EFFECT OF BECLAMIDE ON STRIATAL MONOAMINE LEVELS AND TURNOVER

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Though originally employed as an anti-epileptic agent, beclamide (Nydrane) has been found to be of benefit in stabilizing mood (Delay et al., 1958), reducing aggressive antisocial behaviour and enhancing concentration and co-operation (Peuch et al., 1962). Such abnormal behaviour patterns may be associated with changes in indolamine and catecholamine systems in the CNS (Schildkraut, 1965).
In view of this, the present study was undertaken to examine the effect of beclamide on striatal monoamine levels and turnover in rats.

In the first experiment, male Wistar rats (150-200g) received oral doses of either beclamide (400mg/kg suspended in 0.75% sodium carboxymethylcellulose) or vehicle. Animals were decapitated 1h later and the striata were rapidly removed and frozen in liquid N_2. In the second experiment, rats (150-200g) were similarly treated but in addition received pargyline (75mg/kg, i.p.) 10, 20 or 30 min before sacrifice. Each tissue sample was homogenized in ice-cold perchloric acid (0.1M, 1ml) and centrifuged for 25 min at 3000 x g. Dopamine (DA), homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were determined by HPLC with electrochemical detection.

Striatal DA content was reduced approximately 3-fold by beclamide while DOPAC and HVA levels were increased by a similar factor (Table 1). Striatal indoleamine levels fell below the detection limit of the assay (2.5 pmoles) following beclamide treatment.

Table 1. Effect of beclamide on monoamine and monoamine metabolite levels in rat striatum

Group	DA	DOPAC	HVA	5HT	5HIAA
Control	565±35	103±9	23±1.8	23±2.7	28±3
Beclamide	176±40*	399±43*	67±11*	ND	ND

Values are means ± s.e. (n=6) in pmoles/mg protein; ND = not detectable; *P<0.01

DA turnover, as assessed by DA accumulation in pargyline-treated animals, was unaffected by beclamide. However, DA turnover. as measured by metabolite depletion after pargyline, was increased (Table 2).

Table 2. Effect of beclamide on DA turnover in the striatum of pargyline-treated rats as determined by DA accumulation and metabolite depletion

Group	DA	DOPAC	HVA
Control	1520±94	536±47	52±4
Beclamide	1470±334	2021±217*	134±22*

Values are means ± s.e. (n=6) in pmoles/mg protein/h. *P<0.01

The results suggest that beclamide may possess some type of monoamine-depleting property. Whether such an effect is related to the clinical efficacy of beclamide remains to be established.

Beclamide was a gift from Lipha Laboratories, Hitchin, Herts.

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STEREOSPECIFIC CNS EFFECTS OF THE GABA-TRANSAMINASE INHIBITOR L-CYCLOSERINE ARE POTENTIATED BY DIAZEPAM

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L-cycloserine (L-CY) dose-dependently inhibits GABA-transaminase activity and thus increases the GABA level in whole mouse brain, with a peak effect 2-3 h after either p.o. or i.p. administration. The time course of this GABA elevation in the CNS parallels quite well different neuropharmacological effects of L-CY in mice, rats and cats (Polc et al., 1986). We report now on further studies investigating the potentiation by benzodiazepines of low doses of L-CY. Additionally, the lack of effect of D-CY, alone or with diazepam (DZ), is shown here.

L-CY (both p.o. or i.p.), but not its D-enantiomer, dose-dependently inhibited the tonic convulsions elicited by 3-mercaptopropionic acid (3-MPA) in mice (Polc et al., 1986). The pretreatment with a just subthreshold dose (versus 3-MPA) of L-CY (10 mg/kg i.p., 2 1/2 h before 3-MPA) elicited a parallel shift to the left of the dose-response curve of DZ for protection from 3-MPA seizures. The relative potency for this parallel shift (Bowman & Rand, 1980), which was statistically significant ($P<0.01$), was 3.1. L-CY (60 mg/kg i.p.) time-dependently potentiated the rectal temperature-lowering effect of DZ (6 mg/kg p.o.) in mice, with a peak effect at 3-4 h after administration. The doses used in this experiment for L-CY and DZ were virtually inactive by themselves. D-CY had no effect on rectal temperature, either alone or combined with DZ.

In cats, L-CY (3 mg/kg i.p.) in combination with DZ (0.3 mg/kg i.p.) significantly increased the current threshold for eliciting an attack response on stimulation of the perifornical hypothalamic area. This reduction of the aggressive rage reaction was attained 4 h after administration of L-CY, i.e. 2 h after that of DZ.

In the spinal cord of acute spinal cats, L-CY (10 mg/kg i.v.) enhanced segmental dorsal root potentials (DRPs) and the GABA content, measured 2 h after injection. An inactive dose of DZ (0.05 mg/kg i.v.), given 2 h after L-CY, increased DRPs further. D-CY (followed by DZ) was without effect on DRP and did not affect by itself the GABA content in the lumbo-sacral spinal cord of spinal cats.

These results indicate a clear-cut stereospecificity of L-CY in augmenting GABA mediated phenomena and GABA content in the CNS, and in enhancing various neuropharmacological effects of DZ. The potentiation by DZ of L-CY further supports the extensive body of evidence showing the enhancement by benzodiazepines of GABAergic transmission in the CNS (Haefely & Polc, 1986).

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COMPARATIVE SENSITIVITY OF CATECHOL-INDUCED SENSORY EVOKED CONVULSIONS TO DIFFERENT ANTICONVULSANTS

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Recently both spontaneous and sensory evoked convulsive activity induced by the administration of catechol (1,2 dihydroxybenzene) to anaesthetised rodents has been shown to be blocked by diazepam and valproic acid (Dawson and Dewhurst, 1984; Dewhurst, 1985) which are primarily effective against tonic-clonic convulsions. This study compares the effects of other anticonvulsants effective against animal models of tonic-clonic convulsions with those more effective against absence seizures, on catechol-induced sensory evoked convulsions.

All experiments were performed on female albino rats (Sheffield strain, 190-210g) anaesthetised with urethane (1.2-1.4g/kg i.p.). Catechol was infused into a jugular vein throughout the experiment at a dose of 2.5 mg/kg/min i.v. Electromyographic activity was recorded from Flexor carpii, and evoked by applying electrical stimuli (10-20V; 20μs width; 0.17Hz) at the wrist. Characteristically this electromyographic activity consists of three temporally distinct components which are absent in the non-catechol treated animal (Angel and Dewhurst, 1978; Dewhurst, 1984) and which are the result of i) a propriospinal reflex (M1), ii) a transcortical reflex (2), and iii) a cerebellar reflex (M3) respectively. After recording a suitable control period, anticonvulsants were administered via a femoral vein and recording was continued for a further 40-60 min.

The probability of occurrence of each component was determined in the pre-drug control period and in the post-drug test period, the effects of anticonvulsants being evaluated by determining the differences in the mean percentage probability of occurrence of each component before and after drug administration. Phenytoin (50mg/kg) and carbamazepine (1.5mg/kg) both significantly ($P<0.02$; n=5) decreased the mean percentage probability of occurrence of M2 (44.6±6.1%; 45.0±11.5%) and M3 (49.3±7.3%; 18.8±8.4%), but were without effect on M1, indicating a supraspinal site of action of the anticonvulsants. Trimethadione (100,200mg/kg) and ethosuximide (60,120 mg/kg) had no significant effect ($P>0.1$; n=5) on M1, M2 or M3.

Preliminary experiments indicate that spontaneous convulsions induced by administration of catechol to anaesthetised mice are also unaffected by anticonvulsants such as ethosuximide and trimethadione.

In conclusion these results show that spontaneous catechol convulsions and those components of the catechol-induced, sensory-evoked muscle response, dependent on supraspinal structures (M2 and M3) are only sensitive to anticonvulsants most effective against tonic-clonic seizures supporting the view that catechol convulsions may be a useful model for studying tonic-clonic seizures (Angel et al., 1977).

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OPTIMISING THE PENTYLENETETRAZOL INFUSION METHOD FOR MEASURING SEIZURE THRESHOLDS.

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Pentylenetetrazol (PTZ) a convulsant that acts at the GABA receptor complex is the most widely used chemical screen for anticonvulsant and anxiolytic drugs. It is particularly useful for benzodiazepines, as their relative potency against PTZ seizures closely parallels their clinical potency (Randall & Kapell, 1973). PTZ may also be used to test for proconvulsant effects. PTZ may be given i.p., s.c. or i.v. There are reasons for preferring the i.v. route as seizure thresholds can be determined using small numbers of animals (see Nutt et al, 1980) although changing concentration of infusate and rate of infusion yields different values for these. The present study was designed to determine the optimal rate of PTZ delivery for the detection of anticonvulsant and proconvulsant effects of drugs acting at the benzodiazepine receptor.

PTZ in saline was infused into the tail vein of male Charles River CD1 mice (wt 30-35 g). Latency to the onset of repeated myoclonic jerking of the neck and forelimbs was measured and from this time and the rate of drug delivery a seizure threshold was calculated. PTZ concentration and rate of infusion was varied as in Table. Flurazepam (10 mg kg-1 in saline) was used as the anticonvulsant, and the betacarboline FG 7142 (40 mg kg-1 in 1 drop of Tween 80/10 mls distilled water)as the proconvulsant drug (Little et al, 1984). Both were given i.p. at a volume of 10 ml kg-1. Controls received saline.

Table 1 PTZ Seizure Thresholds Concentration of PTZ

Rate of Infusion (ml min-1)	10 mg ml-1			2.5 mg ml-1			1 mg ml-1		
	C	FZ	FG	C	FZ	FG	C	FZ	FG
1.1	70±8	141±40	34±8	37±5	115±11	24±4	42±9	81±6	n.d.
0.55	73+9	142±24	n.d.	46±7	138±17	33±4	55±4	n.d.	n.d.
0.275	44±4	150±30	25±7	38±4	n.d.	n.d.		n.d.	
0.138	50±10	n.d.	n.d.		n.d.			n.d.	

Numbers are mean ± S.D., n=7-8; n.d. = not determined FZ=flurazepam
Treatments given 30 min pre infusion of PTZ FG=FG 7142

All drug effects were significantly different from control values, however the infusion rate of 1.1 ml min-1 gave the easiest discrimination of end points with those at 0.138 ml min-1 being particularly difficult to determine.At the lower concentrations and slower rates it was hard to detect reliably end points in control mice so drug studies were not carried out. The 2.5 mg ml-1 concentration of PTZ was optimal for detecting threshold elevation, giving good end points, and the largest flurazepam/control ratio. 10 mg ml-1 of PTZ produced the best ratio for detecting threshold lowering. These findings may help improve the value of the PTZ infusion method for drug screening.

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EFFECT OF HISTAMINE ON THE RELEASE OF ENDOGENOUS NORADRENALINE AND 5-HYDROXYTRYPTAMINE FROM RAT CEREBRAL CORTICAL SLICES

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Histamine has been shown to stimulate the release of ^{3}H-catecholamines and ^{3}H-indoleamines from rat brain slices and synaptosomes following prelabelling with ^{3}H-noradrenaline or ^{3}H-5-hydroxytryptamine (Subramanian & Mulder, 1977; Tuomisto & Tuomisto, 1980). In this communication we report the effect of histamine on the release of endogenous catecholamines and indoleamines from rat cerebral cortical slices.

Slices (300 x 300 µm) of rat (Wistar, 210-330 g) cerebral cortex were incubated for 40 min at 37°C in Krebs Henseleit solution containing pargyline (50 µM) under an atmosphere of O_2/CO_2 (95:5%). Following addition of histamine (0.01 - 1 mM, final concentration) or transfer of slices to high K^+ medium (56 mM - with appropriate reduction of Na^+ concentration), incubations were continued for 40 min and terminated by brief centrifugation. Supernatants were then assayed for noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) and their metabolites using HPLC with electrochemical detection (glassy carbon electrode maintained at 0.65 V). 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were assayed using Spherisorb 5ODS reverse phase columns (Mobile phase - 0.1 M citrate-acetate buffer, pH 4.0, containing 1 mM EDTA and 10% methanol). NA, DA and certain metabolites were also assayed by reverse phase chromatography (Spherisorb 5ODS2 column; mobile phase - 35 mM citric acid, 12.5 mM Na_2HPO_4, 0.25 mM sodium octyl sulphate, 0.05 mM EDTA, methanol 6%, pH 3.5) following alumina extraction. Alumina extraction was necessary to remove electrochemically-active exogenous histamine which otherwise co-eluted with NA. Protein content of slices was determined by the method of Lowry et al (1951).

K^+ (56 mM) stimulated the release (pmol/mg protein) of NA (10.1±0.6), DA (19.4±2.5) and 5-HT (21.1±1.4) in a calcium dependent manner ($P<0.001$, values expressed as the difference between stimulated and control levels, mean ±s.e. mean; 11 experiments). Histamine (1 mM) also stimulated the accumulation (pmol/mg protein) of NA (7.3±1.7, $P<0.02$; 5 experiments) and 5-HT (12.7±1.0, $P<0.001$; 20 experiments) but not that of DA. However, the histamine-stimulated accumulation of NA and 5-HT was unaffected by removal of external calcium. Dose-response experiments indicated that a concentration of 0.1 mM histamine or greater was normally required for stimulation of amine release. The mean basal accumulation of NA, DA and 5-HT in these experiments was 1.4, 1.7 and 3.9 pmol/mg protein respectively.

These results show that high concentrations of histamine can produce a marked stimulation of NA and 5-HT release from rat cerebral cortical slices in a calcium independent manner. The possibility of the release of NA and 5-HT from the cerebral cortex by high concentrations of histamine may therefore need to be borne in mind in biochemical and behavioural studies involving histamine.

