BRAIN BY NICOTINE-TOLERANT RATS

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Nicotine (0.1 - 0.4 mg/kg, base sc) may initially depress the activity of non-tolerant rats trained to respond for electrical stimulation of the brain and it subsequently stimulates or reduces responding in a rate-dependent way (Clarke & Kumar, 1981a; Pradhan & Bowling, 1971). Nicotine does not seem to alter the rewarding properties of brain stimulation in non-tolerant rats but this possibility has not been tested in chronically treated rats.

Male hooded rats (n = 7) were trained to shuttle between two preselected and regularly changed arms of a Y maze in order to obtain electrical stimulation of the median forebrain bundle (see Clarke & Kumar, 1981a). This procedure tested both the rate and the accuracy of responding for a given current intensity (25 - 141 μA) as well as measuring responding during periods when no current was available ("time-out").

Two weeks were allowed to elapse after tests of acute doses of nicotine (Clarke & Kumar, 1981a) and of the effects of mecamylamine. The stability of responding was checked over four daily tests with saline and then the rats were given a constant daily dose of nicotine (0.4 mg/kg sc) for 30 days. During the first two weeks, each rat was tested daily, alternately either just before or just after its daily injection of nicotine. During the second fortnight of chronic medication, responses to 0.05 - 0.4 mg/kg of nicotine were tested. The injections of nicotine were then stopped and the rats were retested at intervals for up to 22 days of withdrawal, following which they were again given nicotine 0.4 mg/kg to test for signs of residual tolerance. Multivariate analysis of variance was used to compare trends across days, doses and current intensities.

The initial depression of responding (0 - 20 min) waned rapidly over successive days ($p < 0.001$) and concurrently, a stimulant action of nicotine became increasingly apparent 20 - 80 min after injection ($p < 0.005$). Rewarded responding was stimulated in a dose-related way ($p < 0.005$) except at high currents where baseline rates were high. Responses during "time-out" were also enhanced in a dose-related manner ($p < 0.0001$) reminiscent of tests of locomotor activity (Clarke & Kumar, 1981b). The response rates fell gradually over successive tests on alternate days, which were given before the daily injections of nicotine ($p < 0.05$), and tended to recover when the injections were stopped after 30 days; recovery in the case of "time-out" responding was significant ($p < 0.01$). Some tolerance to the depressant effect of nicotine was still present after three weeks of withdrawal ($p < 0.05$) and marked stimulation of the rate of responding was also seen 20 - 80 min after injection.

The measure of the proportion of "correct" responses failed to detect any changes in the tolerant rats' ability to discriminate the rewarding effects of brain stimulation either when their rates of responding were elevated by nicotine or in withdrawal.

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EVIDENCE FOR EXCITATORY AMINO ACID NEUROTRANSMISSION IN THE HABENULO-INTERPEDUNCULAR PATHWAY

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A considerable amount of neurochemical evidence suggests that the main fibre input to the interpeduncular nucleus (IPN) is cholinergic. Using a brain slice preparation which contains both the IPN and its main neural input, the fasciculus retroflexus of Meynert (FRM) (Brown & Halliwell, 1980), we have investigated the physiology and pharmacology of the FRM input to the IPN *in vitro*. Slices of rat brain were incubated at 30°C in Krebs' solution. Spontaneous activity was recorded extracellularly from IPN neurons using conventional recording techniques and drugs were administered by ionophoresis. A high proportion of IPN neurons were synaptically activated by stimulation of the FRM, responding with unit discharges time-locked to the stimulus with a short but variable latency. Acetylcholine (ACh) often stimulated IPN neurons as did the amino acid glutamate. The nicotinic blocking agent hexamethonium blocked responses to ACh but not those to FRM stimulation or to glutamate. In contrast, a specific antagonist of excitatory amino acids γ-D-glutamylglycine (γDGG) (Davies et al., 1980) reduced the synaptic response and the response to glutamate or D,L-homocysteic acid (DLH) (see Figure 1) but did not reduce responses to ACh. These observations suggest that at least a proportion of the FRM input to the IPN may use an excitatory amino acid as a neurotransmitter rather than ACh.

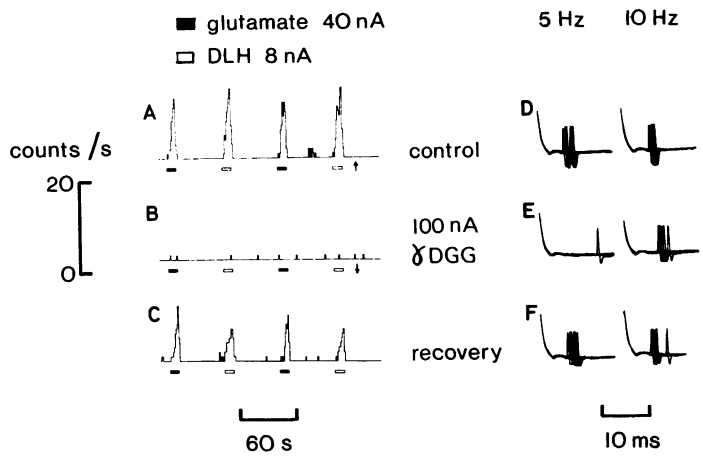

Figure 1 Responses of an IPN neuron to glutamate and DLH (A to C) and to FRM stimulation (D to F) before (A & D), during (B & E) and after (C & F) administration of γDGG. Records A to C are continuous with a 2 min break between B and C. D to F show 20 superimposed stimuli at either 5 or 10 Hz. γDGG was ionophoresed at the upward pointing arrow and terminated at the downward pointing arrow in records A to C. Nerve spikes (D to F) are retouched.

We would like to thank Dr. J.C. Watkins for his generous gift of γDGG.

