SPONTANEOUS APPEARANCE OF BEHAVIOURS ASSOCIATED WITH INCREASED 5-HT ACTIVITY DURING CHRONIC LITHIUM TREATMENT IN RATS

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Increased 5-HT release associated with downregulation of 5-HT receptors in the hippocampus occurs after chronic lithium administration in rats (Treiser et al., 1981). Behavioural studies with fenfluramine and reserpine in lithium (Li) pretreated rats suggest increased neurogenic transmitter release in 5-HT systems involved in drug-induced fore-paw treading, hyperreactivity to handling and inhibition of 'wet-dog' shakes (Harrison-Read, 1983). We now report the spontaneous appearance during chronic Li treatment of behaviours which may reflect increased brain 5-HT activity.

Male Lister hooded rats (5 per cage) were injected i.p. each morning with 0.154 M solutions of LiCl or NaCl (2 mmol/kg) for 28 days. Different periods of Li pretreatment and withdrawal were achieved by substituting NaCl for LiCl as appropriate. On day 25, half the rats received p-chlorophenylalanine methylester (PCPA, 400 mg/kg) dissolved in the NaCl or LiCl injection (13 ml/kg). Before the last injection on day 28, each rat was observed for 1 min in its home cage, and the following behaviours were rated (0-2) by an observer 'blind' to rats' pretreatment : fore-paw treading; rubbing the snout and chin along the cage floor using a weaving movement of the head; mouthing activity distinct from licking & biting ('munching' Dawbarn et al, 1981). None of these behaviours were present in rats receiving saline alone, or in those tested on day 3 of Li pretreatment. Mean (± s.e.m.) rating scores for groups of rats tested on Li days 7 & 28, and on day 4 of Li withdrawal following 21 days of Li treatment (Li21/4W) are shown in Table 1. Mean plasma concentrations of Li measured 8 h after Li injection were between 0.45 & 0.55 mmol /L, but fell to < 0.05 mmol/L on day 4 of Li withdrawal.

Table 1 Spontaneous lithium-induced behaviours and the effect of PCPA

Days on Li	TREADING			RUBBING			MUNCHING		
	Li 7	Li28	Li21/4W	Li 7	Li28	Li21/4W	Li 7	Li28	Li21/4W
CONTROL	0.88* ±0.23	1.88 ±0.12	0.67* ±0.24	0.38* ±0.18	1.50 ±0.19	0.67* ±0.24	0.75 ±0.16	1.25 ±0.25	0.11* ±0.11
P C P A	0.44* ±0.18	1.38 ±0.26	1.12 ±0.30	0.22* ±0.15	1.25 ±0.16	1.50Δ ±0.27	0.11Δ ±0.1	1.00 ±0.19	0.75Δ ±0.25

Mann-Whitney 2-tailed tests: * $P<0.05$, difference from Li 28 day group; Δ $P<0.05$, control versus PCPA groups; n = 8 or 9 per group.

Behavioural ratings showed an increase between Li days 7 & 28, and rapidly fell on Li withdrawal. Additional pretreatment with PCPA (an inhibitor of 5-HT synthesis) reduced, but did not abolish Li-induced behaviour. However, PCPA also partially prevented the decline in Li behaviour following Li withdrawal, without affecting the rapid fall in plasma levels of Li. The effect of PCPA in rats on chronic Li suggests that Li-induced behaviours may reflect increased 5-HT release. On Li withdrawal, the rapid reduction in these behaviours may be partly due to recovery of 5-HT release in a second system which is inhibited by Li, and which is functionally opposed to the first. PCPA and Li may both have an inhibitory effect on transmission in this second system. In the case of PCPA, this will tend to mitigate its inhibitory action on the 5-HT system mediating Li-induced behaviours, particularly during Li withdrawal when the second system may be recovering.

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THE EFFECTS OF PROMETHAZINE AND TWO OF ITS METABOLITES ON OXOTREMORINE-INDUCED CHOLINERGIC SIGNS IN VIVO

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Promethazine (PMZ) is a widely used drug but its systemic availability after oral dosing is low, despite rapid and complete absorption from the gastrointestinal tract (Moolenar et al, 1981). This is consistent with extensive first-pass metabolism mainly to its S-oxidized (promethazine sulphoxide, sPMZ) and N-demethylated (monodesmethylpromethazine, nPMZ) derivatives (Taylor and Houston, 1982).

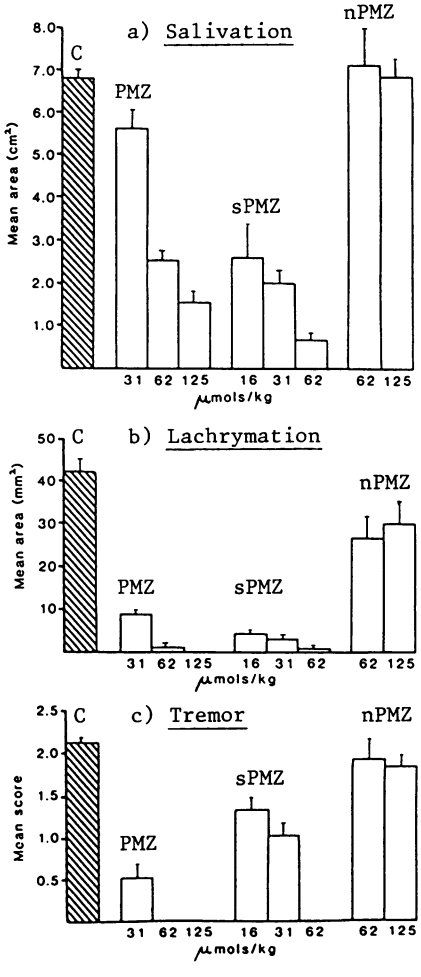

Fig. Effects of PMZ, sPMZ and nPMZ on the salivary and lachrymal secretions, and tremor responses induced by oxotremorine. C = control

In this study, the relative anticholinergic activities of PMZ, sPMZ and nPMZ were assessed in mice in which peripheral (Cho et al, 1962) and central (George et al, 1962) cholinergic phenomena had been induced by oxotremorine (OT).

Groups of 10 MF1 mice (20-30g) were injected i.p. with PMZ, or a metabolite or 0.9% saline (control). Thirty minutes later the condition of the animals was observed and then 250 μg OT /kg given i.p. Salivary and lachrymal secretions were absorbed on paper tissue at 5 min intervals thereafter for 30 min and measured by planimetry. Tremor was assessed visually on a scale 0-3 at the same time intervals.

PMZ and sPMZ produced a dose-related inhibition of OT-induced salivation (Fig.a) and lachrymation (Fig.b). Inhibition was greater with the sulphoxide than with the parent compound, but nPMZ had only marginal effects. In contrast, the central anticholinergic activity of sPMZ appeared less than PMZ (Fig.c). No effect of nPMZ was observed. Some suppression of the signs of central cholinergic overactivity may have been caused by a greater sedative effect which was observed with PMZ.

This study indicates that the anticholinergic activity of PMZ may be enhanced or prolonged in vivo by its metabolic conversion to sPMZ.

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STIMULATION OF FOOD INTAKE BY YOHIMBINE

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Parenteral administration of the indole alkaloid yohimbine induces a marked and reproducible increase in food consumption in satiated rats. The effect is comparable in intensity and duration to that produced by diazepam and is not subject to tolerance on repeated administration. The mechanism of the yohimbine induced feeding was studied with reference to its known pharmacological properties.

Yohimbine causes preferential blockade of presynaptic (α_2) adrenoceptors (Starke et al, 1975). Piperoxan, also an α_2 antagonist, stimulates food intake but it is less effective than yohimbine. This is consistent with the observation that clonidine, an α_2 agonist, is a potent anorexiant in the rat. However, clonidine is reported to produce hyperphagia and weight gain in monkeys (Schlemmer et al, 1981). Yohimbine also raises brain 5-hydroxytryptamine (5-HT) levels (Papeschi et al, 1971), but the yohimbine induced eating was only slightly attenuated by subcutaneous (s.c.) pretreatment with methergoline (1mg/Kg), whilst methysergide (1mg/Kg) had no effect. In addition to the above effects yohimbine releases noradrenaline (NA) peripherally (Crevelling et al, 1968) and inhibits monoamine oxidase (McIsaac and Estevez, 1966). This may contribute to the mechanism since inhibition of catecholamine synthesis by α-methyl-para-tyrosine (αmpt) abolished the yohimbine hyperphagia. Pretreatment with phentolamine, an α-adrenergic antagonist, attenuated the response to yohimbine, whilst propranolol was without effect.

Endorphins have been implicated in various forms of overeating. Naloxone has been shown to antagonise the appetite stimulation induced by diazepam (Stapleton et al, 1979), 2-deoxyglucose (Sewell & Jawaharlal 1980) and stress due to tail pinching (Morley α Levine, 1980). Naloxone pretreatment (0.5 - 1mg/Kg s.c.) was similarly effective in antagonising the yohimbine induced eating at doses which do not affect unstimulated food intake. This effect is independent of an action on δ-opiate receptors since the selective δ-opiate antagonist ICI 154129 (Gormley et al, 1982) was without effect.

The above results imply that yohimbine induced feeding involves both NA and endorphin mediated components. Grandison & Guidotti (1977) have proposed that GABA is also involved in feeding induced by NA and endorphins. In our experiments the GABA antagonist picrotoxin inhibited the yohimbine hyperphagia, although it also reduced food intake in control animals.

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PHARMACOLOGY OF THERMOGENESIS IN THE RAT, ASSESSMENT BY MEANS OF INTERSCAPULAR BROWN ADIPOSE TISSUE (IBAT) TEMPERATURE

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In adult rodents, BAT has recently been identified as the major thermogenic effector tissue responding to both cold exposure (Foster α Frydman, 1979) and overfeeding (Rothwell α Stock, 1979). Thermogenesis, resulting in a rise in IBAT temperature, can be stimulated by parenterally administered catecholamines as well as by electrical stimulation of the nerves supplying the tissue. The response to nerve stimulation is biphasic. An initial small transient decrease in IBAT temperature, possibly related to vasoconstriction, is followed by a more prolonged rise above pre-stimulus levels. It has been reported that the α-blocker phentolamine attenuates both phases of the response whilst the β-blocker propranolol primarily inhibits the secondary rise in temperature (Flaim et al, 1977). In the present study we have investigated the effect of a range of adrenoceptor agonists and antagonists on IBAT temperature in Lister hooded rats as a means of elucidating the functional role of α and β-adrenoceptors in the control of BAT thermogenesis.

