A COMPARISON BETWEEN THE EFFECTS OF GABA UPTAKE INHIBITORS ON THE ACTION OF GABA, MUSCIMOL AND ISOGUVACINE

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Isoguvacine and muscimol are very poor substrates for the GABA carrier (Johnson et al, 1978; Krogsgaard-Larsen et al, 1977). Pharmacological studies of the GABA receptor in the olfactory cortex slice preparation using bath applied GABA have been difficult because of rapid GABA uptake (Scholfield, 1981). To circumvent this problem, muscimol has been used instead of GABA on the assumption that muscimol is free from potency distortion due to uptake. To assess this, isoguvacine and muscimol have been tested on the membrane conductance of neurones in the olfactory cortex in the presence of agents which reduce the cellular uptake of GABA.

Slices of guinea-pig olfactory cortex were superfused with Krebs solution and neurones impaled with microelectrodes to record the membrane potential and conductance. GABA agonists were applied to the bathing solution for 4 min periods and their effectiveness was assessed as the agonist concentration required to double resting input conductance of the neurone. The influence by uptake on agonist potency was assessed by: (a) the effect of reducing the Na concentration of the medium to 20 mM by substitution with Mg, (b) adding the GABA uptake inhibitor nipecotic acid and (c) examining the variation in neurone sensitivity at different depths from the cut surface.

Decreasing the Na concentration from 144 to 20 mM increased the effectiveness of GABA (Table 1) but less so for isoguvacine and muscimol. Nipecotic acid (0.5mM) also potentiated the effects of all three agonists, but there was less difference between GABA and muscimol and isoguvacine (Table 1).

TABLE 1. Effect of uptake inhibition on GABA agonist potency

Inhibitor	Agonist	Control mM	Test mM	Ratio	n
Low Na	GABA	0.64±0.17	0.070±0.017	9.1±1.1	7
Low Na	isoguvacine	0.016±0.004	0.006±0.0012	3.3±1.0	3
Low Na	muscimol	0.004±0.0003	0.0024±0.0018	2.4±0.7	3
Nipecotic	GABA	0.74±0.18	0.39±0.13	2.7±0.8	7
Nipecotic	isoguvacine	0.019±0.007	0.013±0.007	1.9±0.3	4
Nipecotic	muscimol	0.0006±0.0002	0.0003±0.00007	2.2±0.17	3

Neurones deep in the slice showed very poor sensitivity to GABA. Regression analysis was applied to plots of cell depth versus the effective GABA concentration. There was a 200-fold variation in GABA potency between the slice surface and 300 μm deep in the slice compared with a three-fold variation for muscimol over the same depth range.

These results indicate that muscimol and isoguvacine are probably subject to some cellular uptake albeit at a much lower rate than GABA and this reduces their potency when applied via the bathing solution.

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$GABA_A$ AND $GABA_B$ BINDING SITES ON CRYOSTAT SECTIONS OF RAT ATRIA

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γ-Aminobutyric acid (GABA) activates two distinct populations of receptors on peripheral unmyelinated nerve fibres. One type is bicuculline-sensitive and its activation depolarizes the nerve fibre (Brown & Marsh, 1978). The other is bicuculline-insensitive and its activation reduces neurotransmitter release (Bowery et al, 1981) possibly by decreasing Ca++ transport (Desarmenien et al, 1982). These 2 receptor types have been designated $GABA_A$ and $GABA_B$ sites respectively (Hill & Bowery, 1981). We now show that they can be detected in cryostat sections of rat atria using radiolabelled ligand binding techniques.

Atria were immersed in 0.1% paraformaldehyde (0.01 M phosphate buffer pH 7.4) for 30 min prior to freezing in isopentane in liquid nitrogen. 10 μm sections were mounted on glass slides (6/slide) and stored at -20°C. For the assay the sections were brought to ambient temperature and allowed to dry before immersion for 45 min in tris-HCl solution (50 mM) containing sucrose (190 mM) with or without 2.5 mM $CaCl_2$ (for $GABA_B$ and $GABA_A$ respectively, Hill & Bowery, 1981). After drying the sections were then incubated at ambient temperature in fresh solution containing ^{3}H-GABA (100 nM, 50 Ci/mmole, Amersham International) or ^{3}H-baclofen (100 nM, 8.8 Ci/mmole, CIBA-Geigy) with or without unlabelled displacing ligand. In $GABA_B$ assays using ^{3}H-GABA, isoguvacine (40 μM) was present to suppress binding to $GABA_A$ sites (Hill & Bowery, 1981). Tritium bound to the slices was measured by liquid scintillation spectrometry.

The amount of ^{3}H-GABA bound under either incubation conditions was reduced by excess of unlabelled $GABA_A$ or $GABA_B$ receptor ligands. The specific portion of ^{3}H-GABA bound in the absence of Ca^{++} ($GABA_A$ sites) determined with 100 μM isoguvacine comprised 59 ± 1.6% of total equivalent to 592 cpm/6 slices (mean ± s.e., n = 10 triplicate comparisons). The specific portion bound in the presence of Ca^{++}($GABA_B$ sites) determined with 100 μM (±)baclofen comprised 52 ± 1.7% of total equivalent to 303 cpm/6 slices (n=12, triplicate comparisons). Bicuculline methobromide (100 μM) completely suppressed binding to $GABA_A$ sites but was without effect on $GABA_B$ site binding. By contrast (±)baclofen (100 μM) produced <15% displacement from $GABA_A$ sites but displaced ^{3}H-GABA from $GABA_B$ sites to the same extent as GABA. Saturable binding of ^{3}H-baclofen to atrial slices could also be detected in accordance with $GABA_B$ site characteristics (Hill & Bowery, 1981).