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PHENCYCLIDINE: EFFECTS ON ACTIVATION OF NEUROTRANSMITTER RECEPTORS ON MAMMALIAN CENTRAL NEURONES

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Phencyclidine has anaesthetic, analgesic and psychotomimetic properties but "little is known in an unambiguous way" about its interaction with brain neurotransmitters (Johnson, 1978).

In a recent communication to this Society, Anis et al. 1981, reported that ketamine, a closely related substance selectively blocked responses of spinal neurones to N-methyl-aspartate (NMA).

We have extended this study to the effects of both local and systemic administration of phencyclidine on excitation of central neurones by electrophoretically administered amino acids and acetycholine in pentobarbitone-anaesthetised cats and rats.

On 20 dorsal horn neurones in the cat and rat, phencyclidine HCl (2-40 nA; 10 mM in 200 mM NaCl) reduced responses to NMA by 88 $\pm$ 18%, those to quisqulate by 5 $\pm$ 9% and those to kainate by 7 $\pm$ 12%.

On 6 cat Renshaw cells, phencyclidine similarly reduced responses to NMA by 74 $\pm$ 18% and those to acetylcholine by 33 $\pm$ 20%.

On 12 rat trigeminal neurones phencyclidine had similar but somewhat less specific effects on amino acid-induced excitation.

These actions of phencyclidine were dose-dependent in both effect and duration lasting up to one hour. They were not accompanied by changes in spike amplitude or configuration.

When compared on 15 of the above neurones, (+)-ketamine was found to be approximately 10 times less potent than phencyclidine but the selectivity of the two substances in reducing responses to excitatory amino acids and to acetylcholine was almost identical.

Following intravenous administration, phencyclidine (0.2-0.5 mg/kg) reduced responses of all 9 spinal neurones to NMA by 73 $\pm$ 22% with little or no effect on the action of the other excitatory amino acids. Recovery from intravenous phencyclidine took several hours. This dose level of phencyclidine and that of ketamine 2.5 mg/kg i.v. which also blocks NMA-induced excitation, is below that needed for surgical anaesthesia. But since subanaesthetic doses of the dissociative anaesthetics are analgesic (Ryder et al. 1978), it is possible that their NMA-blocking action underlies this aspect of their anaesthetic properties.

This work was supported by an MRC Project Grant to D.L. S.C.B. is an MRC Scholar. N.R.B. is a Wellcome Trust Research Training Scholar. Phencyclidine HCl was a kind gift from the Metropolitan Police.

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ANALEPTIC EFFECT OF INTRACEREBRAL INJECTION OF TRH AND STABILIZED ANALOGUES IN THE PENTOBARBITONE-ANAESTHETIZED RAT

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Thyrotrophin-releasing hormone (TRH) exerts an analeptic action following injection into discrete loci in the rat CNS, including the septum and nucleus accumbens (Kalivas and Horita, 1980). Other workers, however, failed to observe such an effect in the nucleus accumbens (Costall et al., 1979). This study investigates the comparative analeptic potencies of TRH and two biologically stable analogues RX 77368 (p Glu-His-(3,3'-dimethyl) Pro NH_2) and CG 3509 (orotyl-His-Pro NH_2) after injection into the nucleus accumbens (NA), septum or striatum of the pentobarbitone-anaesthetized rat.

Male Sprague-Dawley rats (280-300 g) were anaesthetized with sodium pentobarbitone (PB), 40 mg/kg, i.p. and sleeping time was measured as the duration of the loss of righting reflex (LRR). Intracerebral injections were made bilaterally (0.5 µl) 20 mins after induction of LRR. Each rat was used repeatedly, with an inter-test interval of at least 3 days. Drug effects were assessed against an initial and final corresponding saline (vehicle) control value. All injection sites were verified histologically. Injection of TRH or either analogue into the NA produced a dose-dependent reduction in sleeping time (Fig. 1). The analogues were approximately equipotent and significantly more potent than TRH. Similar effects were observed after intra-septal injection of the peptides, in the same concentration range. In agreement with Kalivas and Horita, 1980, TRH (5 µg) injected into the striatum had no analeptic effect. However, both analogues did provoke a significant but less marked analeptic effect in this region compared with the NA and septum. Increased respiration and reversal of P.B. induced hypothermia were observed after injection of the tripeptides into the NA and septum.

The results show that the analeptic action of the analogues was more marked than that of TRH. Furthermore the effect of the analogues and TRH was greater when they were injected into the NA or septum than into the striatum

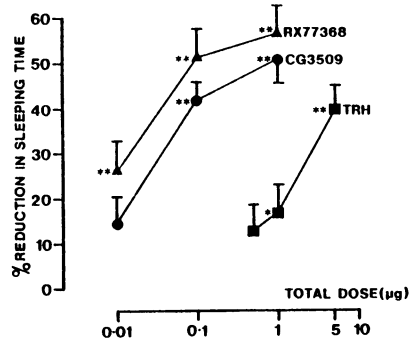

Figure 1.

The effect of intra-accumbens injection of TRH, CG 3509 and RX 77368 on PB-induced sleeping time. The results are the mean of 6 - 11 rats ± s.e.m.

* $P < 0.05$
** $P < 0.01$
Dunnett's test.

T.S. is an SERC CASE student in association with Reckitt and Colman Ltd.

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INFLUENCE OF 6 MONTH TREATMENTS WITH METOCLOPRAMIDE, PIFLUTIXOL AND HALOPERIDOL ON BEHAVIOUR AND STRIATAL (^{3}H)-SPIPERONE BINDING

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The different classes of neuroleptic drugs do not show a unitary pharmacological profile; in addition to their well-known blockade of cerebral dopamine (DA) receptors they interact to varying extents with other neurotransmitter systems including those utilising serotonin, noradrenaline, acetylcholine and histamine (Fjalland & Boeck, 1978; Peroutka & Synder, 1980). To study any functional sequaelae of these diverse actions we have treated rats for 6 months with 3 neuroleptics of widely differing activity spectra.