Basal IBAT temperature was generally in the range 35.5-37.5°C; with core temperatures slightly higher between 36 and 38°C. The results shown are expressed as the mean maximum change in IBAT temperature ± s.e. mean. Noradrenaline (NA) infusion (2μg/Kg/min i.v. for 10 mins) produced a significant increase in IBAT temperature (+0.55 ± 0.08°C). Isoprenaline at the same dose produced a similar effect (+1.29 ± 0.29°C). Dose response curves for the agonists were parallel but isoprenaline was approximately 3x more potent than NA. The thermogenic response to this submaximal dose of NA was unaffected by pretreatment with the α-receptor antagonist phentolamine (5mg/Kgiv.) but was completely antagonised by propranolol (0.3mg/Kg, i.v.). A subsequent finding that stimulation of the sympathetic nerves supplying the tissue produces an increase in IBAT temperature which is stereospecifically blocked by propranolol (L-isomer approx.15x potency of D isomer) suggests that noradrenaline is acting via a β-receptor. The ability of propranolol (β_1/β_2 non-selective, IC_{50} .055mg/Kg i.v.) to inhibit this response to noradrenaline was compared with both the specific $\beta_1$antagonist atenolol (IC_{50} 0.7mg/Kg,i.v.) and the β_2 antagonist ICI 118551 (IC_{50} 1.6mg/Kg i.v.). Across a range of doses neither atenolol nor ICI 118551 achieved a 100% inhibition of the response to NA. Although Flaim et al, 1977 reported that phentolamine partially inhibitied the rise in IBAT temperature after nerve stimulation, infusion of the α-agonist phenylephrine 45μg/Kg/min,i.v. for 10 mins) only produced a fall in IBAT temperature (-0.46 ± .12°C) which was blocked by pretreatment with phentolamine (5mg/Kg,i.v.). Administration of phenylephrine increases GDP binding to rat BAT mitochondria, a measure of thermogenic activity of the tissue. However, in the present study α-receptors would not appear to be involved in the NA induced increase in IBAT temperature.

In conclusion these data are consistent with noradrenergic stimulation of BAT thermogenesis being under the control of a mixed population of β_1 and β_2-receptors or a hybrid receptor such as that proposed for rat white adipose tissue (De Vente et al, 1980). Support for the latter comes from the observation that increases in IBAT temperature produced by selective agonists (Salbutamol, $\beta_2$and prenalterol,β_1) can be at least partially blocked by both atenolol and ICI 118551.

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COMPARISON OF IN VIVO AND IN VITRO ACTIONS OF TIAPRIDE IN RODENTS

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The weak dopamine receptor blocking properties of tiapride may be due to poor brain penetration since it shows potent activity on direct intracerebral injection (Costall et al,1978). However, tiapride inhibits dyskinetic phenomena in rodents that are resistant to classical neuroleptic drugs (Costall et al,1977) suggesting a different mode of action. We have compared the in vivo and in vitro actions of tiapride in rodents.

Tiapride decreased apomorphine (2.0 mg/kg ip)-induced locomotor activity in mice (ID50 95 mg/kg ip) and inhibited apomorphine (2.0 mg/kg ip) induced rotation in mice with a prior unilateral 6-hydroxydopamine lesion of the striatum (ID50 110 mg/kg ip). Tiapride also inhibited apomorphine (0.5 mg/kg sc)-induced stereotypy in rats (ID50 48 mg/kg ip). Striatal HVA and DOPAC concentrations in mice were elevated between 2-3 fold following administration of tiapride (100 mg/kg ip 2 h previously).

Tiapride (48 mg/kg ip 1 h previously) reduced the in vivo binding of ^{3}H-spiperone (25 uCi) by 25% in striatum and 33% in tuberculum olfactorium but had no effect on frontal cortex.

Tiapride (10^{-9}-10^{-5}M) did not displace the specific binding of ^{3}H-haloperidol or ^{3}H-spiperone to rat striatal membranes compared to the effects of both sulpiride and haloperidol (Table 1). Tiapride did, however, displace the specific binding of ^{3}H-sulpiride to striatal tissue. Tiapride (10^{-9}-10^{-5}M) was ineffective also in displacing radioactive ligands labelling noradrenaline, 5HT and muscarinic receptors (Table 1).

Table 1 Receptor profiles of tiapride, sulpiride and haloperidol

Ligand	Site	IC_{50} (M) Tiapride	Sulpiride	Haloperidol
^{3}H-haloperidol	D-2	$>10^{-5}$	1.6×10^{-7}	2.4×10^{-9}
^{3}H-spiperone	D-2	$>10^{-6}$	1.0×10^{-6}	6.0×10^{-9}
^{3}H-sulpiride	D-2	1.35×10^{-7}	2.5×10^{-8}	7.0×10^{-9}
^{3}H-5HT	5HT-1	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
^{3}H-ketanserin	5HT-2	$>10^{-5}$	$>10^{-5}$	7.4×10^{-8}
^{3}H-WB 4101	α-1	$>10^{-5}$	$>10^{-5}$	2.2×10^{-8}
^{3}H-clonidine	α-2	$>10^{-5}$	$>10^{-5}$	$>10^{-6}$
^{3}H-dihydroalprenolol	β	$>10^{-5}$	$>10^{-5}$	-
^{3}H-dexetimide	muscarinic	$>10^{-5}$	$>10^{-5}$	$>10^{-6}$

Poor brain penetration alone may not explain the pharmacological actions of tiapride. The failure of tiapride to displace ^{3}H-haloperidol or ^{3}H-spiperone from dopamine receptors suggests a selective action on sites labelled by ^{3}H-sulpiride may be involved.

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ASYMMETRIC MOTOR BEHAVIOUR REVEALED AFTER UNILATERAL INTRASTRIATAL INJECTIONS OF NEUROLEPTIC AGENTS

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Whilst it is appreciated that most neuroleptic agents presently available have a potential to cause Parkinson-like side effects, and there have been approaches to an appraisal of this potential, the most direct comparison of ability to cause functional change on discrete intrastriatal administration has not been attempted in depth, and this is therefore the purpose of the present studies.

Male Sprague-Dawley rats were subject to standard stereotaxic surgery for the implantation of chronically indwelling guide cannulae for subsequent (14 days recovery) bilateral intrastriatal injection. The experimental approach was then three-fold, 1) to inject neuroleptic agent into one striatum, its solvent into the other, and measure spontaneous asymmetry/circling, 2) to then challenge the rats in 1) with s.c. apomorphine to exacerbate any striatal imbalance, or 3) to inject neuroleptic agent into one striatum, dopamine into the other, and determine whether any spontaneous asymmetry towards the side of the neuroleptic injection can be enhanced by obviating any compensatory mechanisms of the opposing striatum. Spontaneous and apomorphine-induced asymmetry/circling was scored: 0-response of rats indistinguishable from normal, 1-definite tendency for rat to move in one direction only when handled, but still retains an ability to move in the opposite direction, 2-spontaneous movements and body asymmetry in one direction only, exaggerated by handling, 3-marked body asymmetry and active circling in one direction only. Initial studies showed a striatal imbalance on unilateral intrastriatal injection of a neuroleptic to be revealed by peripherally administered apomorphine (0.25 mg/kg s.c., selected as causing a maximal effect with minimal interference from stereotyped responding): challenge with apomorphine at varying times following intrastriatal neuroleptic injection revealed maximal effects at 30 min (fluphenazine, haloperidol, tiapride, metoclopramide, clozapine) or 60 min (α-cis-flupenthixol, thioridazine). These pretreatment times were therefore adopted for all subsequent studies.

Apomorphine revealed a dose-dependent ability for all neuroleptic agents to cause motor asymmetry (ipsilateral to the side of neuroleptic injection) shown both as asymmetric body posturing and circling behaviour. Haloperidol was the most potent neuroleptic in this respect with a threshold dose of 0.5µg (score 0-1) and maximal dose of 10µg (score 3); conversely, thioridazine was the least potent agent tested (threshold dose 5µg, score 2 only achieved at 10µg). Threshold responding was recorded to 2.5µg fluphenazine and clozapine, or to 1µg tiapride, metoclopramide and α-cis-flupenthixol. None of the doses of neuroleptics tested alone gave spontaneous asymmetry of score greater than 1. However, spontaneous responding to threshold doses of the neuroleptic (as regards responding to apomorphine) was enhanced (to score 2) when dopamine was administered into the contralateral striatum (25µg, subthreshold dose for causing contralateral asymmetry in its own right).

The failure of unilateral intrastriatal injections of neuroleptics alone to cause marked spontaneous asymmetric body posturing or circling may be indicative of 'compensation' in mechanisms of the contralateral striatum. However, this may be obviated by dopamine administration into the contralateral striatum or, more markedly, the induced asymmetry can be revealed on peripheral apomorphine administration. These behavioural models can be used to determine relative potencies of neuroleptic agents to antagonise at striatal dopamine receptor mechanisms.

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FURTHER STUDIES ON CICLAZINDOL AS A THERMOGENIC AGENT IN RATS

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Ciclazindol has been shown to produce an energy-wasting (thermogenic) effect in rats at doses which do not induce overt behavioural effects (Rothwell et al, 1981) At higher doses, a greater thermogenic effect is observed, but this cannot be completely separated from a reduced food intake and CNS stimulation (Chart et al 1982). These low dose effects may be accounted for by the selectivity of ciclazindol for monoamine trasnport systems in the autonomic nervous system relative to the CNS (Rothwell et al, 1981). We describe here the pharmacology in the rat of DDC, the 3,3-dimethyl-deschloro analogue of ciclazindol.*

The activity of ciclazindol and DDC as inhibitors of noradrenaline re-uptake resided predominantly in the (-) isomers. All the studies reported below were, however, conducted with the racemic mixture.

DDC was a more potent inhibitor of peripheral uptake than ciclazindol {IC_{50} (nM) inhibition of noradrenaline uptake into brown adipose tissue, 28 ± 3 (6) and 120 ± 20 (6) respectively}. In contrast DDC was much less active in the CNS than ciclazindol {IC_{50} (nM) inhibition of noradrenaline uptake into brain slices, 3400 ± 30 (6) and 420 ± 40 (6) respectively}. On the basis of this *in vitro* data, it would be expected that DDC, with a relative selectivity for brown adipose tissue, would increase resting oxygen consumption at lower doses than ciclazindol and require higher doses to produce overt behavioural stimulation. This assumes that the primary pharmacological action of this type of compound is as a noradrenaline re-uptake inhibitor (For more detailed discussion see Rothwell et al, 1981).

DDC did increase oxygen consumption in conscious and anaesthetised animals at lower doses than ciclazindol {lowest effective dose 1mg kg^{-1} ip and 3 mg kg^{-1} ip, respectively}. The resting oxygen consumption was increased by up to 20% by single oral doses of either drug. In acute dosing studies there was no evidence of overt behavioural effects of ciclazindol at doses up to 10mg kg^{-1}po and DDC at doses up to 90 mg kg^{-1}po. The overt behavioural effects of ciclazindol were qualitatively dissimilar from those observed after acute administration of amphetamine - like stimulant drugs, and there was no evidence of an interaction of ciclazindol or DDC with dopaminergic systems.

In chronic dosing studies (test compounds mixed with drinking water) DDC induced weight loss and reduced rate of weight gain at lower doses than ciclazindol. {Lowest effective dose (inhibition of weight gain : DDC 3.5 mg kg^{-1} day^{-1}, ciclazindol 7.1 mg kg^{-1} day^{-1}) : (weight loss : DDC 10.8 mg kg^{-1} day^{-1} ; ciclazindol 21.5 mg kg^{-1} day^{-1}}. All animals were male, Sprague-Dawley rats. ($550g^{+}$). At these doses there was no significant reduction in food intake.

Overall these results suggest that ciclazindol and DDC produce weight loss by a peripheral action. DDC may offer some advantages over ciclazindol due to a lowered potency on, and selectivity for peripheral monoamine uptake systems.

* DDC = 3,4-dihydro-3,3-dimethyl-10-phenylpyrimido-1, 2a-indol-10 {^{2}H}-ol.