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THE ACTION OF ω-PHOSPHONO-2-AMINO CARBOXYLIC ACIDS ON RELEASE OF [^{3}H]-D-ASPARTATE FROM HIPPOCAMPAL MINISLICES

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ω-phosphono-analogues of glutamate provide a group of antagonists that selectively block the neuronal excitation induced by dicarboxylic amino acids (Watkins and Evans, 1981) with 2-amino-7-phosphonoheptanoic acid [2APH] and 2-amino-5-phosphonovaleric acid [2APV] being particularly potent antagonists of excitation at the N-methyl-D-aspartate [NMDA] preferring receptor. For both 2APH and 2APV the D(-) enantiomer is the more potent antagonist. 2-amino-4-phosphonobutyric acid [2APB] is only a weak antagonist at the NMDA receptor. However L(+)2APB appears to act at a distinctive "2APB receptor" (Fagg et al., 1983).

We have compared the effects of 2APB, 2APV and 2APH on the release of preloaded [^{3}H]-D-aspartate from rat brain minislices and examined the stereo-specificity of the effects of 2APB and 2APV to provide information about the receptors involved. Potassium-evoked release of [^{3}H]-D-aspartate from preloaded hippocampal minislices was carried out according to a modification of the procedure of Chapman et al., (1985).

Table 1 Inhibitory effect of phosphonate analogues of excitatory amino acids on [^{3}H]-D-aspartate release from rat hippocampal slices

ANALOGUE		n	Evoked Release Ratio [$S_2/2_1$ ± S.E.]
Control		85	0.84 ± 0.014
2-amino-4-phosphonobutyric acid [2APB]:			
D(-)2APB	250 μM	8	0.90 ± 0.063
L(+)2APB	100 μM	10	0.72 ± 0.032*
	250 μM	7	0.66 ± 0.053*
2-amino-5-phosphonovaleric acid [2APV]:			
D(-)2APV	10 μM	10	0.70 ± 0.022***
	100 uM	10	0.72 ± 0.027***
L(+)2APV	100 μM	10	0.81 ± 0.065

The basal release of [^{3}H]-D-aspartate remained unchanged following addition of any of the analogues tested. However all three analogues reduced the K^+-induced release of preloaded [^{3}H]-D-aspartate from hippocampal minislices; 2APH being the least potent of the inhibitors. Comparison of enantiomers showed that whereas D(-)2APV was the active form of 2APV, L(+)2APB was the active isomer of 2APB (Table 1). The data for the 2APB analogue can be explained in terms of a pre-synaptic L(+)2APB sensitive receptor decreasing the synaptic release of excitatory amino acids. In the case of 2APV and 2APH synaptic depression may be caused by antagonism of presynaptic NMDA-type receptors.

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INJECTION OF γ-D-GLUTAMYLAMINOMETHYLSULPHONIC ACID (GAMS) IN SUBST. NIGRA PROTECTS AGAINST GENERALISED AND LIMBIC SEIZURES IN RATS

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GAMS preferentially blocks excitation at the kainate-receptor in the spinal cord (Davies & Watkins, 1985). Anticonvulsant properties of GAMS have been demonstrated against sound-induced seizures in DBA/2 mice (Croucher et al, 1984) and kainate-induced myoclonic seizures (Turski et al, 1985). Focal injection of the excitatory amino acid antagonist 2-amino-7-phosphonoheptanoic acid into the substantia nigra (SN) suppresses tonic extension induced by electroshock (De Sarro et al, 1984) or limbic seizures induced by pilocarpine (Turski et al, 1986). The present study reports the effect of focal injections of GAMS into the SN on seizures produced by electroshock or pilocarpine.

Male Wistar rats were stereotaxically implanted with bilateral guide cannulae into the SN (pars reticulata or pars compacta) and tested 48 h later with electroshock or pilocarpine. Electroconvulsions were induced by stimulation via ear electrodes with 50 mA a.c. for a duration of 0.2 sec. The onset and occurrence of tonic extension and clonic convulsions of fore- and hind- limbs was recorded. Electro-convulsions in rats were produced 30, 150 and 270 min following bilateral injection of vehicle (0.5 µl/side) or GAMS (0.5-10 nmol/0.5 µl) into SN, pars reticulata or compacta.

Limbic seizures in rats were induced by systemic injection of a large dose of pilocarpine (380 mg/kg i.p.). To prevent peripheral effects of pilocarpine, the animals were pretreated with scopolamine methylnitrate (1 mg/kg s.c.). The resulting motor limbic seizures were scored on a five-point scale as described by Racine (1972). All animals were tested 30 min after bilateral infusion of vehicle (0.5 µl) or GAMS (1-50 nmol/0.5 µl) into the SN, pars reticulata or compacta. All animals were perfusion-fixed and the brains processed for histological examination.

Intranigral infusion of GAMS reduced the duration of tonic-extension of hind-limbs after electroshock in a dose dependent manner (significant protection being obtained after 5 or 10 nmol.)

Motor limbic seizures following pilocarpine administration were usually apparent within 30 min. Nigral injection of GAMS produced a dose-dependent suppression of motor limbic seizures (ED50 = 18 nmoles). A low dose of GAMS (1 nmole) injected into the pars reticulata did not change the sequence of pilocarpine-induced seizures. However, 10 nmoles of GAMS produced significant protection, and 50 pmoles totally abolished the motor (limbic) convulsions. Less notable protective effects were observed following infusion of GAMS into the pars compacta. (50 nmoles of GAMS completely suppressed pilocarpine-induced seizures in only 50% of animals).

Nanomole amounts of GAMS injected into the SN (pars reticulata or compacta) produces an anticonvulsant action against electroshock and pilocarpine induced limbic seizures. The role of kainate receptors in the SN in the development of seizure activity require further investigation.

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BENZODIAZEPINE AND 5-HT LIGANDS IN A RAT CONFLICT TEST

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Reduction of central 5HT-mediated neurotransmission can lead to anxiolytic-like effects in animal models (for reviews see Iversen, 1984; Gardner, 1985). Benzodiazepines decrease 5HT turnover, and this is a possible mode of their anxiolytic action. New putative anxiolytics (e.g. ritanserin and TVXQ 7821) have been associated with activities on 5HT mechanisms (Engel et al., 1984; Traber et al., 1984; Colpaert et al., 1985). We have compared 5HT and benzodiazepine receptor ligands on a conflict model, consisting of a food-reinforced operant schedule with 5 alternating cycles of unpunished (4min FI 30s) and footshock punished (3min FR5) components. The footshock was titrated during training for each rat to produce 80% suppression and data are shown as mean changes in lever presses/min compared to stable baseline responses on two previous control days.

Compound	Dose mg/kg 25min pre-test	Route	n	Mean Rate Increase (No significant*) Unpunished		Punished	
CDZP	10	p.o.	6	11.1	(4)	36.0	(6)
CGS9896	10	p.o.	4	7.1	(0)	9.9	(2)
ZK91296	20	i.p.	4	17.4	(3)	23.1	(4)
RU24969	2	p.o.	4	-3.4	(1↑,1↓)	4.0	(1)
8OH-DPAT	0.25	i.p.	7	-1.5	(1)	0.8	(0)
Ritanserin	10	i.p.	4	0.7	(0)	0.7	(0)
Metergoline	20	p.o.	4	-2.4	(1↓)	0.2	(0)
Cyproheptadine	10	p.o.	4	3.3	(1↑,1↓)	1.3	(2)
TVXQ 7821	2.5	i.p.	5	0.6	(1)	0.0	(0)

* $p < 0.05$ Mann-Whitney U test, 2 tailed ↑ = increase, ↓ = decrease.

Full and partial benzodiazepine receptor agonists increased punished responding but neither 5HT agonists nor antagonists had marked effects in this model.

The operant schedule, type of reinforcement, response requirements and length of training may be factors in determining activity of 5HT modulating drugs. We conclude that a general role for 5HT mechanisms in producing anxiolytic-like effects in animal models remains to be established.

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SOME PUTATIVE NON-SEDATING ANXIOLYTICS IN A CONDITIONED LICKING CONFLICT

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Many licking conflict procedures suffer from considerable response variability. Recently a procedure involving the re-use of the same rats showed some improvement (Petersen and Lassen,1981), and we have developed this further by training a colony of rats to a conditioning stimulus. The colony was tested every Tuesday and Thursday, using the previously titrated baseline response of each rat as control for drug effects. Male Lister rats(Olac,Bicester) were housed in pairs and deprived of water for 18h prior to every test. At test, each rat was placed in a perspex enclosure (23x21x35 cm high) with a grid floor and a water nozzle in one wall, connected to a capacitance-based lick detector and electronic counters. This apparatus was within a sound attenuating chamber with internal lighting similar to that in the laboratory. Rats were observed via closed circuit television.

After a 1 min unpunished period of drinking, a timer turned on an intermittent tone (3KHz 2 sec on,2 sec off) which continued throughout a 3 min footshock-punished period (200 msec of 50 Hz,20msec 0.2-0.8 mA pulses,one shock per 30 licks). After training (2-3 weeks) the current was adjusted for each rat to obtain stable control responding of 20-200 licks during punishment.

Some putative non-sedative anxiolytic agents have been tested in this procedure in comparison with reference agents:-

DRUG	MEDIAN INCREASE IN PUNISHED LICKS						
	2	5	10	15	20	50	mg/kg p.o.
CDZP	7	532*	569*	469*			
Oxazepam			173*		310*		
Premazepam			67		463*		
CL218872			417*				
PK8165						73	
PK8165			327*		251*		i.p.
PK9084						-6	
PK9084			96			737*	i.p.
Tracazolate					148	168	
Buspirone	-81	-3	-5				

n = 5-11 rats except chlordiazepoxide (CDZP) where n = 10,32,62,21 at increasing doses. * $p < 0.05$ Mann-Whitney U test.

Premazepam and CL218872, two putative benzodiazepine partial agonists, showed good activity in this test. Tracazolate was weak and PK8165, PK9084 and buspirone were inactive after oral administration. The two PK compounds were very active after ip administration but PK9084 subsequently proved toxic at 50mg/kg.

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Gifts of premazepam (Lepetit), PK8165 and PK9084 (Pharmuka), tracazolate(ICI) and buspirone (Mead-Johnson) are gratefully acknowledged.

THE ANXIOLYTIC EFFECT OF SUBSTITUTED BENZAMIDES

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Clinical evidence indicates that the antipsychotic and antidyskinetic agents, sulpiride and tiapride, have additional anxiolytic activity although this has not been systematically assessed in animal models (Shaw et al, personal communication). The present study reports on the first step of an investigation establishing an anxiolytic profile for sulpiride and tiapride, compared with reference agents, in a simple mouse model.

Naive male Albino BKW mice, 25-30g, received i.p. injections of drug or vehicle and after 30-45 min were placed in the testing apparatus. The apparatus consisted of a box (81 x 36 x 27 cm high) 1/3 painted black and illuminated with red light and partitioned (with a connecting 'door') from the remainder of the box which was brightly illuminated and painted white. The floor area was divided by painted lines into 9cm squares. The model is based on the observation that open fields appear to have aversive properties which inhibit rodent exploratory behaviour. Briefly, mice will be averse to the white illuminated area, where a reduction in anxiety is revealed as more frequent line crossings and exploratory rearing in the white area (see Crawley & Goodwin, 1980).

These two parameters, and the time spent in the white and black areas, were observed by remote video recording in the 5 min period following placement of the mouse in the white area. The parameters found most useful for detecting anxiolytic action were line crossings and rearings in the white area, which mirrored each other, and therefore rearings only are shown on Table 1.

Table 1. Modification of rearing in the white area following drug treatment.

Drug treatment mg/kg i.p.		Rearings/ 5 min	Drug Treatment mg/kg i.p.		Rearings /5 min
Control		16.8±1.2	Control		17.1±1.4
Sulpiride	0.13	17.9±1.9	Diazepam	0.06	18.7±1.7
	0.5	31.5±2.8*		0.13	35.1±3.6*
	1.25	37.6±3.5*		0.25	39.1±3.1*
Tiapride	0.13	16.4±1.3	Triazolam	0.025	16.5±1.5
	0.5	28.6±2.4*		0.05	29.6±3.0*
	1.25	36.5±2.9*		0.1	39.1±4.2*
Haloperidol	0.05	16.2±1.5	Amphetamine	1.25	6.9±0.7^{+}
	0.1	9.9±0.8^{+}		2.5	14.6±1.6
	0.2	7.6±0.7^{+}			

n = 6-7 for drug treatments. n = 40 for control values. Significant enhancements relative to control values shown as *P<0.001, significant reductions as $^{+}$P<0.01-P<0.001 (Dunnett's t test).

The reference anxiolytic agents diazepam and triazolam caused an approximate 2 fold increase in rearing in the white section (maximal effects shown on Table 1) which was equalled by sulpiride and tiapride. It is unlikely that the neuroleptic and/or stimulant effects of these agents is relevant to such actions since haloperidol and amphetamine reduced rearing in the white section. It is concluded that the substituted benzamides sulpiride and tiapride have anxiolytic effect in the mouse model.

Crawley, J. & Goodwin, F.K. (1980) Pharmac. Biochem. Behav. 13, 167-170

BUSPIRONE INHIBITS THE METABOLISM OF [^{3}H]-FLUNITRAZEPAM IN RATS

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The observation that the non-benzodiazepine anxiolytic agent buspirone potentiated the binding of 3H-flunitrazepam and 3H-diazepam in-vivo in rats (Oakley and Jones, 1983; Weissman et al., 1984) prompted the suggestion that it may interact with the GABA-benzodiazepine receptor complex (Eison and Eison, 1984). However, evidence from in-vitro experiments suggests that this is not the case (Riblet et al., 1982). We were concerned that the enhancement of 3H-flunitrazepam binding in-vivo by buspirone could be due to interference with metabolism, especially as 3H-flunitrazepam is radiolabelled on the metabolically labile N-methyl group and buspirone also undergoes N-dealkylation.