Male Sprague-Dawley rats (300 g) were treated via drinking water with neuroleptics at doses proportional to clinical antipsychotic potency : metoclopramide (MET, 17 mg/kg/day); piflutixol (PTX, 0.04 mg/kg); haloperidol (HAL, 1.5 mg/kg). After 6 months, while still receiving drugs, they were observed before and after challenge with 0.15 mg/kg s.c. of the DA agonist apomorphine (APOM); neuroleptics were than withdrawn, spontaneous behaviour assessed 6 days later and animals killed the next day for assay of striatal ^{3}H-spiperone (^{3}H-SPIP, 0.8nM) binding (Waddington & Gamble, 1980; Waddington et al, 1982). After 6 months of treatment, while still receiving neuroleptic, APOM stereotypy was indistinguishably antagonised ($p < 0.05$) by PTX and HAL; responses in MET animals were not antagonised but rather a trend towards facilitation observed. Prior to APOM challenge a syndrome of spontaneous perioral movements (predominantly lateral jaw movements) was noted in MET (70% incidence, $p < 0.05$) and PTX (67%, $p < 0.05$) animals over a control incidence of 27%; no such effect was seen in HAL animals (11%) (N's = 9-19). At 6 days after neuroleptic withdrawal excess spontaneous perioral movements endured only in PTX animals (67%; controls 7%, $p < 0.05$). After 7 days of withdrawal striatal ^{3}H-SPIP binding was indistinguishably elevated in all neuroleptic groups (+27-35%, each $p < 0.05$).

All 3 neuroleptic treatments induced indistinguishable 'supersensitivity' of the striatal D-2 DA receptor as exemplified by ^{3}H-butyrophenone (^{3}H-SPIP) binding (Laduron, 1980; Seeman, 1980). Despite this, these treatments were behaviourally dissociable. This is consistent with our previous report that 6 month treatments with the phenothiazines trifluoperazine and fluphenazine can be similarly dissociated behaviourally from HAL treatment despite unitary actions on the D-2 receptor; this dissociation extended to the promotion of spontaneous perioral movements by the phenothiazines but not HAL (Waddington et al, 1982). We have previously noted the striatal binding of ^{3}H-cis (Z) - flupenthixol to a site linked to DA sensitive adenylate cyclase (the D-1 site) to be elevated after phenothiazine but not HAL treatment (Waddington et al, 1982). However, the present effects were not correlated with neuroleptic potencies in influencing the D-1 site. Neuroleptics may exert a common action at the D-2 receptor. However, interactions with non-DAergic systems may be involved in some of their heterogeneous influences on behaviour, including the promotion of spontaneous perioral movements.

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SHORT TERM CHANGES IN CEREBRAL DOPAMINE RECEPTOR FUNCTION DURING CONTINUOUS HALOPERIDOL ADMINISTRATION

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Repeated administration of neuroleptic drugs to rats rapidly causes tolerance to their acute dopamine receptor blocking actions (Asper et al., 1973), such that following drug withdrawal there is behavioural (Tarsy & Baldessarini, 1974) and biochemical (Creese et al., 1976) evidence of dopamine receptor supersensitivity. We report now short term changes occurring in striatal dopamine receptor function during the first four weeks of continuous haloperidol administration to rats.

In two separate studies haloperidol (1.8±0.1 mg/kg/day) dissolved in distilled water was administered to male rats (150±10 g at the start of the experiment) as drinking water for 28 days. Control animals received distilled water only. After 1,2 and 4 weeks of continuous drug intake animals were examined for the presence of catalepsy, and the intensity of apomorphine (0.125-2.0 mg/kg sc 15 min previously) -induced stereotypy. At the same time, striatal tissue from other animals was analysed for the content of dopamine, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), for dopamine (1-150 uM) -stimulated adenylate cyclase activity, and for the specific binding of ^{3}H-spiperone (0.125-4.0 nM; 21 Ci/mmole) and ^{3}H-N,n-propylnorapomorphine (NPA) (0.0625-2.0 nM; 59.8 Ci/mmole) as defined using (+)-butaclamol (10^{-6}M).

Weak catalepsy was observed throughout the 4 week period of haloperidol administration. At 1,2 and 4 weeks stereotypy induced by apomorphine (0.125 and 0.25 mg/kg sc) was inhibited, but the effect of higher doses (0.5-2.0 mg/kg sc) was unchanged compared to control animals. No change in striatal dopamine or HVA content was observed and DOPAC levels were only elevated after 2 weeks haloperidol intake. Striatal dopamine-stimulated adenylate cyclase activity was inhibited after 1 week drug intake but was not altered thereafter.

The Bmax and K_D values for ^{3}H-spiperone and ^{3}H-NPA binding were altered by haloperidol administration as shown in Table 1.

Table 1 Bmax (pmoles/g tissue) and K_D (nM) values for ^{3}H-spiperone and ^{3}H-N,n-propylnorapomorphine (NPA) binding.

	^{3}H-Spiperone				^{3}H-NPA			
	Control		Haloperidol		Control		Haloperidol	
Week	Bmax	K_D	Bmax	K_D	Bmax	K_D	Bmax	K_D
1	25±2	0.26±0.03	38±5*	0.73±0.10*	12±1	0.53±0.01	8±1*	0.40±0.01*
2	27±3	0.30±0.04	33±4	0.38±0.07	12±2	0.55±0.11	12±2	0.62±0.11
4	21±2	0.20±0.02	33±2*	0.41±0.03*	12±2	0.43±0.06	6±1*	0.56±0.12

* $p < 0.05$ compared to control animals.

Continuous neuroleptic intake in the rat at a level producing only modest change in classical markers of cerebral dopamine receptor blockade can result in pronounced early changes in dopamine receptors, as judged by the specific binding of ^{3}H-spiperone and ^{3}H-NPA. The reciprocal changes occurring in the number and dissociation constant for the sites identified by these two ligands suggests they are not independent.