To test the hypothesis that the binding sites are on nerve fibres atrial slices were prepared from adult rats chronically treated with 6-hydroxydopamine (50 mg/kg i.v. day 1 and day 2, 100 mg/kg i.v. day 8 and day 9, excision day 13). This treatment reduced the uptake of ^{3}H-noradrenaline (0.4 μM) by intact whole atria from 9.22 ± 1.04 to 1.71 ± 0.010 (mean tissue:medium ratio ± s.e. n=6 in each group). The tissue was incubated for 40 min at 32°C in Krebs-Henseleit solution containing ascorbic acid (0.1 mM) and pargyline (3 μM). 6-Hydroxydopamine treatment abolished specific binding to $GABA_A$ and $GABA_B$ sites in 6 out of 11 and 8 out of 10 animals respectively. In the remaining 5 and 2 animals, some saturable binding was detected. In conclusion $GABA_A$ and $GABA_B$ sites in atria can be detected by radiolabelled binding to tissue slices. The binding sites are on adrenergic nerve fibres. We thank the MRC for financial support.

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INHIBITORY INFLUENCE OF GABA ON STRIATAL SEROTONERGIC TRANSMISSION AS EVALUATED BY DIFFERENTIAL PULSE VOLTAMMETRY IN THE RAT

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Biochemical evidence suggests that GABA exerts an inhibitory influence on striatal serotonergic transmission (Scatton et al, 1982 ; Collinge et al, 1982) probably by acting on dorsal raphe cells (Gallager and Aghajanian, 1976). Recently, differential pulse voltammetry with electrochemically treated carbon fiber electrodes has been successfully applied to the in vivo measurement of extracellularly released 5-hydroxyindoleacetic acid (5-HIAA) in the rat brain (Cespuglio et al, 1981). In the present study, we have used this technique to evaluate the effects of acute and repeated treatments with the GABA agonist agent progabide (Kaplan et al, 1980) on serotonergic transmission in the rat striatum.

Experiments were performed on anaesthetized and immobilized male Sprague Dawley rats (250g) artificially ventilated with a mixture of N_2O/O_2 (3:1) containing 0.5% fluothane. Electrochemical measurements were performed by using a working carbon fiber electrode (diameter 7 µm, length 500 µm) treated electrochemically before implantation, and auxiliary platinum and reference Ag/AgCl electrodes (ramp potential-50 to +450 mV). The working electrode was calibrated before and after each experiment in a 10 µM 5-HIAA solution. In vitro, 5-HIAA, serotonin and 5-hydroxytryptophan (5HTP) produced an oxidation peak at +300 mV. In vivo, electrochemical measurement in the rat striatum and cerebral cortex revealed the existence of a major electrochemical signal at the same potential which was stable over a period of at least 5 h.

A first set of experiments was designed to identify the nature of the striatal electrochemical signal detected in vivo. L-5HTP (50 mg/kg ip) and reserpine (5 mg/kg ip) increased the peak height by 250% (at 30 min post injection) and by 50% (at 3h post injection), respectively. In contrast, the monoamine oxidase inhibitor pargyline (75 mg/kg ip), the tryptophan hydroxylase inhibitor α-propyldopacetamide (500 mg/kg ip) and the L-aromatic amino acid decarboxylase inhibitor NSD 1015 (100 mg/kg ip) reduced the electrochemical signal by 46, 32 and 33% respectively at 3 h post injection. A good correlation was observed between the drug-induced alterations of the electrochemical signal and of the striatal concentrations of 5-HIAA (measured by HPLC). These data therefore confirm the view (Cespuglio et al, 1981) that 5-HIAA mainly contributes to the striatal electrochemical signal measured under these experimental conditions.

Acute administration of progabide (400 - 1200 mg/kg ip) failed to affect the peak height (over a period of 4 h). However, after repeated treatment with progabide (400 mg/kg ip, bid for 14 days), 2 h after the last injection, a 32% decrease in the striatal electrochemical signal was observed.

These results indicate that prolonged enhancement of GABA synaptic activity by progabide decreases striatal serotonergic transmission and corroborate results obtained by measuring other biochemical parameters related to striatal 5-HT transmission. This confirms the validity of the in vivo differential pulse voltammetry technique.

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SIMILARITIES AND DIFFERENCES BETWEEN EFFECTS OF LITHIUM AND RESERPINE ON 5-HT-DEPENDENT RESPONSES INDUCED BY FENFLURAMINE IN RATS

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Rats given lithium (Li) for 5-6 days show increased behavioural sensitivity to the 5-HT releasing drug fenfluramine (F), although effects on exploratory behaviour suggest reduced neurogenic release of 5-HT (Harrison-Read, 1981). Abnormal 5-HT storage after Li may reduce stimulus-release coupling (Collard, 1978), but increase 5-HT release by F. Reserpine (R) inhibits granular storage of amines and reduces aminergic transmission, and given acutely, may act like Li on F-induced behaviours.

Adult rats were injected daily with NaCl or LiCl (2 mmol/kg i.p.) for 5 or 6 days. On the last day, rats were given R (2.5 mg/kg i.p. in 0.5% acetic acid), or acetic acid alone, and 2½ or 6 h later, an injection of F (7.5 mg/kg i.p.). The following behaviours were scored 'blind' (0 to 3) after 20,24,28 & 120 min, and the scores totalled: hind-limb splay (Sp), tremor and myoclonus (Tm), reactivity to handling (RH), fore-paw treading (Td), and head & body shakes (HBS). Total scores for Sp & Tm, and for RH & Td were combined as they showed similar drug effects. Data for the 2½ and 6 h R-F intervals were similar, and so were pooled.