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STIMULATION OF PHOSPHATIDYLINOSITOL TURNOVER IN RAT BRAIN BY GLUTAMATE AND ASPARTATE

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The "phosphatidylinositol response" is associated with the stimulation of cell-surface receptors, and changes in cellular calcium (Michell, 1975, 1979). A possible involvement of guanosine 3'5'-cyclic monophosphate (cyclic GMP) has also been suggested (Michell, 1975, 1979). The receptor-mediated actions of the excitatory dicarboxylic amino acid neurotransmitters, L-glutamate and L-aspartate are associated with transient changes in cellular calcium and it has been demonstrated that these amino acids evoke large increases in the concentration of cyclic GMP in immature rat cerebellum (Foster and Roberts, 1980, 1981). We report here that the phosphatidylinositol response is also associated with the actions of glutamate and aspartate in rat brain.

Using the methodology described by Downes & Michell (1981) and Berridge et al (1982), rat striatal and cerebellar slices were incubated for up to one hour with 1 mM glutamate or aspartate in the presence of lithium chloride (5 mM) at 37 °C in a Krebs Ringer bicarbonate medium. The incorporation of myo-2-^{3}H-inositol into total inositol phosphates, was 10-25% higher than that observed in the presence of lithium alone.

Typical values for the lithium control were 4540 ± 306 dpm ($\bar{x}$ ± σn-1) and for stimulation by glutamate 7427 ± 734 dpm ($\bar{x}$ ± σn-1). A significant increase in label was found for concentrations of glutamate above 1 mM. Results obtained for aspartate were similar. The tissue response to carbachol (0.1 mM) was used as a 'standard'. This, like glutamate and aspartate showed higher blank values and a lower % stimulation than those reported by Nahorski & Willcocks (1983) and Berridge & Downes (1982). One possible explanation for this is damage to the slices on preparation and during the long incubation times reported.

Viability of slices was monitored by incorporation of [^{3}H]-inositol into [^{3}H]-phosphatidyl inositol. This was linear up to 1 hour (250 dpm/min/50 µl slices). After 1 hour the slope tended to increase suggesting spontaneous depolarization of nerve cells. It is worth noting that brain slices are not metabolically or electrically stable and responses measured may not be primarily related to the stimulus.

These preliminary findings suggest that excitatory amino acid recpetor stimulation is associated with an enhanced turnover of phosphatidyl inositol.

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ASCORBIC ACID AND THE UPTAKE OF CATECHOLAMINES IN RAT BRAIN SLICES

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The reducing agent ascorbic acid is routinely added to the incubation media in pharmacological experiments as catecholamines are susceptible to oxidation (Iversen, 1963). It has recently been shown that ascorbic acid will potently inhibit the binding of dopamine to dopamine receptors in the striatum (Heikkila et al. 1981). It is thus essential to establish whether ascorbic acid has any effect on other processes involving catecholamines, for example uptake. We have therefore investigated the interaction of ascorbic acid with the high affinity uptake systems for catecholamines.

Uptake of [^{3}H]- dopamine ([^{3}H]-DA) and [^{3}H]- noradrenaline ([^{3}H]-NA) were studied using slices (0.5 mm thick) of rat striatum and cerebellum respectively. The tissue was preincubated at 37°C in Krebs solution (pH 7.4) containing different concentrations of ascorbic acid. After 10 min [^{3}H]-DA or [^{3}H]-NA was added to give a final catecholamine concentration of 0.1μM. Incubation was for 2 min during which time uptake was linear. Slices were removed by filtration, washed with 2ml of ice cold Krebs solution, blotted, weighed, digested with Soluene - 350 and the radioactivity measured by liquid scintillation.

Table 1 Catecholamine uptake at different ascorbic acid concentration

Ascorbic acid M	Uptake n mol/g wet wt/min DA	NA
0	0.186 ± 0.019 (5)	0.031 ± 0.004 (6)
10^{-6}	0.197 ± 0.010 (6)	0.028 ± 0.001 (6)
10^{-4}	0.203 ± 0.017 (6)	0.026 ± 0.002 (6)
10^{-3}	0.236 ± 0.024 (6)	0.030 ± 0.003 (6) *
10^{-2}	0.226 ± 0.016 (6)	0.013 ± 0.001 (6)

Values are mean ± s.e.mean (n)
* $p<0.05$

There is no significant difference between the uptake of DA in different concentrations of ascorbic acid. Only NA uptake in an ascorbic acid concentration of 10^{-2}M was significantly different from the others. The results suggesting inhibition.

Ascorbic acid is widely used at concentrations of 20mg/l, (approx 10^{-4}M) (Iversen 1963) to 200mg/l, (approx. 10^{-3}M) (Snyder and Coyle, 1969). It is therefore possible to conclude that the concentrations of ascorbic acid normally employed in studies of DA, and of NA uptake are unlikely to have any inhibitory effect, but care should be exercised in the use of concentrations in excess of 10^{-3}M.

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ON THE INTERFERENCE WITH (^{3}H)-ADTN BINDING TO RAT STRIATAL MEMBRANES BY ASCORBIC ACID

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A controversial finding of ^{3}H dopamine agonist binding studies is that ascorbic acid, a constituent of most assay buffers, may modify agonist binding per se. We report here on detailed studies of ascorbic acid interference with ^{3}H.ADTN binding to rat striatal tissue.

Female Sprague-Dawley rats were stunned, the striata dissected out and homogenised in Tris-HCl buffer (50 mM, pH 7.4, 25°C), twice centrifuged and resuspended, then incubated for 12 min at 37°C in Tris-HCl buffer (50 mM, pH 7.4), followed by a further double centrifugation/resuspension, final resuspension being in Tris-HCl buffer (50 mM, pH 7.4, 25°C) containing 5 mM Na_2EDTA and 10 µM nialamide. Immediately after preparation 0.5 ml of the membrane suspension (10 mg/ml wet wt. tissue, equivalent to approximately 300µg protein) was placed in each assay tube: to this was added ascorbic acid/buffer 0.2 ml, displacing drug/buffer 0.2ml, ligand 0.2ml to a total volume of 1.1ml. Final incubation was for 15 min at 25°C; bound and free ligand were separated by rapid filtration over Whatman GF/B filters washed with 2 x 5 ml ice-cold buffer.

Buffer containing ascorbic acid (10^{-4}M, 5.7 x 10^{-3}M) was prepared 5 or 60 min before addition of 0.125 - 4 nM ^{3}H.ADTN; 10^{-5}M dopamine and 10^{-5}M α-cis-flupenthixol were used as displacing agents. Scatchard analyses show variations in K_D and B_{max} values with 'age' of the ascorbic acid solution (Table 1).

Table 1 Displacement of ^{3}H.ADTN binding: effect of ascorbic acid

Ascorbic acid (preparation)	Displacing agent	$K_{D(nm)}$	$B_{max(fm/mg)}$
None	dopamine	1.26	138
10^{-4}M (5 min)	dopamine	0.98	85
10^{-4}M (5 min)	α-cis-flupenthixol	1.17	86
10^{-4}M (60 min)	dopamine	1.58	95
10^{-4}M (60 min)	α-cis-flupenthixol	1.40	85
5.7 x 10^{-3}M (5 min)	dopamine	7.20	207
5.7 x 10^{-3}M (60 min)	dopamine	3.73	254

15-20% of the total binding of a saturating concentration of 2 nM ^{3}H.ADTN(ascorbic acid 10^{-4}M, 5 min) was displaced by 8 x 10^{-12} - 2.5 x 10^{-10}M dopamine, ADTN and (-)NPA; this displacement was increased with increasing concentration of displacing agents (60-70% at 10^{-6}M). Isoapomorphine and catechol were ineffective (10^{-11}-10^{-7}M). Fluphenazine, α-cis-flupenthixol and (+)butaclamol caused 60% displacement of total binding at 10^{-6}M, concentrations causing a displacement of 30% total binding being in the range of 1-3nM, with a high potency and plateau of displacement occurring at 6 x 10^{-11}- 10^{-9}M. For both the dopamine agonists and antagonists the suggestion of more than one displacement site precludes calculation of meaningful, single IC_{50} values. 10^{-11} - 10^{-7}M piperoxan, yohimbine and α-trans-flupenthixol were ineffective.

Thus, ^{3}H.ADTN binding to rat striatal membranes can be modified by ascorbic acid, dependent on both the concentration and 'age' of the ascorbic acid solution. Using a 'fresh' solution of 10^{-4}M ascorbic acid a saturable, high affinity, specific ^{3}H.ADTN binding to rat striatal tissues, displaceable by low nM concentrations of dopamine agonists and antagonists, can be demonstrated.

This work was supported by the Wellcome Trust.

THE FATE OF NEUROTRANSMITTER IN THE RAT ISOLATED SPLENIC STRIP PREPARATION

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In a previous communication (Ahmed & Naylor 1982) evidence was presented that the rat splenic strip preparation could be used to study both α_1 and α_2 adrenergic receptors. In an attempt to provide evidence that rauwolscine exerted its effect other than by uptake inhibition splenic strips stimulated in the presence of desmethylimipramine (DMI) (10^{-7}M) or cocaine (3×10^{-5}M) and then DMI and rauwolscine (5×10^{-8}M) or cocaine and rauwolscine both showed that rauwolscine significantly ($P < 0.001$) potentiated the response to low frequency stimulation (1-6Hz). This suggested that rauwolscine did not act via uptake$_1$ inhibition. Further investigations of the rauwolscine effect were then attempted using a method to determine the transmitter output in the absence and presence of this α_2 antagonist. Although transmitter outputs during nerve stimulation have been reported for the cat (Brown & Gillespie, 1957) no reported cases could be found for the rat. Since endogenous release was the area of interest no 'loading' with ^{3}H noradrenaline (NA) was performed and the overflowing NA was to be detected using a cascade of four rat tissues: fundic strip, duodenum, colon and anococcygeus muscle.

To obtain a superfusate of the isolated splenic strip, three flow through baths (volume 3-4 ml) fitted with parallel s.s. electrodes (25 mm length, 0.9 mm dia.) were designed and fitted above the first tissue in the cascade. Flow rates of 2-5 ml min^{-1} were used depending upon bath design. All assay tissues were sensitive to NA with threshold sensitivities of: fundic strip 2-4ng, duodenum 0.4-1 ng, colon 1-2 ng, and anococcygeus 0.4-1 ng. Fundic strip, duodenum and colon responded to PGE_2 and F_2 with threshold sensitivities for PGE_2 of: 0.5-1ng, 2-4 ng and 1-2 ng respectively and for $PGF_{2\alpha}$ of 1-2 ng, 2-4 ng and 0.5-1 ng respectively. The superfusate of the stimulated (1-25H_2; n=88) splenic strip when passed over the tissues, no detectable activity in the superfusate was present. In further experiments usi ng duodenum and anococcygeus muscle, the inclusion of DMI or cocaine, although increasing the duration of the response to stimulation and increasing the cascade tissues sensitivity to both NA and adrenaline, did not yield a detectable material in the superfusate from stimulated splenic strips (1-25Hz, n = 30). The addition of pyrogallol (10^{-5}M), benserazide (10^{-5}M), pargyline (10^{-5}, 10^{-4}M), corticosterone (8.6×10^{-5}M) and oxytetracycline (10^{-4}M) either individually or in combination all failed to produce a detectable material in the superfusate from stimulated splenic strips. Both adrenaline and NA added to the flow through bath were always detected by the cascade tissues. In further experiments dopamine (DA) threshold dose (1-2µg) on tissues and 5µg on spleen and the addition of GBR13098 did not produce a detectable response during nerve stimulation.