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THE ROLE OF THE GLOBUS PALLIDUS AND ENTOPENDUNCULAR NUCLEUS IN DOPAMINE-MEDIATED CIRCLING BEHAVIOUR

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The major output pathways from the striatum in the rat are to the zona reticulata of substantia nigra and to the pallidal complex, including the entopeduncular nucleus. Circling behaviour initiated by an unilateral 6-hydroxydopamine (6OHDA) lesion of the nigro-striatal pathway is known to employ the strio-nigral pathway (Tulloch et al., 1978). We report now the effect of kainic acid lesions of the globus pallidus and entopeduncular nucleus on such circling behaviour in rats.

Unilateral kainic acid (0.5 ug in 0.5 ul 0.9% saline pH 6.9) lesions of the globus pallidus (A 6.4; L 3.3; V 6.0; de Groot 1959) produced only weak spontaneous ipsiversive rotation in wide circles 3 days following surgery; it disappeared within 1 month. Administration of apomorphine (0.5 mg/kg sc) induced tight ipsiversive circling (7.4±0.8 turns/min. at 3 days), which was less after 1 month (3.3±1.2 turns/min.).

Prior unilateral 6OHDA (8 ug in 3 ul 0.9% saline containing 2 ug ascorbic acid) lesions of the medial forebrain bundle (A4.6; L 1.9; V 3.0; de Groot 1959) caused contraversive rotation (22.0±1.4 turns/min.) on administration of apomorphine (0.5 mg/kg sc) and ipsiversive rotation (8.3±1.1 turns/min.) to (+)-amphetamine sulphate (3 mg/kg ip 30 min. previously). A subsequent kainic acid lesion of the globus pallidus on the same side as the 6OHDA lesion reversed the direction of apomorphine-induced circling 3 days following surgery to ipsiversive (5.9± 1.2 turns/min.), and enhanced amphetamine-induced ipsiversive rotation (11.8± 1.7 turns/min.). By 30 days some animals had reverted to a low rate of contraversive rotation to apomorphine (5.4±1.2 turns/min.), but amphetamine-induced ipsiversive rotation was further enhanced (16.1±2.4 turns/min.).

Unilateral kainic acid (0.5 ug in 0.2 ul 0.9% saline pH 6.9) lesions of the entopeduncular nucleus (A 5.8; L 2.5; V 7.3; de Groot 1959) caused no spontaneous asymmetry and only weak ipsiversive posturing and rotation (6.0±1.2 turns/min.) in response to apomorphine (0.5 mg/kg sc) 3 days after surgery; no change occurred with time. In animals with a prior unilateral 6OHDA lesion of the medial forebrain bundle, a subsequent kainic acid lesion of the entopeduncular nucleus on the same side reduced apomorphine-induced rotation (before 18.0+1.1 turns/min.; after 1.0±0.7 turns/min.) and reduced or reversed the direction of amphetamine-induced circling (before 6.0±0.6 turns/min. ipsiversive; 3 days after 1.0±0.8 turns/min. contraversive; 1 month after 3.0±1.7 turns/min. ipsiversive).

Lesions of either the globus pallidus or entopeduncular nucleus were confirmed histologically and both failed to alter GAD activity in the substantia nigra.

The data suggests that striatal output to both the entopeduncular nucleus and the globus pallidus is important in the expression of dopamine-initiated circling behaviour.

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RELEASE OF ENDOGENOUS DOPAMINE FROM STRIATAL SLICES 'IN VITRO': COMPARISON OF RELEASE FOLLOWING HIGH K^+ AND ELECTRICAL STIMULATION

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Recent improvements in the methods for detecting catecholamines have allowed the measurement of endogenous transmitter overflow from slices of rat brain superfused 'in vitro' (Bennett et al, 1981; Nahorski & Strupish, 1981; Kapoor, 1981).

The release of dopamine (DA) and its deaminated metabolite 3,4 dihydroxyphenyl-acetic acid (DOPAC) from small slices of neostriatum from rat brain superfused 'in vitro' was estimated after High Performance Liquid Chromatography (5μ,ODS-Hypersil Column, 0.1 M Phosphate - Citrate buffer pH 4.0 with 20mg/l Sodium Octyl Sulphate, 10% Methanol & 0.1mM EDTA) and electrochemical detection on a Carbon paste electrode (+0.65V). During superfusion with Krebs-bicarbonate buffer (KBB) the output of DA and DOPAC was approximately 0.5pmole/mg protein/min and 1.9 pmole/mg protein/min respectively. Superfusion for 2 min with KBB containing 25mM K^+ (K^+ replacing 20mM Na^+) raised these to about 6.5 pmole/mg protein/min (DA) and 3.7 pmole/mg protein/min (DOPAC) (averaged over 6 min from the onset of stimulation). Reducing the Ca^{++} in the superfusate to zero, reduced the output in the presence of high K^+ to control levels.

Electrical stimulation of the slices was performed through silver electrodes at either end of the superfusion chamber. DA and DOPAC levels were increased by stimulation (200 biphasic pulses 2ms wide and 8mA at 2 - 200Hz) but the effects of such stimulation were comparatively small. The overflow of DA increased in the presence of Nomifensine (10^{-6}M). Figure 1 illustrates the frequency dependence of transmitter overflow under these conditions.

In contrast to this marked effect on electrically induced release Nomifensine had no detectable effect on the overflow of DA and DOPAC when high K^+ was the stimulus.

The effects of muscarinic agents on DA overflow which were demonstrated with K^+ stimulation (Kapoor, 1981) can also be seen with electrical stimulation which suggests that the release of DA is still sensitive to some kinds of presynaptic modulation even when increased extracellular K^+ is used as stimulus.