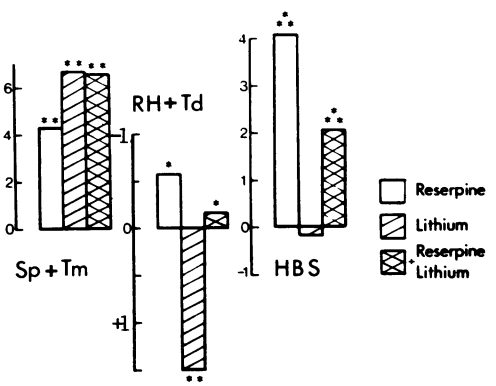

Figure 1 Mean scores of rated behaviour after fenfluramine in reserpine and lithium pretreated rats (difference from saline group), 12 rats per group. Mann-Whitney 2-tailed test: v. lithium group, *P<0.05; v. saline group,**P<0.02

Li & R both increased F-induced Sp & Tm (Figure 1). Although responses were submaximal, effects of Li & R were not additive. Like R, Li may inhibit granular storage of 5-HT within some nerve endings, reducing stimulus-release coupling, but increasing an extragranular pool of 5-HT released by F. Li increased F-induced RH & Td (note inverted scale in Figure 1), and over half these rats subsequently died. R reversed Li effects on RH & Td, and prevented mortality, possibly by disrupting an F-releasable granular store of 5-HT. Li may raise F-induced 5-HT release from this pool. This may reflect enhanced rather than reduced neurogenic release of 5-HT in some pathways, and explain the appearance of spontaneous Td behaviour in rats during long-term lithium treatment (Harrison-Read, 1981). Thus Li may enhance the 5-HT releasing action of F by different mechanisms in at least two functionally distinct 5-HT systems. The effects of Li & R on HBS suggest that this behaviour is influenced in opposite directions by the two systems. Li caused a slight reduction in the HBS score, so the predominant effect of Li on F-induced 5-HT release may be in the system which inhibits HBS. A granular storage pool of 5-HT seems to be involved in this action of Li because R reversed the inhibitory effect of Li on F-induced HBS, and caused a large increase in HBS scores in both NaCl and LiCl rats.

This work was aided by grants from The Wellcome Trust and The Nuffield Foundation. Fenfluramine was donated by Servier Laboratories Ltd.

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'WET-DOG SHAKE' BEHAVIOUR IN RATS MAY REFLECT FUNCTIONALLY-OPPOSED INDOLEAMINERGIC SYSTEMS INVOLVING DIFFERENT 5-HT RECEPTORS

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Paroxysmal head and body shakes (HBS, 'wet-dog shakes') can be induced in rodents by systemic administration of indirect and direct acting indoleaminergic mimetic drugs such as 5-hydroxytryptophan (5-HTP) and tryptamine (T). Inhibition of 5-HTP-induced HBS by indoleamine antagonists in mice is correlated with inhibition of specific (^{3}H)spiroperidol binding in frontal cortex, a measure of affinity for a possible species of 5-HT receptor (5-HT$_2$) (Peroutka & Snyder, 1981). T injected i.p. after MAO inhibition reduces 5-HTP-induced HBS in mice (Jones, 1981). In rats we find that T (4 mg/kg) increases HBS induced by a low dose of L-5-HTP (5 mg/kg), but decreases HBS after a higher dose (10 mg/kg). Other 5-HT-dependent behaviours (tremor, hind-limb splay etc.) are increased by T at both doses of 5-HTP. T may have mixed agonist/antagonist actions at HBS-inducing (5-HT$_2$) receptors, but may also activate other receptors (5-HT$_1$?) which are inhibitory to HBS.

A range of antagonists was given to rats in doses producing partial inhibition of HBS (≈15% relative to a vehicle control group). HBS were scored for 5 min starting 75 min after an injection of L-5-HTP (7.5 mg/kg i.p., 3 h after 2 mg/kg tranylcypromine). The effect of adding T (2 mg/kg i.p.), which on its own caused a slight fall in 5-HTP-induced HBS after 5 min, was increased by some antagonists, and decreased or reversed by others. The % inhibition (+100) of 5-HTP-induced HBS due to antagonists was expressed as a proportion of the % inhibition or disinhibition (+100) of the effect of T on HBS. The negative correlations between mean (± s.e.m.) values listed in Table 1 and the effects of antagonists on radioligand binding at 5-HT$_1$ sites (labelled by (^{3}H)5-HT in cortex or hippocampus) relative to their effects at 5-HT$_2$ sites in cortex suggest that the binding sites represent functional receptors with opposing influences on HBS. Of the drugs tested, only (-)propranolol appears to reduce T effects more than 5-HTP effects, and has affinity for 5-HT$_1$ sites greater than that for 5-HT$_2$ sites. The actions of mianserin on HBS suggest a greater effect on 5-HT$_1$ receptors than is indicated by the binding data, possibly because mianserin, like other antidepressant drugs, influences 5-HT$_1$ receptor 'sensitivity' indirectly (Fillion and Fillion, 1981).

Table 1 Correlations between radioligand binding data and effect on HBS behaviour.

Antagonist	dose	(n)	pIC_{50} (^{3}H)5-HT minus pIC_{50} (^{3}H) spiroperidol†¶ (a)	Inhibition of 5-HTP effect rel. to inhibition of T effect on HBS	
				HBS frequency‡	HBS intensity‡
Haloperidol	0.8 mg/kg	(5)	-1.83	1.66 ± 0.18	1.51 ± 0.11
Cyproheptidine	0.1 mg/kg	(7)	-1.60	1.36 ± 0.09	1.49 ± 0.12
Mianserin	0.2 mg/kg	(6)	-1.50	1.23 ± 0.08	1.05 ± 0.06
Methergoline	0.03 mg/kg	(6)	-0.80	1.22 ± 0.11	1.17 ± 0.12
Methysergide	0.4 mg/kg	(6)	-0.55	1.20 ± 0.17	1.14 ± 0.12
(+)Propranolol	4.0 mg/kg	(6)	+0.09	1.21 ± 0.08	1.37 ± 0.12
(-)Propranolol	4.0 mg/kg	(6)	+1.30	0.97 ± 0.05	0.94 ± 0.02
Correlation coefficient r, versus (a), df=5:				r = -0.87, P≤0.01	r = -0.61, P>0.1

† transformed log(x+3) and ‡ log(x+1) before calculating r; ¶ from published data

This work was aided by grants from The Wellcome Trust and The Nuffield Foundation.