Transmission electron micrographs using the method of Tranzer and Richards (1976) will be shown illustrating the type of adrenergic nerves in rat spleen.

The question arises therefore as to the fate of the transmitter liberated during field stimulation. The results suggest that in rat spleen an uptake process is present which is resistant to DMI, oxy tetracycline, corticosterone and GBR13098 and is not affected by MAO or COMT inhibitors. This appears to have some similarity to that reported for cat spleen (Blakeley et al., 1973).

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NORADRENERGIC (NA) RECEPTOR BINDING IN MICE SUSCEPTIBLE AND RESISTANT TO AUDIOGENIC SEIZURES (AS)

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DBA/2 mice have a high genetically determined susceptibility to audiogenic seizures. This susceptibility is age related, being maximal at 21-28 days of age and considerably reduced or absent at earlier and later ages. The basis of this age-related susceptibility to AS is not known, although an involvement of NA mediated neurotransmission has been implicated. (Kellogg 1976). The incidence and severity of AS in DBA/2 is reduced in a dose dependent manner by α_2 adrenoceptor agonists such as clonidine, (Horton et al, 1980). This effect is antagonised by α_2 adrenoceptor antagonists (Horton et al, 1980). The protective effect of propranolol shows only slight stereoselectivity and is probably unrelated to β blockade (Anlezark et al, 1979).

We now report the quantitation of NA receptors in DBA/2 mice at various ages, before (8-9 days, 13-15 days) during (21-23 days and 27-28 days) and after (40-43) days the period of maximal susceptibility to AS. For comparison experiments were performed concurrently on age-matched C57 BL/6 mice, a strain resistant to audiogenic seizures at all ages. Radioligand binding was performed by standard techniques on freshly prepared well washed whole brain membranes at 25°C. β NA receptors were defined with ^{3}H dihydroalprenolol (0.25-10nM, displacer 1μM ($\pm$) propranolol) α_2 NA receptors with ^{3}H clonidine (0.15-15nM, displacer 10μM (-) NA) and α_1 NA receptors with ^{3}H Prazosin (0.05-2.5nM, displacer 1 μM phentolamine). Results are the means ($\pm$ s.e.m.) of pooled data from 2-4 Scatchard plots repeated on separate occasions. The K_D and R_T values of ^{3}H DHA or ^{3}H clonidine binding (data not shown) were not significantly different at any age between the two strains of mice. The K_D of ^{3}H Prazosin binding was higher in DBA/2 mice at 13-15 and 27-28 days but not at other ages. However, there were fewer binding sites in DBA/2 mice than C57 at all ages (Table 1)

Table 1. ^{3}H Prazosin binding in DBA/2 and C57 mice.

	DBA/2		C57	
age (days)	K_D	R_T	K_D	R_T
8- 9	0.13 $\pm$ 0.01	90 $\pm$ 2***	0.12 $\pm$ 0.01	99 $\pm$ 3
13-15	0.15 $\pm$ 0.01**	136 $\pm$ 4***	0.11 $\pm$ 0.01	154 $\pm$ 3
21-23	0.12 $\pm$ 0.01	152 $\pm$ 4***	0.12 $\pm$ 0.01	181 $\pm$ 5
27-28	0.16 $\pm$ 0.02*	144 $\pm$ 6***	0.12 $\pm$ 0.01	162 $\pm$ 3
40-43	0.12 $\pm$ 0.01	143 $\pm$ 3***	0.12 $\pm$ 0.01	173 $\pm$ 3

K_D = nM R_T = fmoles/mg protein * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. (Student's t test).

The fewer number of ^{3}H Prazosin binding sites in DBA/2 than C57 mice does not show a clear time relationship with the period of maximal susceptibility to AS, and may reflect a permanent strain difference.

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THE INTERACTION OF IMPRAMINE WITH THE 5-HT RE-UPTAKE PROCESS

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Recent evidence suggests that the high affinity {^{3}H}-imipramine (IMI) binding site is closely associated with the neuronal 5-HT uptake mechanism. Thus {^{3}H}-IMI binding is sodium dependent (Briley and Langer, 1981) and is modulated by exogenous 5-HT (Briley et al, 1981). To determine whether the site of 5-HT uptake inhibition by IMI and the {^{3}H}-IMI binding site are identical, we have studied the effects of sodium ions and 5-HT on the 5-HT uptake blocking action of IMI.

The accumulation of 5-HT was studied in purified rat cortical synaptosomes (Wood and Wyllie, 1981). These were incubated for 4 min at 37^o with varying {^{3}H}-5-HT concentrations (30-200nM; 100nM for drug inhibition studies) and various concentrations of inhibitor. Uptake at low sodium concentrations (54mM NaCl, isotonicity maintained using lithium chloride) used a 6 min incubation. To determine active 5-HT accumulation, results were corrected for the energy-independent accumulation of {^{3}H}-5-HT observed in the absence of sodium ions (NaCl substituted by LiCl).

Imipramine was a potent inhibitor of 5-HT uptake (IC_{50} after 4 min = 221nM ± 12, ± SEM, n=4). Imipramine inhibited 5-HT uptake in a complex manner, with low concentrations of IMI reducing the apparent affinity (Ku) and increasing the transport capacity (Vu). IMI at 200nM increased the Ku from 268nM ± 58 to 817nM ± 213 and increased the Vu from 8214 fmoles/4min ± 1246 to 14338 fmoles/4min ± 3211. The Hill coefficient did not deviate from unity in the presence or absence of IMI.

The inhibition of 5-HT uptake by IMI was also modulated by sodium ions. Reduction of the sodium ion concentration to 54mM resulted in a decrease in the inhibitory potency (IC_{50}) of IMI from 249nM ± 20 (± SEM, n=4; 6 min in 120 mM NaCl) to 545 nM ± 51 (± SEM, n=4; 6 min, in 54mM NaCl; $p < 0.05$ paired t-test). Inhibition by unlabelled 5-HT in a low sodium medium was unchanged compared to controls. There was also no change in the slope of the IMI concentraion-inhibition curves with low {Na^+}.

The inhibitory action of imipramine on neuronal 5HT uptake, was affectd by exogenous 5-HT and displayed a marked sodium dependence. This provides further evidence that {^{3}H}-IMI binding and the site of uptake inhibition by IMI, are closely associated with, but topographically distinct from the 5-HT re-uptake site

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CEREBELLAR GLUTAMATERGIC FUNCTION: EFFECTS OF DEPLETION OF GRANULE CELLS AND OF GLIA

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Evidence from studies with granuloprival rodents (McBride et al., 1976a,b; Sandoval & Cotman, 1978; Young et al., 1974) indicates that the transmitter of the cerebellar parallel fibres is likely to be L-glutamate. The rat cerebellum contains a high density of glutamate binding sites (Foster & Roberts, 1978), and incubation of tissue slices with glutamate and other excitatory amino acids resulted in a large accumulation of cyclic GMP (Foster & Roberts, 1980). Application of the depolarising agent protoveratrine (PTV), which releases endogenous transmitters, produced a similar accumulation of cyclic GMP, which could be prevented by inclusion of suitable excitatory amino acid antagonists, implicating the involvement of a glutamate-like substance (Foster & Roberts, 1981). In the present study we have investigated the effects on the cyclic GMP response of depleting the granule cell population by x-irradiation, and of acute destruction of cerebellar glial cells (likely sites of transmitter inactivation) with 6-aminonicotinamide (6-AN).

Irradiation of the rat cerebellum (200 rads on days 8 and 9, and 150 rads on days 11,13 and 15 postnatally) resulted in at least a 69% depletion in the granule cell population (assessed using a camera lucida and digiplot area calculator in serial tissue sections). Concomitant with this, was a small but non-significant reduction of D-^{3}H-aspartate uptake into cerebellar P_2 fractions. The K^+-induced, Ca^{2+} -dependent release of endogenous glutamate from superfused slices was reduced by 60% from 8.47 nmol/mg protein/5 min in control cerebella ($P < 0.02$). The effects of glutamate on cyclic GMP levels in 16 day old rat agranular cerebella did not differ from controls. However, the response to PTV was markedly (45%) attenuated. These data demonstrate that the receptors mediating the endogenous (probably glutamatergic) stimulation of cyclic GMP accumulation are not present on granule cells formed after day 8, and that the transmitter involved in the response emanates in part from the parallel fibre terminals.

To assess the role of glutamate uptake as a factor in regulating its postsynaptic actions, 14 day old rats were injected with 6-AN (8 mg/kg i.p.), which selectively destroys Bergmann glia and other astrocytes (Schaarschmidt, 1975). After two days, the V_{max} for ^{3}H-β-alanine uptake (a glial marker) was reduced by approx 35%. At the same time, the cyclic GMP response to glutamate was enhanced. Thus the glutamate stimulation of cyclic GMP in the cerebellum probably does not occur on glial cells, and the data suggest that these cells may limit the actions of synaptically released glutamate from parallel fibre terminals.

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DISCRIMINATION OF (^{3}H)-SPIPERONE AND (^{3}H)-N,n-PROPYLNORAPOMORPHINE BINDING SITES IN RAT STRIATUM USING PHENOXYBENZAMINE

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The non-specific and irreversible binding of phenoxybenzamine to receptor sites has been used to discriminate between the binding sites occupied by dopamine agonist and antagonist ligands (Hamblin & Creese,1982). We have investigated the effects of *in vitro* incorporation and *in vivo* administration of phenoxybenzamine on the *in vitro* binding of ^{3}H-spiperone and ^{3}H-N,n-propylnorapomorphine to rat striatal membranes.

In *in vitro* competition experiments, phenoxybenzamine (10^{-9}-10^{-4}M) more potently displaced the specific binding of ^{3}H-spiperone (0.5 nM) than the specific binding of ^{3}H-NPA (0.25 nM) (Ki ^{3}H-spiperone 4.9 x 10^{-8} M; ^{3}H-NPA 2.4 x 10^{-7} M). Pre-incubation of striatal membranes with phenoxybenzamine (10^{-8}-10^{-4} M), followed by extensive washing, also inhibited the specific binding of ^{3}H-spiperone (Ki 4.9 x 10^{-8} M) and ^{3}H-NPA (Ki 1.4 x 10^{-7} M). In both competition and pre-incubation experiments total inhibition of the specific binding of ^{3}H-spiperone (as defined by 10^{-5} M (-)-sulpiride) was obtained but 20-40% of specific ^{3}H-NPA (as defined by 10^{-6} M (±)-ADTN) binding remained.

Administration of phenoxybenzamine (2 x 4 mg/kg ip 12 h apart) to rats reduced the number of binding sites (Bmax) and increased the dissociation constant (K_D) for specific ^{3}H-spiperone (0.125-4.0 nM) binding to striatal preparations for up to 24 h following the second drug administration (Table 1). The $t_{\frac{1}{2}}$ for recovery of Bmax and K_D to control levels was approximately 8-9 h. In contrast, only a transient decrease in both Bmax and K_D for specific ^{3}H-NPA binding was observed at 0.5h (Table 1). After 1 h both Bmax and K_D were increased but thereafter values were not different from those obtained for striatal tissue from control animals.

