444P

BEHAVIOURAL EFFECTS OF 5-HT_1 AND 5-HT_2 AGONISTS ON NALOXONE PRECIPITATED MORPHINE ABSTINENCE

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The abrupt withdrawal of treatment in chronically morphinized rats produces a characteristic abstinence syndrome with a diverse behavioural profile. There is substantial evidence that morphine withdrawal is modulated but may not be caused by alterations in neurotransmitter function, particularly 5HT. In the light of the recent characterisation of subtypes of 5HT receptors (Peroutka & Snyder 1981) the present study sets out to evaluate the effects of both chronic and acute administration of specific 5HT agonists on precipitated morphine abstinence.

Male Wistar rats (UWIST breeding stock) were maintained on a 12h light-dark cycle and allowed free access to diet and tap water. Dependence was induced by twice daily i.p. injections of morphine. The initial dose of morphine was 20mg/kg, this was increased by 20mg/kg on each successive day until a maintenance dose of 200mg/kg twice a day was reached on the 10th day. In Schedule A rats received simultaneously with morphine one of the following treatments s.c.: Quipazine, 6-chloro-2-(1-piperazinyl)pyrazine (MK212), 5-Methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1H-indole (RU24969) and 8 Hydroxy-dipropylaminotetralin (8-OHDPAT). In Schedule B the 5HT agonists were administered as single doses prior to precipitation of abstinence with naloxone (1mg/kg i.p.). In both schedules a range of behavioural signs were recorded for 30 minutes after naloxone injection whilst changes in body weight and temperature were measured at 0,30,60 and 120 minutes. In the table below the behavioural signs and weight changes are expressed as an abstinence index score (AIS) along with separate temperature effects (Ben-Sreti et al. 1984).

		Schedule A (Acute Interactant)		Schedule B (Chronic Interactant)	
Interactant*	Dose(mg/kg sc)	AIS	Temp °C†	AIS	Temp °C†
Saline	-	56	-0.33 ± 0.18	67	-0.36 ± 0.16
Quipazine	10	64	-0.13 ± 0.17	76	-0.25 ± 0.35
MK212	10	97	+0.86 ± 0.18	62	-1.3 ± 0.46
RU24969	10	65	-2.05 ± 0.35	72	-0.41 ± 0.2
8OHDPAT	1	49	-2.0 ± 0.24	75	+0.13 ± 0.2

*All groups received chronic morphine. †-=hypothermia, +=hyperthermia, 30 minutes after naloxone

Both acute and chronic treatment with the 5HT agonists produced differential effects on the abstinence behaviours. The individual signs which have been enhanced or attenuated may not be reflected in the AIS but both MK212 and 8OHDPAT produced biphasic effects on the behavioural signs and temperature after acute and chronic administration. The data suggests a differential modulatory role of 5HT receptor subtypes in the behavioural expression of morphine abstinence.

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IN VIVO VOLTAMMETRIC EVIDENCE THAT THE 5-HT AUTORECEPTOR IS NOT OF THE 5-HT_{1A} SUB-TYPE

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It has been demonstrated that neuronal release and metabolism of 5-hydroxytryptamine (5HT) is decreased by the $5HT_1$ receptor agonist 5-methoxy-3(1,2,3.6-tetrahydro-4-pyridinyl)-H-indole (RU 24969). In vitro (Middlemiss, 1985) and in vivo (Marsden & Martin, 1985) evidence has suggested that these $5HT_1$ receptors are located on the nerve terminals and Middlemiss (1984) has further suggested that they are not of the $5HT_{1A}$ subtype. We now present in vivo evidence that 8-hydroxy-2-(di-n-propylamino)tetralin (8OH-DPAT), a $5HT_{1A}$ receptor agonist, does not decrease 5HT release and metabolism in the suprachiasmatic nuclei (SCN) when injected into the nerve terminal region (SCN) or onto the cell bodies in the dorsal raphe nucleus (DRN).

Male Wistar rats (290-310 g) were anaesthetised with chloral hydrate (600 mg/kg i.p) and held in a stereotaxic frame. Carbon fibre working electrodes were implanted into the left SCN. In addition injection cannulae were implanted into the contralateral SCN or the DRN. Differential pulse voltammograms were obtained every 5 mins and the height of the oxidation peak at +300 mV (peak 3) was taken as an indication of the extracellular concentration of the 5HT metabolite 5-hydroxyindoleacetic acid (5HIAA) (Crespi et al, 1983).

Administration of 0.9% w/v sodium chloride (1 µl) into the SCN or DRN did not affect the height of peak 3 over a period of 1 h post-administration. Injection of 8OH-DPAT at doses of 1, 2, 4 and 8 µg (0.5 or 1 µl) into the SCN did not alter the height of peak 3 recorded in the SCN. For example, 15 mins after the injection of 8 µg (1 µl) of 8OH-DPAT the height of peak 3 was 101+2% (n=4) of the preinjection control height compared with 97±3% (n=4) for animals given 0.9% saline.

The administration of 4 µg (0 5 µl) or 8 µg (0.5 µl) of 8OH-DPAT into the DRN did not change the height of the 5HIAA oxidation peak in the SCN. Peak 3 height was 97±3% (n=4) of preinjection control 15 mins after injection of 8 g of 8OH-DPAT and 94±5% (n=4) 30 min after injection. 15 or 30 mins after saline the height of peak 3 was 103+6% and 104±7% (n=4) respectively.

These data are in marked contrast to those reported previously (Marsden & Martin, 1985) when we demonstrated that 1 h after administration of 10 g of RU 24969 into the SCN peak 3 height had decreased by 82±7% (n=4). They provide, therefore, further evidence that the $5HT_1$ autoreceptor is not of the $5HT_{1A}$ subtype. In addition, they also suggest that the previously reported decrease in 5HT release and metabolism (Maidment et al, 1985) following peripheral administration of 8OH-DPAT is the result of a mechanism other than an agonist action at autoreceptors on the nerve terminals or cell bodies.

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446P

8-OH-DPAT STIMULATES INOSITOL PHOSPHOLIPID BREAKDOWN IN RAT BRAIN SLICES AND RABBIT PLATELETS

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5-Hydroxytryptamine (5-HT) is one of a growing number of neurotransmitters whose intracellular effects may be mediated by the breakdown of the inositol phospholipids).The 5-HT stimulated formation of inositol phosphate (IP) in both brain (Conn and Sanders-Bush,1984) and platelets (Schacter et al.,1985) can be inhibited by ketanserin, and it was concluded that this response was a 5-HT_2 mediated effect. We recently observed that RU24969, a putative 5-HT_1 agonist , stimulated the formation of inositol phosphates in rat brain slices (Godfrey et al.,1985). This suggests that the effects of 5-HT_1 receptors may also be mediated through stimulation of inositol lipid metabolism. 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) another agonist with 5-HT_1 activity has recently been characterised (Goodwin and Green, 1985) and we report here that this can also stimulate breakdown of inositol lipids.

Preparation and stimulation of slices from rat cerebral cortex was identical to that described previously for RU24969 (Godfrey et al.,1985). Platelets were obtained from rabbits by cardiac puncture and were prepared as described in Schacter et al.(1985).Both brain slices and platelets were incubated with 8-OH-DPAT for 30 min and the reactions were terminated with chloroform:methanol (2:1). The inositol phosphates were extracted with 1 M ammonium formate/0.1 M formic acid as described previously (Godfrey et al.,1985).

8-OH-DPAT stimulated the formation of inositol phosphates in a dose-dependent manner in both cortical slices and intact platelets. In brain the effect was not maximal at 3mM 8-OH-DPAT and at this concentration the level of IP was increased by 120% over control.In the platelet significant responses to 8-OH-DPAT could be elicited at lower concentrations of the drug, with a maximal response being elicited at 300µM. Ketanserin (1µM) and L-propranolol (5µM) failed to inhibit the response to 8-OH-DPAT in brain and platelets, suggesting that the response is not mediated through the 5-HT_2 ketanserin sensitive receptor or the 5-HT_1 propranolol sensitive receptor. The response in brain was almost completely abolished by removing $Ca^{2+}$and increasing the Mg^{2+} to 10mM which may mean that, in this tissue, the response may be an indirect effect involving some other substance. The possibility that 8-OH-DPAT may be stimulating phospholipase-C by a non-specific mechanism was tested by treating isolated rat parotid cells with 8-OH-DPAT; no stimulation of IP formation was seen in this system. Thus 8-OH-DPAT stimulates inositol phospholipid metabolism via a 5-HT receptor which we have not yet been able to characterise.

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IS RU24969 A 5-HT_2 ANTAGONIST ON THE RAT FUNDIC STRIP PREPARATION?

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Previous work has shown that the 5-HT "D" receptor located on the rat fundic strip has the properties that would be expected of a 5-HT_2 receptor (Gregg & Osborne, 1985). Thus, the 5-HT_2 antagonists mianserin and ketanserin were far more potent than N,N-dimethyltryptamine (N,N-DMT) in reducing 5-HT induced contractions while the potent 5-HT_1 agonist RU24969 (Hunt et al, 1981) had little or no contractile effects. However, a more detailed study of the effects of RU24969 revealed that this compound powerfully inhibited 5-HT induced contraction of the rat fundus. The antagonistic effects of RU24969, mianserin, ketanserin and N,N-DMT on the isolated rat fundic strip preparation (Vane, 1957) are described in this report.

Fundic strips were incubated at 35°C in Ringer-Locke solution containing 1μM atropine to eliminate the possibilty of 5-HT contractile effects caused via activation of "M" receptors. Dose response curves were constructed for 5-HT in the presence and absence of 10^{-7}M RU24969. IC_{50} values (the concentration of antagonist required to cause 50% inhibition of contraction) were determined using a fixed concentration of 5-HT (10^{-7}M) and increasing the concentrations of the antagonists. In each case standard responses were obtained to 5-HT and then the tissue was allowed to equilibrate with each dose of the antagonist for 30 min prior to retesting for the effects of 5-HT. Use of fundic strips not exposed to 5-HT antagonists showed that, when administered at 5 min intervals, the response to 10^{-7}M 5-HT became constant after 3 - 4 additions and remained stable for at least 3 h.

RU24969 (10^{-7}M) increased the ED_{50} value for 5-HT from 2.3 $\pm$ 0.4 X 10^{-8}M to 7.5 $\pm$ 0.8 X 10^{-7}M (n = 6, significantly different with $P < 0.001$) as well as increasing the maximum response by 15%

Table 1: concentration of antagonist causing 50% inhibition

Compound	IC_{50} (M)	
Mianserin	8.00 $\pm$ 0.5 X 10^{-10}	(n = 8)
Ketanserin	8.51 $\pm$ 0.9 X 10^{-10}	(n = 7)
RU24969	5.64 $\pm$ 0.6 X 10^{-9}	(n = 6)
N,N-DMT	9.60 $\pm$ 0.9 X 10^{-6}	(n = 6)

The data in Table 1 suggests that on the isolated rat fundic strip preparation, the 5-HT_1 agonist RU24969 is a potent antagonist of 5-HT_2 receptors but unlike mianserin, ketanserin and N,N-DMT (Gregg & Osborne, 1985) it exhibits competitive kinetics.

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448P

β-ADRENOCEPTOR AGONISTS STIMULATE THE RELEASE OF 5-HT FROM RAT SEROSAL MAST CELLS

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β-Adrenoceptor agonists inhibit the release of histamine and SRS-A from fragments of human lung challenged with antigen (Orange et al, 1971; Butchers et al, 1980) but have been reported to have weak and variable effects on rat serosal mast cells (Butchers et al, 1979; Marquardt & Wasserman, 1982). We have now examined the effect of a range of these agonists on rat pleural mast cells in more detail.

Unpurified rat pleural cells were passively sensitised with DNP-ovalbumin antiserum and challenged as previously described (Butchers et al, 1979) using the release of ^{14}C 5-HT from preloaded mast cells as a marker of degranulation. Except in experiments studying the duration of action, β-adrenoceptor agonists were added simultaneously with antigen.

Surprisingly, β-adrenoceptor agonists given simultaneously with antigen, enhanced release by as much as 300%, while failing to stimulate basal release in the absence of antigen. However, if cells were exposed to β-agonists for as little as 15 seconds before antigen, enhancement did not occur. Cells pre-exposed to agonists did not respond to a second exposure to agonist given with antigen. The rank order and relative potencies of β-adrenoceptor agonists to enhance 5-HT release were isoprenaline (pD_2=7.57) > adrenaline (pD_2=6.91) > salbutamol (pD_2= 6.14) > noradrenaline (pD_2=5.53), indicating that the effect was mediated through β_2-adrenoceptors. The β-adrenoceptor antagonists, propranolol (4-100nM), ICI 118551 (4nM) and atenolol (10μM) given 5 minutes before agonist and antigen, caused shifts to the right of concentration-effect curves to isoprenaline. The effective concentrations of these antagonists are also indicative of interactions with β_2-adrenoceptors. However, the shifts were non-parallel and maximum responses to isoprenaline were depressed, suggesting that antagonism was not of a simple competitive nature.

We conclude that occupation of β_2-adrenoceptors on rat mast cells enhances antigen-induced release of 5-HT and that the variable effects previously reported reflect different periods of preincubation. The present observations illustrate further the heterogeneity of mast cells and supports the view that rat serosal mast cells are inappropriate as models of human lung mast cells both for the evaluation of modulators of mast cell function and the assessment of biochemical mechanisms involved in the release process.