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THE EFFECTS OF SOME CNS STIMULANTS ON DOPAMINE AND 5HT RELEASE FROM RAT STRIATAL SLICES

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Both methylphenidate and pemoline have amphetamine-like stimulant properties inducing increased locomotor activity and stereotyped behaviour (Mueller and Hsaio, 1980; Scheel-Kruger, 1971). The stereotypy induced by amphetamine is associated primarily with dopamine (DA) release (Creese and Iversen, 1975). Methylphenidate and pemoline have been shown to have some DA-releasing activity but neither compound is potent (Dyck et al. 1980; Molina and Orsingher 1981). 5HT may also be involved in amphetamine induced stereotypy but it is not known whether it is involved in the action of methylphenidate or pemoline.

In this study the effects of amphetamine, methylphenidate, and pemoline on DA and 5HT release were compared using a brain-slice superfusion system. Slices were incubated in oxygenated, physiological medium (containing 10^{-5}M pargyline) at 37°C for 10 min after which (^{14}C)-DA or (^{3}H)-5HT was added (final concentration 10^{-6}M - 10^{-7}M) and incubation continued for a further 20 min. Slices were then perfused at a rate of approximately 0.5 ml min^{-1} and after a 30 min washout period 2 min samples of perfusate were collected and their radioactive content estimated. Tissue was exposed to the drugs for 10 min periods. Pemoline (10^{-4}M) was dissolved in dimethyl sulphoxide (DMSO) and diluted with physiological medium to give a 0.01% final dilution of DMSO.

Amphetamine (10^{-4}M - 10^{-7}M) caused a dose-dependent release of both DA and 5HT from striatal slices. Methylphenidate (10^{-3}M - 10^{-5}M) also induced the release of DA and 5HT but a 10-fold higher concentration was required in order to obtain responses of similar magnitude to amphetamine. Pemoline (10^{-4}M) had little effect on DA release and no effect on 5HT release at this concentration. The data demonstrate that methylphenidate, although less potent than amphetamine, induces DA and 5HT release from the striatum. Such an effect could contribute to its behavioural actions. In contrast, pemoline is virtually devoid of DA- and 5HT-releasing activity in this brain region.

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ACTIONS AND INTERACTIONS OF 3-ACETYLPYRIDINE, HARMALINE, NICOTINAMIDE AND 6-AMINONICOTINAMIDE ON BENZODIAZEPINE RECEPTORS

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3-acetylpyridine can produce lesions of rat central nervous system which can be altered by the presence of harmaline and nicotinamide (Llinas et al., 1975). 6-aminonicotinamide can also produce lesions of the CNS (Horita et al, 1981). Both nicotinamide (Skolnick et al, 1979) and harmaline (Rommelspacher et al, 1980) are possible ligands for central benzodiazepine (BZD) receptors and thus we have examined the effects of 3-acetylpyridine alone and in combination with these compounds on benzodiazepine binding.

The binding assay was carried out according to a modified method of Möhler and Okada (1977) by incubating (15 minutes) a 4-times washed and once frozen crude synaptosomal membrane preparation (protein concentration approximately 0.5 mg/ml) of rat cerebral cortex with ^{3}H-diazepam (specific activity 85 Ci/mmol and at a free concentration of 1.5 nM) in a 5 mM TRIS-citrate (pH 7.6) medium at 0°C. Non specific binding was estimated using 1 μM flurazepam and constituted approximately 10% of total binding.

All of the compounds tested displaced specific ^{3}H-diazepam binding in a dose dependent manner, but were all less effective displacers in the presence of GABA (10^{-4}M). IC_{50}'S determined by linear regression analysis of log probit plots (n = 3) over the concentration range 10^{-7}M to 10^{-2}M were (μM, in the absence and presence of GABA:) harmaline, 137 ± 24 and 190 ± 49; 3-acetylpyridine, 4,500 ± 950 and 9,100 ± 2,700; Nicotinamide, 19,000 ± 4,100 and 23,500 ± 8,600; and 6-aminonicotinamide, 158 ± 21 and 151 ± 66 (mean ± s.e. mean; nicotinamide values obtained by extrapolation).

IC_{20}'S were interpolated from the same log probit plots and used to study the displacement activity of combinations of these compounds in the absence and presence of 10^{-4}M GABA (which itself potentiated control binding 55.2 ± 5.2% (n = 5). Displacement activity of all combinations were not significantly different from those predicted from the sum of the activities of each component compound (Wilcoxon tests, n = 5).

We conclude that all of the compounds tested displace ^{3}H-diazepam but it is of great interest that 6-aminonicotinamide is 100-fold more potent than nicotinamide. Finally it appears that significant interactions between the ligands tested do not occur at the BZD receptors studied.

P.F.M. is an M.R.C. Student. We thank the SK & F Foundation and London University CRF for grant support.

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Ro 15-1788 AND CHLORDIAZEPOXIDE EACH REVERSE THE REDUCTION IN EXPLORATION CAUSED BY CGS 8216

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A pyrazoloquinoline, CGS 8216, binds potently to benzodiazepine receptors (Czernik et al, 1982) and reverses the effects of benzodiazepines in a variety of behavioural tests (Bernard et al, 1981). It has also been found to have an anxiogenic action (File & Lister, 1983) and in this respect resembles two other benzodiazepine antagonists, ethyl B-carboline-3-carboxylate (B-CCE) and the imidazodiazepine RO 15-1788 (File et al, 1982a). The purpose of the present study was to investigate the effects of CGS 8216 alone and in combination with chlordiazepoxide (CDP) in a holeboard, which allows an animal's exploration (Head-dipping) to be measured independently of its locomotor activity and rearing.

Male hooded rats weighing approximately 350 g were individually tested for 7.5 min in a holeboard, 30 min after an i.p. injection of either : water/Tween vehicle, 10 or 20 mg/kg CGS 8216 alone, or in combination with CDP (5 mg/kg), or CGS 8216 (10 mg/kg) in combination with RO 15-1788 (10 mg/kg).