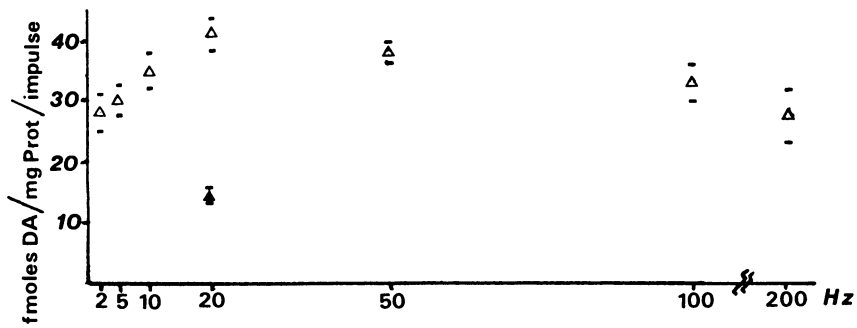

FIGURE 1. Dopamine overflow in the presence (△) & absence (▲) of Nomifensine.

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RX 781094 INCREASES THE α-mpt-INDUCED RATE OF DECLINE OF THE NORADRENALINE CONTENT OF RAT CORTEX

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RX 781094 has been identified as a potent and highly selective α_2 -adrenoceptor antagonist (Chapleo et al.1981) with activity in the rat CNS: the EEG synchrony and behavioural depression produced by the α_2-agonists clonidine and guanoxabenz were reversed by RX 781094 (Dettmar et al.1981). Yohimbine increased and clonidine decreased the α-methyl-p-tyrosine (α-mpt) induced decline of rat brain noradrenaline (NA) concentrations (Anden et al. 1976; Hedler et al. 1981). This report describes the effects of RX 781094 on the cortical NA, MHPG (3-methoxy 4-hydroxyphenylglycol) and striatal dopamine (DA) concentrations in the rat.

NA and DA were extracted from brain tissue homogenised in perchloric acid by adsorption to, then elution from alumina (Wagner, Palfreyman and Zraika, 1979). Catecholamines in the eluate were routinely separated and quantified using hplc with electrochemical detection. MHPG was assayed as in Dettmar, Cowan and Walter (1981). Either RX 781094, 5-20 mg/kg in distilled water or vehicle alone was dosed orally. In the α-mpt experiments, rats were dosed with D,L-α-mpt methyl ester HCl, 320 mg/kg i.p. NA and DA were measured every 15 min in groups of 4 rats from 0-2 h following dosing. The data were adequately described by a single exponential model, from which the mean rate of decline (k) and the mean pool size were determined. The product of the pool size and k was the apparent turnover rate.

After RX 781094, the α-mpt-induced rate of decline of cortical NA increased from -0.167/h (control) to -0.261/h (10 mg/kg) and from -0.188/h (control) to -0.476/h (20 mg/kg), which were increases in the apparent turnover rate from 52 ± 8 to 82 ± 8 ng/g/h ($P<0.05$) and from 55 ± 10 to 138 ± 11 ng/g/h ($P<0.01$) after the 10 and 20 mg/kg p.o. doses of RX 781094 respectively. There was no change in the rate after 5 mg/kg. None of these doses of RX 781094 altered the α-mpt induced rate of decline of striatal DA. In the absence of α-mpt, there was a temporary reduction in the NA content 30 min after the 10 mg/kg dose of RX 781094 (19%, $P<0.05$) and 60 min following the 20 mg/kg dose (22%, $P<0.01$) but no change after 5 mg/kg. The steady state concentration was re-established 2h after dosing. One hour following the 20 mg/kg (but not the 10 or 5 mg/kg) dose of RX 781094, there was also a marked increase in the concentration of MHPG in rat cortex (100% $P<0.01$).

In summary, RX 781094 accelerated the rate of disappearance of cortical NA in α-mpt treated rats, temporarily decreased the steady state NA concentration and increased the MHPG concentration. These results are consistent with RX 781094 having an inhibitory effect on an α-adreno-receptor feedback mechanism controlling NA release in rat brain.

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A COMPARISON OF EPILEPTIC KINDLING IN RATS INDUCED BY FOLIC AND KAINIC ACIDS

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Recently, binding studies (Ruck et al, 1980), and neurotoxicity observations (Olney et al, 1981), have suggested that both folates, known to be convulsant in laboratory animals (Obbens & Hommes, 1973), and kainic acid, a neuroexcitant and neurotoxic agent, may act via a common receptor pathway in the mammalian central nervous system. To further examine the convulsant actions of these agents, we have compared the ability of folic acid (FA) and kainic acid (KA) to induce epileptic kindling in rats; this phenomenon being an accepted model for epilepsy (Girgis, 1981).

Epidural screw electrodes were implanted over the frontal and parietal cortex, in rats (Wistar, male 200-250g), anaesthetised with halothane. An intraventricular (i.c.v.) cannula was also implanted into the left lateral ventricle of each animal (Goff et al 1975). FA (11.3 nmol, n=8) or KA (23 pmol, n=8), dissolved in artificial CSF (Leusen, 1949), pH 7.4, were injected via the i.c.v. cannula, in a dose volume of 10 ul, once daily (Monday - Friday) for 12 consecutive occasions. These doses were chosen to be approximately 25% of the convulsive ED_{50} when given i.c.v.. Control rats (n=10) received only artificial CSF.

Both FA and KA induced kindling (behavioural clonus with epileptic EEG) after a similar number of doses (9 ±1, mean ± s.e.m.). Some behavioural effects after FA and KA were similar, progressing from intermittent restless locomotor activity with normal EEG after the initial doses, to facial clonus with epileptic EEG and, at a later stage, forelimb clonus and contralateral limb jerking. KA differed from FA in also inducing salivation, blinking, staring and the wet dog shakes syndrome.

The epileptic EEG activity produced by FA and KA was different. Prior to the onset of clonus after FA large spikes (0.5 to 1 mV) were seen on both frontal and parietal leads whereas after KA, bursts of high frequency epileptic EEG occurred on the parietal leads only. After both drugs, high voltage epileptic EEG was recorded from all leads during clonus, the parietal EEG after KA again being of higher frequency.