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THE INHIBITION OF AN ACETYLCHOLINE-INDUCED CALCIUM CURRENT IN CELL F-1 OF HELIX ASPERSA BY ADENOSINE

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Adenosine, 0.6 μM, has previously been found to depress the acetylcholine (ACh) induced depolarization ('D' response) in cell F-1 in the right parietal ganglion of Helix (Cox & Walker 1985). Adenosine has been shown to inhibit three calcium-dependent potentials in the postganglionic neurones of the rat superior cervical ganglion (Henon & McAfee 1983). The adenosine derivative AMP has also been found to depress the depolarization evoked by L-glutamate in rat cerebral cortex (Phillis et al, 1979). This depression has been attributed to AMP inhibiting the calcium component of the L-glutamate depolarization. In the light of these observations it was decided to investigate whether there was a calcium component in the ACh 'D' response on cell F-1 and if so, whether it could be blocked by adenosine.

Microelectrode recordings were made from cell F-1 in the isolated suboesophageal ganglionic mass of the snail, Helix aspersa. The cell was voltage clamped using a Dagan 8100 single electrode clamp. All experiments were performed at least 5 times. It was observed that in normal sodium but high calcium (20mM) Ringer, the ACh 'D' response was significantly increased ($p = 0.01$). The percentage increase was 99.2 $\pm$ 18.0, mean $\pm$ S.E., $n = 5$. For example, in a typical experiment the increase in the ACh 'D' response was from 7 mV to 13 mV. Adenosine had a significantly greater effect ($p = 0.05$) in depressing the ACh 'D' response in this high calcium Ringer than in Ringer with normal calcium (7 mM). The percentage depression of the ACh 'D' response in normal Ringer by adenosine was 28.8 $\pm$ 6.62, mean $\pm$ S.E., $n = 5$, whereas in high calcium Ringer this depression by adenosine increased to 48.2 $\pm$ 2.40, mean $\pm$ S.E., $n = 5$. Partial current dose-response curves to ionophoretically applied ACh were obtained using this Ringer and then repeated in sodium free, high calcium Ringer where the sodium was replaced with Tris. Under these conditions there was a small residual inward current which had a longer time course than the current obtained in normal sodium Ringer. This residual current in sodium free Ringer was approximately one third of that obtained in Ringer containing normal sodium, high calcium. The percentage of the original current remaining was 33.3 $\pm$ 3.05, mean $\pm$ S.E., $n = 5$. For example, in a typical experiment the decrease in current was 0.88 nA to 0.31 nA. This residual current could be blocked by the addition of 3 mM cobalt or 50 μM verapamil, indicating that it was a calcium current. This current could also be completely blocked by 0.6 μM adenosine.

It is concluded from these experiments that the inward current associated with the ACh 'D' response of cell F-1 consists of two components, one sodium based and one calcium based. Adenosine reduces the ACh 'D' response by depressing the calcium component of this ACh 'D' response.

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450P

EFFECTS OF INTRACEREBROVENTRICULAR (ICV) KAINIC ACID ON ACETYLCHOLINESTERASE-CONTAINING NEURONES IN RAT HIPPOCAMPUS

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Acetylcholinesterase (AChE) histochemistry performed after in vivo pretreatment with the irreversible cholinesterase inhibitor DFP, reveals three distinct populations of neurones within the rat hippocampal formation. Many AChE-positive neurones are found within the hilus of the area dentata, stratum oriens of CA1 and at the transition between stratum radiatum and moleculare of CA1. As well as containing AChE, these cells also may contain somatostatin (Zimmer et al., 1983) and/or GABA (Davies & Roberts, unpublished; Wainer et al., 1984). ICV Injections of kainic acid (KA) result in a unilateral lesion of the CA3-CA4 pyramidal cells (Wheal et al., 1984). Electrophysiological analysis, 7 days post lesion, suggests that there is either a marked reduction, or absence of GABA-mediated IPSPs in ipsilateral CA1 pyramidal neurones. This may be due to a loss of both feed forward and recurrent inhibition (Wheal et al., 1984). The aim of this study was therefore to determine the neurotoxic effects of KA on histochemically-defined populations of hippocampal interneurones.

0.5ug of KA in 0.5ul PBS was infused stereotaxically (over 30 min) into one lateral ventricle of pentobarbitone-anaesthetised rats (180-200 g). 7 days later, the animals received DFP (2.0 mg/kg I.M.) in arachis oil, together with atropine (10 mg/kg I.P.). After a further period of 17h, the animals were anaesthetised and perfused via the ascending aorta with 100 ml saline followed by 500 ml of ice-cold 4% paraformaldehyde in 0.1M sodium phosphate buffer (pH 7.4). Brains were post-fixed in 4% paraformaldehyde and 50 um coronal or horizontal sections processed for AChE (Karnovsky & Roots, 1964; Mesulam, 1982). Alternate sections were stained with thionin. No loss of AChE positive neurones within the hilus of the area dentata or the subfields of CA1 could be detected ipsilateral to the KA injection. The hippocampal formation both ipsilateral and contralateral to the KA lesion contained a similar distribution of cells to non-injected controls. These results are consistent with a previous study showing no loss of glutamate decarboxylase (GAD) immunoreactive neurones within the hippocampus following an identical lesion (unpublished).

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THE EFFECT OF DICYCLOMINE AND TRIFLUOPERAZINE ON MUSCARINIC RECEPTORS

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The muscarinic receptor has been subdivided into M1 and M2 receptor subtypes on the basis of the differential affinity of pirenzepine for muscarinic receptors present in the cortex and the heart (Hammer et al, 1980). While differences in agonist affinities in the two tissues also support heterogeneity in muscarinic receptor subtypes (Birdsall & Hulme, 1976; Birdsall et al, 1978), there have been few reports of other ligands which discriminate between these subtypes. In the present study the muscarinic subtype affinity of trifluoperazine and dicyclomine have been investigated.

All experiments were performed using EDTA washed (Cheung et al, 1982) rat cardiac and cerebral cortical membrane preparations. [^{3}H]-N methyl scopolamine ([^{3}H]-NMS) was used as the radioligand and assays were conducted at 32^{o}C in a total volume of 3 ml of 50 mmol.litre^{-1} Tris, 0.5 mmol.litre^{-1} EDTA assay buffer (pH 7.4). Incubations were for 3 h at 32^{o}C, after which time bound and free ligands were separated by vacuum filtration. Data were analysed using iterative curve fitting techniques (Munson & Rodbard, 1980; Michel & Whiting, 1984). All data are mean values from 4 separate experiments.

Dicyclomine was a potent inhibitor of [^{3}H]-NMS binding in both cardiac and cerebral membranes. Although dicyclomine displayed a higher affinity for cerebral (pIC_{50} corr. 8.5) than for cardiac (pIC_{50} corr. 7.4) membranes, the interaction of this ligand with muscarinic receptors in the cerebral cortex was atypical. Thus, while the interaction of dicyclomine with cardiac receptors was competitive (nH 0.97), that in the cortex exhibited a steep displacement curve (nH 1.05). In contrast, pirenzepine, which showed similar differences in affinity between cortex and heart (pIC_{50} corr. 7.36 cortex; 6.58 heart), exhibited a low Hill coefficient in the cortex (0.78) and the displacement isotherm in this tissue could be resolved into high (pKi 7.64) and low (pKi 6.48) affinity components.

Trifluoperazine inhibited binding of [^{3}H]-NMS to both cerebral (pIC_{50} corr. 6.25) and cardiac (pIC_{50} corr. 5.70) membranes. As with pirenzepine, this agent displayed a low Hill coefficient (0.81) in the cerebral cortex, and displacement isotherms could be resolved into high (pKi 6.61) and low (pKi 5.6) affinity components.

The results of the present study demonstrate that in addition to pirenzepine, dicyclomine and trifluoperazine display selective, albeit limited, M1 muscarinic receptor blocking activity.

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A COMPARISON OF THE MUSCARINIC AND HISTAMINE H_1 RECEPTOR BINDING ACTIVITIES OF THIAZINAMIUM CHLORIDE AND ITS MAJOR METABOLITE

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Thiazinamium chloride (T) is an aerosol bronchodilator drug that possesses antihistaminic and anticholinergic properties in animal models (Lewis et al., 1982). We have compared the in vitro muscarinic and histamine H-1 binding activity and the in vivo bronchodilator activity of its enantiomers and dl-T sulfoxide (TSO), a major metabolite (Lewis et al., 1984).

The compounds were administered by aerosol 1 min prior to either acetylcholine or histamine injected i.v. into pentobarbital anesthetized guinea pigs (Lewis et al., 1982). The bronchodilator potencies of the 4 compounds were very similar against both agonists (Table 1). The duration of effect of the dl-, d- and l-forms of T did not differ whereas dl- TSO had a shorter duration of action.

These compounds were evaluated for their inhibition of ^{3}H-quinyclidinyl benzilate (QNB; M-1 and M-2 muscarinic; Watson et al., 1983), ^{3}H-pirenzepine (M-1 muscarinic; Hammer &Giachetti, 1982) and ^{3}H-pyrilamine (H-1 histamine) receptor binding in rat brain striatal homogenates. The resultant K_is are shown in Table 1. The dl-, d- and l- forms of T demonstrated a similar binding affinity to muscarinic receptors and appear to interact with the M-1 muscarinic receptor. In contrast, dl- TSO has a greatly reduced affinity for muscarinic receptors compared to dl-T although the affinity of both compounds to H-1 receptors was similar. A similar finding was observed measuring ^{3}H-QNB binding to mature bovine trachealis muscle, dl-T (K_i 5.03 nM) exhibiting a greater binding affinity than dl-TSO (K_i 671 nM).

These studies suggest that there is a lack of stereospecificity for the enantiomers of T in terms of functional and binding characteristics. In contrast, the dl-sulfoxide metabolite possesses a weaker muscarinic antagonist profile although its antihistaminic activity is similar to dl-T.

Table 1 Comparison of the enantiomers and sulfoxide forms of thiazinamium (T)

	ID_{50} (μg)		K_i (nM)		
	Ach	Histamine	^{3}H-QNB	^{3}H-Pirenzepine	^{3}H-Pyrilamine
dl-T	1.47 (>60)	1.73 (>60)	7.2	0.7	7.3
d-T	1.11 (>60)	1.11 (>60)	6.5	1.1	NT
l-T	1.69 (>60)	2.94 (>60)	5.4	1.2	NT
dl-TSO	1.35 (13.5±2.4)	1.65 (16.2±5.2)	451	31	3.0

Duration of action in min at 15 μg shown in parentheses; NT, not tested

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IN VITRO COMPARISON BETWEEN THE NEUROMUSCULAR BLOCKADE PRODUCED BY ATRACURIUM AND TUBOCURARINE IN FROG SARTORIUS MUSCLE

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Atracurium besylate is a new competitive non-depolarizing neuromuscular blocking agent (Stenlake,1979).Atracurium has an advantage,over tubocurarine,in that the drug undergoes spontaneous degradation at the physiological pH and body temperature (by Hofmann elimination) without the need for hepatic or renal function.

The pharmacology of atracurium in man (Payne & Hughes,1981) and in animal species (Hughes & Chapple,1981) has been studied and a comparison with tubocurarine has been made in vitro preparation (Healy & Palmer,1982;Wali & Flynn,1983).
In the present investigation,the effects of atracurium and tubocurarine on the amplitude of indirectly-elicited twitch contractions were studied and compared in the isolated sciatic nerve-sartorius muscle preparation of the frog.
The preparation was set up in an organ bath (100 ml) containing frog Ringer solution kept at a room temperature of 22-24°C. The sciatic nerve was repetitively stimulated at 0.18 Hz with 5-10 V(maximum) and 0.2 ms pulse duration.The contractile responses produced by motor nerve stimulation (indirect twitch tension) were recorded isometrically by means of a force-displacement transducer (Dl 50 g) and a Washington pen recorder (model 400 MD 2C,Bioscience,U.K.).The time to onset of drug action,time to 50% and maximum block,and to 95% recovery from atracurium and tubocurarine was measured (see Table 1).

Atracurium (0.08-8.0 μM) and tubocurarine (0.127-12.7 μM) reduced,in a dose-dependent manner,the amplitudes of the indirect twitch contractions in the frog sartorius muscle.The mean (±s.e.) IC_{50} values (concentration to produce 50% inhibition of the indirect twitch tension) of atracurium and tubocurarine-induced depression of the twitch tension were: 0.64± 0.1 μM and 1.2± 0.2 μM respectively (n=6, $P < 0.01$). A dose-ratio (tubocurarine/atracurium) of 1.9:1.0 was obtained.

Table 1.Effects of atracurium and tubocurarine on the amplitude of indirectly-elicited twitch contractions in frog sartorius muscle.

	Atracurium (A) mean±s.e.	Tubocurarine (T) mean±s.e.	Dose-Ratio (T/A)	n	P<
IC_{50} (μM)	0.64± 0.1	1.2± 0.2	1.9/1	6	0.001
Time to onset (s)	28± 3	50± 4	1.8/1		
Time to 50% block (s)	66± 4	110± 8	1.7/1		
Time to 95% recovery (min)	10± 2	21± 3	2.1/1		
IC 100 (μM)	6.5± 0.1	16± 1	2.5/1	6	0.001
Time to onset (s)	11± 2	24± 4	2.2/1		
Time to 100% block (s)	27± 2	51± 4	1.9/1		
Time to 95% recovery (min)	22± 2	50± 3	2.3/1		

The amplitude of the control twitch tension was 1.8± 0.2 g tension.Table 1 shows, based on the IC_{50} values,that atracurium was twice as potent as tubocurarine.The time to onset of action,50% block and 95% recovery following atracurium administration was approximately half that of tubocurarine.