CDP (5 mg/kg) reduced locomotor activity ($P < .01$) and rearing ($P < .001$), and these effects were reversed by CGS 8216 (10 & 20 mg/kg, $P < .05$), which alone had no significant effects on these measures. Although both CGS 8216 (10 mg/kg) and CDP (5 mg/kg) individually reduced head-dipping ($P < .05$), in combination they counteracted each other's effects ($P < .05$). The reduction in head-dipping observed after 10 mg/kg CGS 8216 was also counteracted ($P < .05$) by RO 15-1788 (10 mg/kg).

These data make it impossible to consider CGS 8216 as an intrinsically inert compound; however, its intrinsic effects differ from those found with two other benzodiazepine 'antagonists', B-CCE and RO 15-1788. B-CCE causes marked reduction in rearing and only slight reductions in locomotor activity and head-dipping, none of which are reversed by CDP (File & Lister, 1982). RO 15-1788 (4 mg/kg) causes an increase in head-dipping that is potentiated by CDP (File et al, 1982b). Thus none of these three benzodiazepine antagonists can be considered behaviourally inert and each differs from the others in its effects.

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BEHAVIOURAL EFFECTS OF RU24969, A $5HT_1$ RECEPTOR AGONIST, IN THE MOUSE

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Ligand binding studies have identified several sub-types of serotonin (5HT) binding sites. One major division is between sites labelled by ^{3}H-5HT ($5HT_1$ sites) and cortical sites labelled by ^{3}H-spiperone ($5HT_2$ sites). Behavioural changes induced by 5HT or 5HT agonists have generally been associated with the $5HT_2$ sites (Leyson & Laduron, 1977; Peroutka & Snyder, 1979) and the functional significance of the $5HT_1$ sites has not been clearly determined.

RU 24969 (5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1H indole) is a potent, selective $5HT_1$ receptor agonist (Hunt et al, 1981) and its behavioural effects have been compared with the piperazine-containing 5HT agonists MK212, quipazine and trifluoromethyl phenyl piperazine (TMPP).

MK212 (5-20 mg/kg i.p.), quipazine (20-60 mg/kg i.p.) and TMPP (20-40 mg/kg i.p.) each induced a syndrome which included head twitching, reciprocal forepaw treading (myoclonus), hind limb abduction and other minor behaviours in groups of 10 male CD-1 mice (20-25g). Head twitches were counted and myoclonus rated 0-3 (on severity and duration) during a 1 min. observation period every 5 min. for 20 min. and at 30 and 40 min. after administration of the agonist. The total of the mean scores at each time was used to assess each behaviour. Behaviours induced by quipazine and TMPP were less marked than those induced by MK212.

RU 24969 (2.5-40 mg/kg i.p.) induced neither of these behaviours but did evoke marked hyperlocomotion which was assessed between 5 and 15 min. after administration, using Columbus activity meters.

Head twitching and myoclonus induced by MK212 (20 mg/kg i.p.) were antagonised by metergoline (ED_{50}s were respectively 0.65 and 0.84 mg/kg i.p.), mianserine (0.55,0.68 mg/kg i.p.) and BOL 148 (1.4, 8.1 mg/kg i.p.) but $\pm$ propranolol (0.2 - 30 mg/kg i.p.) was inactive. However, hyperlocomotion induced by RU 24969 (10 mg/kg i.p.) was antagonised by $\pm$ propranolol (ED_{50} = 12.3 mg/kg i.p.) as well as by metergoline (ED_{50} = 1.7 mg/kg i.p.), mianserine (ED_{50} = 1.3 mg/kg i.p.) and BOL 148 (ED_{50} = 1.6 mg/kg i.p.) whilst basal locomotion was unaffected by these doses of the antagonists.

Propranolol is active on $5HT_1$ sites (Middlemiss et al, 1977) and this may be the basis of its antagonism of the effect of RU 24969. These data suggest that 24969-induced hyperlocomotion may be mediated via $5HT_1$ receptor activation whilst head twitching and myoclonus are mediated via $5HT_2$ receptor activation.

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THE ATTENUATION OF CLONIDINE-INDUCED HYPOACTIVITY IN MICE BY ADMINISTRATION OF TRH AND ITS BIOLOGICALLY STABLE ANALOGUES

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Peripheral or central administration of TRH (pyroglutamyl-L-histidyl-L-proline-amide) and some of its biologically stable analogues to rodents causes behavioural excitation and locomotor activity and this probably results from dopamine release in specific brain regions such as the n. accumbens (Miyamoto & Nagawa, 1977; Heal et al, 1981a). However TRH also enhances central noradrenaline turnover (Keller et al, 1974) and we have therefore examined the effects of this tripeptide and some of its biologically stable analogues on a behaviour mediated by decreased presynaptic noradrenergic function. Clonidine injection to mice causes hypo-activity, a distinct form of sedation, probably by stimulating presynaptic α_2-adrenoceptors and inhibiting central noradrenaline release. This was rated from 0-3 according to severity, using 5 behavioural parameters (passivity, tactile responsiveness, posture, gait and body sag) and the method is fully described by Heal et al (1981b). Injection of 0.1 - 10 mg/kg of TRH or its biologically stable analogues CG3509 (orotyl-L-histidyl-L-prolineamide; Grunenthal GmbH), CG3703 (6-methyl-5-oxo-thiomorpholinyl-3-carbonyl-L-histidyl-L-prolineamide; Grunenthal GmbH) and RX77368 (pyroglutamyl-L-histidyl-L-3,3'-dimethyl-prolineamide; Reckitt & Colman) 10 min before clonidine (1 mg/kg) all produced a dose-dependent attenu-ation of clonidine-induced hypoactivity responses (CG3703 > RX77368 > CG3509 >> TRH). In contrast, injection of 2 TRH metabolites, 'TRH acid' (pyroglutamyl-L-histidyl-L-proline; 10 mg/kg) and histidyl-proline diketopiperazine (10 mg/kg), 10 min before clonidine (1 mg/kg), did not alter the hypoactivity responses and this agrees with their reported ineffectiveness in other behavioural tests (Heal et al, 1981a). When TRH (10 mg/kg) or its analogues (1 mg/kg) were injected 60 min before clonidine (1 mg/kg), CG3509, CG3703 and RX77368 still potently reduced the hypoactivity responses, while TRH was now without effect. The attenuation by TRH and its analogues was probably not due to the behavioural excitation and locomotor activity caused by central dopamine release because at a dose of 1 mg/kg only CG3703 and RX77368 produced these behavioural effects, while all of the compounds significantly reduced clonidine-induced responses. Further-more, apomorphine (5 mg/kg), which markedly increased mouse locomotor activity, did not alter the hypoactivity produced by clonidine (1 mg/kg) 10 min later. In conclusion clonidine-induced hypoactivity in mice was attenuated by pretreatment with TRH and its biologically stable analogues. The prolonged action and potency of CG3509, CG3703 and RX77368 is probably a reflection of their resistance to enzymic degradation. The attenuation is almost certainly not mediated by the behavioural effects of central dopamine release and suggests that TRH and its analogues may also release noradrenaline in murine brain.