The binding of 3H-flunitrazepam to three brain regions in rats in-vivo was determined as described previously (Oakley and Jones, 1983), but in addition, total radioactivity in the unfiltered homogenate was measured. Total radioactivity in peripheral samples was measured by sample oxidation.

We confirmed that buspirone (10-80 mg/kg orally) dose-dependently increased 3H-flunitrazepam binding in-vivo in cortex (+24 to +120%), cerebellum (+9 to +76%) and hippocampus (+14 to +97). However, it also increased the total radiolabel in the brain by a similar amount. Thus, the proportion of bound radiolabel was in the range 46-66% of total in all regions. Total radioactivity in whole blood, plasma, heart and adrenals was also increased by buspirone. In lung and kidney, concentrations were slightly increased, but in liver there was no change.

The effect of buspirone on the ex-vivo metabolism of 3H-flunitrazepam was examined by incubating 3H-flunitrazepam with liver microsomes taken from rats that had been treated with either buspirone, 80mg/kg orally, or vehicle, 40 minutes previously.

The decline of ether-extractable tritium with time was much slower in microsomes taken from the buspirone-treated rats. Under the conditions of our experiment, the half-life was 6.2 minutes compared to less than 30 seconds in control microsomes. TLC analysis of the ether extractable phase confirmed the slower degradation of 3H-flunitrazepam by the buspirone pretreated microsomes.

Finally, we tested the microsomal enzyme inhibitor SKF525A (50mg/kg orally) on 3H-flunitrazepam binding in-vivo. Like buspirone, it increased both the total and bound radioactivity proportionally in the three brain regions (+169 to +182%).

We conclude that the potentiation of 3H-flunitrazepam binding by buspirone is caused by interference with the metabolism of 3H-flunitrazepam. It is therefore unlikely that the anxiolytic action of buspirone involves an interaction with the GABA-benzodiazepine receptor complex.

Eison, M.S. & Eison, A.S. (1984) Drug Dev. Res. 4; 109-119.
Oakley, N.R. & Jones, B.J. (1983) Eur. J. Pharmac. 87; 499-500
Riblet, L.A. et al (1982) J. Clin. Psychiat. 43; 11-16
Weissman, B.A. et al (1984) Drug Dev. Res. 4; 83-93

ACUTE AND CHRONIC CONSEQUENCES OF PERSISTENT INFUSIONS OF NEUROLEPTIC AGENTS INTO RAT NUCLEUS ACCUMBENS

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The peripheral administration of neuroleptic agents as a chronic treatment leads to an enhanced responsiveness to dopamine agonists, assessed behaviourally and biochemically (Clow et al, 1980). In the present studies we investigate whether a discrete and persistent blockade of dopamine receptors in the limbic system can modify the responsiveness to dopamine agonist challenge.

Male Sprague-Dawley rats having stereotaxically implanted cannulae were subject to bilateral infusion into the nucleus accumbens (ACB, Ant. 9.6, Vert. 0.0, Lat. ±1.6; atlas of De Groot) of clozapine, sulpiride, fluphenazine or vehicle via Alzet osmotic minipumps located subcutaneously and infusing at a rate of 0.48µl/h for 13 days (see Costall et al, 1984 for experimental details; n = minimum of 5 for each treatment). Spontaneous locomotor activity was assessed daily (for a 1h period between 8.00 and 11.00 am) during and post-infusion, and responsiveness to the locomotor stimulant effects of (-)N-n-propylnorapomorphine ((-)NPA, 0.05 mg/kg s.c.) was assessed at the completion of infusion and at weekly intervals post-infusion using individual photocell cages.

The infusion of clozapine (0.5, 5 and 10 ng/day), sulpiride (0.1, 0.5 and 1.25 ng/day) and fluphenazine (0.5, 5 and 10 ng/day) into the ACB failed to modify spontaneous locomotor activity during or for 4 weeks following infusion ($P>0.05$, control locomotor activity 74±13 counts/60 min). On the 13th day of infusion, clozapine (0.5 and 5 ng/day) enhanced (-)NPA induced locomotor activity by 89-167%, $P<0.05$-$P<0.01$, and similar significant enhancements were recorded during the 4 week post-infusion period, $P<0.05$-$P<0.01$. Such enhancements were not consistent following the infusion of the higher dose of clozapine (10 ng/day). Whilst sulpiride (0.1, 0.5 and 1.25 ng/day) infusion on the 13th day failed to modify the locomotor stimulant effects of (-)NPA, 1 to 4 weeks post-infusion an increased responsiveness of 83-185%, $P<0.05$-$P<0.01$, was observed. On the 13th day of infusion of fluphenazine, the higher doses of 5 and 10 ng/day markedly reduced the locomotor stimulant effects of (-)NPA by 65-76%, $P<0.01$. 1 to 4 weeks post infusion, only the intermediate dose of 5 ng/day fluphenazine enhanced (83-89% $P<0.01$) the effects of (-)NPA, the other doses were ineffective. The infusion of the neuroleptics was not associated with any other overt changes in behaviour either during or after infusion.

It is concluded that the infusion of clozapine, sulpiride and fluphenazine into the nucleus accumbens of rat brain failed to modify spontaneous locomotor activity in doses that enhanced (clozapine, sulpiride) or reduced (fluphenazine) the locomotor stimulant effects of (-)NPA at the completion of infusion or post-infusion. The preliminary data suggest that a persistent antagonism of limbic dopamine function may modify the locomotor responsiveness to dopamine agonist challenge and that such changes may persist after discontinuation of the neuroleptic treatment.

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Clow, A. et al (1980) Psychopharmacology 69, 227-233

ASYMMETRIC LESION OF THE MEDIAL RAPHE NUCLEUS CAN LEAD TO BIOCHEMICAL DISTURBANCES IN BOTH HEMISPHERES

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Chandu-Lall et al (1970) showed that lesions of the nigrostriatal pathway in one hemisphere could cause biochemical disturbance in both hemispheres. Here we show that asymmetric damage to the medial raphe nucleus (MRN) can bilaterally disturb brain biochemistry.

Male Sprague-Dawley rats (250-300g) were subject to standard stereotaxic surgery for electrolesion (stainless steel electrode, 0.65mm diameter, insulated except at the tip, 1 mA for 20s) asymmetrically (left hemisphere) of the MRN (Ant. 0.3, Vert. -2.6, Lat. -0.2, electrode angled 65° posterior, atlas of Konig & Klippel. Sham-operated animals were also prepared. 2 days after surgery animals circling in excess of 7 revs/min contralateral to the lesion were selected as having sustained effective asymmetric MRN lesions (confirmed histologically). Sham operated rats failed to circle. The animals were then killed by cervical dislocation and the nucleus accumbens (ACB), striatum (CP) and amygdala (AMG) dissected out for determination of levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) using HPLC with electrochemical detection.

Table 1. Changes in the levels of DA, 5-HT and their metabolites in the right (R) and left (L) forebrain areas following lesion of the MRN in the left hemisphere.

Transmitter/ Metabolite		ACB L / R	CP L / R	AMG L / R
5HT	Control	685±38	271±13	580±30
	Lesion	489±46*/437±56*	135±5***/142±11***	547±15/352±36**
5HIAA	Control	231±14	237±13	226±11
	Lesion	160±19*/195±20	192±13/208±22	216±8/197±15
DA	Control	3.56±0.37	12.36±0.39	1.11±0.03
	Lesion	4.11±0.45/2.56±0.26	10.89±0.4/13.0±0.17	0.37±0.08***/1.15±0.07
DOPAC	Control	651±36	1185±18	104±8
	Lesion	864±64+/408±51*	927±56**/1058±42*	ND/ND

Values expressed as pg/mg wet weight of tissue except for DA, ng/mg. n = 4-6. Control values were obtained from tissues pooled from both hemispheres. A significant reduction indicated *P<0.05, **P<0.01, ***P<0.001 and a significant increase +P<0.05. Student's t test. ND - not detectable.

The major biochemical findings following lesion of the MRN in the L hemisphere were significant reductions in 5-HT in the ACB and CP in both hemispheres and in the AMG of the R hemisphere. Whilst not directly disrupting the midbrain DA projections, the MRN lesions also caused reductions in DA and/or DOPAC in the R and L hemispheres in the ACB and AMG respectively. It is concluded that a lesion placed asymmetrically in the MRN may effect serotonergic function in both hemispheres, the degree of change depending on the brain area considered, and may also result in changes in dopamine biochemistry in forebrain regions. It is suggested that these findings relate to the facility of the brain to compensate for injury.

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Chandu-Lall, J.A. et al (1970) Exp. Neurol. 29, 101-110

TOLERANCE TO THE ANXIOLYTIC ACTION OF DIAZEPAM IN RATS

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We recently demonstrated that tolerance to the antipunishment properties of diazepam (DZP) in mice was not associated with changes in benzodiazepine (BZ) receptor binding nor with an uncoupling of BZ from GABA receptors (Stephens and Schneider, 1985). Others have reported an uncoupling of the BZ and GABA receptors following chronic DZP treatment of rats (Gallager et al, 1984) but no evidence for tolerance to the anxiolytic effects was presented. We have therefore investigated the ability of GABA to stimulate ^{3}H-BZ binding in membranes prepared from rats which were tolerant to the anxiolytic effects of DZP. The ability of DZP (0.625, 1.25 and 2.5 mg/kg, i.p.) or vehicle (10 % Cremophor EL in 0.9 % saline) to increase exploration of the open arms of an elevated plus-maze (Handley and Mithrani, 1984) was studied in the same animals acutely and following 8 and 15 days treatment with DZP (5 mg/kg/day, i.p.). Acutely, DZP increased the number of entries into and time spent in the open arms of the maze ($F(3,28) = 5.26$, and 3.93, respectively, $p < 0.05$) without affecting total entries. This effect was reduced at 8 days and following 15 days treatment DZP exhibited no significant effects, indicating tolerance to its anxiolytic activity.

Following behavioural testing, each rat received a supplementary dose of DZP to make its daily dose up to 5 mg/kg and 2 rats from each group were killed 1, 24 or 48 h following this supplementary dose (i.e. n = 8 at each time point), together with 6 untreated rats. The ability of GABA (3×10^{-7} - 10^{-4} M) to stimulate ^{3}H-lormetazepam (LMZ) binding to repeatedly washed membranes prepared from frozen forebrains (Stephens and Schneider, 1985) from these groups was studied. There were no differences among the groups in basal LMZ binding values and a small non-significant increase in stimulated values in only the group killed 1 h following the last dose of DZP at any GABA concentration (Values for maximal stimulation, at 10^{-5} M GABA: Untreated 220 $\pm$ 3.7; 1 h 231 $\pm$ 3.5; 24 h 225 $\pm$ 8.5; 48 h 226 $\pm$ 4.6 % of basal values).

These results support our previous conclusion that tolerance to the anxiolytic properties of DZP is not due to a change in the coupling of BZ and GABA receptors.

Gallager, D.W. et al (1984). Nature 308 : 74

Handley, S.L. and Mithrani, S. (1984). Naunyn-Schmiedeberg's Arch. Pharmacol. 327 : 1

Stephens, D.N. and Schneider, H.H. (1985). Psychopharmacol 87 : 322

ACCUMULATION OF L-DOPA IN MUSCLE IS ENHANCED BY CARBIDOPA ADMINISTRATION

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Alterations in plasma L-DOPA levels may be responsible for changes in drug efficacy during the chronic treatment of Parkinson's disease. However, following acute or chronic administration of L-DOPA, a large proportion of the amino acid accumulates in skeletal muscle (Ordonez et al 1972; Romero et al 1983). Using a sensitive HPLC procedure we have compared the effects of acute oral administration of L-DOPA in the presence and absence of a peripheral decarboxylase inhibitor on the accumulation and metabolism of L-DOPA in plasma and skeletal muscle.

Male Wistar rats (140 ± 2g) were treated with L-DOPA (200 mg/kg po) with and without carbidopa (α-methyldopahydrazine; 25 mg/kg po). Animals were killed at intervals upto 8h following drug administration and the concentrations of L-DOPA, dopamine, HVA and DOPAC were determined in plasma and muscle from the upper foreleg by HPLC with electrochemical detection.

Administration of L-DOPA (200 mg/kg po) produced elevated plasma and muscle concentration of the amino acid for 3h and 4h respectively (Table 1). In the presence of carbidopa (25 mg/kg po) plasma and muscle concentrations of DOPA were higher than those observed following administration of L-DOPA alone and were maintained for 4h and 8h respectively. Following administration of L-DOPA alone elevated dopamine concentrations were detected in plasma but carbidopa treatment diminished the increase observed. In contrast, the elevation of dopamine levels observed in muscle following administration of L-DOPA alone was not decreased by carbidopa administration, and was of longer duration.

In plasma, the increase in HVA and DOPAC concentrations observed following administration of L-DOPA alone was not altered by carbidopa treatment. Similarly carbidopa treatment had little effect on muscle HVA and DOPAC concentrations but tended to increase the duration of effect.

Table 1 Plasma and muscle concentration of L-DOPA

	Plasma (ug/ml)		Muscle (ug/g tissue)	
Time (hr)	L-DOPA	L-DOPA + Carbidopa	L-DOPA	L-DOPA + Carbidopa
0	0.1±0.1	0	0.1±0.1	0.1±0.1
1	4.6±1.4	7.1±1.2	6.4±1.1	11.4±2.9
2	1.8±0.4	7.0±1.8*	5.6±1.4	11.8±3.2
3	1.4±0.6	5.2±1.8	1.2±0.3	10.8±2.0*
4	0.4±0.2	2.9±0.3*	2.3±1.3	11.6±4.2
8	0.1±0.1	1.7±1.5	0.4±0.1	5.0±2.1*

* Denotes significant difference from L-DOPA alone ($p < 0.05$)

The results suggest 1) that L-DOPA accumulation in muscle is enhanced by carbidopa treatment; 2) that carbidopa does not inhibit decarboxylase activity in muscle; and 3) that plasma HVA and DOPAC concentrations may reflect the degradation of dopamine in tissues other than plasma.