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454P

ACTION OF PIRETANIDE ON ACETYLCHOLINE RESPONSES OF CENTRAL NEURONES OF HELIX ASPERSA

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Piretanide (4-Phenoxy-3-(pyrrolidin-1-yl)-5-sulphamoylbenzoic acid) has been shown to modify chloride transport in epithelial cells of the fish gut and in corneal epithelium and is reported to act by blocking an inwardly directed chloride pump (Zeuthen et al, 1978; Candia et al, 1981). Piretanide has also been shown to abolish dorsal root depolarizing responses of GABA in frog spinal cord (Wojtowicz & Nicoll 1982). In the present study an attempt has been made to investigate whether piretanide had any effect on acetylcholine (ACh) responses of Helix central neurones, particularly the chloride-dependent inhibitory events.

Intracellular recordings were made from identified neurones in the visceral ganglion of the isolated brain of the snail, Helix aspersa. In particular cell E-4 which is hyperpolarized ('H' response) by ACh through a chloride mediated conductance increase and cells E-1 and E-2 which are depolarized ('D' response) by ACh through a sodium mediated conductance increase. The dissection was prepared according to the method of Walker (1968) and placed in recording bath, 20 ml volume. Conventional electrophysiological recording techniques were used to amplify and display the results. Permanent records were made using a Watanabe pen recorder. ACh was applied ionophoretically from a separate electrode containing 0.5 M ACh, pH 4.0. ACh was applied as one second pulses using currents in the range 50 - 1000 namps. Both standard ACh responses and ACh dose-response curves were obtained and the effect of piretanide on them analysed over time periods of up to 3 hours. Stock solution of piretanide was made up as 280 µM. All experiments were performed at least 5 times unless otherwise stated.

Neurones hyperpolarised by ACh showed a 50% reduction in the ACh response after exposure to 2.8 µM piretanide for periods between 30 and 60 minutes. For example, in a typical experiment a standard ACh 'H' response was reduced from 7.8 mV to 3.2 mV. Increasing the concentration of piretanide 10 fold did not alter the time of onset of the response. Over the same time period control experiments showed no appreciable change in the amplitude of the ACh response. Neurones depolarized by ACh showed no appreciable change in the ACh 'D' response after exposure to 28 µM piretanide for periods up to 180 minutes. In an attempt to determine whether piretanide altered the equilibrium potential value for ACh (E-ACh) on 'H' cells, which provides an indication of the chloride equilibrium potential (E-Cl), (since the ACh 'H' response is a pure chloride event) the reversal potential for ACh was determined before and in the presence of piretanide. Control values for E-ACh and hence E-Cl were -69.7 $\pm$ 1.7 mV, mean $\pm$ S.E., n = 4. In the presence of piretanide, 2.8 µM, there was no appreciable change in the value. However it was noted that at membrane potentials more negative than E-Cl, the ACh 'H' response failed to reverse in the presence of piretanide.

These studies suggest that piretanide can reduce the influx and efflux of chloride ions across the membrane of Helix neurones. But since the E-Cl value was not changed over the time period studied, no evidence was obtained for the blocking by piretanide of a chloride pump which in the case of ACh 'H' cells would involve an outward directed chloride pump.

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THE EFFECT OF REPEATED ELECTROCONVULSIVE SHOCK ON THE BEHAVIOURAL RESPONSE TO A TRH ANALOGUE (CG 3509)

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Within the nucleus accumbens, evidence suggests that a close relationship exists between thyrotrophin-releasing hormone (TRH) and 5-hydroxytryptamine (5HT) (Lighton et al, 1984a). Following chronic antidepressant treatment, levels and ex vivo release of TRH in the nucleus accumbens are increased, whilst the behavioural response to intra-accumbens injection of a TRH analogue is attenuated (Bennett et al, 1985). In contrast repeated electroconvulsive shock (ECS) results in a decrease in levels of TRH in the nucleus accumbens (Lighton et al, 1984b). The well documented, reciprocal effects of the two antidepressant treatments upon the $5HT_2$ receptor, may further suggest an interaction between TRH and 5HT in the nucleus accumbens.

In the present study, the effect of repeated ECS on behaviour induced by intra-accumbens injection of the TRH analogue CG 3509 (orotyl-histidyl-prolineamide), has been measured. Response to CG 3509 was assessed in the rat by the analogue's ability to induce hyperactivity and to reduce the duration of pentobarbitone sleeping time (Bennett et al, 1985).

CG 3509-induced hyperactivity: rats received intra-accumbens injections of CG 3509 (2x2.5 µg in 0.5 µl saline). Hyperactivity was monitored in 15 min periods for 2 h, using an Actimat doppler shift radar activity meter.

CG 3509-reduction of pentobarbitone sleeping time: sleeping time was measured as the duration of the loss of righting reflex (LRR) following sodium pentobarbitone (35 mg/kg, i.p.). 20 min after LRR, animals received intra-accumbens CG 3509 (2x2.5 µg in 0.5 µl saline). Response to central saline was assessed 24 h later. Rectal temperature and respiration rate were measured upon LRR and every 10 min thereafter.

Repeated ECS was administered to male Wistar rats as 5 shocks over 10 days (125 V, 1 s, 50 Hz; days 1, 3, 5, 8 and 10) through earclip electrodes, whilst anaesthetised with halothane. Control animals received halothane and electrode placement, but no current was passed. CG 3509-reduction of pentobarbitone sleep time was assessed prior to and 24 h following the final ECS. Hyperactivity was only determined 24 h following repeated ECS.

Repeated ECS significantly enhanced CG 3509-induced hyperactivity, when compared to sham-shocked controls (+11%; $P<.05$). Following ECS, CG 3509-induced reduction of pentobarbitone sleep time was not significantly different from that of sham-shocked animals. However, repeated ECS significantly increased the reversal of pentobarbitone hypothermia compared to sham-shocked controls ($P<0.001$) and enhanced the reversal of respiratory depression, induced by CG 3509.

These results indicate that in the nucleus accumbens, the decreased levels of TRH previously measured following ECS, are associated with an increased responsiveness to injection of a TRH analogue into the same region.

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456P

VALIDATION OF A TEST OF ANXIETY IN THE RAT BASED ON EXPLORATORY ACTIVITY IN A PLUS-MAZE

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With the search for new classes of anxiolytics that would provide advantages over the benzodiazepines (BDZs), the need for new and simple tests of anxiety has become pressing. Limitations with current tests, particularly those using conditioned behaviour and requiring food or water deprivation, led us to develop a test for anxiolytic and anxiogenic drugs relying on spontaneous behaviour in the rat. It is important that such a test be validated not only pharmacologically but behaviourally and physiologically; this was the aim of the present investigations. The test was based on a procedure described by Montgomery (1955) and Handley and Mithani (1983). The apparatus consisted of a +-maze elevated to a height of 50cm, with 2 open arms (50x10cm) opposite to each other, and 2 closed arms (50x10x40cm) with an open roof. The rat was placed in the centre of the maze and the following measures taken during 5min: the number of entries into and the time spent in either open or closed arms. Total exploration was given by the total number of arm entries; the percentage of open arm entries provided a measure of fear-induced inhibition of behaviour (Montgomery 1955, behavioural validation below). To provide a comparative measure of exploratory and locomotor activity in a test that does not primarily measure anxiety, rats were placed in a holeboard for 5min before placement on the maze.

There was significantly more anxiety-related behaviour (freezing, defaecation, high plasma corticosterone concentrations) when rats were confined to the open arms compared to those confined to the closed, suggesting that anxiety is greater on open arms. Neither novelty nor illumination was an important factor in the rats' behaviour; test-retest correlations were high for one retest (% open arm entries: r=0.99). The following compounds had 'anxiolytic' activity (i.e. increased the % of open arm entries/time spent on open arms compared to controls); both acutely and after 5 days pretreatment: diazepam (1&2mg/kg) and chlordiazepoxide (5&7.5mg/kg); the barbiturate phenobarbitone (25&35mg/kg) was less effective. The following had'anxiogenic' activity (i.e. decreased the % of open arm entries/ time spent on open arms compared to controls): yohimbine (1.25&2.5mg/kg), caffeine (15&30mg/kg), amphetamine (1&2mg/kg) and, less selectively, pentylenetetrazole (10&20mg/kg). All of these results are consistent with the reported activity of these compounds in man. No specific effects on 'anxiety' were observed with the major tranquiliser haloperidol (0.1&0.25mg/kg) or the antidepressants imipramine (5&15mg/kg) and mianserin (10&20mg/kg), acutely. The direction of the effects obtained in the +-maze did not clearly correlate with those on exploratory head-dipping or locomotor activity and rearing in the holeboard.

In conclusion, the results suggest that the percentage of open arm entries/ time spent on open arms of an elevated +-maze may provide a valid and reliable test of anxiety in the rat.

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BEHAVIOURAL INDICES OF WITHDRAWAL FOLLOWING A SINGLE DOSE OF SODIUM PHENOBARBITONE

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There are reports that epileptic children treated with sodium phenobarbitone can become fractious and irritable, but the root cause of this behaviour is unknown. In order to determine a possible role of drug withdrawal in increased aggression, a single dose of phenobarbitone (20 or 50 mg/kg, injected i.p.) was administered to rats 6, 8 or 12 hours before testing for aggressive behaviour. Aggressive encounters were scored between a resident and an intruder. Either the resident or the intruder had been treated with phenobarbitone, the opponent was always untreated.

Decreased fighting was observed six hours after a 50 mg/kg dose of phenobarbitone, a time at which the rats were still sedated, as measured by spontaneous locomotor activity in a holeboard. No other groups showed any significant changes in motor activity. However, six hours after a dose of 20 mg/kg had been administered to intruders, they showed an increase in aggression (kicking and pushing) towards the undrugged residents and these residents, in return, showed a decrease in kicking and pushing accompanied by an increase in submissions to the intruders. Eight hours after a dose of 50 mg/kg had been administered to residents, they showed an increase in aggressions (kicking and pushing and aggressive grooming) to undrugged intruders who, in response, showed an increase in submissions. No significant changes were observed at 12 hours after phenobarbitone.

No change in dominance, as assessed by competition for a preferred food, were found at six hours after 20 mg/kg of phenobarbitone or at eight hours after 50 mg/kg of phenobarbitone.

Finally, there was evidence that the drug withdrawal at these times was aversive to the rats. Untreated rats were placed for ten minutes in a distinctive two-chamber compartment and their unconditioned preference measured by the time spent in each side. They were then given four days of conditioning during which they were confined for one hour to one side of the chamber following injection with phenobarbitone (six hours after 20 mg/kg or eight hours after 50 mg/kg) and to the other side following saline injections. Their undrugged preference was again tested on day five. Rats avoided the side in which they had been placed after drug treatment, indicating that drug withdrawal at these times was aversive.

458P

INVOLVEMENT OF OTHER NEUROTRANSMITTERS IN TRH-INDUCED WET-DOG SHAKING

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Injection of thyrotrophin-relesing hormone (TRH) into rat brain periaqueductal grey region (PAG) produces wet-dog shaking (WDS) (Widdowson et al.1983). To investigate neurotransmitter interactions in this behaviour, the effects of neurotransmitter antagonists, opioid agonists and chemical lesions to 5-HT and dopamine systems have been investigated. TRH (in 0.5 µl saline) was injected into the PAG of male Sprague-Dawley rats (180-200g) 15 min after an intraperitoneal drug injection or 5 min after intra-PAG injection of opioid agonist. 5,7-dihydroxytryptamine (5,7-DHT) dissolved in 10 µl saline was injected into the lateral ventricles in 2 separate doses 2 days apart followed by 10 day recovery period. Pargyline (5 mg/kg) and desipramine (25 mg/kg) were injected i.p. 2h before surgery, followed by 6-hydroxydopamine (6-OHDA) in 10 µl saline into the lateral ventricles and a 10 day recovery period. The number of WDS / 30 min was counted and injection sites were confirmed histologically.

Phenoxybenzamine (2 mg/kg), propranalol (1 mg/kg), atropine sulphate (1 mg/kg), naltrexone (2 and 10 mg/kg) and methysergide (4 mg/kg) injected i.p. did not alter TRH-induced WDS. Haloperidol and morphine gave a dose-dependent reduction in WDS, as did intra- PAG [D-Ala^2,D-Leu^5]-enkephalin (DADLE, 1.5 µg), [D-Ala^2,MePhe^4, Gly-ol^5]-enkephalin (DAGO, 1 µg) and β-endorphin (2 µg). Intra-PAG dynorphin(1-8) and ethylketocyclazocine had no effect. Lesioning of the 5-HT system with 5,7-DHT also had no effect but 6-OHDA -lesioning produced a significant reduction.

Table 1 Effects of drugs on TRH-induced WDS

Injection	WDS/30 min
Control TRH (1 µg)	69.6 ± 5.9
Haloperidol (1 mg/kg) + TRH (1 µg)	10.0 ± 3.2[a]
Morphine (3 mg/kg) + TRH	8.2 ± 5.2[a]
DADLE (2.5 µg) + TRH	16.4 ± 3.0[a]
DAGO (1 µg) + TRH	16.1 ± 3.8[a]
β-endorphin (2 µg) + TRH	12.8 ± 3.2[a]
Control TRH (5 µg)	80.5 ± 6.4
5,7-DHT-lesioned + TRH (5 µg)	80.3 ± 4.2
6-OHDA-lesioned + TRH (5 µg)	26.0 ± 6.5[a]

[a] $p<0.01$ vs. control TRH; n = 6-8 S.E.M.

These findings suggest an involvement of the dopamine system in TRH-induced WDS, as well as µ- and δ - but not κ - opioid receptor-mediated systems; blockade of 5-HT, adrenergic and cholinergic receptors was without effect.