Table 1 Alteration in Bmax (pmoles/g) and KD (nM) for in vitro specific ^{3}H-spiperone and ^{3}H-NPA binding produced by in vivo administration of phenoxybenzamine (2 x 4 mg/kg ip)

Time after phenoxybenzamine	^{3}H-spiperone Bmax	K_D	^{3}H-NPA Bmax	K_D
Control	22.0±2.1	0.16±0.03	16.8±2.3	
0.5 h	5.7±0.9*(26%)	0.38±0.10*(238%)	8.7±0.7*(51%)	0.73±0.8*(67%)
1 h	4.8±0.4*(22%)	0.42±0.05*(263%)	26.3±2.7*(156%)	1.63±0.15*(150%)
2 h	10.3±0.8*(47%)	0.37±0.03*(231%)	17.3±1.9 (102%)	1.11±0.12 (102%)
4 h	12.1±1.1*(55%)	0.27±0.03*(169%)	15.8±1.9 (94%)	1.07±0.14 (98%)
12 h	14.4±0.9*(65%)	0.25±0.02*(156%)	16.4±2.2 (97%)	1.08±0.13 (99%)
24 h	17.8±1.6 (81%)	0.24±0.02*(156%)	15.5±2.0 (92%)	1.05±0.12 (96%)
72 h	19.1±1.6 (98%)	0.21±0.02 (117%)	14.9±1.6 (101%)	1.07±0.10 (103%)

* $p < 0.05$ compared to values from saline-treated control animals. The values in parentheses are percentages of Bmax or K_D compared to values measured in tissue from control animals

Phenoxybenzamine discriminates between the binding sites labelled by ^{3}H-spiperone and ^{3}H-NPA both *in vitro* and *in vivo*, suggesting they are not identical. The rate of recovery of receptor numbers following *in vivo* administration of phenoxybenzamine may provide some index of receptor turnover.

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COMPARISON OF TREATMENT OF RATS FOR 9 MONTHS WITH HALOPERIDOL OR CLOZAPINE ON STRIATAL AND MESOLIMBIC DOPAMINE RECEPTOR FUNCTION

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Clozapine, an atypical antipsychotic agent, does not induce extrapyramidal disturbances in man (Gerlach et al,1974). We report the effects of chronic administration of clozapine on cerebral dopamine function compared to haloperidol.

Male Wistar rats (205±14 g at the start of the experiment) received either haloperidol (1.6-1.7 mg/kg/day; HPL) or clozapine (23-25 mg/kg/day; CLOZ) dissolved in distilled drinking water for up to 9 months. Age-matched control animals (CON) received distilled water alone.

Stereotyped behaviour induced by low doses of apomorphine (0.125 or 0.25 mg/kg sc 15 min previously) was persistently inhibited by one month's HPL administration. The stereotyped response to higher doses of apomorphine (0.5-2.0 mg/kg) was unaffected. Administration of CLOZ for up to 9 months did not alter the stereotyped response to apomorphine (0.125-2.0 mg/kg). The number (Bmax) of striatal ^{3}H-spiperone binding sites was increased throughout the 9 month period by haloperidol treatment (Table 1). In the mesolimbic area Bmax was elevated after 1, 3 and 6 months but not after 9 months drug intake. Biphasic changes in the dissociation constant (K_D) were observed. Bmax for ^{3}H-NPA binding in striatum was decreased after 1 month of haloperidol treatment but was not different from control values after 3 or 6 months and was elevated after 9 months drug intake. K_D showed a similar pattern of change in striatal tissue. In mesolimbic tissue Bmax and K_D were not altered by drug treatment. Administration of clozapine caused an initial decrease in Bmax and K_D for striatal ^{3}H-spiperone binding but thereafter values were not different from those for control animals. Striatal ^{3}H-NPA binding was unaffected by clozapine administration for 9 months. In the mesolimbic area, Bmax was decreased for ^{3}H-spiperone binding after 6 and 9 months and for ^{3}H-NPA binding at 6 months. Otherwise no changes were observed. K_D for both ^{3}H-spiperone and ^{3}H-NPA binding in the striatal and mesolimbic area again showed biphasic change.

Table 1 Bmax (pmoles/g tissue) for ^{3}H-spiperone and ^{3}H-NPA binding

	Striatum			Mesolimbic		
	CON	HPL	CLOZ	CON	HPL	CLOZ
^{3}H-spiperone						
1 month	19.0±0.8	26.0±2.0*	16.4±0.5*	7.5±0.4	14.7±2.6*	6.7±0.5
3 months	18.8±1.5	26.3±1.2*	16.6±1.2	8.4±0.2	11.2±0.6*	8.0±0.8
6 months	18.5±1.0	29.1±2.4*	18.4±1.3	5.4±0.2	6.0±0.3*	4.8±0.2*
9 months	19.7±0.7	31.7±0.8*	18.8±0.5	9.5±0.4	8.2±0.6	5.6±0.6*
^{3}H-NPA						
1 month	16.0±2.3	9.1±1.3*	14.9±2.6	7.3±1.5	8.3±2.3	6.4±1.6
3 months	15.6±2.8	14.8±0.9	15.4±1.0	8.6±1.8	7.9±1.7	6.9±1.3
6 months	16.3±3.4	13.9±3.6	15.0±1.9	8.1±1.7	7.9±2.0	3.2±0.6*
9 months	10.5±0.6	16.3±1.8*	10.6±0.6	10.3±2.2	9.2±1.1	5.9±1.4

* $p < 0.05$ compared with tissue from age-matched control animals

The effects of chronic clozapine administration on brain dopamine function are consistent with lack of change in striatal dopamine receptor function and a selective action on mesolimbic dopamine receptors.

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SELECTIVITY OF DOPAMINE AGONISTS AT D-1 AND D-2 RECEPTORS DOES NOT DEPEND ON LIPID SOLUBILITY

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The selectivity of dopamine antagonists for D-1 and D-2 dopamine receptors in rat brain is partially dependent on lipid solubility (Fleminger et al,1982). Recently, a selective D-1 agonist, SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine) and a selective D-2 agonist, LY 141865 (trans-(+)-4a,5,6,7,8,9-octohydro-5-propyl-2H-pyrazole-(3,4-g)quinoline) have been described (Setler et al, 1978; Tsuruta et al,1981). We have investigated the relationship between the lipid solubility of these and other dopamine agonists and their selectivity at D-1 and D-2 receptors.

Dopamine agonist (10^{-10}-10^{-4}M) were examined for displacement of the specific binding of ^{3}H-spiperone (0.15 nM; as defined using 3 x 10^{-5} M sulpiride) from D-2 sites and ^{3}H-piflutixol (0.3 nM; as defined using 10^{-6} M cis-flupenthixol in the presence of 3 x 10^{-5} M sulpiride) from D-1 sites on rat striatal membranes. The apparent lipid partition coefficients at pH 7.4 (log P') were determined from the partitioning of each drug (approx.10^{-5} M) between 50 mM Tris HCl buffer,pH 7.4 and n-octanol at 20°C.

Bromocritpine,lisuride and apomorphine potently displaced ^{3}H-spiperone (0.15 nM) for rat striatal membranes (Table 1). Dopamine, SKF 38393 and LY 141865 were only weakly active. Only lisuride was reasonably effective in displacing the specific binding of ^{3}H-piflutixol (0.3 nM). The other dopamine agonists were only weakly effective. The ratio of D-2/D-1 activity showed all drugs, with the exception of SKF 38393, to possess a greater ability to displace the specific binding of ^{3}H-spiperone than ^{3}H-piflutixol. SKF 38393 alone was selective for D-1 sites.

Determination of log P' showed apomorphine, lisuride and bromocriptine to be highly lipophilic in comparison to the other dopamine agonists examined (Table 1). There was no correlation between agonist lipid solubility and displacement of specific binding of ^{3}H-spiperone or ^{3}H-piflutixol.

Table 1 Comparison of lipid solubility of dopamine agonists with action at D-1 and D-2 receptors

Drug	log P'	IC_{50} (nM) ^{3}H-spiperone	^{3}H-piflutixol	Ratio D2/D1
Dopamine	-2.58	1,200	190,000	0.006
SKF 38393	0.25	27,000	2,800	9.64
LU 141865	0.30	2,900	>100,000	>0.029
Apomorphine	2.4	63	7,100	0.009
Lisuride	>2	0.94	380	0.002
Bromocriptine	>3.5	1	1,500	0.0007

D-1 and D-2 receptor selectivity of dopamine agonist drugs is unrelated to lipid solubility. This is highlighted by the similar lipid solubility of SKF 38393 and LY 141865 but the markedly different D-2/D-1 receptor selectivity of these compounds.

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PROLONGED TREATMENT WITH THE SPECIFIC 5-HT-UPTAKE-INHIBITOR CITALOPRAM. AMPHETAMINE HYPERSENSITIVITY WITHOUT RECEPTOR CHANGES

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Chronic treatment with antidepressant drugs is followed by changes in several brain receptors, the most commonly reported being a down-regulation of β-adrenergic and serotonin$_2$ (S_2) receptors, whereas dopamine (DA) receptor number does not change (Peroutka & Snyder, 1980). Behavioural supersensitivity to mesolimbic DA receptors have been described (Spyraki & Fibiger, 1981). In this study we have examined the effect of prolonged treatment with the clinically effective specific serotonin (5-HT)-uptake-inhibitor, citalopram (Hyttel, 1982; Lindegaard Pedersen et al., 1982).

Male Wistar rats received citalopram in the diet, 99 μ mol/kg daily, for 13 days. One day after withdrawal the motility response to d-amphetamine (5.4 and 8.2 μmol/kg s.c.) was measured for 90 min in photocell motility cages. In vitro changes of DA D-2, S_2, β_1-adrenergic and α_1-adrenergic receptors were examined using ^{3}H-spiroperidol (striatum), ^{3}H-spiroperidol (cortex), ^{3}H-dihydroalprenolol (cortex) and ^{3}H-prazosin (remaining forebrain) as ligands. B_{max} and K_d were determined by Eadie-Hofstee analysis. Kinetics of 5-HT uptake was measured in forebrain tissue minus cortex and striatum. Content of 5-HT in whole blood as well as plasma and brain levels of citalopram were measured fluorimetrically.

Test model			(mean ± s.e.m.) Control		Citalopram		N
D-amphetamine hyperactivity	5.4 μmol/kg		785	± 215	1847	± 233**	8
(Motility counts/90 min)	8.2 μmol/kg		1711	± 156	2151	± 201*	8
Receptor numbers	(B_{max}, fmol/mg protein)						
DA D-2, striatum	^{3}H-SPI		268	± 17	300	± 22	5
S_2, cortex	^{3}H-SPI		191	± 26	190	± 8	5
β_1, cortex	^{3}H-DHA		217	± 14	226	± 12	5
α_1, whole brain	^{3}H-PRAZ		222	± 20	235	± 9	6

* $p < 0.05$, ** $p < 0.01$ according to van der Waerden's X-test

No changes in B_{max} or K_d-values were observed one day after withdrawal in any of the receptor systems studied (table), but hyperactivity induced by low doses of d-amphetamine was increased in the citalopram group. This effect was seen also 2 h after withdrawal. Similar results were obtained one day after 2 weeks treatment with citalopram (49 μmol/kg, p.o., twice daily). No change was found after acute citalopram (1.6-25 μ mol/kg p.o., 2 h before d-amphetamine). The content of 5-HT in whole blood was decreased by 78 per cent in the citalopram group indicating pronounced uptake-inhibition during treatment. Kinetics of 5-HT-uptake in brain was unchanged one day after withdrawal (V_{max}, K_m and K_i for citalopram). Citalopram was not present in plasma or brain at that time, but 2 h after withdrawal the concentrations were 435 nM in plasma and 4.7 μ mol/kg wet weight brain tissue, the plasma levels being close to those reported clinically (Lindegaard Pedersen et al., 1982).