Ordonez, L.A. et al (1972) (Abstract) Fed. Proc. 31, 589
Romero, J.A. et al (1973) J.Pharmacol.Exp.Ther. 184, 67

LONG TERM CHANGES IN α-ADRENOCEPTOR AND 5-HT RECEPTORS IN RAT BRAIN INDUCED BY AMINE DEPLETING DRUGS

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Monoamine depleting drugs, such as reserpine and oxypertine, are used to treat hyperkinetic movement disorders. This action is associated with decreased brain dopamine function but these drugs also affect noradrenaline and 5HT systems (Nakahara et al,1980). We report on the effect of long term oxypertine and reserpine treatment on brain α-adrenergic and 5HT receptors compared with the actions of chlorpromazine

Male Wistar rats were treated for 6 months with oxypertine (6.3-7.3 mg/kg/day), reserpine (0.28-0.30 mg/kg/day) or chlorpromazine (33-36 mg/kg/day) via their distilled drinking water. Control animals received distilled water alone. Washed membrane preparations of brains were resuspended in 100 volumes of 50 mM, Tris-HCl buffer (pH 7.6). Initially the ability of each drug added *in vitro* to the tissue preparations was assessed for activity on the receptor populations identified. The specific binding of ^{3}H-WB4101 (0.3 nM; defined using 10^{-4}M noradrenaline) to α-1 sites in rat cerebral cortex preparations and of ^{3}H-ketanserin (0.5 nM; defined using 10^{-5}M cinanserin) to 5HT-2 sites in rat frontal cortex preparations was potently displaced by both oxypertine and chlorpromazine (10^{-9} - 10^{-4}M) but not by reserpine. Oxypertine and chlorpromazine were only weakly effective in displacing specific ^{3}H-clonidine (2.5 nM; defined using 2×10^{-6}M noradrenaline) binding to α-2 sites in rat cerebral cortex membranes but reserpine had no effect (Table 1). Administration of chlorpromazine (33-36 mg/kg/day) for 6 months increased the number (Bmax) of specific binding sites for ^{3}H-WB4101 but decreased Bmax for specific ^{3}H-ketanserin binding. Chlorpromazine was without effect on the number of specific ^{3}H-clonidine sites. Chlorpromazine increased K_D for ^{3}H-WB4101 and ^{3}H-ketanserin but not ^{3}H-clonidine binding. Administration of oxypertine (6.3-7.3 mg/kg/day) or reserpine (0.28-0.30 mg/kg/day) increased Bmax for both ^{3}H-WB4101 and ^{3}H-clonidine binding; ^{3}H-ketanserin binding was unaffected. K_D values for all ligands was unaltered by oxypertine and reserpine.

Table 1 Alterations in α-1 and α-2 adrenergic receptors and 5HT-2 receptors in rat brain

	^{3}H-WB4101	^{3}H-Clonidine	^{3}H-Ketanserin
In vitro (IC_{50}; mM)			
Oxypertine	4.1×10^{-8}	3.9×10^{-6}	2.5×10^{-8}
Chlorpromazine	3.4×10^{-9}	2.6×10^{-6}	1.6×10^{-8}
Reserpine	$> 10^{-4}$	$> 10^{-4}$	$> 10^{-4}$
6 months administration (Bmax; pmol/g tissue)			
Control	4.2 ± 0.2	2.3 ± 0.1	7.6 ± 1.9
Oxypertine	6.7 ± 0.3*	3.2 ± 0.3*	7.4 ± 0.6
Chlorpromazine	6.0 ± 0.4*	2.3 ± 0.02	2.9 ± 0.6*
Reserpine	5.5 ± 0.1*	3.4 ± 0.2*	9.2 ± 0.8

* $p < 0.05$ compared to controls, Dunnett's test.

Although oxypertine like chlorpromazine, interacts directly with brain α-1 and 5HT-2 receptors, the effect of long term administration resemble those of the monoamine depleter, reserpine, in causing an increase in both α-1 and α-2 binding sites, but not 5HT-2 sites.

Nakahara, T. et al (1980) Biochem.Pharmacol. 29, 2681.

AFFINITY CHROMATOGRAPHY OF SOLUBILISED DOPAMINE D_2 RECEPTORS USING IMMOBILISED DOPAMINE ANTAGONISTS

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Dopamine D-2 receptors identified by ^{3}H-spiperone from rat striatal tissue can be solubilised using the zwitterionic detergent CHAPS (Kilpatrick et al 1985). Molecular characterisation of D-2 receptors requires purification from such soluble tissue preparations. Attempts to purify solubilised D-2 receptors by affinity chromatography using immobilised haloperidol resulted in low enrichment and a low yield (Ramwani & Mishra 1982). We now report on preliminary attempts to purify solubilised D-2 receptors by affinity chromatography using immobilised substituted benzamide drugs with high D-2 receptor selectivity.

Sulpiride, metoclopramide, N-[1-methyl-cyclopropyl-methyl-2-pyrrolidinyl-methyl]-2-methoxy-4-amino-ethylsulphonyl benzamide (2366) and for comparison the butyrophenone compound pipamperone were coupled selectively via their primary amino group to Sepharose 4B gel (Pharmacia). The resulting gels were poured into LKB (1.5 x 10 cm) columns. Gels were equilibrated with 50 mM Tris HCl buffer containing 1 mM EDTA (pH 7.4; 4°C). CHAPS (5 mM) solubilised extracts of rat striatal tissue (7 ml; 4°C) prepared as described previously (Kilpatrick et al 1985) were applied to the columns and continuously reapplied via a peristaltic pump for 2h. Columns were then eluted with Tris-EDTA buffer and 3 x 20 ml fractions of eluate collected. For each of the immobilised drugs the majority of protein (> 95%) and specific ^{3}H-spiperone (0.35 nM; defined by the inclusion of 3×10^{-5}M sulpiride) binding sites (> 85%) were eluted in these first 3 fractions. Sulpiride, to a final concentration of 10^{-4}M, was then applied and continuously reapplied in the column over a period of 1.5h to displace bound protein. The column was then eluted with Tris-EDTA buffer and 2 x 20 ml fractions of eluate collected. Fractions were dialysed to remove excess sulpiride. Using the immobilised substituted drugs sulpiride, metoclopramide and 2366, a small percentage (7-13%) of specific ^{3}H-spiperone binding sites were observed in these fractions (Table 1). Using immobilised pipamperone no specific ^{3}H-spiperone binding could be observed in these fractions. A purification factor (by comparison of bound: protein ratios in these fractions to mean bound:protein ratios in all fractions) for preparations exposed to immobilised substituted benzamide drugs of between 14-22 fold was obtained (Table 1).

Table 1 Affinity chromatography of solubilised striatal preparations

Immobilised drug		D-2 receptor retention (%)	Purification factor
Sulpiride	(3)	13	14
Metoclopramide	(3)	10	20
2366	(4)	7	22
Pipamperone	(2)	0	0

Results represent mean of (n) experiments

In conclusion, these preliminary studies reveal that some purification of solubilised dopamine D-2 receptors may be achieved using immobilised substituted benzamide drugs, however, the recovery was small.

Kilpatrick, G.J. et al (1985) J.Pharm.Pharmacol. 37, 320.
Ramwani, J. & Mishra, R.K. (1982) Fed.Proc. 41, 1325.

ANTIPARKINSONIAN ACTIVITY FOLLOWING SUBCUTANEOUS OR ORAL ADMINISTRATION OF (+)-PHNO TO MPTP-TREATED MARMOSETS

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(+)-4-Propyl-9-hydroxynaphthoxazine ((+)-PHNO) is a highly potent dopamine agonist of novel structure, which is selective for D-2 receptors in brain (Martin et al, 1984). We have studied the potential antiparkinsonian effects of (+)-PHNO in marmosets rendered parkinsonian by administration of the selective dopamine cell neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Adult common marmosets of either sex (weight range 289-390g) were treated daily with MPTP in doses between 1-4 mg/kg so as to render them obviously parkinsonian as previously described (Nomoto et al, 1985). Marmosets treated with MPTP exhibited fewer and less coordinated movement than control animals when examined in the home cage. Administration of (+)-PHNO (1-4 μg/kg sc) to MPTP-treated marmosets caused a dose-dependent behavioural activation, including checking movement and general movement from place to place within the cage. Onset of drug action occurred within 10 min of (+)-PHNO administration. At 2, 3 or 4 ug/kg sc (+)-PHNO activity was maximal by 45 min and the effects lasted for between 90-120 mins. Movements were well coordinated and included normal running on the floor of the cage, jumping up to high perches and down to the floor. Most of this repertoire of movement appeared normal, but at high doses there were sometimes periods of explosive motor action. Oral administration of (+)-PHNO (10 or 20 μg/kg) to the MPTP-treated animal also caused behavioural activation. The onset of motor activation occurred 10 min following the administration of (+)-PHNO and persisted around 90 mins. (+)-PHNO also caused motor activation in normal control animals after subcutaneous or oral administration but this was less than that observed in MPTP-treated marmosets (Table 1).

Table 1 Cumulative movement counts following (+)-PHNO administration

Subcutaneous (dose μg/kg)	Saline	Movement count 150/min 1	2	3	4
MPTP	13 ± 11	143 ± 65	1382 ± 215*†	1835 ± 547*	2518 ± 452*
Control	67 ± 22	55 ± 40	576 ± 148◇	909 ± 130◇	1848 ± 251◇
Oral (dose μg/kg)	Saline	10	20		
MPTP	-	1371 ± 298*	1602 ± 335*		
Control	-	1022 ± 292◇	1153 ± 204◇		

n = 4-5, * $p < 0.01$ compared to saline-treated MPTP group, ◇$p < 0.01$ compared to saline-treated control group, †$p < 0.05$ compared between MPTP and control group.

Subcutaneous or oral administration of (+)-PHNO reversed the persistent motor deficits observed in MPTP-treated marmosets in microgram doses. The drug was approximately seven times more effective subcutaneously than orally.

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INHIBITION OF PILOCARPINE-INDUCED PURPOSELESS CHEWING BY MIANSERIN SUGGESTS CENTRAL ANTICHOLINERGIC ACTIVITY.

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The clinical use of the atypical antidepressant mianserin is associated with a low incidence of anticholinergic side effects compared to the classical antidepressant amitriptyline (Brogden et al 1978). Indeed mianserin appears to have little or no central anticholinergic activity since in doses of upto 20 mg/kg it does not antagonise oxotremorine-induced tremors in rats (Maj et al 1978). However, in vitro mianserin displaces specific ^{3}H-atropine binding to homogenates of whole rat brain (Golds et al 1980). We now investigate the actions of mianserin in a model of central cholinergic activity, namely pilocarpine-induced purposeless chewing in the rat, in comparison to drug activity on central 5HT-2, α-2, α-1 and H-1 receptors.

Male Wistar rats (150-220g) received pilocarpine (1-8 mg/kg ip) and the number of purposeless chewing movements was recorded over a 5 min period, 15 mins following drug administration. In some experiments animals were pretreated with methylscopolamine (1 mg/kg sc), scopolamine (1 mg/kg sc), mianserin (0.5-5.0 mg/kg sc), ketanserin (2 mg/kg sc), idazoxan (2 mg/kg sc), prazosin (2 mg/kg sc) or mepyramine (5 mg/kg sc) 30 mins prior to the administration of pilocarpine (4 mg/kg ip).

Pilocarpine (1-8 mg/kg ip) administration to rats induced a dose related increase in purposeless chewing. Pilocarpine (4 mg/kg ip)-induced chewing was inhibited by scopolamine (1 mg/kg sc) but not by methylscopolamine (1 mg/kg sc). Pretreatment with mianserin (0.5-5.0 mg/kg sc) caused a dose related inhibition of pilocarpine (4 mg/kg ip)-induced chewing (ED_{50} 0.82 mg/kg; Table 1). Pretreatment with ketanserin, idazoxan, prazosin or mepyramine did not inhibit pilocarpine-induced chewing but produced a non-significant potentiation.

Table 1 Pilocarpine (4 mg/kg ip)-induced purposeless chewing in rats

Drug (mg/kg sc)		Number of chewing movements ± SEM Vehicle	Drug-treatment
Methylscopolamine	(1)	140±26	136±23
Scopolamine	(1)	147±27	3±2*
Ketanserin	(2)	75±25	136±35
Mepyramine	(5)	107±27	147±34
Prazosin	(2)	130±22	173±31
Idazoxan	(2)	86±33	107±16
Mianserin	(0.5)	101±21	58±17
	(1)	161±35	88±29*
	(2.5)	155±38	34±17*
	(5.0)	111±20	34±11*

n = 6-12; * $p < .05$ Mann Whitney U-test

Pilocarpine-induced chewing behaviour is mediated via central cholinergic receptors. The antagonism of chewing by mianserin may reflect an interaction with central cholinergic receptors rather than central 5HT2, α-2, α-1 or H1 receptors.

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EFFECT OF CHRONIC ADMINISTRATION OF MIANSERIN AND CLOMIPRAMINE ON TRYPTOPHAN-5-HYDROXYLASE ACTIVITY IN RAT BRAIN.