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THIORPHAN AND BESTATIN: EFFECT ON FOOD INTAKE?

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Leu- and met-enkephalin undergo rapid enzymatic degradation in vivo. The behavioural effects of these peptides, however, may be reflected by the activity of enzyme-resistant enkephalin analogues. Thus, D-Ala^2-D-Leu^5-enkephalin has been shown to increase food intake in non-deprived rats suggesting that endogenous enkephalins may play a role in the control of ingestive behaviour in these animals (Tepperman & Hirst, 1983; Jackson & Sewell, 1985). More recently, compounds have been developed which selectively inhibit the major enkephalin-metabolizing pathways such as the enkephalinase inhibitor thiorphan and the aminopeptidase blocker bestatin (see Schwartz et al. 1982 for review). It was of interest in the present investigation, therefore, to examine the appetitive effects of thiorphan and bestatin - both alone and in combination - using the freely-feeding rat model.

Male Wistar rats (250-300g) were implanted with intracerebroventricular (i.c.v.) cannulae and allowed at least 7 days recovery from surgery before use. Individually-housed animals were then randomly allocated to treatment groups of six and allowed free access to a powdered standard rat diet and tap water at all times. Experiments were carried out during the daylight period when the food intake of control animals was minimal. Thiorphan (DL-3-mercapto-2-benzylpropanoyl-glycine; gift from Wellcome Research Laboratories) and bestatin ([(2S,3R)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine; Sigma) were dissolved in 0.9% sterile saline and injected directly into the lateral ventricle in a dose volume of 10µl/rat. Feeding jars were weighed at the time of drug administration and after 1,2 and 4 hours to enable the calculation of mean group cumulative food intake (g/kg rat weight±sem).

The food intake of rats injected with either thiorphan (1-100µg/rat i.c.v.) or bestatin (1-50µg/rat i.c.v.) was not significantly different to that of vehicle-treated controls over the 4hr test period. Moreover, combinations of thiorphan with bestatin did not appear to influence ingestive behaviour as animals injected concurrently with doses of each compound (up to 50µg/rat i.c.v.) ate similar amounts to the corresponding control group. Neither thiorphan, bestatin, nor their combination exerted any overt behavioural erfects which might have interfered with a possible stimulant action on food intake.

The lack of appetitive effect of thiorphan, bestatin, and a mixture of thiorphan and bestatin suggests that during the day, at least, these agents cannot modulate enkephalinergic levels to the extent that is required for the initiation of ingestive behaviour. These findings are supported by reports that thiorphan and bestatin produce antinociception only in those analgesic tests thought to involve a concomitant release of endogenous enkephalins (Schwartz et al. 1982). Hence, it would appear that the effects on feeding of these enzyme inhibitors may be more usefully studied in situations where levels of the endogenous opioid peptides are enhanced.

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460P

ARE SOCIALLY ISOLATED RATS ANXIOUS?

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The socially isolated rat exhibits several characteristic behaviours, including neophobia (Morgan et al, 1975), which may be related to an increased anxiety state. Hyponeophagia has been shown to be potentiated by 5-hydroxytryptamine (5-HT) agonists, and attenuated by diazepam treatment (Shephard and Broadhurst, 1982). Biochemically, anxiogenic conditions produce an increased brain 5-HT turnover which is reversed by anxiolytic drugs (File and Vellucci, 1978). In the present study the effects of social isolation on anxiety was assessed using both behavioural (hyponeophagia) and biochemical (5-HT turnover) indices.

Male Sprague-Dawley rats weaned at 21 days were either housed singly in opaque plastic cages (ISOL) or in groups of four to a cage (SOC). After 20 days they were tested for their neophagic response following 24 hour food deprivation (Shephard and Broadhurst, 1982). Five days later, striatal and hippocampal 5-HT and 5-hydroxyindole acetic acid (5HIAA) concentrations were measured fluorimetrically after separation on Sephadex G-10 microcolumns (Earley and Leonard, 1978).

ISOL rats showed an increased hyponeophagic response in all three parameters measured (Table 1) and a reduced 5-HT turnover in the hippocampus (Table 2).

Table 1 Effect of social isolation on the hyponeophagic response

	Approach Time(s)	Food Eaten (g)	Time spent eating(s)
SOC	165 ± 4	1.06 ± 0.03	88 ± 16
ISOL	384 ± 45*	0.37 ± 0.09*	47 ± 11*

Each value is the mean ±SEM for 15 rats (obtained from 2 separate experiments)
* $P<0.05$ SOC v ISOL.

Table 2 Effect of social isolation on brain 5-HT turnover

	Corpus Sriatum	Hippocampus
SOC	0.98 ± 0.08 (14)	3.15 ± 0.05 (12)
ISOL	0.73 ± 0.06 (13)	1.50 ± 0.24 (13)*

Each value is the mean [5HIAA]/[5-HT] ±SEM with number of determinations (n).
* $P<0.05$ SOC v ISOL.

The behavioural data suggested a raised level of anxiety in ISOL rats in the novel environment, while hippocampal 5-HT turnover suggested a reduced level of anxiety. 5-HT turnover may relate to the undisturbed home cage situation, while the behavioural responses may be related to the anxiety provoking novel environment. Thus, the ISOL rat in its home cage may have a lower level of anxiety but show a greater increase in anxiety in the novel environment. Work is in progress to resolve this discrepancy by measuring brain 5-HT turnover immediately after exposure to an unfamiliar environment.

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BEHAVIOURAL PARAMETERS OF 5-HT_1 RECEPTOR STIMULATION DO NOT SHOW CIRCADIAN VARIATION

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We have previously demonstrated that the 5-methoxy-N,N-dimethyltryptamine induced head-twitch, a behavioural measure thought to indicate activity at 5-HT_2 receptors, shows a marked circadian variation (Moser and Redfern, 1985a). In the same experiments, another measure of central 5-HT activity, the 5-HT behavioural syndrome, showed no such variation. It has been suggested that the 5-HT syndrome is mediated by 5-HT_1 receptors although the evidence is at present equivocal. We now present data showing that other behavioural measures also thought to reflect 5-HT_1 receptor activation show no circadian variation. In drug discrimination studies we have previously shown that the stimulus properties of 5-HTP are mediated by 5-HT_1 receptors (Moser and Redfern, 1985b); using this technique the time taken for an animal to learn to discriminate between a standard dose of 5-HTP and saline can therefore be used as a measure of the activity of 5-HT_1 receptors. Eight groups of 8 male Wistar rats (300-350g at the start of the experiment) were trained to discriminate 5-HTP from saline (Moser and Redfern, 1985b). Each group was trained at a different point of the 12:12 light-dark cycle, training of different groups thus taking place at 3 hourly intervals. Carbidopa (25 mg/kg ip) was given 60 min before testing; 5-HTP (50 mg/kg ip) or saline (2 ml/kg ip) was given 30 min before testing. Training sessions, carried out on weekdays only, consisted of 8 trials one minute apart. 5-HTP or saline administration, according to a double alternation sequence, continued until a criterion of 8 correct first-trial responses out of 10 consecutive sessions was reached. The overall 24-hour mean for the time taken to reach this criterion was 26.3 days (∓ 1.5 sem) and analysis of variance showed no evidence of any significant 24 hour variation about the mean.

Another behavioural model that has recently been developed to study 5-HT_1 receptors is the hyperactivity following administration of RU 24969 (Gardner and Guy, 1983), a 5-HT agonist which shows selectivity for the 5-HT_1 binding site. Different groups of 8 mice received RU 24969 (0.625 mk/kg ip) at 3 hourly intervals throughout a 12h light - 12h dark cycle and were tested 30 minutes later in an open field, in parallel with vehicle treated mice. The 24-hour mean for the percentage increase in activity following RU 24969 was 160 (∓ 5.3 sem), and the degree of hyperactivity induced by this agent showed no circadian variation.

These results thus confirm and extend our earlier observations which indicated that behavioural responses mediated by 5-HT_1 receptors do not show circadian variation, unlike the 5-HT_2 receptor mediated 5-HTP head-twitch. The physiological significance of this difference remains to be elucidated; in the meantime it may provide a useful means of categorising events mediated by the two receptor subtypes.

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462P

THE USE OF A THERMAL STIMULUS DEVICE TO ASSESS ANALGESICS IN THE SHEEP

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We have previously reported on the construction of a thermal stimulus device to measure nociception in the sheep (Livingston, Morris & Nolan, 1985) and this study concerns the effectiveness of two groups of analgesic compounds in modifying the nociceptive response. The classical analgesics, the opioids, were represented by two compounds, pethidine and buprenorphine, whilst the α_2 adrenoceptor agonist xylazine represented the second group. Adult female clun sheep, bodyweight 60-75 kg were anaesthetised with halothane and catheters were placed in the carotid artery to record blood pressure and the jugular vein for administration of drugs. The animals were allowed to recover and the thermal stimulus device placed on the pinna of one ear. The device incorporated an inertia cut out system which reversed the thermal ramp by the sheep flicking its ear or shaking its head, and a thermal cut out which operated before tissue damage could occur. Readings were taken on a 0-100 scale, which represented a temperature range of approximately 35-75^{o}C, every 15 minutes. The scale was calibrated for temperature after every experiment. A series of readings were taken in the conscious sheep to obtain a baseline and then the analgesic drug was given intravenously. Readings were then taken until values returned to the baseline pre-drug values.

The opioid drug pethidine at a dose of 2.5 mg/kg showed only a short-lived and mild analgesia representing an increased tolerance of only about 10^{o}C which lasted for about 30 minutes whilst buprenorphine at dose of 6.0 µg/kg produced a maximal response which persisted for several hours. However, both opioids produced agitation and restlessness in the sheep which made reliable recording difficult.

The α_2 adrenoceptor agonist xylazine at a dose of 50 µg/kg was very effective at producing analgesia giving a maximal response which persisted for 60 mins and then was rapidly reversed, returning to baseline by about 90 minutes. Thus from these studies it is possible to evaluate analgesia in the sheep using a thermal stimulus and it would appear that the pethidine produced only a partial and transient analgesia in the sheep when used at a dose of 2.5 mg/kg, whilst buprenorphine at a dose of 6 µg/kg produced a marked and long lasting effect, however both opioids caused agitation in the sheep. Xylazine on the other hand did not cause agitation but gave a degree of sedation at a dose of 50 µg/kg and gave a good analgesia which persisted for sixty minutes. Control experiments using both saline and the sedative tranquillizer acepromazine at 0.2 mg/kg showed no increase in the animals' responsiveness to the stimulus.

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THE PARTIAL INVERSE BENZODIAZEPINE AGONIST Ro 15-4513 ANTAGONIZES ACUTE ETHANOL EFFECTS IN MICE AND RATS

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Ro 15-4513 (Ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4] benzodiazepine-3-carboxylate,synthesized by Dr Hunkeler) the azido analogue of the benzodiazepine antagonist Ro 15-1788,showed , like the latter, a high affinity for central benzodiazepine binding sites (Möhler et al.,1984).In the horizontal wire test and in the cat spinal cord, Ro 15-4513 behaved as a partial inverse agonist at the benzodiazepine receptor (Bonetti et al.,1984).In mice,Ro 15-4513 is active against phenobarbitone in the horizontal wire test (ED50 = 0.89 mg/kg p.o.),is not convulsant (up to 5000 mg/kg p.o.)and of very low acute toxicity (LD50 >5000 mg/kg p.o.).

In the horizontal wire test,Ro 15-4513 was now found to reverse the impairment of motor performance induced by ethanol (3000 mg/kg p.o. in mice, 6000 mg/kg p.o.in rats) with an ED50 of 0.84 mg/kg p.o. in mice and 8.62 mg/kg p.o.in rats.These effects were antagonized by Ro 15-1788 (3 mg/kg p.o.)when given both before or after Ro 15-4513. Also electrophysiological experiments in the cat spinal cord and behavioural observations in the rat demonstrated an interaction between Ro 15-4513 and ethanol (Polc,1985).

The β-carbolines β-CCE (ethyl-9H-pyrido[3,4-b]indole-3-carboxylate) and β-CCM (methyl-9H-pyrido[3,4-b]indole-3-carboxylate) were tested against ethanol in the horizontal wire test in mice, in doses which were effective against phenobarbitone.A slight activity against ethanol was seen after a subconvulsant dose of β-CCM only(4 mg/kg i.v.). This effect was antagonized by Ro 15-1788 (3 mg/kg p.o.).

Ethanol dose-dependently decreased the level of cerebellar cGMP in mice (ED50= 2000 mg/kg, 1/2 h after oral administration).This decrease was antagonized by Ro 15-4513 (ED50= 8 mg/kg p.o.) but not by β-CCM when given in a dose (0.3 mg/kg i.v.)which was not convulsant. The antagonistic effect of Ro 15-4513 against ethanol was almost completely abolished by the specific benzodiazepine antagonist Ro 15-1788 (100 mg/kg p.o.).

β-CCE and β-CCM,as well as Ro 15-4513,did not affect the in vitro ^{35}S-TBPS (t-butylbicyclophosphorothionate)binding(IC50 >10 µmol/l).

The effect of Ro 15-4513 on ethanol(and phenobarbitone) and its reversal by Ro 15-1788 is in line with an inverse agonistic activity of Ro 15-4513.However,the β-carbolines β-CCM and β-CCE,which are also inverse agonists at the benzodiazepine receptor,were only slightly active or not active at all against ethanol in these experiments.The reason for this differential activity of inverse agonists is not clear. An action through the chloride ionophore part of the GABA-benzodiazepine receptor complex as suggested by Mendelson et al.(1985) does not seem to be a prerequisite.