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CRITERIA FOR DETECTING LOCOMOTOR STIMULATION TO LOW DOSE NEUROLEPTIC TREATMENT IN THE RAT

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Although neuroleptic agents are reported to both reduce and, at low dosage, enhance motor activity of rodents, the consistency of the motor depression contrasts with the irregularity and/or non-dose related stimulation of locomotion (Puech et al., 1981). The present study in the rat investigates whether the technique of measurement and the selection of animals according to basal locomotor responsiveness may be important factors for allowing the detection of locomotor stimulation to low-dose neuroleptic treatment.

Sprague-Dawley rats were either i) non-selected or ii) selected as high (HA), moderate (MA), low (LA) activity or non-responders (NA) to the hyperactivity effect of 0.05 mg/kg s.c. (-)N-n-propylnorapomorphine, (-)NPA, measured in photocell cages (see Costall et al., 1982). The locomotor activity responses of these animals to low and high doses of (-)sulpiride (0.625-40 mg/kg i.p.) and haloperidol (0.02-0.32 mg/kg i.p.) were assessed using i) individual photocells and ii) treadwheels (Costall et al., 1972).

Firstly, it is emphasised that although animals were selected according to their responses to (-)NPA, they could not be differentiated by their spontaneous responding in treadwheels or photocell cages even though, as described below, the spontaneous locomotion of the selected groups was clearly differentiated by the locomotor stimulatory effects of low doses of the neuroleptics. This selection, however, was not evidenced by the photocell measure (all values for (-)sulpiride and haloperidol in the range 71-91 counts/60 min.), although locomotor depression occurred at 40 mg/kg (-)sulpiride (31-39 counts/60 min., $P < 0.001$) and 0.32 mg/kg haloperidol (28-37 counts/ 60 min., $P < 0.001$). This is in marked contrast with data obtained using treadwheels which not only revealed the stimulatory properties for lower doses of both neuroleptics in non-selected (2.5-10 mg/kg (-)sulpiride, 0.04-0.16 mg/kg haloperidol) and selected (10 mg/kg (-)sulpiride, 0.04 mg/kg haloperidol) rats, but also differences in effects of the two neuroleptics. Thus, sulpiride selectively enhanced the locomotion of LA rats (460% of control, $P < 0.001$) whilst haloperidol enhanced the responding of both LA and MA rats (295 and 265% of control respectively, $P < 0.001$). Further, low dose sulpiride, but not haloperidol, depressed the locomotion of HA rats (16% of control, $P < 0.001$). Responses of NA rats were unaffected. The treadwheel technique also detected locomotor depression by larger doses of sulpiride (40 mg/kg) and haloperidol (0.32 mg/kg) which, in contrast to the effects of lower doses, were non-selective in that depression occurred in all groups, HA, MA, LA and NA.

Thus, whilst neuroleptic depression of locomotor activity could be detected regardless of animal selection and measurement technique, locomotor stimulation by low dose neuroleptic could only be detected using treadwheels, and could differentially occur in carefully selected animals. Generally, animals less responsive to the locomotor stimulant action of the dopamine agonist (-)NPA were more sensitive to locomotor stimulation by low doses of neuroleptic agent.

This work was supported by the Wellcome Trust.

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ALTERATIONS IN CEREBRAL PEPTIDE LEVELS FOLLOWING 18 MONTHS ADMINISTRATION OF CIS-FLUPENTHIXOL TO RATS

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Administration of neuroleptic drugs to rodents alters putative peptide transmitters in basal ganglia. Repeated, but not acute, administration of haloperidol for a few weeks increased met-enkephalin levels in striatum but decreased substance P concentrations in substantia nigra (Hong et al,1978a,b). We have previously reported that continucus chronic neuroleptic administration to rats reverses initial cerebral dopamine receptor blockade so as to produce functional dopamine receptor supersensitivity (Murugaiah et al,1982). We report now the effect of such treatment on levels of met-enkephalin, substance P and cholecystokinin (CCK-8) in basal ganglia.

Rats received either cis-flupenthixol (0.8-1.2 mg/kg/day) or trans-flupenthixol (0.9-1.2 mg/kg/day) for 18 months in drinking water. At this time,animals receiving cis-, but not trans-, flupenthixol showed an enhanced stereotyped response to administration of apomorphine hydrochloride (0.125-2.0 mg/kg sc) and increased numbers of specific ^{3}H-spiperone (0.125-40 nM) binding sites in striatal preparations.