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It has been suggested that the clinical efficacy of antidepressant drugs may be linked to an ability to alter circadian rhythms in the CNS (Wehr and Wirz-Justice 1982). Most antidepressant drugs primarily affect 5-HT-containing neurones, and the synthesis and release of 5-HT shows a marked circadian rhythm (Hillier and Redfern 1977; Martin and Marsden 1985). Since conversion of tryptophan to 5-HTP by tryptophan 5-hydroxylase (TRY-OHase) is the rate limiting step in the synthetic process, and since the activity of this enzyme also displays a marked circadian rhythm, (Cahill and Ehret, 1981) we have examined the effect of two antidepressants, mianserin and clomipramine on TRY-OHase activty at the nadir and zenith of enzyme activity.

Groups of 8 male Wistar rats, 90-130g, were housed for two weeks under controlled environmental conditions including a 12:12 light:dark cycle, and free access to food and water. Mianserin, 20 mg/kg, or clomipramine 7.5 mg/kg, were administered i.p. twice daily for two weeks. Animals were killed at either mid-light or mid-dark, and the activity of TRY-OHase in brain stem homogenates were determined using the 'CO_2-trapping' method of Ichiyama et al (1971).

The results, expressed as pmol total CO_2 evolved in 30 min per mg wet wt are shown in Table 1.

Table 1 Activity of Tryptophan-5-hydroxylase in brain stem homogenates (pmol total CO_2 per 30 min per mg wet wt)
mean ∓ sem (n); difference from control * $p<0.05$; ** $p<0.01$

		Mid-light	Mid-dark
Acute mianserin	treated	4.00 ∓ 1.64 (5)	4.51 ∓ 0.21 (4)
20 mg/kg ip	control	3.35 ∓ 0.32 (5)	4.41 ∓ 0.15 (4)
		**	*
Mianserin	treated	2.68 ∓ 0.20 (4)	3.63 ∓ 0.12 (5)
20 mk/kg bd x 14	control	1.97 ∓ 0.07 (4)	2.87 ∓ 0.25 (3)
		**	**
Clomipramine	treated	6.90 ∓ 0.35 (4)	8.20 ∓ 0.34 (4)
7.5 mg/kg bd x 14	control	4.71 ∓ 0.32 (4)	5.89 ∓ 0.29 (4)

It can be seen that, after chronic, but not acute, administration both drugs significantly increased enzyme activity, supporting the belief that chronic antidepressant administration enhances activity in 5-HT neurones (Willner 1985). However there is no evidence from our experiments of any quantitative difference in response at the two time points.

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A DIRECT ASSAY FOR TYROSINE HYDROXYLATION IN SUPERFUSED RAT STRIATAL SLICES

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A variety of neurotransmitters, including dopamine, have been shown to regulate tyrosine hydroxylase, the enzyme catalysing the rate limiting step in catecholamine biosynthesis (Zigmond, 1985). In at least some cases, these effects have been shown to be mediated by cell-surface receptors and in an attempt to examine effects of dopamine agonists and antagonists on the synthesis and consequent release of dopamine, we have developed a method to directly assay tyrosine hydroxylation in superfused striatal slices. The method is based on that described by Hirata et al. (1983) in which the accumulation of tissue L-DOPA is assayed following inhibition of DOPA decarboxylase with NSD-1055 (p-bromo-benzyl-oxyamine). However, in this assay DOPA is detected in superfusate allowing kinetic analysis and various drug treatments to be examined in the same tissue.

Rat striatal slices (350 x 350 μM) were superfused with Krebs buffer (0.5 ml/min) and fractions collected and assayed for DOPA using HPLC-electrochemical detection. Addition of NSD-1055 (10 μM) increased DOPA 10-20 fold in the superfusate with a $t_{\frac{1}{2}}$ of about 10 min., reaching a steady state production of 3-4 pmoles DOPA/mg protein/min. 30-50 min. after addition of the inhibitor. Under these conditions, DOPA production remained constant for at least 90 min. Addition of the tyrosine hydroxylase inhibitor alpha-methyl-p-tyrosine (50 μM) rapidly ($t_{\frac{1}{2}}$ 15-20 min.) and completely suppressed DOPA accumulation, establishing that tyrosine hydroxylation was being monitored in this system. Further experiments were designed in which both tissue and superfusate DOPA were assayed in the same experiment. The accumulation of DOPA after NSD-1055 and suppression by alpha-methyl-p-tyrosine in superfusates was only a small fraction of the slice content but at all times closely reflected changes in the tissue.

The effects of dopamine receptor agonists and antagonists on tyrosine hydroxylation was examined by pre-incubating the tissue for 60 min. with NSD-1055 and then collecting superfusate fractions prior to and following drug addition. 7-Hydroxy-2-(N,N-dipropyl-amino)-tetralin (7-OHDPAT, 1 μM) suppressed DOPA accumulation in superfusates by 50-60% ($P < 0.001$) with a $t_{\frac{1}{2}}$ of 20-25 min. and again changes closely reflected tissue DOPA content. These effects were not blocked by the uptake inhibitor GBR 12921 (0.1 μM), suggesting mediation by a cell-surface receptor. The D_2 antagonist (-)-sulpiride (1 μM) induced a small increase (20%) in DOPA accumulation but was only weakly effective in blocking the effect of 7-OHDPAT. This novel assay system should allow further investigation of receptor-mediated effects on dopamine synthesis and release.

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THE EFFECT OF DIAZEPAM AND RO15-1788 ON EXTRACELLULAR ASCORBIC ACID, DOPAC AND 5-HIAA IN THE RAT STRIATUM IN VIVO.

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Benzodiazepine receptors have been found in the rat brain, and the stimulation of these receptors appears to be responsible for the major pharmacological effects of these drugs. Several studies have shown that benzodiazepines modify the turnover of cerebral dopamine and 5-hydroxytryptamine. The activity of these two neuronal systems can now be monitored simultaneously in vivo by the determination of the extracellular concentrations of their respective metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA), using the technique of differential pulse voltammetry (Crespi et al, 1984). This technique also allows the detection of extracellular ascorbic acid, whose physiological function is not known at present. We have now used differential pulse voltammetry to determine the effects of diazepam and the benzodiazepine receptor antagonist Ro15-1788 on the extracellular concentration of these acids in the rat striatum in vivo.

Male Sprague-Dawley rats (250-300g) were anaesthetised with chloral hydrate (400mg/kg i.p.), held in a stereotaxic apparatus, and implanted with a specifically pretreated carbon-fibre electrode in the striatum (Crespi et al, 1984). In these anaesthetised animals (n=5), diazepam (10mg/kg i.p.) did not significantly alter extracellular concentrations of ascorbic acid or 5-HIAA, but led to a 25% reduction of extracellular DOPAC, which became significant ($P < 0.05$) 165 min after the injection. As we had previously noted that the effects of sodium valproate on these parameters were perturbed by anaesthesia (Crespi et al, 1985), we repeated our experiments in freely-moving rats that had recovered from the anaesthesia. In these animals (n=5), diazepam (10mg/kg i.p.) produced significant reductions ($P < 0.05$) in extracellular ascorbic acid (-70% after 120 min), DOPAC (-50% after 105 min) and 5-HIAA (-70% after 180 min). In order to determine the role of the benzodiazepine receptor in these effects of diazepam, another group of 5 freely-moving rats was administered diazepam (10mg/kg i.p.), and 2 h later the receptor antagonist Ro15-1788 (30mg/kg i.p.). After this treatment, the reduction in extracellular 5-HIAA was significantly less (-20%, 180 min after diazepam, $P < 0.05$) than that observed in rats treated only with diazepam. In contrast, the diazepam-induced reductions in extracellular ascorbic acid and DOPAC were not significantly altered by the administration of Ro15-1788.

These results indicate that anaesthesia reduces or abolishes the effects of diazepam on extracellular ascorbic acid, DOPAC and 5-HIAA. This supports the observation of Trulson (1984), who found that anaesthesia abolished the effect of diazepam on the firing of raphe dorsalis neurones The results also support a role for the benzodiazepine receptor in the diazepam-induced reduction in extracellular 5-HIAA. However, the involvement of this receptor in the reduction of ascorbic acid and DOPAC remains uncertain.

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AUTORADIOGRAPHIC LOCALIZATION OF [^{3}H]-ZOLPIDEM BINDING SITES IN THE RAT BRAIN

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Zolpidem is a novel non-benzodiazepine hypnotic with rapid onset short duration of action, which interacts in vivo with central benzodiazepine receptors (Arbilla et al., 1985 ; Depoortere et al., 1986). Recent binding studies with ^{3}H-zolpidem have revealed that this radioligand labels the GABA-benzodiazepine macromolecular complex with preferential affinity for the type I benzodiazepine receptor (Arbilla et al., 1986). In the present study, we have used the technique of autoradiography to localize precisely ^{3}H-zolpidem binding sites in the rat brain.

Coronal and sagittal sections (15 µM in thickness) from the rat brain were prepared at - 20°C using a cryostat and were thaw-mounted onto subbed microscope slides. For binding assay, incubation was performed for 30 min at 4°C in a 50 mM Tris-HCl buffer, pH 7,4 containing 120 mM NaCl and 5 mM KCl in the presence of 0.6-15 nM ^{3}H-zolpidem (specific activity : 60.6 Ci/mmol). Non-specific binding was defined by Ro 15-1788 (2 µM). Sections were then rinsed (3 min) in cold buffer and dried rapidly under a stream of air. Autoradiograms were generated by apposing tissue sections to tritium-sensitive film for one month at 4°C. The optical density of the autoradiograms was quantified by using an automatic image analyser and densitometer (Quantimet 920, Cambridge Instruments) connected to a PDP 11/23 computer with a variable frame.

Scatchard analysis of saturation data on sagittal sections revealed that ^{3}H-zolpidem binds to a single population of sites with an apparent dissociation constant (Kd) of 6 nM and a maximal capacity of 719 fmol/mg protein. Autoradiographs prepared from coronal sections incubated in the presence of 5 nM ^{3}H-zolpidem revealed a highly heterogenous distribution of the ^{3}H-ligand. The highest levels of specific binding were found in the olfactory bulb (glomerular layer), islets of Calleja, cerebral cortex (especially in layer IV), ventral pallidum, inferior colliculus, medial septum, nucleus of the diagonal band of Broca, cerebellum (molecular layer), substantia nigra reticulata and some amygdaloid nuclei. Intermediate levels of binding were found in the CA1 field of the hippocampus (molecular layer), primary olfactory cortex, dorsal tegmental nucleus, dorsal raphe, central gray, superior colliculus, endopiriform nucleus, subthalamic nucleus and in some thalamic and hypothalamic nuclei. Low levels of ^{3}H-zolpidem binding were found in the caudate putamen and dentate gyrus of the hippocampus.

In conclusion, the regional brain distributin of ^{3}H-zolpidem binding sites is compatible with the view that zolpidem binds to benzodiazepine recognition sites. The fact that the highest concentrations of the ^{3}H-ligand were present in brain areas containing type I benzodiazepine receptors (e.g. substantia nigra reticulata, globus pallidus, cerebellum, cortex) reinforce the contention that zolpidem preferentially labels this receptor subtype.

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AUTORADIOGRAPHIC LOCALISATION OF [^{3}H] BUPRENORPHINE IN THE SPINAL CORD OF THE SHEEP

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Reports of the binding of buprenorphine to rat brain and spinal cord homogenates have been published (Villager & Taylor 1981,1982) and also some in vivo studies (Dunn & Herz 1981), which indicate that this compound shows affinity for mu, delta and kappa opioid receptor sites.

In parallel to an in vivo study of the action of this drug in sheep (Nolan et al 1986), we have undertaken an autoradiographic investigation of the localisation of buprenorphine in the spinal cord of sheep. Samples of spinal cord from cervical, lumbar and sacral sites were obtained 20-60 minutes after death and kept in ice cold Tris buffer pH 7.5 for transport to the laboratory. Cryostat sections were prepared as previously described (Bouchenafa & Livingston 1985), and incubated with 25 nM [^{3}H] buprenorphine, control sections were incubated in the presence of 10μM naloxone to displace the mu component and 10μM dynorphin to displace the kappa component. Sections were exposed to LKB Ultrofilm for four weeks to obtain autoradiographs. The sections from the various spinal cord regions showed a similar distribution of autoradiographic density with a fairly uniform amount of radioactivity over both dorsal and ventral horns, with possibly a slightly greater density over the dorsal horn. Control sections incubated with dynorphin showed very little alteration in binding whereas incubation with naloxone caused a general reduction in the level of binding throughout the grey matter. These findings would suggest that the majority of the buprenorphine binding seen in the grey matter was associated with the mu rather than the kappa sites.

Further experiments using preincubation with cold ligands and U50,488 instead of dynorphin as a kappa ligand are in progress.

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DECREASED HIGH-AFFINITY [^{14}C]-2-OXOGLUTARATE UPTAKE IN STRIATAL SYNAPTOSOMES AFTER FRONTAL CORTICAL ABLATION IN THE RAT

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The synthesis of neurotransmitter glutamate from glucose, its release and subsequent uptake by glia entails the loss of neuronal Krebs cycle intermediates, which have to be replaced. This requires the use of an anaplerotic process such as CO_2 fixation, which is however primarily localized in glia (Shank and Campbell, 1984). It has therefore been proposed that there is a net flux of glial-synthesized Krebs cycle intermediates from glia to neurones to balance the neuronal to glial flux of amino acids derived from neuronal glucose (Shank and Campbell, 1984; Carter, 1985). This is supported by the existence of a synaptosomal high-affinity uptake site for the Krebs cycle intermediate 2-oxoglutarate, which is distinct from the high-affinity glutamate/aspartate uptake site (Shank and Campbell, 1981, 1982, 1984). [^{14}C]-2-Oxoglutarate incorporated into synaptosomes is preferentially metabolized to glutamate (Shank and Campbell, 1984), suggesting that [^{14}C]-2-oxoglutarate uptake might be used to selectively mark glutamatergic nerve terminals. We have tested this assumption by analysing [^{14}C]-glutamate, [^{14}C]-2-oxoglutarate and [^{14}C]-GABA uptake in rat striatal synaptosomes following destruction of the cortical glutamatergic input to the striatum.