Although epileptic kindling was induced by both FA and KA, the characteristic differences in EEG activity between the two compounds would suggest that they act through different mechanisms. In view of the observation of Reynolds on the possible involvement of folates in epilepsy, it is interesting to note that FA, a normal dietary constituent, was able to induce epileptic kindling, (Reynolds, 1967). Also, it is important to note that the doses of FA and KA required to induce kindling were smaller than those required to produce hippocampal lesioning following local intrastriatal injection in rats (Olney et al, 1981). It remains to be determined if such lesions were produced by the kindling doses of KA and FA used in the present study.

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GABA MODULATION PREDICTS BIOLOGICAL ACTIVITY OF LIGANDS FOR THE BENZODIAZEPINE RECEPTOR

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Ethyl β-carboline-3-carboxylate (β-CCE) was isolated by Braestrup et al (1980) in their attempts to identify the endogenous ligand for the benzodiazepine receptor. We have found that ^{3}H-βCCE exhibits saturable binding to an apparently homogeneous population of sites in well washed rat brain membranes and that this binding obeys mass action kinetics at ligand concentrations <6nM with a Kd of 1nM. The equilibrium dissociation constant for β-CCE is however unaffected by concentrations of up to 10^{-4}M GABA or 150mM NaCl, either alone or in combination, a behaviour which is markedly different from that of the benzodiazepines, the affinity of which increases by a factor of 2-3 under these conditions (Tallman et al 1978; Martin and Candy 1978).

β-CCE exhibits behavioural characteristics opposed to those of the benzodiazepines; it is a pro-convulsant and is able to reverse the sedative effects of flurazepam. It is possible therefore that the behavioural actions of compounds which are able to displace the benzodiazepines from their binding sites can be predicted from the effect of GABA and NaCl on their apparent IC_{50} values against ^{3}H-βCCE binding.

Well washed cerebellar membranes were prepared as described previously (Martin & Candy 1978) and aliquots (50-100μg protein) were incubated with 0.5nM ^{3}H-βCCE (SA 24Ci/mmol; Radiochemical Centre, Amersham) in a total volume of 1 ml 0.1 Tris-citrate buffer pH 7.1. The incubation was allowed to proceed for 60 min on ice after which the membranes were separated by filtration and washed with a further 10 ml ice cold buffer. IC_{50} values were obtained with at least 5 concentrations of the compound of interest both in the presence (condition A) and absence (condition B) of 10^{-4}M GABA and 150mM NaCl. The results are shown in the Table.

Table

Compound	$\frac{IC_{50}\ \text{condition B}}{IC_{50}\ \text{condition A}}$
flunitrazepam	2.1
diazepam	1.9
flurazepam	1.7
nitrazepam	1.7
chlordiazepoxide	2.1
methyl β-carboline-3-carboxylate	0.8
ethyl β-carboline-3-carboxylate	1.0
n-propyl β-carboline-3-carboxylate	1.6

All of the benzodiazepines so far investigated showed a decrease of about 2 fold in their apparent IC_{50} values in the presence of GABA and NaCl while methyl-β-carboline 3-carboxylate showed a significant increase. However the propyl ester exhibited a decrease of about 60% indicating that this compound may possess "benzodiazepine-like" activity. It has now been shown that the methyl ester is in fact a convulsant and the propyl-ester has anticonvulsant properties similar to the benzodiazepines (Jones & Oakley 1981). These data support the hypothesis that the action of GABA /NaCl on the affinities of different ligands for the benzodiazepine receptor seems to be an accurate predictor of their effects on convulsant thresholds.

A.D. is an MRC Scholar.

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THE EFFECTS OF CHLORDIAZEPOXIDE AND CL 218,872 ON CONDITIONED SUPPRESSION IN RATS

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While anxiolytic agents ameliorate punishment-induced suppression in the rat, their effects on conditioned suppression are disputed (see Rawlins et al, 1980). Conventional conditioned emotional response (CER) procedures (Estes & Skinner, 1941) involve extensive training and suffer from baseline disruption during on-the-baseline conditioning, and other complications arise when examining drug effects (Millenson & Leslie, 1974). A CER procedure has been developed which allows rapid shaping and stabilisation of the operant response on a FR 10 schedule, where footshock is applied off-the-baseline (Yeo, 1974). This has been adapted to on-line computer control. Pre-exposure of 4 kHz (75 dB) tone was followed by off-line pacing of tone with a footshock (0.5mA for 1 s, interstimulus interval 10 s, intertrial interval 3 min).

In the present study the effects of the selective BZ_1 receptor ligand CL 218,872 (Klepner et al, 1979) and the non-selective benzodiazepine chlordiazepoxide have been examined on the CER procedure. Random groups of 8 male hooded rats (230-260g) were pretreated orally for 60 min with drugs, and were tested with the conditional stimulus (CS) alone presented during operant responding. The time taken to complete FR 10 response runs under baseline conditions and in the presence of the CS were recorded.

The FR 10 latency of control animals increased from baseline value of 17.2 ± 1.9 s to 246.3 ± 24.0 s in the presence of the CS. Both chlordiazepoxide (1.25-10mg/kg p.o.) and CL 218, 872 (5-20mg/kg p.o.) produced a significant disinhibition of conditioned suppression ($F(4,35) = 3.51$, $p < 0.05$ and $F(3,34) = 7.95$, $p < 0.01$ respectively, ANOVA). The effects were dose-dependent, with a minimum effective dose of 10 and 5mg/kg p.o. for chlordiazepoxide and CL 218, 872 producing a 65.5 and 61.3% decrease in CS FR10 latency respectively ($p < 0.05$, Newman-Keuls test). Neither agent disrupted baseline responding.