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THE EFFECTS OF β-CARBOLINES IN INFANT RATS

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Two recent studies have suggested that benzodiazepines can cause behavioural excitation and possibly seizures in infant rats (Barr and Lithgow, 1983; Pappas and Walsh 1983). The former authors suggested that benzodiazepines can cause convulsions in rat pups through interaction withbenzodiazepine Type 2 receptors, which develop postnatally before type 1 (Lippa et al., 1981). We have investigated the effects in infant rats of two β-carboline compounds, methyl-β-carboline -3-carboxylate (BCCM) and methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) which bind to benzodiazepine receptors. These drugs cause convulsions which are antagonised by the benzodiazepine receptor antagonist Ro 15-1788 (Nutt et al., 1982) but they do not have consistent selectivity for either receptor subtype. (Affinity of BCCM greater for type 1 than Type 2; affinity DMCM greater for type 2 than type 1, Braestrup & Nielsen 1981; Braestrup et al. 1983). We compared their effects with those of a benzodiazepine, flurazepam, and with the classical convulsant pentylenetetrazol (PTZ).

Homebred litters from Sprague-Dawley rats were used throughout. All testing was done on day 3, the day of birth being day 0. Weights were 3-6g. Drugs were administered i.p. and the pups observed (in groups of 4) for the following 15 min. Ratings were made of four behaviours on a 0-3 scale (0 = not seen, 1 = mild, 2 = moderate, 3 = severe) by an observer unaware of the treatments given. Ambient temperature was 33°C. The β-carbolines were dissolved in 0.1N HCl then diluted with distilled water; flurazepam and PTZ were dissolved in saline.

	Controls		BCCM	DMCM	Flurazepam	PTZ
	Saline	Acid/water	5mgkg^{-1}	5mgkg^{-1}	10mgkg^{-1}	100mgkg^{-1}
	(n=9)	(n=8)	(n=11)	(n=5)	(n=8)	(n=5)
Shaking	0.8	0.3	1.7	2.8^{b}	0.1	2.8^{b}
Twitches	0.7	0.3	0.1	0	2.0	0
Locomotor activity	0.5	0.1	3.0^{a}	3.0^{a}	0	2.8^{a}
Loss of righting relfex	0	0	1.4	0	1.0	2.4^{c}

(mean values)

a = $P < 0.001$ v vehicle controls and v flurazepam treatment; b = $P < 0.05$ v vehicle controls and v flurazepam treatment; c = $P < 0.001$ v vehicle controls and $P < 0.05$ v flurazepam treatment (Mann -Whitney 'U' test).

These results show that the β-carbolines caused very different behavioural effects from those of flurazepam and that the effects of the latter are not likely to be seizure activity. Our findings suggest that increased locomotor activity in infant rats may be equivalent to seizures in adults.

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INTERACTIONS OF PARTIAL INVERSE BENZODIAZEPINE AGONISTS Ro 15-4513 AND FG 7142 WITH ETHANOL IN RATS AND CATS

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Ro 15-4513 (ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate), the azide analogue of the benzodiazepine antagonist Ro 15-1788, has been shown to have a partial inverse agonist activity at benzodiazepine receptors (Bonetti et al, 1984) and to photolabel central benzodiazepine binding sites for antagonists (Möhler et al, 1984). FG 7142 (N-methyl-β-carboline-3-carboxamide) is another high affinity benzodiazepine receptor ligand with a partial inverse agonist activity in animals and anxiogenic effect in man(Braestrup et al, 1984).

In the present study, Ro 15-4513 (3-30 mg/kg p.o.) and FG 7142 (10-100 mg/kg p.o.) were devoid of intrinsic effects on the spontaneous locomotor activity in rats. However, Ro 15-4513 antagonized the reduction of motility caused by ethanol (3 g/kg p.o.) without affecting the depressant effect of phenobarbitone (100 mg/kg p.o.). The selective action of Ro 15-4513 against ethanol is supported by the finding that FG 7142 was unable to antagonize the ethanol-produced decrease of locomotor activity.

In the cat spinal cord, Ro 15-4513 (0.3-3 mg/kg i.v.) dose-dependently reduced segmental dorsal root potentials and increased spontaneous activity of γ-motoneurones (Bonetti et al, 1984). Although FG 7142 (1-10 mg/kg i.v.) produced similar effects as Ro 15-4513, only the latter drug prevented the depression of ongoing γ-motoneurone activity induced by ethanol (300-500 mg/kg i.v.) and reversed this ethanol effect if administered afterwards.

In conclusion, the present results suggest a rather specific antagonism by Ro 15-4513 of the ethanol-induced depression of spontaneous behaviour in rats and of neuronal excitability in cats. The lack of effect of the proconvulsant β-carboline FG 7142 against ethanol indicates that the inverse agonistic property at benzodiazepine receptors, at least the one which is associated with the proconvulsant activity, is not sufficient to fully explain the antagonism between Ro 15-4513 and ethanol. Furthermore, the inactivity of Ro 15-4513 in the binding of [^{35}S] t-butylbicyclo-phosphorothionate (Bonetti et al, 1985) excludes the involvement of the Cl^--ionophore part of the GABA-benzodiazepine receptor complex in the interaction between Ro 15-4513 and ethanol.

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SUBSENSITIVITY OF CEREBELLAR PURKINJE CELLS TO IONOPHORESED NORADRENALINE INDUCED BY CHRONIC ADMINISTRATION OF DOTHIEPIN

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Chronic administration of the antidepressant dothiepin reduced cortical β-adrenoceptor binding (Buckett & Thomas, 1982) and noradrenaline receptor-coupled adenylate cyclase activity (Buckett & Diggory, 1982). The present work was undertaken to determine whether chronic administration of dothiepin also reduces sensitivity to ionophoresed noradrenaline.

Adult male Wistar rats were given dothiepin HCl orally (chronic: 10 $mg.kg^{-1}$, n=10), saline orally (n=10) daily for 10-42 days, or a single dothiepin dose (acute: 10 $mg.kg^{-1}$, n=5). Adult albino guinea-pigs (Hartley strain) were given dothiepin orally (10 $mg.kg^{-1}$, n=5) or saline (n=8) orally daily for 17-42 days.

Rats and guinea-pigs were anaesthetised with urethane (1.3-1.5 $g.kg^{-1}$, i.p.) 22-26 h after the last dothiepin or saline administration. Five or 7-barrel micropipettes were used for extracellular recording from cerebellar Purkinje cells and the ionophoretic application of: DL-noradrenaline HCl (NA: 20 mM, pH 4.5), γ-aminobutyric acid (GABA: 20 mM, pH 5.0) and dothiepin HCl (20 mM, pH 5.0). The responsiveness of neurones to NA and GABA was evaluated from the ionophoretic charge (ejection current I(nA).time T(s)) required to obtain a 50% reduction in firing (I.T50).

Ionophoresis of NA and GABA suppressed the firing rate of both rat and guinea-pig Purkinje cells. Dothiepin ejected at currents of 0-10 nA increased the magnitude and duration of suppressed firing induced by NA, but not that induced by GABA. Ejection of dothiepin at higher currents produced a current-dependent suppression of firing.

Table 1. The effect of chronic dothiepin administration on spontaneous simple-spike discharge and responsiveness to NA.

		Firing Rate	(n)	I.T50 NA (nC)	(n)
RAT	Control	54.6 ± 9.9	(76)	237.9 ± 49.3	(46)
Chronic	10 $mg.kg^{-1}$	18.6 ± 4.5	(38)#	687.1 ± 52.9	(31)*
Acute	10 $mg.kg^{-1}$	42.6 ± 7.4	(27)	215.1 ± 43.5	(24)
GUINEA-PIG:	Control	50.4 ± 3.6	(42)	203.8 ± 48.6	(31)
Chronic	10 $mg.kg^{-1}$	22.3 ± 4.6	(27)*	548.1 ± 67.8	(19)*

Values are mean ±s.e. mean; # $p<0.02$, * $p<0.001$ (unpaired t test).

Following chronic administration of dothiepin, Purkinje cells exhibited a marked reduction in firing rate (Table 1). The sensitivity of ionophoresed NA was reduced 2-3 fold, as reflected by the higher I.T50 estimations (Table 1), while no sigificant change in the sensitivity to ionophoresed GABA was found.

The reduced spontaneous discharge and sub-sensitivity to NA may be attributable to uptake blockade by dothiepin (Richelson & Pfenning, 1984) resulting in elevated extracellular NA levels.

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NORADRENERGIC RECEPTOR BINDING IN BRAIN REGIONS OF MICE SUSCEPTIBLE AND RESISTANT TO AUDIOGENIC SEIZURES

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DBA/2 mice have a high genetically determined susceptibility to audiogenic seizures (AS). This susceptibility is age related, being maximal at 21-28 days of age and considerably reduced or absent at earlier and later ages. The basis of this age-related susceptibility to AS is not known, although an involvement of noradrenaline (NA) mediated neurotransmission has been implicated (Kellogg 1976). The incidence and severity of AS in DBA/2 is reduced in a dose dependent manner by α^2 adrenoceptor agonists such as clonidine. This effect is antagonised by α^2 adrenoceptor antagonists (Horton et al, 1980). The protective effect of propranolol shows only slight stereoselectivity and is probably unrelated to ß blockade (Anlezark et al, 1979).

We have previously compared NA receptor binding in DBA/2 mice at various ages before, during and after the period of maximal susceptibility to AS, with age-matched C57 BL/6 mice, a strain resistant to audiogenic seizures at all ages (Horton et al, 1983). In membranes derived from whole brain, the affinity and number of ß NA and α^2 NA binding sites (labelled with 3H dihydroalprenolol and 3H clonidine) did not differ between the two strains of mice at any age, whereas there were fewer α^1 NA sites (labelled with 3H prazosin) in DBA/2 mice at all ages.

We now report the binding of 3H prazosin and 3H clonidine to membranes prepared from cerebral cortex, fore-brain, mid-brain, hippocampus, cerebellum and pons-medulla of DBA/2 mice, before (13-15 days), during (21-24 days) and after (40-43 days) the period of maximal susceptibility to AS, and age-matched C57 BL/6 mice. The Bmax of 3H prazosin binding was constant or increased slightly between 13 and 42 days in cerebral cortex, fore-brain, hippocampus and cerebellum in both strains of mice, whereas Bmax decreased in mid-brain and pons-medulla (particularly between 13 and 21 days). The Bmax of 3H clonidine binding was constant or increased between 13 and 42 days in cerebral cortex, fore-brain, mid-brain and cerebellum of both strains of mice. In hippocampus and pons-medulla there was a decrease in Bmax from 13 to 21 days followed by an increase between 21-42 days. There were no significant differences between K_D or Bmax of 3H prazosin or 3H clonidine binding in DBA/2 and C57 mice, at any age in any brain region studied. It seems unlikely that the age-related susceptibility of mice to AS is related to an abnormality in NA binding site sensitivity or number.

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468P

INCREASED LOCOMOTOR ACTIVITY CAUSED BY DOPAMINE INFUSED INTO THE NUCLEUS ACCUMBENS OF PRIMATE BRAIN

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Dopamine infused into the nucleus accumbens of rat brain causes an enhanced locomotor activity which is sensitive to neuroleptic antagonism (Costall et al, 1982; 1984). In the present study a technique was developed to allow intracerebral infusion in the primate brain to establish that in this species, as in the rodent, a persistent infusion of dopamine into the mesolimbic nucleus accumbens leads to an increased locomotor activity which can be prevented by neuroleptic treatment.

Common marmosets (Callithrix jacchus, male, 350-400g), anaesthetised with Saffan(R), 1 ml/kg body weight, i.m., were subject to standard surgical and stereotaxic techniques for the implantation of bilateral stainless steel guide cannulae (constructed as for the rat, Costall et al, 1982) with tips at Ant. 12.5, Lat. ±2.0 and Vert. +13.3 (4 mm above the centre of the nucleus accumbens) (see Stephan et al, 1980). After a 14 day recovery period, the cannulated animals were re-anaesthetised (0.5-1 ml/kg i.m. Saffan) for subcutaneous implantation in the scapula region of two Alzet(R) osmotic minipumps attached to stainless steel injection units made to terminate at the centre of the nucleus accumbens. The design of the units allowed the infusion of dopamine or its vehicle (N_2 bubbled 0.1% sodium metabisulphite) to proceed for 13 days with no obvious disturbance to the free movement of the animal. For measurement of locomotor activity marmosets were placed in individual cages (760 cm high, 500 cm wide, 600 cm deep) having 4 computer-linked infrared units strategically placed to measure movement on or between two perches and the cage floor (counts summated over 60 min). Remote video recording allowed subsequent visual analysis of marmoset behaviour.

Marmosets having chronically indwelling intracerebral cannulae and receiving intracerebral infusions remained in excellent health. Their spontaneous activity measured prior to dopamine/vehicle infusion was in the range 40-140 counts/60 min. Dopamine infusion (25 µg/24h, 0.48 µl/h) enhanced the locomotor activity of all 6 animals by 92-758%. Time taken to achieve a maximum response varied considerably (from 1-7 days), as did the spectrum of change over the 13 day period (from a persistent hyperactivity to a fluctuating response). The raised levels of locomotor activity caused by the dopamine infusions were highly significant ($P<0.001$) both relative to the original basal locomotor activity levels and those of the marmosets receiving vehicle infusion (n=6). In further experiments, marmosets (n=4) subject to dopamine infusion into the nucleus accumbens received neuroleptic treatment throughout the period of infusion (twice daily dosing with 0.1 mg/kg i.p. fluphenazine or 5 mg/kg i.p. sulpiride) and the dopamine hyperactivity was prevented throughout the 13 day treatment period. Histological analyses indicated that infusion sites had been correctly located within the area of the nucleus accumbens.