Cortical ablation was performed in 250g male Sprague-Dawley rats according to McGeer et al. (1977). Two weeks after surgery the high-affinity uptake ($5x10^{-7}$ M) of L-U[^{14}C]glutamate, L-U[^{14}C]2-oxoglutarate or L-U[^{14}C]GABA (293, 271, 231.6 mCi/mMole; Amersham) was measured in striatal synaptosomes prepared from a washed P_2 pellet of control and lesioned rats (Carter, 1982).

Table 1.: High-affinity [^{14}C]-glutamate, [^{14}C]-2-oxoglutarate and [^{14}C]-GABA uptake in striatal synaptosomes of control and lesioned rats.

Treatment	nmoles/mg protein/hr		
	[^{14}C]-glutamate	[^{14}C]-2-oxoglutarate	[^{14}C]-GABA
CONTROL	4.76 ± 0.33	0.52 ± 0.06	2.48 ± 0.18
CORTICAL ABLATION	3.17 ± 0.26*	0.18 ± 0.03*	2.44 ± 0.22
% CONTROLS	67 %	34 %	98 %

* $p<0.001$, N=8 (Individual Striata)

The lesions produced the expected reduction in striatal [^{14}C]-glutamate uptake (≃33%) without affecting [^{14}C]-GABA uptake. Striatal [^{14}C]-2-oxoglutarate uptake was reduced by 66%.

In conclusion, the data support the contention that 2-oxoglutarate uptake sites exist on striatal glutamatergic nerve terminals. The magnitude of the reduction (twice that observed for glutamate) suggests that [^{14}C]-2-oxoglutarate uptake might be a very sensitive marker for excitatory amino acid nerve terminals.

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INTERACTION OF RAUWOLSCINE WITH 5-HT BINDING SITES IN THE RAT BRAIN.

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The α_2-adrenoceptor antagonist rauwolscine also possesses serotonergic blocking properties, both in the periphery (Kaumann, 1983) and in the CNS (Fluerstein et al, 1985). Tritiated-rauwolscine binds to two sites in the rat cortex, and it has been suggested that the low affinity site may represent an interaction with a serotonergic binding site (Broadhurst and Wyllie, 1986). Recent studies have revealed the presence of multiple binding sites for 5-HT, namely 5-HT_{1A}, 1_B and 1_C (Blurton and Wood, 1986) and 5-HT_2. We have therefore studied the interaction of rauwolscine with these binding sites in the rat brain.

Binding was studied in membranes prepared from various rat brain regions as described by Blurton and Wood (1986). (Table 1). Displacement data were analysed using the ALLFIT program (De Lean et al, 1978) to obtain IC_{50}'s.

Table 1. Binding parameters for multiple 5-HT sites

	5-HT_2	5-HT_{1A}	5-HT_{1B}	5-HT_{1C}
region	frontal cortex	hippocampus	striatum	striatum
ligand (nM)	^{3}H-ketanserin (1)	^{3}H-5-HT (3)	^{3}H-5-HT (3)	^{3}H-5-HT (3)
displacer (μM)	methysergide (10)	8-OH-DPAT (1)	1-propranolol (.5)	mianserin(.25)

Rauwolscine inhibited the binding of ^{3}H-ketanserin to the 5-HT_2 site and of ^{3}H-5-HT to the 1B and 1C sites at micromolar concentrations (Table 2). In contrast, rauwolscine was a potent inhibitor of ^{3}H-5-HT binding to the 1A site.

Table 2: Interaction of rauwolscine with 5-HT binding sites.

IC_{50} (nM)			
5-HT_2	5-HT_{1A}	5-HT_{1B}	5-HT_{1C}
2150±853	44±16.6	7650±2550	3060±1480

Results are mean ± SEM from 3-4 experiments

The present study has shown that the α_2-adrenoceptor antagonist rauwolscine interacts potently with 5-HT_{1A} sites. This is consistent with the observation that low affinity ^{3}H-rauwolscine binding is blocked potently by spiperone (IC_{50} 35nM ± 8.3 (n=4) which has a high affinity for 5-HT_{1A} sites and less so by ketanserin (IC_{50} 261 nM ± 35 (n=4) which is a selective 5-HT_2 ligand. It is also consistent with the absence of low affinity ^{3}H-rauwolscine binding in platelets (Kawahara & Bylund, 1985) which lack 5-HT_1 receptors.

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LOCALISATION AND CHARACTERISATION OF [^{3}H]-5-HT BINDING SITES IN THE AMYGDALA OF RAT BRAIN.

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[^{3}H]-5-Hydroxytryptamine (5-HT) binds preferentially and with high affinity to specific sites, termed 5-HT$_1$, in rat brain membranes in vitro (Peroutka & Snyder, 1979). Previous studies (see, for example, Whitaker & Deakin, 1981) have found that lesioning serotoninergic cell bodies either has little effect on, or may even increase, the number of 5-HT$_1$ binding sites in brain areas such as frontal cortex, denervated as a result of the lesions. This suggests that binding to presynaptic sites is only a fraction of total specific binding. We have pharmacologically characterised and investigated the synaptic localisation of [^{3}H]-5-HT binding in rat brain amygdala, an area with a high density of [^{3}H]-5-HT binding sites (Pazos & Palacios, 1985).

[^{3}H]-5-HT binding assays were carried out by a rapid filtration method as described by Bennett & Snyder (1976). Saturation analysis of [^{3}H]-5-HT binding to crude amygdaloid membranes suggested that the ligand binds with high affinity (K_D 3.78 ± 0.47 nM, m ± s.e.m., n=3) in a saturable manner (Bmax 203 ± 15 fmol/mg protein, m ± s.e.m., n=3). The potency of some 5-HT agonists and antagonists in displacing [^{3}H]-5-HT binding (3.5nM) was determined. For the agonists the order of potency (IC_{50} values, nM) was: 5-methoxytryptamine (10.6) ≃ 5-carboxamidotryptamine (12.6) ≃ RU24969 (13.4) >α-methyl 5-HT (111.9) >8-hydroxy-2-[N-dipropylamino]tetralin (2186) > 2-methyl 5-HT (>10000), and for antagonists: methergoline (37.4) > methiothepin (101.6) > methysergide (823) > AHR1709 (2619) > ketanserin (14659). Most of the compounds tested gave Hill slopes of less than unity, indicating interaction at more than one binding site. However, these results are in general agreement with the relative potency of these compounds at the '5-HT$_1$-like' receptors which regulate 5-HT release in the CNS (Engel et al., 1983).

Electrolytic lesioning of the dorsal raphe nucleus was accomplished stereotaxically. Fourteen days after lesioning when compared to sham-operated animals it was found that the 5-HT content of the amygdala had fallen by 53% ($p < 0.001$) and the number of [^{3}H]-5-HT binding sites (Bmax) had also decreased, by approximately 47% ($p < 0.05$) without any statistically significant change ($p > 0.05$) in the affinity of the ligand (K_D) for its binding sites.

The present results indicate that the amygdala of rat brain has a high density of presynaptic [^{3}H]-5-HT binding sites which resemble '5-HT$_1$-like' receptors in terms of their affinity for 5-HT receptor stimulants and blockers.

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AUTORADIOGRAPHY OF [^{3}H]-KAINIC ACID BINDING SITES IN BRAIN

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Kainic acid (KA), a heterocyclic analogue of glutamate, is a potent excitant of some mammalian neurones, and also exerts widespread neurotoxic actions. These effects are thought to be mediated by a subclass of excitatory amino acid receptors, at which KA is a selective agonist. Previous studies have demonstrated that ^{3}H-KA labels a specific binding site in rat brain with the pharmacological properties of the receptor mediating the excitatory and neurotoxic effects of KA (Slevin et al, 1983). Whilst the KA receptor has been characterised in the brains of higher species, the distribution of binding sites has not been investigated. In the present study we have used the technique of high-resolution receptor autoradiography to map the distribution of ^{3}H-KA binding sites in the brain of the cynomologous monkey.

Brains were frozen in isopentane at -35°C and 20μ sections cut in a cryostat at -15°C. Brain sections were preincubated with 50 mM tris-acetate pH 7.4 for 20 min at room temperature to remove endogenous glutamate. Sections were then incubated with 50 mM tris-acetate pH 7.4 containing 5 nM kainic acid [vinylidene-^{3}H] (60 Ci/mmol, NEN) for 30 min at room temperature. Adjacent sections were incubated with 10 μM unlabelled KA to define non-specific binding. Following incubation sections were washed and processed for autoradiography as described by Mitchell et al, (1984). Quantification of the autoradiographs was performed using a computer-based image analysis system (Slater, 1985). No correction was made for the differential quenching of white matter.

Table 1 Distribution of ^{3}H-kainate binding in monkey brain

^{3}H-kainate bound (fmol/mg protein)		
<50	50-150	>150
Thalamus	Caudate nucleus	Dentate gyrus
Globus pallidus	Cingulate cortex	CA3 hippocampus
S. nigra	Nucleus accumbens	Pyriform cortex

^{3}H-KA binding sites were unevenly distributed throughout the brain, with highest levels in the hippocampus, particularly region CA3. Other areas of the limbic system, and particularly "limbic cortex" also had high levels of ^{3}H-KA binding. The pattern of ^{3}H-KA binding was distinct from that of ^{3}H-glutamate binding in monkey brain. The distribution of ^{3}H-KA binding sites in the monkey is similar to that of the rat (Monaghan & Cotman, 1982). The areas with the highest concentration of ^{3}H-KA binding (e.g. hippocampus) are those which are particularly sensitive to the neurotoxic effects of KA.

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THE EFFECT OF ACRYLAMIDE ON THE RETROGRADE AXONAL TRANSPORT OF SPECIFIC PROTEINS IN RAT SCIATIC NERVE

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The neurotoxic compound acrylamide produces a dying-back neuropathy characterised by axonal swellings in the distal parts of peripheral nerves. One explanation for its effect is its known ability to interfere with retrograde axonal transport (Jakobsen and Sidenius, 1983). We undertook this study to determine which retrogradely transported proteins were most affected by acrylamide intoxication.

Eight female Wistar rats received twice-weekly i.p. injections of 50 $mg.kg^{-1}$ acrylamide to a cumulative dose of 150 or 350 $mg.kg^{-1}$ (four in each group). Eight control rats received only saline. The extent of neuropathy in the rats was assessed by the method of Edwards and Parker (1977). They were then anaesthetised with ether and the left sciatic nerve crushed at mid-thigh level with a pair of ligatures 5mm apart. Eighteen hours later, 2mm pieces of sciatic nerve immediately rostral, immediatly caudal, and between, the ligatures were removed along with an equivalent piece from the contralateral nerve. The nerve proteins were separated by two-dimensional polyacrylamide gel electrophoresis (McLean, 1985) and stained with silver.

Proteins which were anterogradely or retrogradely transported were detected on gels from nerve pieces rostral or caudal to the ligatures respectively by their increased staining intensity relative to those on gels from nerve pieces between the ligatures. Gels from the contralateral nerve served as a control that the protein content of the nerve was not altered by the ligation. Staining intensity was assessed by eye by an experienced observer unaware of the experimental protocol.

Neuropathy was apparent in only those rats which received 350 $mg.kg^{-1}$ acrylamide. Acrylamide led to a reduction in the retrograde transport of the following proteins in all the nerves studied (Table 1).

Table 1 Effect of acrylamide to reduce the accumulation of transported proteins

Acrylamide ($mg.kg^{-1}$)	Proteins reduced (molecular weight (kdaltons) (pI))
150	37(6.0);37.5(5.7);38.5(5.4);39 (6.5)
350	36.5(5.9);37(6.0);37.5(5.7);38.5(5.4);39(6.5);45(4.2);47.5(4.5);49(4.7)

The above proteins represent about half of the retrogradely transported proteins detectable by the above method. We conclude that acrylamide inhibits the retrograde transport of specific proteins in a dose-dependent manner, even before signs of neuropathy are apparent.

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THE EFFECTS OF THE IRREVERSIBLE ANTAGONIST BENEXTRAMINE ON α_2-ADRENORECEPTOR LIGAND BINDING AND RESPONSES IN RABBIT BRAIN

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Recovery of binding sites and responses after treatment with the irreversible alpha adrenoceptor antagonist phenoxybenzamine (P) has been suggested as an index of receptor turnover (1,2). However P is lipophilic and relatively selective for $alpha_1$ adrenoceptors. Benextramine (B) another irreversible alpha adrenoceptor antagonist is hydrophilic and has a greater affinity for $alpha_2$ adrenoceptors than P (3,4). We have evaluated the use of B in the study of $alpha_2$ adrenoceptor number and responses in the brain stem.

Male New Zealand white rabbits received a freehand intracisternal (IC) injection of either B or P (1 ug/kg). Groups of animals (n=6) were killed 1, 24, 48 or 72 hours later. Specific binding of the $alpha_2$ antagonist ligand [^{3}H] idazoxan to forebrain and hindbrain membranes was studied (5). In other groups of rabbits a small polyvinyl catheter was implanted into the cisterna magna through the atlanto occipital membrane (6). 8-10 days later clonidine (1 ug/kg) was administered IC to conscious animals and blood pressure measured. The animals then received B through the same catheter; clonidine injections were repeated 1, 24, 48 and 72 hours later. B had no effect on [^{3}H] idazoxan binding to forebrain (Bmax 68 $\pm$ 6, 70 $\pm$ 10 and 76 $\pm$ 6 fmoles/mg protein in controls and 1 and 24 hours after B). In contrast reductions in the binding in hindbrain were observed (Table 1). Bmax was lowest 12-24 hours after B and had returned to pretreatment levels by 3 days. B given IC also attenuated depressor responses to clonidine (Table 1) with a similar time course to that observed in the binding studies.