These results indicate that citalopram differs from other antidepressants regarding receptor regulation, but that the behavioural supersensitivity corresponds to that reported for desipramine.

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FURTHER STUDIES ON THE SELECTIVE ANTAGONISM OF BOMBESIN-INDUCED GROOMING IN RATS BY BENZOMORPHAN ANALGESICS

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Bombesin elicits dose-related excessive grooming when given i.c.v. to rats. This behaviour is attenuated by several benzomorphan analgesics but is essentially unaffected by a wide range of opioids, including alkaloids and peptides (Gmerek & Cowan, 1982a). In the present communication, we provide more information on (i) the interaction between bombesin and benzomorphans, and (ii) the nature of the binding sites that mediate the behaviour.

Male, Sprague Dawley rats (180-200 g) were implanted stereotaxically with stainless steel cannulae in the right lateral cerebral ventricle. Five to seven days later, rats (n=6-8) were place singly in Plexiglas observation boxes (22 cm long; 18 cm wide; 25 cm high) and pretreated s.c. or i.c.v. with vehicle or test compound 15 min before a standard i.c.v. dose of bombesin (0.10 μg in 3-4 μℓ saline). Rat behaviour was then monitored for 5 sec, every 20 sec, for 30 min with the help of a microcomputer (Murray et al, 1981). Results were calculated as percent of the maximum number of grooming episodes (Gmerek & Cowan, 1982b). A 50 values (and 95% confidence limits) were determined by linear regression analysis.

The s.c. A 50 for ethylketocyclazocine (EK) against bombesin-induced grooming was 0.36 (0.33-0.40) mg/kg. This antagonism was stereospecific, with (-)-EK being active. (-)-Naloxone, but not (+)-naloxone, reversed the antagonizing effect of EK (0.50 mg/kg); the A 50 for (-)-naloxone was only 76 (68-86) μg/kg. These findings suggest the involvement of stereospecific opiate receptors.

EK (10 μg) and phenazocine (10 μg) both lost their ability to attenuate the grooming if they were given i.c.v. The benzomorphan-bombesin interaction is clearly site-specific.

Buprenorphine (0.50 mg/kg, s.c.), an oripavine derivative that dissociates very slowly from morphine receptors, had no marked effect on bombesin-induced grooming. This dose of buprenorphine, given 30 min before EK or phenazocine (both at 0.5 mg/kg), did not counter the ability of the benzomorphans to antagonize the grooming. Mu opiate receptors are therefore probably not involved in the interaction between benzomorphans and bombesin. This suggestion is supported by our finding that twice daily s.c. injections (at 0800 h and 1700 h) of morphine (10-100 mg/kg) for 4 days did not influence the ability of EK or phenazocine (both at 0.50 mg/kg) to antagonize the grooming. Tolerance did develop, however, to the antagonizing action of EK after twice daily injections of EK (5-20 mg/kg) for 4 days.

In summary, we have used classic pharmacological approaches for inferring opiate receptor mediation in vivo. Since bombesin-induced grooming in rats is selectively and stereospecifically antagonized by benzomorphan analgesics, our results support the idea of specific binding sites for benzomorphans (e.g. Chang et al, 1981). The model provides a simple, yet novel, behavioural endpoint that may be helpful in the continuing search for better agonists and antagonists from the benzomorphan class.

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SUBSTRATE SPECIFICITY OF A MEMBRANE BOUND PROLINE ENDOPEPTIDASE FROM RAT BRAIN

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A number of neuroactive peptides contain the Pro-X sequence in the biologically active portion of the peptide. These peptides include oxytocin, angiotensin II, and neurotensin, so that in these peptides cleavage of the Pro-X bond by a proline endopeptidase (E.C. 3.4.21.26) present at the synaptic membrane could represent one mechanism of inactivation of the neuroactive peptide. We have used a sensitive fluorimetric assay for proline endopeptidase, with the synthetic substrate benzyloxycarbonyl-glycyl-L-proline-4 methylcoumarinyl-7-amide (Z-Gly-Pro-7AMC), to show that synaptic membranes prepared by discontinuous sucrose gradient separation (Cotman and Taylor, 1972) were enriched in proline endopeptidase activity relative to total rat brain membrane preparations. (Brain membranes 3.3 nmol 7AMC/mg protein/hour, synaptic membranes 30.7 nmol 7AMC/mg protein/hour.) The membrane bound enzyme appears to have similar properties to those described previously for the soluble enzyme (Walter et al, 1980). The ability of a number of neuroactive peptides to act as competitive inhibitors/substrates of the membrane bound proline endopeptidase was examined and Ki values are shown in Table 1.

Table 1. Ki values for the effects of peptides on proline endopeptidase from rat brain membranes

Peptide	Ki	Peptide	Ki
Neurotensin	0.047 µM	Angiotensin II	0.11 µM
Neurotensin 1-8	3.81 µM	Thyrotropin releasing hormone	>100 µM
Neurotensin 1-11	1.13 µM		
Neurotensin 8-13	0.68 µM	Luteinizing hormone releasing hormone	6.0 µM
Xenopsin	0.38 µM		
Bradykinin	0.13 µM	Oxytocin	4.0 µM

Of the peptides tested neurotensin had the highest affinity for the endopeptidase (Ki 47 nM). Comparison of the Ki values for the different portions of the neurotensin sequence showed that the carboxy-terminal portion of the neurotensin sequence has a higher affinity for the enzyme (NT_{8-13} Ki 680 nM), than the amino-terminal sequence (NT_{1-8} Ki 3.81 µM). Apart from degrading neurotensin the rat enzyme will also degrade bradykinin, angiotensin II, oxytocin and luteinizing hormone releasing hormone and in all these cases it will inactivate the peptide. Thyrotropin releasing hormone has only a poor affinity for the endopeptidase so that it seems unlikely that proline endopeptidase would contribute to the inactivation of this peptide (Griffiths et al, 1982). The present results suggest that a synaptically localized proline endopeptidase could contribute to the inactivation of several neuroactive peptides.

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CERTAIN SUBSTANCE P-LIKE IMMUNOREACTIVE DORSAL ROOT GANGLION CELLS POSSESS OPIATE AND/OR HISTAMINE BINDING SITES

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We have previously demonstrated that a proportion of small diameter dorsal root ganglion cell bodies possess specific binding sites for opiates and the H_1 antagonist mepyramine (Ninkovic et al, 1982). In some cases, the same neurons possessed both types of receptor binding sites. We now provide evidence to show that certain of the small neurons with these binding sites also contain the peptide substance P.

Dorsal root ganglia were taken from two rhesus monkeys, perfused with 0.1% paraformaldehyde in phosphate buffer pH7.4. Five-micron thick sections were cut on a cryostat and collected in groups of 3 serial sections. The second serial section was processed for substance P immunohistochemistry using the immunoperoxidase technique while the first section was incubated in ^{3}H-etorphine (to detect opiate binding sites) and every third section in ^{3}H-mepyramine (to detect histamine H_1 receptor sites) before autoradiographic processing (Young & Kuhar, 1979). Of 30 substance P labelled cells randomly chosen for study, examination of adjacent sections revealed that 4 possessed both histamine H_1 and opiate binding sites, 2 had opiate receptors only and 3 only histamine receptors. No convincing evidence for receptor labelling on the remaining 21 cells was seen. While this may suggest that some substance P neurons do not possess opiate or histamine receptors, it seems reasonable to assume that the opiate binding sites on some substance P containing neurons represent the receptor substrate for the observed *in vitro* inhibition of substance P release by opiates in slices of the rat trigeminal nucleus (Jessell & Iversen, 1977).

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EFFECTS OF SP AGONISTS AND PUTATIVE ANTAGONISTS ON THE GUINEA-PIG ISOLATED TRACHEA PREPARATION

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The trachea of the guinea pig is known to contain substance P (SP) (Nilsson et al, 1977) and SP and some of its analogues have been shown to contract the guinea pig tracheal strip in vitro (Mizrahi et al, 1982). We now describe attempts to characterise the SP receptors in the guinea pig trachea by examining the rank orders of potency of C-terminal fragments of SP, the related tachykinins, and SP methyl ester and determining the effects of the putative SP antagonists [D-Pro^{2}-D-Phe^{7}-D-Tryp^{9}]SP (PPT.SP) and [D-Pro^{2}-D-Tryp^{7,9}]SP (PTT.SP) (Folkers et al, 1982). Male guinea pigs (700-900g) were killed by cervical dislocation. The trachea was immediately removed, cleaned and cut as a spiral strip. 15mm lengths were mounted under 1g tension in 1ml organ baths containing Krebs-Henseleit solution at 37^{o}C gassed with 95% O_2/5% CO_2. All contractile responses were measured isometrically.

All of the peptides produced a slow, dose-dependent contraction of the muscle (SP ED_{50} $\sim$ 3 x 10^{-6}M) which returned to baseline over a period of 15-20 mins. Comparison of the relative potencies of the SP C-terminal fragments (SP=1.0) showed the hexa-(34 ± 2) and hepta-(20 ± 3) peptides to be most potent followed by the octa- (2.3 ± .01) and the nona-peptides (0.83 ± .1) which were approximately equipotent with SP and the decapeptide (0.27 ± .03) which was less potent than SP. The activity of the penta-peptide was too low to enable a relative potency to be estimated. This order of potency differs from those described for guinea pig and rat ileum, bovine pupillary sphincter and guinea pig urinary bladder, (Teichberg et al, 1981), but is similar to that described for the isolated superior cervical ganglion (SCG) of the rat, (Brown et al, 1982). However on the trachea the order of potency of the tachykinin peptides was Eledoisin (83 ± 12) > Kassinin (43 ± 7) > Physalaemin (11 ± 1) and is therefore not identical to that reported for the SCG of the rat (Brown et al 1982).

Unlike the guinea pig ileum (Hawcock et al, 1982) atropine, 3 x 10^{-6}M, did not alter the responses produced by any of the C-terminal fragments, tachykinins or SP methyl ester on the trachea.

The putative SP antagonists PPT.SP and PTT.SP 3 x 10^{-6}M both produced a contraction of the trachea which completely desensitized after a single dose. However such desensitization of the trachea had no effect on the response to SP. These contractions were partially (30%) reduced by atropine, 3 x 10^{-6}M. Higher doses of PTT.SP 10^{-5}M also reduced responses to SP.

According to the classification of Iversen et al (1982) the results obtained with the tachykinins may suggest the presence of an SP-E receptor sub-type on the tissue. However substance P methyl ester was approximately equipotent (1.1 ± 0.4) with SP on the trachea suggesting the presence of the SP.P receptor sub-type. Whether both receptor sub types are present remains to be shown.