It is considered that the present procedure has a major advantage over conventional CER procedures in that the footshock is applied off-the-baseline. The technique allows a much greater control of the temporal and familiarity dimensions of the stimuli used and gives a reliable basis for evaluation of anxiolytic agents. The conditioned suppression was modified by both chlordiazepoxide and CL 218,872. However delineation of interaction with the BZ_1 receptor subtype for anxiolytic activity is premature since the in vivo receptor selectivity of CL 218,872 at equivalent doses in mice is poor (Jones & Oakley, 1982 this meeting).

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Ro 15-1788 ANTAGONIZES THE PROTECTIVE EFFECTS OF FLURAZEPAM IN THE HIGH PRESSURE NEUROLOGICAL SYNDROME

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We have demonstrated previously that drugs which facilitate GABA transmission oppose the high pressure neurological syndrome (HPNS) (Bichard, Little and Paton, 1981). One of the most effective drugs used was flurazepam. We have now studied the effects of the specific benzodiazepine antagonist Ro 15-1788 (Hunkeler et al., 1981) on this protective effect of flurazepam in order to determine whether its effects were due to its interaction with the benzodiazepine receptor or to a non-specific action (such as is thought to be the basis of the effects of general anaesthetics).

Male CDI mice were injected (i.p., s.c.) with the drugs (using coded solutions) before being placed individually in a pressure chamber. The pressure was raised (3 atm min^{-1}) using helium gas, and the pressures noted for the onset of tremors and of convulsions. The rectal temperatures were maintained at 37°C ± 1°C and the oxygen partial pressure was close to 1 atm initially. The dose of Ro 15-1788 used was that which antagonised the effect of flurazepam on the convulsion threshold to intravenous bicuculline, when the same time interval between onset of convulsions and administration of Ro 15-1788 was used. The Ro 15-1788 was given 5 min before the flurazepam.

Ro 15-1788, at 20 mg kg^{-1}, abolished the protective effect of flurazepam against both tremors and convulsions (Table 1). No effects of Ro 15-1788 alone were observed.

Table 1

Drug	Dose (mg kg^{-1})	Onset Pressures (atm + S.E.M.) Tremor	Convulsions	(n)
Flurazepam plus Tween Vehicle	10 -	97 ± 1 *	118 ± 1 *	(5)
Flurazepam plus Ro 15-1788	10 20	71 ± 2	87 ± 2	(5)
Tween Vehicle plus saline	- -	67 ± 3	85 ± 3	(6)
Ro 15-1788 plus saline	20	72 ± 3	83 ± 2	(5)

(n) = number of mice. * P 0.001 (Mann-Whitney 'U' test)

Ro 15-1788 or Tween given s.c., flurazepam or saline given i.p.

We conclude that these results provide evidence that the effect of flurazepam in protecting against the behavioural effects of high pressure is due to its action at benzodiazepine receptors (leading to facilitation of GABA transmission) and not to a nonspecific action.

We are grateful to Hoffmann-la Roche & Co. Ltd. for the gift of Ro 15-1788. A.R.B. is an M.R.C. Scholar.

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AN IONTOPHORETIC STUDY OF Ro 15-1788, A SELECTIVE BENZODIAZEPINE ANTAGONIST, ON RAT CEREBELLAR NEURONES

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The benzodiazepines produce their main pharmacological and therapeutic effects on the central nervous system by interacting with a specific benzodiazepine receptor. This receptor is a membrane protein localised in synapses utilising GABA as a transmitter (Costa et al, 1975). Electrophysiological studies have shown that the primary mode of action of benzodiazepines is the facilitation of GABAergic inhibitions (Geller et al, 1978). RO 15-1788 is an imidazodiazepine which has been shown to inhibit specific high affinity binding of 3H-diazepam to brain synaptosomal fractions but which failed to exert any behavioural effects typical of benzodiazepines (Hunkeler et al, 1981). The possibility that RO 15-1788 is a selective antagonist at the benzodiazepine receptor prompted an 'in vivo' iontophoretic study of this compound on Purkinje cells of the rat cerebellum, an area of high receptor density (Lippa et al, 1978).

Female Wistar rats weighing 180g were anaesthetised with chloral hydrate 350mg/Kg and maintained on 1-2% halothane in 100% oxygen and positioned in a stereotaxic frame. Extracellular recordings were made from the central barrel of a 7 barreled microelectrode. The remaining barrels were used for the application of the following compounds: GABA, flurazepam, loprazolam (RU 31158), nicotinamide, noradrenaline and RO 15-1788, all at 0.1M, pH 4.5, with the exception of RO 15-1788 which was used as a saturated solution. One barrel filled with 1.5M NaCl was used for current balancing.

10 to 30nA flurazepam and loprazolam markedly enhanced GABA-mediated inhibitions in 17 out of 20 and 9 out of 13 cells respectively but failed to enhance noradrenaline or nicotinamide mediated inhibitions. The effects of flurazepam and loprazolam were readily reversed and could be antagonised by picrotoxin and bicuculline. Neither antagonist had any effect on noradrenaline or nicotinamide inhibitions. RO 15-1788 at 50nA specifically antagonised the direct inhibition mediated by the two benzodiazepines in 15 out of 22 cells and reduced the benzodiazepine potentiated GABA response in all cells examined. Onset of antagonism was slow, 2-10 minutes, but recovery was rapid and complete. RO 15-1788 did not block the inhibitory responses to noradrenaline, nicotinamide or GABA applied alone. RO 15-1788 had no direct effect on cell firing rate. These results suggest that RO 15-1788 is a specific antagonist at the cerebellar benzodiazepine receptor. Its lack of activity against nicotinamide opposes the suggestion that nicotinamide is a putative endogenous ligand at the benzodiazepine receptor (Mohler et al, 1979). This imidazodiazepine should prove an important tool in defining the characteristics of the benzodiazepine receptor and in studying the possible existence of an endogenous ligand.