TABLE The effect of intracisternal benextramine on specific [^{3}H] idazoxan binding in hindbrain and depressor responses to clonidine.

Hours after injection	Control	1	12	24	48	72
Bmax for [^{3}H] idazoxan (fmoles/mg protein)	95 ± 21	55 ± 16*	49 ± 12*	48 ± 7*	59 ± 8*	99 ± 11
Depressor response to IC clonidine (mmHg)	21 ± 2	14 ± 2*	--	11 ± 2*	14 ± 2*	20 ± 2

* Significantly less than control values

Unlike B, P caused similar reductions in Bmax in both forebrain and hindbrain to 49 $\pm$ 3 and 37 $\pm$ 6 fmoles/mg protein at 1 hour with a slow recovery over days.

These results suggest that B may be useful for studying central $alpha_2$ adrenoceptors. As in previous studies with P reductions in receptor number and responses to IC clonidine were of similar magnitude (7) suggesting little receptor reserve for $alpha_2$ adrenoceptors involved in cardiovascular control in the brain stem. However recovery was more rapid after B. Redistribution of lipophilic P could contribute to its longer half life for recovery and over estimate recovery time. Thus B may have advantages over P in studies of this type.

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CHRONIC TRICYCLIC ANTIDEPRESSANT TREATMENT DOES NOT ALTER THE CARDIOTOXICITY OF HIGH DOSES OF IDAZOXAN IN THE RABBIT

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The selective α2-adrenoceptor antagonist, idazoxan elicits an increase in rat cortical 3-methoxy-4-hydroxyphenyl glycol (MHPG) concentration which is potentiated by desipramine pretreatment (Walter and Haynes, 1984). Doses of idazoxan two orders of magnitude greater than those required to produce α2-adrenoceptor antagonism in the rabbit, have been shown to be cardiotoxic in this species (Hersom and Mackenzie, 1984). This study therefore investigates whether chronic desipramine or amitriptyline treatment potentiates the cardiotoxicity associated with high intravenous doses of idazoxan in the rabbit.

Male, NZW rabbits were treated twice daily for 14 days with saline (0.2 ml/kg, i.p.; n=6), desipramine (5.0 mg/kg i.p.; n=6) or amitriptyline (2.5 mg/kg, i.p.; n=6). These doses were the largest which did not themselves induce overt changes in the ECG. The effects of idazoxan (3.0 mg/kg/min, i.v.) infusion were then compared 5-8 hours after the last treatment injection. Rabbits were anaesthetised with pentobarbitone (30 mg/kg, i.v.), mechanically ventilated and prepared for drug infusion (jugular vein) and ECG measurement (Lead II). In parallel experiments (n=6), the respective tissue concentrations of desipramine and amitriptyline at this time were 170 ± 58 and 36 ± 18 ng/g in the heart and 430 ± 190 and 97 ± 39 ng/g in the brain. These values are of the same order of magnitude as have been reported to inhibit monoamine uptake$_1$ *in vitro* (Moller Nielsen, 1980).

Intravenous infusion of idazoxan (3.0 mg/kg/min, i.v.) elicited rapid changes in ECG (usually variations in R wave amplitude or slight S wave depression. The doses of idazoxan eliciting the first change in ECG in saline, desipramine and amitriptyline treated groups were 3.0 ± 0.3, 2.7 ± 0.3 and 2.4 ± 0.3 mg/kg i.v. respectively. The intravenous doses of idazoxan eliciting changes in ECG probably indicative of cardiotoxicity (deepened and widened S wave: Elonen et al, 1974) in saline, desipramine and amitriptyline treated groups were 22.2 ± 4.2, 17.4 ± 2.4 and 17.4 ± 2.1 mg/kg, i.v. respectively. There was no statistically significant difference between these values ($P>0.05$).

Thus although desipramine has been reported to potentiate the idazoxan-induced increase in brain MHPG in the rat, neither desipramine nor amitriptyline potentiated the cardiotoxic effects of high infusion rates of idazoxan in the anaesthetised rabbit model.

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MOTOR IMPAIRMENT AS A FACTOR AFFECTING DRUG DISCRIMINATION TRIALS

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Drug discrimination studies are so designed that the performance of a motor task is fundamental to the experimental design. As such, any drug with a debilitating effect on motor performance may well produce misleading results. In the present investigation an assessment of motor impairment has been made involving a variety of opiate drugs which featured in a previously reported drug discrimination study (Blackman et al., 1985). Untreated rats (male, Sprague-Dawley, 90-110g) were trained to remain on a drum set to revolve at a fixed rate of 16 r.p.m. (rotarod) for a period of at least 60 seconds. Trained rats (n=10) were then dosed subcutaneously with test drug (dose volume 1 ml/kg) and 30 minutes later retested for motor impairment. Inability to remain on the rotarod for 30 seconds was taken as indicative of motor incapacity. Opiates tested were morphine, buprenorphine, ethylketocyclazocine (EKC), cyclazocine and dl-N-allylnormetazocine (NANM). Rotarod results for these compounds were compared with their respective drug discrimination results (latency to make the first press of the operant lever). The results showed that morphine produced a dose-related and effective motor incapacity in the rotarod test (0.3 - 30 mg/kg) but produced only short and non dose-related latencies during drug discrimination trials (0.1 - 6 mg/kg). Buprenorphine (0.0003-30 mg/kg) induced motor impairment with some evidence of a biphasic dose-response relationship. Latencies to lever press after buprenorphine (0.005 - 3 mg/kg) were generally short and non dose-related in drug discrimination trials. After EKC (0.03 to 1 mg/kg) rats trained to discriminate morphine from saline exhibited exceptionally lengthy, dose-related latencies (max > 9 minutes). These results for EKC contrasted with those in rats trained to discriminate NANM from saline where EKC, in doses up to 0.3 mg/kg failed to induce longer latencies than 7.9 seconds. Dosed at 1 mg/kg EKC however these rats showed a complete lever response failure. In the rotarod test EKC (0.1 - 1 mg/kg) produced a dose-related motor incapacity and was twice as potent as morphine in this respect. Cyclazocine (0.002 to 2 mg/kg) produced similar results to those of EKC with the exception that the maximum latencies were shorter (maximum of 2 minutes) and the slope of the rotarod dose-response curve more shallow. Response failure was not a feature of cyclazocine generalisation trials. NANM (0.1 to 5 mg/kg) had very little effect on either rotarod performance or on onset to the first lever press. The data indicate that under the conditions of the drug discrimination studies described, there is no apparent relationship between motor incapacity and lever responding for the drugs studied. Results with EKC suggest that long latencies observed during drug-discrimination trials are unlikely to be caused by adverse effects on motor performance. At high doses (> 1mg/kg), EKC-induced 'sedation' (Martin et al., 1976) rather than motor incapacity remains a possibility in view of the complete lever response failure in the NANM trained rats.

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THE EFFECTS OF ETHYLCHOLINE MUSTARD AZIRIDINIUM (ECMA) IN RAT BRAIN REAGGREGATE CULTURES.

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The injection of ECMA into rat brain causes reductions of choline transport (ChT) and choline acetyltransferase (ChAT) activity, accompanied by cognitive defects similar to those seen in Alzheimer's disease (see Walsh et al., 1984). In rat brain synaptosomes, ECMA causes direct inhibition of ChAT, gaining access via the high and low affinity carriers for choline (Pedder & Prince, 1985). Foetal rat brain reaggregate cultures contain comparable concentrations of ChAT, but choline is accumulated only by low affinity ChT and acetylcholine (ACh) synthesis is minimal (Atterwill et al., 1984). We therefore investigated the compartmentalisation of ChAT in relation to ChT in cultures of this kind.

Cultures were prepared in serum-supplemented medium from 15-16 day rat foetuses as previously described (Atterwill et al., 1984). ECMA was freshly prepared from the precursor acetylethylcholine mustard. It was then added directly to the cultures on either the ninth day in vitro (9 DIV) or 12 DIV (final concentrations 50 μM). In some experiments hemicholinium-3 (HC-3, final concentration 40 μM, sufficient to inhibit high and low affinity ChT) was added to the cultures 30 min prior to ECMA. Aggregates were harvested by sedimentation and washed. Samples were homogenised and treated with Triton X-100 (0.5%, 1h, 0^{o}C). ChAT activity was measured as previously described (Atterwill et al., 1984). The stability of ECMA in the culture medium at 37^{o}C was monitored by thiosulphate titration.

ECMA was rapidly degraded having a half life of approximately 15 min at 37^{o}C. Cultures treated with ECMA at 12 DIV and harvested after 2,4,27 and 48 h showed essentially constant reductions (approx 38%, $p < 0.05$) in ChAT specific activity. Those treated at 9 DIV with ECMA showed similar reductions after 2 h (ie 33%, $p < 0.01$), which were prevented by HC-3 pretreatment. After 72 h ChAT activity had fallen by a total of 61% ($p < 0.01$), an effect not prevented by HC-3. Comparable results were obtained after 120 h. ChAT activity in the cultures was not affected by HC-3 alone.

Thus, ECMA appears to have two effects in these cultures. The loss of ChAT activity established within 2 h and remaining unchanged for 48 h would seem to be mediated by HC-3 sensitive choline carriers. This may be similar to the inhibition of ChAT within synaptosomes. The choline carriers in reaggregate cultures therefore do give access to ChAT, even though ACh synthesis is minimal. The loss of ChAT over the longer time intervals (ie, 72 and 120 h) appears independent of choline carriers. Since the survival time of ECMA in the culture medium was only 2 h (6-7 half lives) this later effect must also have been initiated within the initial 2 h period and may represent a more general cytotoxic response.

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AF-DX 116, AN M_2 CARDIOSELECTIVE ANTAGONIST, EXHIBITS LOW ANTI-SECRETORY PROPERTIES.

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Receptors classified as belonging to the M_2 muscarine subtype mediate functions as diverse as negative inotropic and chronotropic effects, increase in smooth muscle contractility, and stimulation of exocrine glands secretions. Discriminative properties between cardiac and smooth muscle muscarine receptors have been evidenced for a number of compounds (gallamine, 4-DAMP, hexahydro-siladiphenidol) (Birdsall & Hulme). AF-DX 116 (11[[2-[(diethylamino)methyl]-1-piperidinyl] acetyl] -5,11-dihydro-6H-pyrido [2,3-b][1,4] benzodiazepine-6-one) is a muscarinic antagonist with a ten fold higher affinity for the cardiac M_2 receptors than for those on smooth muscle (R. Micheletti et al., this meeting). Therefore it was of interest to investigate its potency in antagonizing secretory responses due to muscarine receptor stimulation. We report the results obtained in "in vitro" and "in vivo" models.

The isolated mouse stomach was prepared according to Angus & Black (1982), acid output being stimulated by 10 µM bethanechol (BCh). Lumen-perfused anaesthetized rats were i.v. infused with BCh (1.55 umol/kg/h) to produce 40% of maximal acid secretion. Salivary secretion was studied in anaesthetized rats with a cannulated submandibular duct, using a submaximal concentration of BCh (12.4 umol/kg/h).

AF-DX 116 dose-dependently inhibited acid output and saliva secretion. The intravenous doses effective in reducing the parameters measured by 50% (ED_{50}) are summarized in Table 1, in comparison with atropine.

Table 1 AF-DX 116 antisecretory potencies (ED_{50})

	acid secretion		saliva secretion
	mouse	rat	rat
	µM	nmol/kg	nmol/kg
AF-DX 116	17.5	1862	1670
	(9.4-32.9)	(1442-2408)	(1430-1950)
Atropine	0.03	4.5	2.38
	(0.02-0.05)	(3.5-5.9)	(1.6-3.5)

It is apparent that AF-DX 116 is a weak inhibitor of the secretory responses investigated, since its potency is from 2 to 3 orders of magnitude as low as that of atropine. In contrast, the potency ratio with atropine observed on cardiac parameters is of 1 order of magnitude or lower (Giachetti et al., 1986). Collectively these results confirm that AF-DX 116 discriminates among M_2 receptors, in favour of the cardiac muscarine subtype.

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ASSESSMENT OF THE MUSCARINIC SUBTYPE SELECTIVITY OF (±)3-QUINUCLIDINYL XANTHENE-9-CARBOXYLATE HEMIOXALATE HYDRATE(QNX)

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It has been claimed, on the basis of binding studies with tissues from different species, that QNX is a selective M_1-muscarinic receptor antagonist (Gibson et al., 1983). This claim has been investigated on a range of in vitro preparations obtained from ~ 250g male Lister hooded rats. (±) Muscarine was used as the standard agonist. Isolated tissues were maintained at 37°C and suspended in a modified Krebs solution equilibrated with 95% oxygen and 5% carbon dioxide. QNX was obtained from Research Biochemicals Inc.. Due to its limited availability, a single 1x10-3M stock solution was prepared in methanol and stored at -20°C.

Concentrations of QNX between 1 x 10^{-9}M and 1 x 10^{-8}M produced parallel shifts of the log-dose response curve to muscarine on isolated ileum contracture and atria (rate). pK_B values were calculated from the dose ratios observed when the tissues appeared to be in equilibrium with the antagonist:- $pK_B = \log[(DR-1)/(B)]$ where B is the concentration of antagonist. The mean values observed were 9.4±0.1 and 8.9±0.1 respectively (mean ± s.e.m., n = 5 tissues).