It is concluded that drug infusion into the marmoset brain offers a practical approach to modifying neurotransmitter function within a discrete brain area. The consequences of raising dopamine activity in the nucleus accumbens is to cause a marked locomotor hyperactivity response which is neuroleptic sensitive.

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LACK OF EFFECT OF OVINE PROLACTIN ON SPECIFIC [^{3}H]-SPIPERONE BINDING SITES IN MALE RAT STRIATUM

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Administration of ovine or rat prolactin to intact or hypophysectomised male rats (Hruska et al, 1982) was reported to increase specific striatal [^{3}H]-spiperone binding. However, tumour-induced hyperprolactinaemia did not alter the density of ^{3}H-spiperone binding sites in striatum of normal female rats (Cronin et al, 1983) and domperidone-induced hyperprolactinaemia did not alter receptor numbers in normal male rats (Rupniak et al, 1983). We examine the effect of ovine prolactin treatment on specific striatal [^{3}H]-spiperone binding in male rats.

Ovine prolactin was administered to male Wistar rats (initial weight 195-210 g) as a depot dose in sesame oil (0.125-1.0 mg s.c.) or as a bolus dose in 0.9% saline (1.0 mg s.c.) daily for 6 days, or as a single intravenous dose (1 mg/kg). Biochemical determinations were performed 1 day following chronic and 2 hours following acute treatment.

The number of specific striatal [^{3}H]-spiperone (0.03-1.0 nM; defined using 10^{-5}M ($\pm$) sulpiride) binding sites (Bmax) was not changed by any treatment used (Table 1).

In vitro incorporation of ovine prolactin into washed striatal membranes was ineffective in displacing the specific binding of ^{3}H-spiperone (0.1 nM) at concentrations up to 10^{-7}M. In the presence of prolactin (10^{-8}M) the potency of haloperidol, sulpiride or dopamine to displace specific ^{3}H-spiperone binding was not altered. IC50 values; haloperidol, vehicle: 8.9 x 10^{-9}M, prolactin: 5.01 x 10^{-9}M; sulpiride, vehicle: 3.16 x 10^{-7}M, prolactin: 2.51 x 10^{-7}M; dopamine, vehicle: 1.58 x 10^{-5}M, prolactin: 1.12 x 10^{-5}M.

Table 1: Effect of ovine prolactin on [^{3}H]-spiperone (0.03-1 nM) binding in male rat striatum

Treatment	Vehicle-treated		Prolactin-treated	
	Bmax (pmol/g)	Kd (nM)	Bmax (pmol/g)	Kd (nM)
1 mg/day sc - depot doses	23.4$\pm$1.4	0.086$\pm$0.004	21.2$\pm$1.2	0.096$\pm$0.032
1 mg/day sc - bolus doses	14.2$\pm$1.8	0.074$\pm$0.010	14.9$\pm$0.6	0.086$\pm$0.009
1 mg/kg iv - single dose	23.7$\pm$0.8	0.058$\pm$0.009	22.9$\pm$0.6	0.065$\pm$0.009

n= 6-8 Specific binding defined using 10^{-5}M ($\pm$) sulpiride

The lack of effect of ovine prolactin in vitro or in vivo upon specific striatal [^{3}H]-spiperone binding suggests this hormone does not influence striatal dopamine D-2 receptors.

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470P

ENHANCEMENT BY VERAPAMIL OF PRESYNAPTIC INHIBITORY EFFECT OF GABA ON SYMPATHETIC TRANSMISSION IN RAT ANOCOCCYGEUS MUSCLE

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Reduction of transmitter release by GABA, via $GABA_B$ receptors, is an important way in which presynaptic inhibition occurs (Bowery et al, 1980). In the rat anococcygeus muscle this effect can be seen as a concomitant reduction in the twitch response of the muscle to electrical stimulation and in the stimulus-evoked release of radiolabel following incubation with (^{3}H)noradrenaline (Muhyaddin et al, 1982,1983). The underlying mechanism may involve a reduced calcium entry to nerve terminals (Dunlap & Fischbach, 1981) and the present investigation explores this possibility by studying the effect of verapamil (an organic calcium antagonist) on the response to GABA in the rat annococcygeus muscle.

Muscles from male Wistar rats were suspended in 2ml of Krebs-Henseleit solution at 37°C, which was bubbled with 95%O_2+5%CO_2 and contained ascorbic acid (0.1mM) and iproniazid (0.5mM). Field stimulation (10V,4Hz,2ms pulse width) was applied via platinum ring electrodes for 5s every 30s and the contractile responses recorded isometrically. In experiments in which the release of (^{3}H)NA was studied, muscles were pre-incubated for 1h in Krebs medium containing (^{3}H)NA (1μM,16 Ci/mmol) and hydrocortisone (1μM). The muscles were then washed and suspended in 2ml Krebs medium containing hydrocortisone as above and, additionally, cocaine (4.4μM). The contents of the bath were changed every 5 min and aliquots assayed for radioactivity. When a steady basal efflux was established, stimulation was applied as above for a 5 min interval. Drugs were added 1 min before the onset of stimulation.

GABA (2-100 μM) produced a dose-dependent inhibition of the twitch response of the muscle to field stimulation; this response was mimicked by baclofen (2-100 μM) and unaffected by bicuculline (75 μM). Doses of verapamil (2-10nM) which had no effect on the twitch response of the muscle when tested alone, nevertheless enhanced the inhibition produced by GABA. Thus, GABA (10 μM) caused a 31±3% reduction in the twitch response (mean ± s.e. mean) while GABA (10 μM) together with verapamil (10nM) produced a significantly greater ($p<0.01$) inhibition (48 ± 3%)(n=5). In muscles preloaded with (^{3}H)NA, field stimulation produced a calcium-dependent increase in the output of radiolabel. In 5 experiments, the mean (± s.e. mean) stimulus-evoked efflux was 4.0 ± 0.2 times the immediately preceding basal value. Application of GABA (100 μM) reduced this ratio to 2.2 ± 0.2 (n=4) and application of GABA (100 μM) together with verapamil (10nM) caused a greater reduction ($p<0.05$) to 1.4 ± 0.5 (n=5).

These findings show that in doses which do not affect the muscle directly verapamil enhances the inhibitory effect of GABA and therefore lend support to the suggestion that $GABA_B$ receptor activation reduces the availability of calcium for transmitter release.

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DIFFERENCES IN RESPONSES TO GABA OF SPONTANEOUSLY ACTIVE CEREBRAL CELLS IN VITRO REVEALED BY INTER-SPIKE INTERVAL ANALYSIS

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Inter-spike interval (ISI) analysis of spontaneously active cells can provide quantitative data describing changes in firing pattern. A shift in the mean or modal intervals indicates a change in the fundamental firing frequency of the cell, whilst the scatter around that point can be used as a measure of the regularity of firing. Cells having complex firing patterns frequently exhibit more than one ISI peak, revealing the presence of two or more fundamental components in their activity.

The firing patterns of 163 spontaneously active cells recorded in 800µm thick slice preparations of rat cerebral cortex in vitro were analysed using an inter-spike interval histogram technique. Cells were recorded in layers 3 and 4 of the cortex and classified into five categories according to firing pattern.

Type 1 - regularly firing cells which showed a normally distributed ISI histogram around a mean value of 100-200ms, (n=27).
Type 2 - cells fired regularly as Type 1 cells, but with additional high frequency bursts of activity (100-200Hz), followed by a silent period. These cells showed two peaks on the ISI histogram with modes near 20ms and 100ms, (n=30).
Type 3 - cells fired only high frequency bursts of spikes lasting 50-200ms, (n=56).
Type 4 - cells fired irregularly, having inter-spike intervals covering a range up to 400ms, but with the mode skewed towards the shorter intervals, (n=30).
Type 5 - cells fired doublets of action potentials, interspersed by more regular single spikes similar to Type 1 cells, (n=20).

Bath application of GABA and bicuculline revealed differences in the pharmacological responses of the cell types. GABA (2mM) increased the rate and regularity of firing of Type 1 cells whilst it suppressed the firing of Type 3 cells completely. On Type 2 cells a complex response was observed, with the mean firing rate of the regular spontaneous activity increased whereas the irregular bursts of activity were suppressed. These effects of GABA could be reversed by the inclusion of 10µM bicuculline in the bathing medium. GABA also reduced the higher firing frequencies of the Type 4 cell ISI histogram, and abolished the single spikes in Type 5 cells, leaving only the doublets remaining.

The application of bicuculline (0.02µM) alone to Type 2 cells showing both low frequency regular activity and intermittent high frequency bursts of action potentials, had the effect of abolishing the low frequency activity whilst leaving the high frequency bursts intact.

The use of ISI analysis in these experiments has revealed the presence in the cortical slice preparation of populations of spontaneously active cells which respond differently to exogenous GABA and bicuculline.

ALTERATIONS IN CEREBRAL GABA PARAMETERS FOLLOWING TREATMENT OF RATS FOR UP TO 1 YEAR WITH HALOPERIDOL, SULPIRIDE OR CLOZAPINE

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Nigral glutamic acid dicarboxylase (GAD) activity is reduced in primates exhibiting haloperidol-induced tardive dyskinesia (Gunne et al, 1984) and in rats with haloperidol-induced persistent peri-oral movements (Gunne & Haggstrom, 1983). We now compare the effects on brain GABA function of continuous chronic administration of haloperidol with those of the atypical neuroleptics sulpiride and clozapine whose use is associated with a lower incidence of tardive dyskinesia.

Male Wistar rats received either haloperidol (1.4-1.6 mg/kg/day), sulpiride (102-109 mg/kg), clozapine (24-27 mg/kg/day) or distilled water alone for upto 12 months via their daily drinking water. As previously reported continuous chronic administration of haloperidol, but not clozapine, or sulpiride, caused an increase in striatal D-2 dopamine receptors (Fleminger et al, 1984).

Treatment with haloperidol, but not sulpiride or clozapine, for 6 or 12 months increased striatal glutamic and decarboxylase (GAD) activity (Table 1). Nigral GAD activity was unaffected by drug treatment. The number of specific ^{3}H-flunitrazepam (FNM; 0.1-15 nM; defined using 2 uM clonazepam) binding sites (Bmax) in striatum was not altered by 12 months drug treatment (Table 1). However, Bmax for cerebellar specific ^{3}H-FNM binding was decreased following 12 months treatment with haloperidol, sulpiride or clozapine compared to tissue from control animals (Table 1). The ability of GABA (0.25-100 uM) to stimulate specific striatal or cerebellar ^{3}H-FNM (0.5 nM) binding in the presence or absence of sodium chloride (200 mM) was not altered by drug treatment.

Table 1: Alteration in cerebral GABA systems after 1 year's treatment with haloperidol, sulpiride or clozapine

Treatment	GAD activity (nmol/hr/mg protein)		Bmax for specific ^{3}H-FNM binding (pmoles/mg protein)	
	Substantia nigra	Striatum	Striatum	Cerebellum
Control	1065 ± 75	300 ± 23	0.83 ± 0.06	1.84 ± 0.12
Haloperidol	1092 ± 83	357 ± 11*	0.67 ± 0.03	1.36 ± 0.05*
Sulpiride	1018 ± 40	328 ± 12	0.77 ± 0.07	1.36 ± 0.07*
Clozapine	883 ± 47	314 ± 12	0.71 ± 0.04	1.29 ± 0.18*

* $p < 0.05$ compared to age-matched control animals. Student's t-test; n = 3-6

Striatal dopamine receptor supersensitivity resulting from continuous chronic neuroleptic intake alters striatal rather than nigral GAD activity. The alterations in cerebellar ^{3}H-FNM binding were surprising but confirm a previous report of altered GABA function in this area following chronic haloperidol or clozapine treatment (Lloyd et al, 1977).

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KAINIC ACID BINDING IN THE RAT CEREBELLUM: EFFECTS OF NOVEL KAINATE ANALOGUES

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The responses to the putative excitatory amino acid transmitters L-glutamate and L-aspartate are believed to be mediated via 3 subclasses of receptors, namely those activated preferentially by N-methyl-D-aspartate (NMDA), quisqualate (QA) and kainate (KA) (Watkins & Evans, 1981). However, the lack of selective antagonists has precluded further detailed characterisation of the KA receptor. In this study we have investigated a series of novel KA analogues in relation to their ability to inhibit KA binding to rat cerebellar membranes.

[^{3}H]KA (specific activity: 60 Ci/mmol; NEN) binding to washed adult rat cerebellar SPMs was sodium-independent, saturable, and attained equilibrium within 90 min at 2°C. Saturation analysis revealed the presence of at least one population of binding sites, with a Kd = 7.2nM and Bmax of approx. 100 fmol/mg protein.

The ability of a number of compounds to inhibit KA binding was as follows (IC50 values in parentheses): KA (25nM), domoic acid (40 nM), carboxykainate (100nM), α-ketokainate (126nM), homokainate (251nM), L-glutamate (630nM), QA (790nM), alloketo-β-kainate (790nM), α-kainyl-glycine (890nM), keto-β-kainate, and α-kainylamino-methylphosphonate (each 1uM). β-kainate was very weak with an IC50 value of 11.22uM, whilst at concentrations of up to 1000nM, the following compounds were ineffective inhibitors of KA binding: kainic acid lactone, β-kainylamino- methylphosphonate, β-kainylGABA, dihydrokainate, β-dihydrokainate, cisPDA, GDEE, NMDA, APV, D-asp, L-asp and ibotenic acid.