Administration of cis-flupenthixol (0.8-1.2 mg/kg/day) for 18 months resulted in a decrease in striatal (ST) but not substantia nigra (SN) met-enkephalin concentrations when compared to age-matched control animals (Table 1). Administration of trans-flupenthixol (0.9-1.2 mg/kg/day) increased SN met-enkephalin levels and caused a borderline increase in ST. Administration of either cis- or trans-flupenthixol for 18 months caused no change in SN substance P levels (Table 1). Following 18 months administration of cis-, but not trans-, flupenthixol ST CCK-8 levels were elevated. Neither cis- nor trans-flupenthixol administration altered CCK-8 concentrations in globus pallidus (GP)

Table 1 Peptide concentration in basal ganglia following 18 months cis- or trans-flupenthixol (FP) administration

Drug Group	pmole/g tissue				
	met-enkephalin		Substance P	CCK-8	
	ST	SN	SN	ST	GP
Control	633±59	65±3	741±72	41±5	39±3
cis-FP	443±22*	61±4	707±74	58±6*	39±5
trans-FP	830±80	87±5*	797±39	46±5	38±5

n = 5-16. * $p < 0.05$ compared to control animals

Chronic cis-flupenthixol administration causes tolerance to or reversal of the short term changes in peptide levels in basal ganglia induced by neuroleptic drugs. After 18 months drug intake selective alterations in striatal peptide content occur at a time when functional striatal dopamine receptor supersensitivity is apparent. Why trans-flupenthixol should cause an increase in met-enkephalin levels is not clear.

PDM is an MRC Scholar

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QUANTITATIVE DETERMINATION OF DOPAMINE ANTAGONIST POTENCIES AGAINST DOPAMINE INHIBITORY RESPONSES ON HELIX CENTRAL NEURONES

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Ergometrine is a potent antagonist of the dopamine inhibitory response on Helix neurones (Walker et al, 1968) and a dopamine agonist in the mammalian brain (Woodruff, 1982). In the present study the potency of ergometrine as an antagonist has been compared with that of bromocriptine, another ergot alkaloid which stimulates central dopamine receptors. The potency of the benzamide sulpiride, a potent and specific dopamine antagonist on mammalian neurones (Woodruff, 1982), has also been determined.

Intracellular microelectrode recordings were made from identified central neurones of the snail, Helix aspersa. The ganglionic mass was prepared as described by Walker (1968) and mounted on a glass slide in a bath of 5ml vol. and viewed with an Olympus stereozoom microscope. Neuronal activity was amplified and displayed using conventional electrophysiological techniques and permanent records made using a Hewlett Packard pen recorder. Antagonists were added to the bath in 1ml of Ringer and the dopamine applied to the cell soma ionophoretically via a second micropipette containing a 0.5M solution at pH 4.0. Log dose response curves to dopamine were constructed both in the absence and presence of antagonist under test and pA_2 values calculated. The pA_2 was expressed as Mean ± s.e. mean.

Table 1 pA_2 values for dopamine antagonists on Helix neurones

Antagonist	pA_2	n
(±) Sulpiride	5.8 ± 0.1	5
(+) Sulpiride	4.7 ± 0.1	5
(-) Sulpiride	6.0 ± 0.1	5
Ergometrine	12.5 ± 0.1	5
Bromocriptine	6.2 ± 0.2	5

From table 1 it can be seen that ergometrine is six orders of magnitude more potent as a dopamine antagonist than either sulpiride or bromocriptine which are approximately equipotent. In 4/5 cells the antagonism produced by bromocriptine was irreversible which is similar to the observation of Marek & Roth (1980), who postulated that bromocriptine may act as an irreversible or noncompetitive agonist at presynaptic dopamine receptors. The (-) enantiomer of sulpiride is 20 times more potent than the (+) enantiomer which agrees well with the data from mammalian studies and this difference is statistically significant with a P value of $< 0.1\%$ from the 'Student's ' t test. However the fact that bromocriptine and sulpiride have similar potencies in terms of antagonising the dopamine inhibitory response in Helix, would suggest that this receptor does not fit into the D-1 and D-2 classification as proposed by Kebabian & Calne (1979).

We are grateful to Southampton University for financial support.

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THE EFFECT OF THREE NOVEL DOPAMINE RECEPTOR AGONISTS ON NEURONAL ACTIVITY IN THE RAT SUBSTANTIA NIGRA

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The dopamine receptor agonist, piribedil, inhibits the firing of neurones in the zona compacta of the substantia nigra (SNC) when administered peripherally (Walters et al 1975). We have compared the effects of S3608 (4-(5-coumaranyl-methyl)-1-(2-thiazolyl) piperazine HCl), a structural analogue of piribedil, and of two N,N-diphenethylamine derivatives, RU 24213 (N-n-propyl-N-phenylethyl-p-(3-hydroxyphenyl) ethylamine HCl) and RU 24926 (N-n-propyl-di-β-(3-hydroxyphenyl)-ethylamine HCl) with those of piribedil on zona compacta cells. The above compounds are all inactive on the striatal dopamine sensitive adenylate cyclase (Miller and Iversen, 1974; Euvrard et al, 1980) but have the characteristics of potent dopamine receptor agonists when tested as displacers of ^{3}H-sulpiride binding in striatal homogenates (Woodruff and Freedman, 1982).

Male Wistar rats (150-180 g) were anaesthetised with chloral hydrate (350 mg/kg). The femoral vein was cannulated for drug administration. The animals were placed in a stereotaxic frame and anaesthesia was maintained with a 1-2% halothane/O_2 mixture. Conventional extracellular recording techniques were used to record from neurones in the SNC, using single barrel glass microelectrodes filled with 2 M NaCl containing 2% pontamine sky blue. The SNC cells were identified by their electrophysiological properties and their sensitivity to intravenous apomorphine (Bunney et al, 1973; Guyenet et al, 1978). The position of the recording electrode was later verified histologically. Haloperidol was dissolved in lactic acid, 1% in 0.9% NaCl. Other drugs were dissolved in 0.9% NaCl, either alone (fluphenazine), or containing 8 mM tartaric acid. All drugs were injected intravenously.