On rat superior cervical ganglion, using a method based on that of Brown et al., (1980),1 x 10^{-8}M QNX took over 3h to reach an apparent equilibrium. At this time the maximum response to muscarine was reduced by about 50%. Assuming that this reduced maximum was associated with the slow onset and offset of QNX on this tissue, the dose ratio that would have been expected had a parallel shift been produced was estimated by the method of Kennedy & Roberts (1985). This corresponded to a mean pK_B of 7.9 ± 0.2 (n=3). This low value is surprising as, when membranes were prepared from rat superior cervical ganglion, a pK_i value of 9.3 was estimated from the displacement of [^{3}H]-N-methyl scopolamine by QNX.

The inhibition of muscarine stimulated phosphatidylinositol breakdown by a range of QNX concentrations was examined on slices of rat cerebral cortex as described by Lazareno et al. (1985). pK_i values were estimated from the observed dose ratio corresponding to the EC_{50} of muscarine as described above ($pK_i = \log[(DR-1)/(B)]$), where B is the estimated IC_{50} of QNX in the presence of 1 x 10^{-3}M muscarine, a concentration producing a maximum response alone. A pK_i value of 9.1 ± 0.3 (n=2) for QNX was obtained.

In conclusion, these observations show that QNX is non-selective for muscarinic receptor sub-types and therefore do not support the claim that QNX is a selective M_1-muscarinic antagonist.

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QUANTIFICATION OF THE EFFECTS OF MUSCARINIC ANTAGONISTS ON THE RELEASE OF [^{3}H]-ACETYLCHOLINE FROM RAT CEREBRAL CORTEX SLICES

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Although the release of acetylcholine from slices of rat cerebral cortex appears to be controlled by presynaptic muscarinic receptors, it is necessary to estimate the apparent dissociation constants of antagonists at this site before receptor subtypes can be distinguished. The following method enables such values to be estimated relatively quickly and without the use of unreasonably high agonist concentrations.

Slices of rat cerebral cortex (350 x 250μm) were prepared using a McIlwain Chopper, dispersed in a Krebs' solution aerated with 95% oxygen and 5% carbon dioxide and labelled for 40 minutes at 37°C with 1 x 10^{-6}M [^{3}H]-choline chloride (40μCi/mmol) as described by Boudois et. al. (1974). They were then washed, placed in baths made from Millipore filter holders and superfused with Krebs' solution containing 1 x 10^{-6}M hemicholinium-3 at a rate of 0.5ml/min. After 30 minutes, samples of superfusate were collected every 2 minutes and the dpm for each estimated by liquid scintillation spectrometry. Increasing the total concentration of potassium in the superfusing fluid to 20mM for two 4 min periods (S_1 and S_2) produced a calcium-dependent release of tritium. Compounds were added to the superfusing medium after the first pulse and 20 minutes before the second, and their effects quantified by comparing the radioacitvity released by S_2 with that in S_1.

The effects of 8 concentrations of dl-muscarine between 3 x 10^{-8}M and 1 x 10^{-4}M were examined with replicates (n>4) of each concentration. ALLFIT (Delean et al., 1978) was used to fit a logistic function to the mean values. The estimated EC_{50} for muscarine was 9.6 ± 1.59 x 10^{-7}M.

Antagonists were investigated by measuring their abilities to reverse the effect of muscarine, 1 x 10^{-5}M. A range of concentrations was examined with replicates and ALLFIT used to estimate the concentration (C) required to reduce the effect of 1 x 10^{-5}M muscarine to that of the EC_{50} of muscarine. The negative logarithm of the apparent dissociation constant (pK_B) for the antagonist was estimated from the equation : pK_B=log[(Dose-Ratio-1)/C], where the Dose Ratio = 1 x 10^{-5}/9.6 x 10^{-7} = 10.42. The following estimates were made : Atropine C = 1.18 ± 0.399 x 10^{-8}, pK_B = 8.90; Pirenzepine C = 1.19 ± 0.20 x 10^{-6}, pK_B = 6.90; Gallamine C > 1 x 10^{-3}M, pK_B < 4.

The relatively low potencies of pirenzepine and gallamine suggest that the presynaptic muscarinic receptors in rat cerebral cortex are more similar to those on ileum preparations than to those on the atrium or superior cervical ganglion and are probably of the M_2 sub-type.

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HIPPOCAMPAL CHOLINERGIC (MUSCARINIC AND PUTATIVE NICOTINIC) RECEPTORS IN HUMAN COGNITIVE DISORDERS.

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Neurochemical and neuropathological changes in the cholinergic system in Alzheimer's and Parkinson's diseases suggest a role for this system in cognitive function (Perry et al, 1986). Muscarinic cholinergic receptor subtypes (including M1,measured by displacement of 1nM ^{3}H-N-methylscopolamine by 2×10^{-6}M pirenzepine and M2, measured in the presence of 1mM $MgCl_2$, by 3×10^{-4}M carbachol displacement - Birdsall et al, 1984) and putative nicotinic receptor binding (specific binding of 4×10^{-9}M ^{3}H-nicotine - Clarke et al, 1984) have been investigated in the hippocampus of normal and cognitively impaired patients. Autopsy tissue, also assayed for activities of the cholinergic enzymes, choline acetyltransferase and acetylcholinesterase, was obtained from 7 groups of cases (n = 4-11) aged between 30 and 90 years, (assessed clinically and pathologically): Normal, Alzheimer's disease (AD), Parkinson's disease (PD), Down's syndrome (DS), Alcoholic Dementia (AL - including Wernicke's encephalopathy), Huntington's and Motoneuron diseases. Statistically significant abnormalities, detected in dispersed membrane preparations of frozen coronal hippocampal sections, are summarised in Table 1.

Table 1 Cholinergic receptor and enzyme abnormalities in human hippocampus

	AD	PD	DS	AL
Muscarinic M1 subtype	85	117a		
Muscarinic M2 subtype	79			
Nicotinic binding	42	74	48	
Choline acetyltransferase	32	48b	40	67c
Acetylcholinesterase	51	63b	58	

*Results expressed as mean percentage normal activity (significantly different, Mann - Whitney U test, $p = <0.05$ or less)
a,b,c, abnormalities more pronounced in respectively non-demented cases, demented cases, and Wernicke's encephalopathy

In addition to the decrease in ^{3}H-nicotine binding in AD, there was also a significant (over 60%) loss in AD, but not the other disorders investigated, of a soluble endogenous factor (Sershen et al,1984) inhibiting nicotine binding in human cerebral membranes. These findings suggest that, in hippocampus, there is a complex relationship between changes in muscarinic receptor subtypes or nicotinic binding sites and abnormalities in the cholinergic system, as reflected by decreased cholinergic enzyme activities.

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THE EFFECT OF BLOCKADE OF 5-HT RECEPTOR SUBTYPES ON THE CHRONIC MORPHINE ABSTINENCE SYNDROME

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In a previous study we examined the effects of both chronic and acute administration of 5HT agonists on precipitated morphine abstinence and demonstrated a differential modulatory role for 5HT receptor subtypes in the behavioural expression of withdrawal phenomena (Gonzalez & Stolz, 1985). The present study sets out to extend these observations to include an assessment of the effects of selective 5HT receptor blockade on abstinence behaviour. For this purpose pindolol and its analogue cyanopindolol were used to block 5HT-1 receptors (Nahorski & Willcocks, 1983), whilst ketanserin and pipamperone were included as 5HT-2 receptor antagonists (Leysen et al., 1981). For comparison, the combination of atenolol and ICI 118511 (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol), β_1 and β_2 adrenoceptor antagonists respectively, were used to block β-adrenoceptors without blocking 5HT sites.

Male Wistar rats (UWIST breeding stock) were maintained on a 12hr 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 B rats received simultaneously with morphine one of the following treatments s.c.: Atenolol/ICI 118511, Pindolol, Cyanopindolol, Ketanserin or Pipamperone. In schedule A the antagonists were administered as single doses prior to precipitation of abstinence with naloxone (1mg/kg s.c.). 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 Table 1 the behavioural signs and weight changes are expressed as an abstinence index score AIS along with separate temperature effects (Ben Sreti et al. 1984).

Table 1

		Schedule A (Acute Interactant)		Schedule B (Chronic Interactant)	
Interactant*	Dose (mg/kg s.c.)	AIS	Temp °C	AIS	Temp °C
Saline		97	-0.55±0.27	84	-1.1 ±0.3
Atenolol/ ICI 118511	5/5	49	-0.81±0.18	64	-1.14±0.4
Pindolol	5	57	-0.95±0.27	67	-1.26±0.3
Cyanopindolol	5	68	-1.2 ±0.15	-	-
Ketanserin	5	65	-1.85±0.65	66	-0.9 ±0.2
Pipamperone	5	61	-1.67±0.25**	55	-0.46±0.4

*All groups received chronic morphine. †=hypothermia 30 minutes after naloxone
**(P<0.01 Two tailed students t-test)

The results suggest little differentiation between the two subclasses of 5HT receptor with respect to their effects on the abstinence syndrome. In addition the effects of β-adrenoceptor and 5HT receptor blockade are similar. This may reflect the involvement of 5HT and catecholaminergic neurones in expression of the abstinence syndrome or the lack of specificity of available 5HT antagonists.

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CONFORMATIONAL ANALYSIS OF DYNORPHINS[1-17] AND[1-8]

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The opioid peptides, dynorphins [1-17] and [1-8] are believed to be ligands for the κ-type receptor, whereas Leu-enkephalin (which corresponds to the N-terminal pentapeptide sequence of the dynorphins) acts at δ-type receptors. Preferred conformations of dynorphins [1-17] and [1-8] have been calculated by global energy minimization incorporating features to simulate solvent effects. These preferred conformations have been analysed using interactive molecular graphics to illustrate structural relationships between themselves and semi-rigid opiates.

Potential energy calculations were applied to Leu-enkephalin from a large number (80) of starting conformations to cover features of secondary structure importance, crystallographic data, previous theoretical studies and active substitutions. Results have been compared with those from NMR (Griffiths et al, 1986). The solvent is modelled as a dielectric continuum using the Onsager reaction field (Ward et al, 1986). Energy minimization of the dynorphins was initiated from the fully-extended structure as well as secondary structure predictions, and solvent effects were represented by an algorithm involving potential water sites linked to potential hydrogen bonding groups. All calculations were also carried out in the absence of solvent effects.

Under conditions simulating interaction with lipid membranes, significant overlap was obtained between stable and metastable conformers of dynorphins[1-17] and [1-8]. The N-terminal pentapeptide sequence of both was relatively extended as compared with a folded conformation which is preferred for Leu-enkephalin. The results calculated for dynorphins [1-17] and [1-8] are similar to those found experimentally for dynorphin [1-13] (Erne et al, 1985). This allows speculation on the importance of the spatial arrangement of the five (Leu-enkephalin) residues, especially the aromatic rings, for determining receptor specificity. Conformational studies such as these may provide information to assist in the rational design of opioid receptor agonists and antagonists.

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THE ROLE OF ACETYLCHOLINE AND 5-HYDROXYTRYPTAMINE IN A NALOXONE INSENSITIVE MODEL OF STRESS-INDUCED ANTINOCICEPTION

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It has been reported that a 30sec swim in water at 30°C reduces the number of abdominal constrictions produced in mice by the i.p. injection of acetic acid and that this stress-induced antinociception (SIA) is insensitive to naloxone and to a delta opioid receptor antagonist (Hart et al 1983). Acetylcholine (ACh) is involved in a naloxone sensitive model of SIA in mice (Hart & Yadav,1985) whilst Tricklebank et al (1982) have shown in a naloxone insensitive model in the rat that SIA is inversely related to 5-hydroxytryptamine (5HT) availability or release. We have, therefore, re-examined the swim-abdominal constriction model of SIA and investigated the possible role of ACh and 5HT.

Male LACA mice (25-35g) were injected with saline or drug by the i.p. route and 15 or 30min later were placed in water at 30°C for 30sec. On removal from the water the mice received an i.p. injection of freshly prepared 0.6% acetic acid (1ml/100g) and the number of constrictions were counted in the period 10-20min after injection by an observer unaware of the drug treatment. In some experiments the interval between removal from the water and injection of acetic acid was varied in order to investigate the duration of the SIA.

In mice receiving saline, acetic acid induced 25 constrictions in naive animals and 7 constrictions when administered immediately after the 30sec swim (Table 1). A similar degree of antinociception was observed when the interval between the swim and acetic acid injection was 1,2,5 and 10min but no SIA was observed at 20 min. It was confirmed that the SIA was not affected by naloxone (5mg/Kg) although the effect of morphine (2.5mg/Kg) was attenuated by 2mg/Kg naloxone. There was no evidence for a role for ACh in this model of SIA for it was unaffected by atropine methyl nitrate (5,10 mg/Kg) or atropine sulphate (1,5,10 mg/Kg) given 15min prior to the swim. The effects of methysergide and cyproheptadine administered 15min prior to the swim are shown in Table 1; similar results were obtained when these antagonists were injected 30min before the swim.

Table 1 Median number of constrictions (with interquartile range) occurring 10-20min after the injection of acetic acid

		No Swim	Swim
Saline		25, 23.5-27 (12)	7, 6-9 (30)
Methysergide	1mg/Kg	24.5, 24-25 (6)	1.5, 1-2 (6)*
	2		0.5, 0-1 (6)*
	5	24.5, 20-26 (6)	0, 0-1 (6)*
	10	2, 0-4 (10)*	0, 0-0 (6)*
Cyproheptadine	1mg/Kg		6.5, 5-9 (6)
	2		7.5, 4-8 (6)
	5	18, 10-23 (11)	3.5, 2-6 (6)*
	10	1.5, 0.5-2.5 (12)*	0, 0-1 (6)*

*$P<0.005$ against appropriate control using Mann-Whitney U test.

Thus muscarinic receptors are not involved in this naloxone insensitive model of SIA and the results with the 5HT antagonists suggest a role for this neurotransmitter which requires further elucidation.

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