We are grateful to Hoffmann-La Roche for a gift of RO 15-1788

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TRYPTAMINE-INDUCED INHIBITION OF SEROTONIN RELEASE FROM RAT HYPOTHALAMIC SLICES IS MEDIATED VIA A CHOLINERGIC INTERNEURONE

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We have previously reported (Cox, Ennis & Lee, 1981) that tryptamine and serotonin produce a concentration-related inhibition of K^+-evoked tritium release from slices of rat hypothalamus preloaded with [^{3}H]-serotonin. However, unlike the response to serotonin, the inhibition produced by tryptamine was sensitive to tetrodotoxin suggesting that the receptors involved in this response were not located on the serotonergic nerve terminal and that the response might involve the release of a second neurotransmitter. Héry, Bourgoin, Hamon, Ternaux & Glowinski (1977) have identified both nicotinic and muscarinic receptors on the serotonin nerve terminals in the rat hypothalamus.

Therefore we decided to investigate the effects of cholinergic agonists and antagonists on the release of [^{3}H]-serotonin from hypothalamic slices and to determine if the response to tryptamine could be modified by drugs that affect cholinergic transmission.

The method used has been previously described (Ennis, Kemp & Cox, 1981) except that [^{3}H]-serotonin was used in place of [^{3}H]-dopamine and hypothalamic slices in place of striatal slices.

As we have previously shown, tryptamine produced a dose-related inhibition of K^+-evoked tritium release with a pD_2 value of 6.86 $\pm$ 0.15(n = 14) and was antagonised by a series of serotonin antagonists with a relative order of potency which suggested that tryptamine was acting on a postjunctional or S_1 receptor (Ennis & Cox, 1981). Atropine produced a parallel rightward shift in the concentration-effect curve to tryptamine with a pA_{10} value of 6.12 $\pm$ 0.45 whilst the nicotinic receptor antagonist pempidine had no effect in concentrations up to 10^{-5}M. Furthermore, both the IC_{50} and the maximum response to tryptamine were enhanced (by approximately 20%) in the presence of physostigmine (10^{-5}M).

Oxotremorine (10^{-9} to 10^{-6}M) produced a concentration-related inhibition of K^+-evoked tritium release with a pD_2 value of 7.56 $\pm$ 0.19 (n = 9). In contrast nicotine in concentrations up to 10^{-5}M had no effect. The inhibition produced by oxotremorine (10^{-7}M) was unaffected by the presence of tetrodotoxin (10^{-7}M) indicating that the receptors mediating the response to oxotremorine were located on the serotonin nerve terminals. The response to oxotremorine was antagonised by atropine and scopolamine with pA_2 values of 6.40 $\pm$ 0.30 and 7.84 $\pm$ 0.23 respectively. These results suggest that an inhibitory muscarinic receptor can modulate the K^+-evoked release of tritium from slices of rat hypothalamus preloaded with [^{3}H]-serotonin and that this receptor may be involved in the mediation of the response to tryptamine which appears to act via the release of acetylcholine.

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COMPLEX CHANGES IN GLUCOSE UTILISATION IN THE EXTRAPYRAMIDAL AREAS FOLLOWING QUIPAZINE ADMINISTRATION

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Microiontophoretic application of serotonin into the extrapyramidal areas of the rat brain has led to the publication of some contradictory data; some investigators finding a mainly inhibitory effect, while others found an increase in cell-firing rate to be the major event in the areas studied (Bevan et al, 1975; Dray et al. 1976). Since some of these differences might be due to different anaesthetic regimes, variations in the methods of recording and the problem of sampling a sufficient number of cells in large brain structures, it was of interest to measure the effects of the stimulation of central serotonin receptors using another parameter of cerebral function, glucose utilisation, in conscious rats.

Male Sprague-Dawley rats had femoral catheters inserted under light halothane anaesthesia. A loose plaster cast was fitted around the lower body and the rats were allowed to recover consciousness. Quipazine maleate (2-(1piperazinyl) quinoline maleate), the putative serotonin agonist, (1,3 or 10mg/kg i.v.) or vehicle (0.4ml saline) was administered ten minutes before the bolus injection of (^{14}C) 2-deoxyglucose (50uCi i.v.). The quantitative autoradiographic measurement of local glucose utilisation was subsequently carried out as described by (Sokoloff et al, 1977).

As can be seen from Table 1, quipazine produces a dose-dependent decrease in glucose utilisation in the substantia nigra (pars compacta). The 3mg/kg dose of quipazine produced 11 $\pm$ 2% and 15 $\pm$ 3% <u>decreases</u> in glucose utilisation in the substantia nigra (pars reticulata) and ventromedial caudate nucleus. While at a dose of 10mg/kg this drug produces 44 $\pm$ 4% and 20 $\pm$ 3% <u>increases</u> in these two structures respectively.

These data are, therefore, suggestive of a complex interaction between the central serotoninergic system and the major extrapyramidal areas. Further, it suggests that care has to be taken in the interpretation of data from experiments involving the use of high doses of quipazine.

TABLE 1

		QUIPAZINE (mg/kg i.v.)			
	STRUCTURE	0	1	3	
Substantia Nigra	pars compacta	83 $\pm$ 2	70 $\pm$ 4	60 $\pm$ 4[1]	61 $\pm$ 4[1,2,3]
	pars reticulata	62 $\pm$ 1	62 $\pm$ 3	55 $\pm$ 2[1,3]	89 $\pm$ 6
Caudate Nucleus	dorsolateral	99 $\pm$ 4	93 $\pm$ 3	94 $\pm$ 5	102 $\pm$ 7
	ventromedial	86 $\pm$ 4	85 $\pm$ 3	72 $\pm$ 3[1,3]	102 $\pm$ 3[1,3]

Data is given as mean $\pm$ S.E. mean (n = 5) in umols/100g/min.
1: different from control. 2: different from 3mg/kg dose.
3: different from 10mg/kg dose - ANOVA and Scheffe (p 0.05).

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