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COMPARISON BETWEEN LOCOMOTOR ACTIVITY AND ROTATIONAL BEHAVIOUR INDUCED BY DOPAMINE RECEPTOR AGONISTS IN THE RAT

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It has been proposed that rotational behaviour induced in rats with unilateral nigrostriatal lesions by dopamine receptor agonists is dependent upon two components: postural asymmetry, mediated by dopaminergic imbalance between the two striata, and a locomotor drive component arising from stimulation of mesolimbic dopamine receptors, particularly within the nuclei accumbens (Kelly & Moore, 1976; Pycock & Marsden, 1978). However, this concept is difficult to reconcile with findings that dopamine receptor agonists cause vigorous rotation when injected locally into the striatum ipsilateral to the nigrostriatal lesion (Gower & Marriott, 1982).

In the work described here the relation between locomotor activity and rotation responses induced by dopamine agonists has been further examined. Agonists were injected bilaterally, via indwelling cannulae, into the nuclei accumbens (A 9.4; H 0.05, L 1.25, De Groot, 1959) or were injected peripherally. Locomotor activity was measured, after allowing a 1h period of free exploration, in photocell cages similar to those described by Dews (1953). Rats were placed singly into these cages and were used once only. The effects of antagonists (i.p.) on agonist responses were determined at the times of peak effect of agonists and antagonists. The results were compared with results previously obtained in rotational studies in rats with unilateral nigrostriatal lesions (Gower & Marriott, 1982).

In the previous studies, dopamine, SKF 38393 and the 2-aminotetralins A-5,6 DTN A-6,7 DTN and N,N-dipropyl-A-5,6 DTN all caused contralateral rotation on intra-striatal injection, on the lesioned side, in doses of 3 - 30 µg per rat. N,N-dipropyl A-5,6 DTN (0.03 - 0.1 mg/kg), SKF 38393 (2 - 4 mg/kg) and apomorphine (0.1 - 1 mg/kg) induced this response following i.p. injection. Rotation induced by SKF 38393 was inhibited by clozapine (5 - 40 mg/kg i.p.) but not by haloperidol (0.8 mg/kg i.p.), pimozide (8 mg/kg i.p.), yohimbine or prazosin (2 mg/kg i.p.).

In the present study, following intra-accumbens injection, dopamine, SKF 38393, A-5,6 DTN, A-6,7 DTN and N,N-dipropyl A-5,6 DTN (3 - 30 µg per rat) all increased locomotor activity but apomorphine (0.3 - 10 mg/kg i.p.) and SKF38393 (1 - 100 mg/kg i.p.) did not. Locomotor activity induced by intra-accumbens SKF 38393 was antagonised by clozapine (10 mg/kg i.p.), haloperidol (0.2 mg/kg i.p.), pimozide (4 mg/kg i.p.), yohimbine (2 mg/kg i.p.) and prazosin (2 mg/kg i.p.).

The induction of rotation but not locomotor activity by i.p. apomorphine or SKF38393 indicates that rotation is separable from locomotor drive. Furthermore, locomotor activity induced by intra-accumbens SKF 38393 was blocked by drugs which failed to inhibit rotation induced by this agonist. The present results do not therefore support the two-component rotation theory. They indicate that SKF 38393-sensitive receptors in the nuclei accumbens may be α-adrenoceptors or that they may be SKF 38393-sensitive dopamine receptors linked to both α-adrenoceptors and haloperidol-sensitive dopamine receptors in the neuronal chain of events leading to the locomotor response.

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DELAYED HYPERSENSITIVITY REACTIONS IN SUBSTRAINS OF WISTAR RATS

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The genetical basis of the resistance of the NR rat (Hanahoe, Tanner and West, 1973) to the dextran anaphylactoid reaction has been traced to an autosomal recessive gene. NR rats are also resistant to adjuvant induced arthritis (Eisen et al, 1973), but the genetical basis of this resistance and the relative susceptibility of NR animals to other forms of delayed hypersensitivity has not been determined.

Tuck Wistar (dextran reactive,R) rats and NELP Wistar (NR) rats were tested for their abilities to produce the following delayed hypersensitivity reactions: skin reactions to, tuberculin (PPD), ovalbumin in Freund's incomplete adjuvant (OA/FIA), and ovalbumin in Freund's complete adjuvant (OA/FCA); adjuvant induced arthritis (AIA); experimental allergic encephalomyelitis (EAE); and oxazalone skin contact sensitivity (OXAZ). In some experiments R rats were pretreated with cyclophosphamide (100 mg/kg.I.P.) 3 days prior to sensitisation. In other experiments the responses to some of these insults, as well as to intraperitoneal dextran (180mg/kg), were monitored in R x NR interbred rats (F_1) and F_1 x NR backcrossed animals (F_2). The results showed that whereas R rats responded well to each of these inflammatory procedures, NR animals did not develop AIA, nor delayed reactions to OA/FIA or EAE. Moreover, cyclophosphamide pretreated R rats behaved like NR animals in that they did not respond to AIA, OA/FIA or EAE. F_1 animals responded to dextran and AIA, but did not give delayed reactions to OA/FIA and only minor responses to EAE. ABout 50% of F_2 rats responded to dextran (F_2 R) and about 50% were resistant (F_2 NR). However, both F_2 R and F_2 NR reacted well to AIA, but only poorly to EAE and did not give delayed reactions to OA/FIA (Table 1).

Table 1 Delayed hypersensitivity reactions in Wistar rats.

	WISTAR SUBSTRAIN				
	R	NR	F_1	F_2 R	F_2 NR
Dextran	+++	0	+++	+++	0
AIA	+++	0	+++	+++	+++
EAE	+++	0	+	+	+
OA/FIA - Arthus	+++	+++	+++	+++	+++
OA/FIA - Delayed	+++	0	0	0	0
OA/FCA - Arthus	+++	+++	NT	NT	NT
OA/FCA - Delayed	+++	+++	NT	NT	NT
PPD	+++	+++	+++	+++	+++
OXAZ	+++	+++	+++	+++	+++

The severity of each response is expressed on a scale of 0-3 (NT = not tested).

These results clearly demonstrate that although NR rats are resistant to AIA, EAE and OA/FIA, they are capable of developing Arthus reactions and full delayed hypersensitivity reactions to other antigenic systems. They further show that the resistance to AIA is not associated with the genetic resistance of NR rats to dextran and further indicate that the responses of R rats may be rendered similar to those of NR by cyclophosphamide pretreatment.

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PLETHYSMOGRAPHIC DETERMINATION OF HISTAMINE INDEPENDENT LUNG DYSFUNCTION FOLLOWING ANTIGEN PROVOCATION OF CONSCIOUS GUINEA-PIGS

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Although SRS-A is the principal mediator of guinea pig bronchoconstriction in vitro (Hand and Buckner 1979; Burka and Paterson 1981), and its bronchospastic role in anaesthetised guinea pigs has been observed following pharmacological manipulation (Ritchie, Sierechio, Capetola and Rosenthale 1981), no data are available on conscious animal allergic lung dysfunction caused by a non-histamine mediator.

We have developed a modified 'pressure box' technique (Dubois, Bothelho and Comroe 1956) to determine non-histaminic lung function changes following antigen challenge of conscious guinea pigs. Animals were placed in a box with two compartments separated with a neck restrainer. The animals were allowed to breath through a face mask which was sealed from the box by an inflatable rubber membrane. Tidal flow ($\dot{V}$), box pressure(P_B) and nose pressure (P_1) were recorded. At one minute intervals, tidal breathing was occluded at end-expiration and nasal occlusion pressure ($-\Delta Pocc$) measured. Division of $-\Delta Pocc$ by $\dot{V}$, immediately preceeding occlusion, derived respiratory resistance (Rr). Boyles Law was used to derive thoracic gas volume (TGV) from a knowledge of ΔP_B, $-\Delta Pocc$ and box volume. Specific airway conductance (S.Gaw) was derived by Rr ÷ TGV. These measurements were repeated at one minute intervals after a five minute provocation with 1% w/v aerosolised ovalbumen.

Two groups of ten animals were used. Group 1 were pretreated with 10mg/kg i.p. mepyramine, 30 mins. prior to antigen challenge; Group 2 were pretreated with 10mg/kg i.p. mepyramine + 5mg/kg i.p. indomethacin. Following provocation of group 1 animals, TGV increased from 14.4±1.1ml to 25.6± 4.6 ml ($p<0.05$), ten mins. after challenge completion. S.Gaw decreased from 0.083±0.004 to 0.05±0.007 cm H_2O sec. ($p<0.05$). In group 2 animals, antigen provocation increased TGV maximally at 4 mins post challenge (16.3± 0.8 to 46.6± 18.4 ml; $p<0.05$). S. Gaw decreased from 0.062± 0.004 to 0.004± 0.005 cm H_2O sec ($p<0.05$). Interestingly, baseline S.Gaw in the indomethacin pre-treated group was significantly lower than in group 1 animals ($P < 0.005$) whereas TGV and respiratory resistance alone were not significantly different.

In conclusion antigen provocation of mepyraminised guinea pigs produce changes in lung function which could be potentiated by indomethacin, suggestive of an SRS-A component. TGV was the most selective parameter measured and may be indicative of a predominant effect on peripheral airway smooth muscle.

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THE EFFECTS OF SOME ANTI-SECRETORY AGENTS ON THE ACTIVITY OF ISOLATED GASTRIC GLANDS AND MICROSOMAL PREPARATIONS

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Preparations of isolated gastric glands are independent of neuronal, hormonal and vascular control, but respond to a variety of stimulants and inhibitors of acid secretion. Berglindh et al (1980) demonstrated that the site of acid secretion is the secretory canaliculus of the parietal cells. The acid secreting function of these cells can be assessed in vitro by measuring either the accumulation of a weak base, aminopyrine, or by the activity of a K^+-dependent adenosine triphosphatase (H^+,K^+-ATPase) located in parietal cells. Effects of some antisecretory agents have been examined in both these systems.

Stimulation of acid production in isolated glands was measured using the secretagogues dibutyryl cyclic AMP (DBcAMP) and potassium ions. Fundic glands were isolated using the method of Berglindh and Obrink (1976): stomachs of anaesthetised rabbits were perfused with oxygenated, hyperbaric, phosphate-buffered saline at pH 7.3. The mucosa was separated from the muscle, minced and incubated in phosphate-buffered medium at pH 7.4 containing collagenase (1mg ml^{-1}), for 60 minutes at 37°C. Glands were filtered, washed and incubated for 60 minutes in buffered medium at pH 7.4 with ^{14}C-aminopyrine (0.25 to 0.35 µCi ml^{-1} of gland suspension) and the secretory stimulant (0.5mM DBcAMP or 100mM K^+) with or without an antisecretory agent. Cells were then separated by centrifugation, radioactivity measured in both cells and supernatant, and the aminopyrine uptake ratio calculated.

ATPase activity was measured in microsomal material from rabbit stomachs. Fundic mucosa was homogenised in 10 vols 0.25M sucrose/50mM Tris-HCl buffer. The homogenate was centrifuged at 10,000 gav, the supernatant recentrifuged at 100,000 gav., and the final pellet suspended by ultrasonication in 20 vols 50mM Tris-HCl buffer. ATPase activity was measured in the presence of $MgCl_2$ (5mM) and various concentrations of KCl. The reaction was initiated by addition of ATP (2mM) and terminated after 20 min at 37° by addition of 0.8% DDS. The phosphate release was measured spectrophotometrically. Antisecretory compounds were preincubated with the enzyme for 30 min. H^+,K^+-ATPase activity was calculated as the increment due to KCl (20mM).