These results confirm that the presence of the isopropylene side chain on the KA moiety is crucial for activity at the KA receptor, and that in the design of potential specific KA antagonists, the presence of a ring structure similar to that of KA, together with substitutions on the carboxyl terminals may be a necessary prerequisite.

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474P

A SELECTIVE N-METHYL-D-ASPARTATE ANTAGONIST REDUCES CONVULSANT-INDUCED EPILEPTIFORM ACTIVITY IN RAT HIPPOCAMPAL SLICES

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Selective NMDA antagonists have been reported to display anticonvulsant activity in experimental models of epilepsy (Croucher et al, 1982). Binding studies using the competitive antagonist D-2-amino-5-phosphonovalerate (APV) have indicated that the region of the hippocampus that is innervated by Schaffer collateral-commissural fibres contains the highest density of NMDA receptors in the rat brain (Monaghan et al, 1984). We have therefore examined the sensitivity to APV of convulsant-induced epileptiform activity in this area using in vitro electrophysiological techniques.

In the presence of physiological concentrations of Mg^{++} (1 mM) 0.1 Hz stimulation of the Schaffer collateral-commissural pathway elicits a single population spike in the CA1 cell body region that is completely insensitive to 20 μM APV. An NMDA receptor mediated component of synaptic transmission can, however, be demonstrated in Mg-free media. Under these conditions identical stimulation elicits multiple population spike discharges of which all but the first are completely eliminated by 20 μM APV. (Coan & Collingridge, 1985).

Multiple population spike discharges in response to Schaffer collateral-commissural stimulation were produced in the presence of 1 mM Mg^{++} by superfusion of pentylenetetrazol (1.5 - 2.0 mM, n = 7), folic acid (1 mM, n = 4) or bicuculline methobromide (2 μM; n = 2). In all cases 20 μM APV caused a partial suppression of the multiple responses without affecting the primary population spike. The relative sensitivity to APV of the epileptiform activity induced by the following treatments were as follows: Mg^{++}-free > pentylenetetrazol > folic acid > bicuculline methobromide.

In view of the extreme susceptibility of the hippocampus to epileptic behaviour and the well characterised role of NMDA receptors in this region (Collingridge et al, 1983; Coan & Collingridge, 1985) this preparation should prove invaluable for the evaluation of NMDA antagonists as anticonvulsants.

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EXCITATORY AMINO ACID BINDING SITES IN HUMAN CEREBELLUM

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High affinity binding sites for excitatory amino acids have been identified in animals, and may be related to sub classes of physiological receptor (Sharif & Roberts,1981; Foster & Fagg,1984). To date, there have been few reported studies of these receptors in human brain. In view of the potential involvement of amino acids in neurodegenerative and psychiatric disorders, we have characterised the binding of [^{3}H]aspartate (Asp) to human cerebellar membranes, and compared this to the binding of [^{3}H]glutamate (Glu) in the absence of calcium and chloride ions.

Human brains were obtained at autopsy from normal subjects and frozen at -40°C. Membranes were produced by homogenisation in 100 volumes of 50 mM Tris-acetate, pH 7.4, and centrifugation at 15,000 g/10 min. This fraction was washed 6 times and frozen before assay of [^{3}H]Asp (100 nM) and [^{3}H]Glu (50 nM) binding using a filtration technique (Cross et al, 1984). [^{3}H]Asp binding was saturable, of medium affinity (K_D 200-500 nM) and was inhibited by several excitatory amino acids and analogues. In cerebellum, [^{3}H]Glu labelled 3-5 times as many binding sites as [^{3}H]Asp.

Table 1 Inhibition of [^{3}H]aspartate and [^{3}H]glutamate binding

Compound	IC_{50} (μM)	
	[^{3}H]Aspartate	[^{3}H]Glutamate
L-Aspartate	0.18	0.25
L-Glutamate	0.36	0.18
Quisqualate	305	5[1]
Ibotenate	32	75
N-Acetylaspartate	11	>500

[1]Maximal 60% inhibition of [^{3}H]glutamate binding

Quisqualate was of high affinity at a proportion of [^{3}H]Glu binding sites, but only weakly active at [^{3}H]Asp binding. N-Acetylaspartate was a potent inhibitor of [^{3}H]Asp binding but was only weakly active at [^{3}H]Glu binding. Analysis of the inhibition of [^{3}H]Glu binding by N-acetylaspartate revealed a 20-30% displacement with relatively high affinity (IC_{50} 30 μM). Moreover, 100 μM N-acetylaspartate displaced 25% of [^{3}H]Glu binding in an apparently non-competitive manner. The number of [^{3}H]Glu binding sites displaced by N-acetylaspartate (5.0 pmol/mg protein) was equivalent to the number of [^{3}H]Asp binding sites (5.8 pmol/mg protein) in the same membrane preparation.

These findings demonstrate high affinity binding sites in human brain for both Glu and Asp. The pharmacological properties of the [^{3}H]Asp binding site are distinct from those relating to the Glu binding site(s), although it is likely that a proportion of [^{3}H]Glu sites include the Asp site. The relationship between these binding sites and the physiological effects of Asp and Glu acids remains to be determined.

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476P

HORDENINE (N,N-DIMETHYL TYRAMINE) IS A SELECTIVE SUBSTRATE FOR MONOAMINE OXIDASE B FROM RAT LIVER

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The sympathomimetic amine hordenine occurs in high levels (1-5 mg/g) in the alga Gigartina stellata which is incorporated into the food product 'Carrageen' (Barwell & Blunden, 1981). It has been reported to be a substrate for monoamine oxidase (MAO) from porcine brain (Williams, 1974) and rat liver mitochondria (Barwell et al. 1984). Rat liver mitochondria are known to contain approximately equal catalytic activities of MAO-A and MAO-B (Fowler et al. 1981). Hordenine could be a substrate for both or only one of these forms of MAO. This has been investigated using isolated rat liver mitochondria.

Livers from male Wistar rats weighing 250-300g were homogenised in 0.3M sucrose. Mitochondria were isolated by differential centrifugation and washed twice. MAO activity was assayed at pH 7.4 and 37°C using an oxygen electrode. Michaelis constants were determined from Hanes plots (s/v against s) of kinetic data. To determine which MAO utilises hordenine, isolated mitochondria were incubated for 30 min at 37°C with Lilly 51641 at concentrations from 10^{-8}M to 10^{-3}M. This compound is an irreversible (Fuller, 1968) selective (Squires, 1972) inhibitor of rat liver MAO-A. At each inhibitor concentration, the MAO activity remaining was assayed with 1 mM tyramine and 10 mM hordenine.

Initially the Michaelis constant of the MAO preparation for tyramine and hordenine was determined under the particular conditions of this investigation. The value was 142 µM for tyramine and 1449 µM for hordenine. In subsequent inhibition studies enzyme activity was assayed with 1 mM tyramine and 10 mM hordenine in order to permit expression of any MAO-A or MAO-B activity towards these substrates.

In inhibition studies using Lilly 51641, with tyramine as substrate a biphasic inhibition curve was obtained with a distinct plateau between 10^{-6}M and 10^{-5}M inhibitor concentrations. Complete inhibition occurred at 10^{-3}M. The plateau occurred when 40% of the total activity had been inhibited, indicating that the preparation contained both forms of MAO in the proportion, 40% MAO-A and 60% MAO-B. With hordenine as substrate no inhibition occurred at inhibitor concentrations between 10^{-8}M and 10^{-5}M. At concentrations above 10^{-5}M a monophasic sigmoidal inhibition curve, with complete inhibition at 10^{-3}M was obtained.

The results indicate that, under the experimental conditions, hordenine was utilised only by MAO-B. Since the concentration of hordenine used (10 mM) was seven times higher than the Michaelis constant (1.4 mM) it appears that it is a highly selective substrate for rat liver mitochondria MAO-B. It is not known at present whether hordenine is also a selective substrate for MAO-B of other tissues and species. However, it is problematic as to whether the generally low activity of MAO-B in mammalian small intestine would be adequate for the pharmacological inactivation of hordenine ingested in high levels.

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MPTP DEMETHYLATION BY MICROSOMES FROM MICE AND GUINEA-PIG LIVER

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Systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) induces Parkinsonian symptoms accompanied by selective destruction of nigrostriatal dopamine neurones in man (Davis et al. 1979), monkeys (Burns et al. 1983) and mice (Heikkila et al. 1984). In contrast rats and guinea pigs are highly resistant to these effects (Chiueh et al. 1983). The neurotoxicity of MPTP is associated with its oxidation by MAO-B to N-methyl-4-phenylpyridine (MPP) (Markey et al. 1984). A species' susceptibility to the neurotoxic effects of MPTP may be related to MAO-B and MPP levels (Javitch & Snyder, 1985). However other aspects of MPTP metabolism may influence its neurotoxicity. Since its demethylated derivative 4-phenyl-1,2,5,6-tetrahydropyridine (PTP) is not neurotoxic (Heikkila et al. 1984), the level of demethylation in vivo could affect a species' sensitivity to the neurotoxic effects of systemically administered MPTP. This was investigated by measuring MPTP demethylation in vitro using liver microsomes from two species which exhibit markedly different sensitivities to MPTP namely mice and guinea pigs (Heikkila et al. 1984; Chiueh et al. 1984).

Livers from five male C57 mice weighing 30-35 g or one male guinea pig weighing 500-600 g were homogenised in 0.3M sucrose and microsomes isolated by differential centrifugation. Demethylation of MPTP was studied at pH 7.4 and 37°C by measuring formaldehyde production with Nash reagent. Kinetic parameters were determined by measuring the rate of formaldehyde production at five different concentrations of MPTP which ranged from approximately half to twice the apparent Michaelis constant. Michaelis constants and maximum velocities were determined from Hanes plots (s/v against s) which were linear in each case.

Table 1 summarises the results. The Michaelis constants were very similar whilst the maximum velocity exhibited by the guinea pig microsomes was seven times greater than that of the mice. Thus the species least susceptible to the neurotoxic effects of MPTP appears to possess the greatest capacity for its detoxification via demethylation.

Table 1. Kinetic constants for MPTP demethylation by mouse and guinea pig liver microsomes

Michaelis constant (μM)		maximum velocity (nmol.h^{-1}.mg $protein^{-1}$)	
mouse	guinea pig	mouse	guinea pig
2527 ± 287	2651 ± 438	61 ± 3	433 ± 47 *

Values are means ± SEM of 4 preparations, * $p < 0.001$: unpaired t-test

The results indicate that a species' sensitivity to the neurotoxic effects of systemically administered MPTP may be determined, at least in part, by its capacity to demethylate the compound.

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478P

EFFECT OF SELECTIVE AND NON-SELECTIVE MONOAMINE OXIDASE INHIBITORS ON THE P,P'-DDT-INDUCED BEHAVIOURAL RESPONSE IN MICE

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The insecticide p,p'-DDT (1,1,1,-trichloro-2,2-bis-(p-chlorophenyl)ethane) produces tremor and myoclonus in mice and rats (Chung Hwang and Van Woert, 1978; Pratt et al, 1985). This response is most effectively antagonised in rats by non-selective monoamine oxidase (MAO) inhibitors such as pargyline and tranylcypromine (Pratt et al, 1985). It is therefore of interest that 1-methyl-4-phenylpyridine (MPP^+), which may be the active metabolite of the selective dopamine neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Markey et al, 1984; Bradbury et al, 1985a), has been used as a herbicide. Furthermore the neurotoxic actions of MPTP in mice are selectively antagonised by deprenyl (a MAO-B inhibitor) but not by clorgyline (a MAO-A inhibitor) (Heikkila et al, 1984; Bradbury et al, 1985b). We now report the effects of selective and non-selective MAO inhibitors on the behavioural response of mice to p,p'-DDT administration.

Male CD1 mice (23-29g; Charles River) were orally administered 200mg/kg p,p'-DDT immediately prior to intraperitoneal treatment with saline or a MAO inhibitor (clorgyline 10 mg/kg; deprenyl 10 mg/kg; pargyline 75 mg/kg; nialamide 100 mg/kg). The intensity of the behavioural response, which largely consisted of tremor, myoclonus and seizures, was assessed on a 0-6 observer rating system (Pratt et al, 1985) at 0.5h intervals.

The p,p'-DDT-induced behavioural response was markedly antagonised by the MAO-B inhibitor deprenyl and the non-selective MAO inhibitors pargyline and nialamide, but not by the MAO-A inhibitor clorgyline (Table 1).

Table 1 Effect of MAO inhibitors on the p,p'-DDT-induced behavioural response in mice.

Treatment	Mean behavioural score (± SEM)	Treatment	Mean behavioural score (± SEM)
Saline	4.18 ± 0.52	Deprenyl	1.92 ± 0.45*
Saline	3.25 ± 0.46	Pargyline	0.17 ± 0.11**
Saline	2.67 ± 0.38	Nialamide	0.58 ± 0.26*
Saline	3.08 ± 0.40	Clorgyline	2.75 ± 0.30

The intensity of the behavioural response was assessed 5h after p,p'-DDT (200 mg/kg p.o.) administration (n=12 mice/treatment).
*$p<0.01$; **$p<0.001$ vs saline treatment (Mann Whitney U-test).

Therefore the behavioural response of mice to p,p'-DDT and the neurotoxic action of MPTP in mice are both selectively attenuated by an inhibitor of MAO-B (deprenyl) but not by an inhibitor of MAO-A (clorgyline) which may suggest a common mechanism in their effects.