^{14}C-aminopyrine accumulation was enhanced in a concentration-related manner by both DBcAMP (to a maximum at about 1.0mM) and K^+ (from normal unstimulated uptake at 5.4mM, to 100mM). ATPase activity was enhanced by K^+.

The compounds examined were cimetidine, an H_2-antagonist, timoprazole, an H^+,K^+-ATPase inhibitor (Fellenius et al, 1980) and tiquinamide, a non-selective antisecretory agent (Beattie, et al, 1979). Cimetidine (10^{-6}M to 10^{-3}M) and tiquinamide (10^{-6}M to 10^{-4}M) showed no inhibitory action in either system. Timoprazole (10^{-7}M to 10^{-3}M) showed concentration related inhibition of both aminopyrine uptake and ATPase activity. The effects of cimetidine and timoprazole are consistent with their stated sites of action, while the lack of effect with tiquinamide in these systems indicates an, as yet, undefined action.

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THE ROTATION INDUCED BY DOPAMINE AGONISTS IN 6-OHDA ANIMALS IS MAINLY MEDIATED BY ACTIONS WITHIN THE DENERVATED STRIATUM

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We have found that apomorphine and ergot derivatives like pergolide and bromocriptine induce different patterns of rotational behaviour in 6O-DHA-denervated rats, which may be explained by selective actions on different dopamine receptor populations. Sulpiride, an antagonist devoid of any action on D1 type of receptor inhibits the ergots action in a dose range 1000 times less than the required amount to inhibit apomorphine. Cis-flupenthixol, an antagonist with high selectivity on D1 and D2 types of receptors inhibits equally well both kind of dopamine agonist effects (Understedt and Herrera-Marschitz, 1981, Ungerstedt et al, 1982)

It has been suggested that rotation may be produced by a simultaneous mediation of striatal and limbic-accumbens dopamine systems controlling a postural-deviation and a locomotion component of the turning behaviour respectively (Pycock and Marsden, 1978), therefore, it was important to elucidate whether the found differences may be due to a selective action on striatal or accumbens nuclei.

These present results show that apomorphine, pergolide, bromocriptine, as well as, dopamine induce contralateral turning when injected directly into the striatum of 6OHDA-denervated rats in volumes as low as 1 ul. The responses show both dose dependency together with a regional specificity, the apomorphine action being more effective in rostro-medial striatal regions, whilst the "ergot" action being more effective in the medial-caudal striatal regions. The spreading of the intrastriatal injected substances had been studied by using labelled compounds e.g. 3H-apomorphine, 3H-bromocriptine and 3H-dopamine. It was found that the spreading from the injected region was minimal at all times. Moreover the amount of labelled substances reaching the nucleus accumbens was of negligible importance.

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INHIBITORY EFFECTS OF LOCAL ANAESTHETICS ON CALCIUM CHANNELS IN SMOOTH MUSCLE

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Blockade by some local anaesthetics of the Na^+ channel is ascribed to an action of the cationic form of the inhibitor after entry into the channel from the inside of the cell. In contrast, neutral lipid-soluble anaesthetics such as benzocaine may block channels by gaining access to their site of action from the cell membrane (Hille, 1977 ; Courtney, 1980). Drugs which bind to the Na^+ channel may interact at equivalent sites on the Ca^{++} channel (Romey & Lazdunsky, 1982). The present study compares some of these agents with established Ca^{++}-antagonists.

Taenia preparations from the guinea-pig caecum were set up in Ca^{++}-free Tyrode solution containing K^+ (40 mM) and the isotonic responses to cumulative addition of Ca^{++} (0.1-30 mM) were recorded at 30 min intervals (Spedding, 1982). Cationic local anaesthetics reversibly displaced concentration-response curves to the right in parallel following 20 min preincubation in Ca^{++}-free media. Thus the drugs resembled calcium-antagonists but had lower potency (Table 1).

Table 1. Apparent pA_2 values for antagonism of Ca^{++}-induced contractions

Drug	Apparent pA_2	slope	n
Quinidine	5.2 ± 0.1	-1.10 ± 0.06	9
Quinine	5.1 ± 0.1	-0.97 ± 0.06	9
Lignocaine	4.0 ± 0.1	-1.00 ± 0.13	4
Procaine	3.6 ± 0.1	-1.01 ± 0.07	4
Verapamil	7.8 ± 0.1	-1.11 ± 0.15	5

All the local anaesthetics when tested at equieffective concentrations (dose ratio $\sim$10) relaxed established Ca^{++}-induced contractions rapidly (optimal effect < 15 min) and in this respect resembled calcium-antagonists like verapamil, but not cinnarizine (Spedding, 1982). The neutral local anaesthetic, benzocaine, abolished contractility at 3 mM, although low concentrations were less effective than might be expected (1 mM, dose ratio 9.2±1.9, n=4). This compound had a very rapid onset and offset of action (equilibrium < 5 min) which is compatible with its high mobility in cell membranes (Courtney, 1980).

In contrast to verapamil (Spedding, 1983), the effects of procaine were increased following incubation with sodium salicylate (10 mM for 20 min, $p < 0.05$). This effect is probably due to increased passage of procaine across the cell membrane (McLaughlin, 1973). The effects of procainamide (1mM) were not increased by salicylate.

Veratridine, which activates Na^+ channels, did not increase sensitivity to Ca^{++} at any concentration tested (3-100 µM) and antagonized Ca^{++}-induced contractions at concentrations greater than 10 µM. Veratridine (100 µM) relaxed established contractions induced by Ca^{++} (100µM) slowly (t50 > 20 min).

These findings confirm that agents acting on Na^+ channels may also act on Ca^{++} channels. However, qualitative as well as quantitative differences exist between these drugs and established Ca^{++}-antagonists such as verapamil.

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THE LATENCY AND TIME COURSE OF MUSCARINIC DEPOLARIZATIONS OF MYENTERIC NEURONES

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Muscarinic responses are characterized by a latency of several hundred milliseconds and a duration of several seconds. In myenteric neurones, the effect of muscarinic agonists is closure of membrane potassium channels. In the present experiments we have sought to investigate the reasons for the latency and prolonged time course of this action.

Intracellular recordings were made from neurones in the myenteric plexus of the guinea-pig ileum in vitro. Muscarinic depolarizations were evoked by brief (1 - 5 ms) ionophoretic applications of acetylcholine (ACh) or other agonists. Nicotinic responses to ACh evoked by the same ionophoretic pulse had short latencies and rapid rise times, appropriate to the close proximity of the tip of the ionophoresis electrode to the cell membrane (2 - 5 μm). In most experiments, hexamethonium was used to block these nicotinic responses. The muscarinic depolarization had a latency of 100 - 1000 ms, and a total duration of several seconds. The response amplitude increased with increasing amounts of ACh but the latency and time constant of decay of the response did not change. This time constant of decay of the muscarinic depolarization was the same whether it was induced by ACh, carbachol, methacholine or oxotremorine.

Hyoscince and barium were ejected onto the neurones by brief (30 ms - 1 s) pressure pulses applied to pipettes positioned close (within 50 μm) to the impaled cell. Hyoscine applied immediately after ACh, during the latency and rising phase of the muscarinic response, did not antagonize the action of ACh. The same application of hyoscine made immediately before ACh caused complete antagonism. Muscarinic depolarization induced by perfusion of ACh was also reversed by pressure application of hyoscine; the time course of this reversal was similar to that of the decline of the muscarinic response following a single brief application of ACh by ionophoresis. Barium induced a depolarization which was similar to that caused by ACh in latency, time course, reversal potential, and temperature sensitivity. However, barium depolarizations were not antagonized by hyoscine even when the hyoscine was perfused at a concentration of 1 μM.

It is concluded that neither diffusion of ACh to the receptor nor the kinetics of the ACh-receptor interaction contribute to the latency and prolonged time course of the muscarinic response.

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μ-TYPE OPIATE RECEPTORS ON SINGLE LOCUS COERULEUS NEURONES

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Opiates and opioid peptides inhibit the firing of neurones in the rat locus coeruleus by opening membrane potassium channels (Williams, Egan & North, 1982). The purpose of the present study was to identify the receptor sub-type responsible for this effect.

Intracellular recordings were made from locus coeruleus neurones located in a superfused slice of rat pons. Agonists (normorphine, Met^5-enkephalin (ME), D-Ala^2-D-Leu^5-enkephalin (DADLE), D-Ala^2-NMePhe^4-Met(O)^5-ol-enkephalin (FK 33,824) and ethylketocyclazocine (EKC)) were applied by superfusion or by pressure ejection from a pipette with its tip (diameter 10-15 μm) in the solution above the slice. Superfusion of agonists caused concentration-related hyperpolarization and conductance increase in the concentration ranges of: normorphine (300 nM - 10 μm), FK 33,824 (10 - 300 nM), DADLE (100 nM - 3 μM), EKC (300 nM - 3 μM). Hyperpolarizations of shorter time course (15 s - 2 min) were evoked by pressure application, and dose response curves were constructed by increasing the number of pressure pulses applied to the pipette. While recording from a single neurone, such dose response curves were repeated several times during superfusion with different concentrations of antagonists. The antagonists used were naloxone, β-funaltrexamine (β-FNA), N,N-bisallyl-Tyr-Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH (ICI 154129) and naloxonazine; none of these had any effects on membrane potential or conductance. Successful experiments produced Schild plots which were linear and had slopes close to unity. The pA_2 for naloxone vs normorphine was 8.48 ± 0.19 (mean ± s.e. of mean, n = 3) and for naloxone vs DADLE it was 8.56 ± 0.19 (n = 6). In one cell, naloxone antagonized ME with a pA_2 of 8.3. The δ-antagonist ICI 154129 (Shaw et al, 1982) had a pA_2 of 5.19 ± 0.13 (n = 4) against DADLE and 5.20 ± 0.14 (n = 2) against normorphine. These values are close to those found by Shaw et al (1982) for ICI 154129 at the μ-receptor in the guinea-pig ileum but different to those at the δ-receptor in the mouse vas deferens. β-FNA (200 - 400 nM), which is a selective irreversible μ-receptor antagonist (Ward, Portoghese & Takemori, 1982), prevented responses to normorphine and DADLE which were initially equal in amplitude. Hyperpolarizing responses to noradrenaline, which are also mediated by potassium activation, were not affected. Naloxonazine (Hahn & Pasternak, 1982) had the same effect as β-FNA. These experiments provide no evidence for the existence of δ-receptors on locus coeruleus neurones.

Dynorphin, cyclazocine and β-FNA had no effect on membrane potential in concentrations up to 1 μM. EKC hyperpolarized the neurones, but this was also prevented by β-FNA (400 nM). This suggests that κ-receptors also do not occur on these cells. The hyperpolarizing action of opiates and opioid peptides on locus coeruleus neurones therefore appear to be mediated exclusively by μ-receptors. Interestingly, these receptors have an affinity for naloxone which is similar to that found in ligand binding studies on brain homogenates.

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