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FAILURE OF VILOXAZINE TO INHIBIT TYRAMINE INDUCED PRESSOR RESPONSES IN THE ANAESTHETISED RAT

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Viloxazine ('Vivalan', ICI) is a selective inhibitor of noradrenaline uptake and in animals potentiates a range of noradrenergic phenomena(1). A clinical report(2) that viloxazine is only weakly active in inhibiting tyramine induced pressor responses, a commonly used test of catecholamine uptake inhibition in man, prompted this study of pressor responsiveness to the direct and indirectly acting sympathomimetics methoxamine (M), noradrenaline (NA) and tyramine (T). Male Wistar rats (200 ± 10g) were anaesthetised with alphaxalone/alphadalone ('Saffan', Glaxo), 18 mg/kg i.v. The jugular vein and carotid artery were cannulated for continuous anaesthetic infusion (0.3 - 2mg/kg/minute), drug injection, and measurement of mean arterial blood pressure respectively. Pressor responses to previously established equiactive doses of NA, T and M were determined in separate groups of five rats both before and after the i.v. administration in saline of viloxazine (5mg/kg) and desmethyl-imipramine (DMI)(1.2 mg/kg). Control rats received saline alone. Qualitatively, the effects on NA and M were identical following each antidepressant. Thus, both DMI and viloxazine caused a pronounced potentiation of NA responses (170 ± 22 and 273 ± 16 percent of control respectively) but had no significant effect on the pressor response to M. In contrast, whereas DMI markedly inhibited T evoked pressor responses (23 ± 4 percent of control pressor response) viloxazine was essentially inactive (85 ± 11 percent of control pressor response).

The results indicate that, at the dose levels tested, both DMI and viloxazine are effective inhibitors of prejunctional NA uptake and have no action on postjunctional α_1 receptors. The failure of viloxazine to inhibit T responses may be a reflection of a weak MAO inhibitory influence or could indicate that N and T are transported into neurones via discrete mechanisms.

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480P

PHARMACOLOGICAL EVIDENCE FOR THE NEURONAL SELECTIVITY OF THE MAO INHIBITING PRODRUG, MDL 72394

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Using biochemical techniques and selective lesions of dopamine containing nerves of the nigro-striatal tract, we have shown that (E)-β-fluoromethylene-m-tyrosine (MDL 72394) is decarboxylated by neuronal aromatic L-aminoacid decarboxylase (AADC) to yield the potent enzyme-activated irreversible inhibitor of monoamine oxidase (MAO), (E)-β-fluoromethylene-m-tyramine (Mely et al, 1984). We now provide pharmacological evidence for this neuronal selectivity.

Male Wistar rats (225-275 g) were unilaterally lesioned with 6-hydroxy-dopamine (8 µg/ 2 µl) in the median forebrain bundle at the level of the substantia nigra (Robin et al, 1985). Two weeks later, successfully lesioned rats were selected on the basis of vigorous contralateral rotations to apomorphine (0.2 mg/kg s.c.). Groups of lesioned rats (n = 3-5) were pretreated orally with MDL 72394 (0.1 mg/kg) plus carbidopa (10 mg/kg), clorgyline (5 mg/kg) or distilled water (5 ml/kg). Four, 72, 144 and 216 h later they were injected i.p. with amphetamine (5 mg/kg) or L-DOPA (5 mg/kg) and rotations recorded over the following 2 h. Separate groups of lesioned animals were treated with the MAO inhibitors and at the above mentioned times MAO A activity was determined in each striatum using [^{14}C]5HT as substrate.

Both MAO inhibitors significantly ($p < 0.05$, paired "t" test) potentiated the ipsilateral rotations to amphetamine at 4 and 72 h after treatment with a return to control rates of rotation at 216 h. In contrast, only clorgyline potentiated the contralateral rotations produced by L-DOPA ($p < 0.05$) 4 h after treatment, with a return to control rates of rotation at 72 h.

Lesioning did not significantly alter the MAO activity of the striatum. MDL 72394, in contrast to clorgyline, produced a greater inhibition of MAO in the intact striatum and this lateralisation was evident up to 144 h after treatment. By 216 h afer MDL 72394, although there was still slight inhibition of MAO (25%), this effect was no longer lateralised.

Amphetamine produces ipsilateral rotations in lesioned rats by releasing dopamine from the intact nigro-striatal pathway (Ungerstedt, 1971). MAO inhibitors acting on the neuronal pool of MAO will thus potentiate these effects. L-DOPA cannot be decarboxylated in dopamine nerves on the lesioned side ; formation of dopamine occurs in capillaries and non-dopamine nerves before diffusing to the supersensitive receptors to produce contralateral rotations (Melamed et al, 1984). The dopamine produced from L-DOPA is thus susceptible to degradation by non-neuronal MAO. The fact that MDL 72394, in contrast to clorgyline, did not potentiate L-DOPA-induced rotations, is consistent with the former being selective for the neuronal pool of MAO.

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BILE ACIDS ACTIVATE HUMAN COLONIC TISSUE KALLIKREIN-LIKE AMIDASE

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Both increased bile acid secretion (Rutgeerts et al, 1979) and raised tissue levels of colonic kallikrein (Zeitlin & Smith, 1973) have been implicated in the aetiology of inflammatory bowel disease. We recently reported that bile acids both release and activate colonic mucosal TKLA in rats (Al-Dhahir & Zeitlin, 1983). We now describe the actions of bile acids on colonic mucosal TKLA in man.

Histologically normal mucosa was obtained from the resection edge of colectomy tissue from 5 patients with colonic tumour and one with ulcerative colitis. Tissue was stored (Krebs solution, 4°C) for up to 1h before being chopped into strips (1 x 3-5mm), washed and incubated (37°C, 30min) in Krebs solution alone or containing sodium deoxycholate (DC), chenodeoxycholate (CDC) or tauro-chenodeoxycholate (TCDC), 1.93 and 3.86mM or Triton-X 100 (TX, 0.1 and 1.0% v/v). Supernatants were assayed for TKLA by incubation (37°C, pH 8.2) with the tissue kallikrein selective tripeptide substrate, S-2266 (Kabi Ltd). When enzyme inhibitors were tested, extracts were pre-incubated with inhibitor for 15min prior to assay.

The 3 bile acids caused a dose-related release of TKLA with a potency order of CDC>DC>TCDC. Neither concentration of TX caused enzyme release ($P > 0.05$). The activity released by CDC (3.86mM) was not significantly inhibited by soybean trypsin inhibitor (SBTI, 250µg/ml), inhibited 91% by tosyl lysyl chloroketone (TLCK, 10mM) and totally by aprotinin (Trasylol, Bayer Ltd, 2000KIU/ml). If allowed a prolonged pre-incubation, TLCK is an active site inhibitor of kallikrein-like enzymes (Chao, 1981). The selective nature of the assay substrate, the alkaline pH of the assay and the response to inhibitors distinguish the amidase released from human mucosae by CDC, from trypsin, plasma kallikrein (both inhibited by SBTI) and cathepsins, and indicate its similarity to a tissue kallikrein.

Following incubation of the chopped tissue with CDC (3.86mM), the SBTI-resistant, TLCK-sensitive tissue kallikrein-like enzyme increased in the supernatant by over 2600% ($P < 0.01$). There was also a small but significant increase ($P < 0.05$) in SBTI-sensitive, TLCK-resistant amidase. The bile acids also induced dose-related increases in TKLA activity in cell-free supernatants from homogenates of human colonic mucosa. However, the increases in activity induced by bile acids in the cell-free extracts ranged only from 60-200%, while those induced by bile acids in chopped tissue incubates ranged from 300 to over 2000. It is thus probable that bile acids have a complex action on human colonic mucosal TKLA, both releasing intracellular enzyme and activating or potentiating released enzyme.

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RESPONSE OF NEUROBLASTOMA HYBRID CELLS TO PHOSPHODIESTERASE INHIBITORS AND PGE_1: CHANGES IN CYCLIC AMP AND OPIOID PEPTIDES

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The neuroblastoma-glioma cell line NG108CC15 has been shown by competitive binding assay to contain enkephalin-like material (Glaser et al. 1982). Using several radioimmunoassays we have confirmed the presence of opioid peptides in this and another neuronal line, NH15-CA2 (Heumann et al. 1979). Eiden & Hotchkiss (1983) have described changes in opioid peptides following changes in cyclic AMP levels in cultured bovine adrenal chromaffin cells. We have used these two cell lines to investigate the effect of phosphodiesterase inhibitors and prostaglandin E_1 (PGE_1) on cyclic AMP and opioid peptide levels.

Results described here use two radioimmunoassays for peptides: one will cross-react with all opioid peptides derived from proenkephalin (total opioid peptide-like IR) and the other cross-reacts with the carboxyl-terminus of proenkephalin, the heptapeptide sequence met-enkephalin(arg^6,phe^7). Cyclic AMP is measured by a protein binding assay.

Elevations in cyclic AMP in both the cell extract and in the supernatant were seen when cells were cultured in the presence of phosphodiesterase inhibitors (isobutyl-methylxanthine, IBMX; RO-20-17-24) with or without prostaglandin E_1. For example, after 24 h treatment, the following levels were seen in the supernatants of NH15-CA2 cells: control 3.74 ± 0.88; 50 μM IBMX, 13.04 ± 1.25; 50 μM IBMX + 1 μM PGE_1, 244 ± 9; 50 μM IBMX + 10 μM PGE_1, 274 ± 15; 50 μM RO-20-17-24, 8.91 ± 1.81, (pmol cyclic AMP/mg cell protein ± SEM, n = 4). These results are consistent with the description by Freedman et al. (1984) of changes in NG108CC15 cells following incubation with IBMX and PGE_1.

In order to investigate the consequences of increased cyclic AMP levels on opioid peptides, we treated both cell lines with 50 μM IBMX and 1 μM PGE_1 for four days. In the NG108CC15 cells the met-enkephalin(arg^6,phe^7) immunoreactivity rose to 152% of controls ($P < 0.01\%$) and the total opioid peptide immunoreactivity to 199% of controls ($P < 0.01\%$). In the NH15-CA2 cells the met-enkephalin(arg^6,phe^7) immunoreactivity rose to 266% of controls ($P < 0.01\%$) while the total opioid peptide immunoreactivity was not significantly different.

It appears then that cyclic AMP levels influence the accumulation of opioid peptides in these cells, including products of the proenkephalin gene, for which the met-enkephalin(arg^6,phe^7) assay is specific. This influence may be at several levels, including that of expression of the gene for pre-proenkephalin.

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NOCICEPTIVE EFFECTS OF GABA-ERGIC AGENTS IN SOCIALLY CROWDED RATS

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The chronic stress of social isolation or crowding in rats alters nociceptive response thresholds (Browne & Pilcher, 1984). These effects appear to be opioid mediated in the former but not in the latter case. Since there is evidence that stress may mobilise putative endogenous ligands of benzodiazepine receptors (Millan & Duka, 1981) and GABA-ergic agents are analgesic (Sivam & Ho, 1983), we have addressed the possibility that crowding-induced changes in nociception reflect involvement of a GABA-ergic system.

Male Hooded rats were raised from weaning under crowded (C) or non-crowded (NC) conditions as previously described (Pilcher & Jones, 1981). C rats were housed in groups of 10 with a floorspace allocation of 95cm^2 per rat. NC animals were allowed 710cm^2 floorspace per rat in groups of 3. Nociceptive pressure thresholds of non-inflamed hind paws were determined using an Analgesy Meter and threshold latencies were determined at 50±0.2°C by a tail immersion method. All drugs were injected (s.c.) in a volume of 1.0 mlkg^{-1}.

Results are summarised in Fig. 1. In NC rats bicuculline and picrotoxin both produced analgesia to pressure and hyperalgesia to heat, effects that resemble those of crowding stress alone. These antagonists had no effects on nociceptive thresholds with either stimulus in C rats. The reverse was observed with the GABA-mimetic muscimol and diazepam, neither of which affected pressure or heat thresholds in NC rats. However, both of these agents produced significant hyperalgesia with pressure and analgesia to heat in C animals.

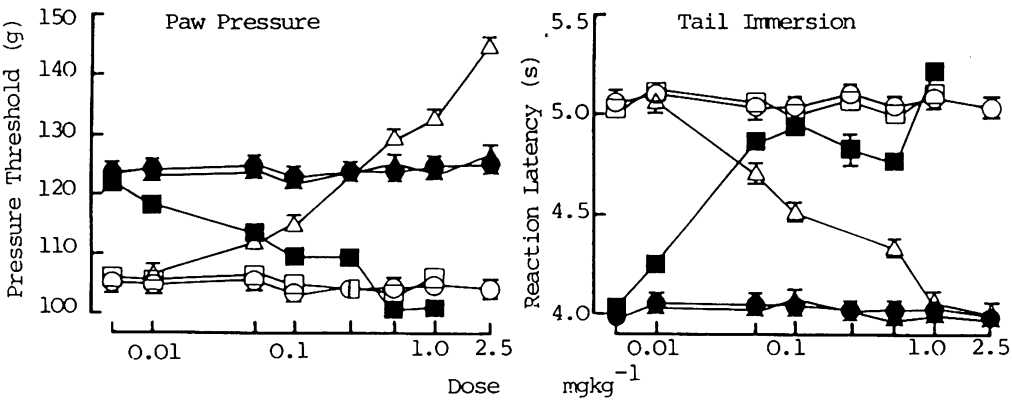

Figure 1. Effects of GABA-ergic agents on nociception

○ NC) control
● C) thresholds

□ NC) muscimol
■ C)

△ NC) bicuculline
▲ C)

These results suggest that a GABA-ergic system is involved in the mediation of crowding stress-induced changes in nociceptive thresholds, and one consequence of crowding appears to be a reduction in GABA-ergic activity.

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