Apomorphine (1-50 µg/kg) caused a dose-related depression of firing of all SNC neurones on which it was tested. Piribedil and S3608 were tested on 13 and 28 cells respectively. Both drugs mimicked the effects of apomorphine and caused a dose-related depression of firing over the dose range 5-50 µg/kg. The depressant effect of S3608 was blocked by fluphenazine, 50 µg/kg (6 out of 7 cells) and by haloperidol, 100 µg/kg (2 out of 2 cells). RU 24213 and RU 24926, each at 50 µg/kg, were tested on 14 and 18 cells respectively. In all cases they caused depression of the firing rate of SNC neurones. Dose dependent responses to these drugs were not obtained, since on repeated administration there was an attenuation of the response, both to the diphenethylamine derivatives and to apomorphine. RU 24213 and RU 24926 both caused inhibition of firing which lasted for up to one hour. The recovery from a single dose was biphasic with a fast initial phase recovering to about 50% of the pre-drug firing rate, followed by a slow second phase of recovery.

In conclusion S 3608 has a similar potency and duration of action to piribedil. RU 24213 and RU 24926 appear to be long-acting dopamine agonists but exhibit tachyphylaxis which could be due to a partial agonist activity.

We thank Dr Yvonne Evrard and Professor J.R. Boissier for gifts of drugs.

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EFFECT OF INCUBATION TEMPERATURE ON (^{3}H)-SPIPERONE BINDING TO SOLUBILISED NEUROTRANSMITTER RECEPTORS

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Although much useful information may be obtained on dopamine receptors from studies in the membrane-bound state, a complete understanding will not be achieved until the relevant receptor proteins have been isolated and characterised. A prerequisite for this is solubilisation of active receptors using detergents and we have shown that lysophosphatidylcholine (LPC) is useful for solubilisation of active D_2 dopamine receptors from bovine brain (assayed by [^{3}H]spiperone binding) (Withy et al., 1981). If LPC is used for solubilisation of bovine caudate nucleus, providing proteinase inhibitors are present during solubilisation, a preparation is obtained containing active solubilised D_2 receptors and a good correlation is observed between the ligand-binding properties of the solubilised D_2 receptors and the membrane-bound species.

Although this preparation is useful for studying D_2 receptors the specific binding of [^{3}H]spiperone constitutes only about 40% of the total binding. If, however, the ligand-binding assays are run at 25°C rather than 4°C as was used for the previous experiments the data are improved considerably. Under these conditions, specific [^{3}H]spiperone binding (defined as the difference in binding between assays containing 1 μM (-) and (+)-butaclamol) constitutes 80% of the total binding. Specific [^{3}H]spiperone binding is displaced with a high affinity by (+)-butaclamol and non-radioactive spiperone whereas (-)-butaclamol is virtually inactive. The selective dopaminergic antagonist domperidone gives a biphasic displacement curve indicating a major class of higher affinity sites and a minor class of lower affinity sites. The selective serotonergic antagonist mianserin displaces a minor portion of the binding with high affinity. Thus specific [^{3}H]spiperone binding under these conditions may be to dopaminergic and serotonergic sites.

In conclusion, therefore, if [^{3}H]spiperone binding is used for assaying solubilised receptors the use of a higher temperature for performing the ligand-binding assays enables specific [^{3}H]spiperone binding to form the major component of the observed binding and allows [^{3}H]spiperone binding to dopaminergic and serotonergic receptors to be detected.

This research was supported by a grant from The Wellcome Trust.

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(^{3}H)-SPIROPERIDOL BINDING IN CORPUS STRIATUM OF ADULT RATS MADE THYROID DEFICIENT WITH PROPYLTHIOURACIL

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Consistent with morphological evidence of reduced axonal and dendritic arborization, and reduced numbers of spines per dendrite, in corpus striatum of neonatal thyroid deficient rats (Lu & Brown, 1977) striatal choline acetyltransferase (ChAT) choline transport (Kalaria et al, 1981a) and spiroperidol-binding (Timiras & Vaccari, 1981) were reduced, suggesting the development of striatal cholinergic neurones is impaired. However the number of spines per dendrite in visual cortex was also reduced after thyroidectomy of adult rats (6 or 17 week-olds, Ruiz-Marcos et al, 1980). We therefore investigated the effect in the striatum of thyroid deficiency induced in adult rats.

Male Wistar rats (6 week-olds, 110-150 g, groups of 5 or 6) were given propylthiouracil (PTU, 0.3%, w/w in 41B meal) ad lib, age matched controls 41B meal only, for 8-10 weeks. ChAT and tyrosine hydroxylase (TOH) were assayed in whole homogenates of striatum, and in some experiments, of adrenals. (+)Butaclamol (2 µM)-sensitive (specific) binding of ^{3}H-spiroperidol (4 nM) was assayed in washed crude membrane preparations of striatum (Fakouhi et al, 1982).

Preliminary experiments had confirmed that PTU-treatment caused thyroid deficiency in adult rats (Kalaria et al, 1981b). Specific binding of spiroperidol in the striatum was reduced by 27-31% in PTU-treatments. ChAT, TOH (Table 1) glutamate decarboxylase, protein concentration, muscimol and QNB (specific) bindings were unaffected. Adrenal TOH activity was increased (1.47 ± 0.1 to 2.15 ± 0.15 µmol/h/g tissue, n = 5+5, P < 0.001) compatible with previous results (Roy et al, 1977) but ChAT activity was unchanged (0.70 ± 0.08 µmol/h/g tissue, to 0.60 ± 0.09, n = 5+5, P > 0.4).

Table 1.

	Assays* of corpus striatum from:	
	PTU-treated rats	age matched controls
^{3}H-spiroperidol binding†	14.54 (0.58)**	20.49 (0.55)
	12.40 (0.58)	12.41 (0.67)
TOH activity‡	0.59 (0.02)	0.60 (0.02)
ChAT activity‡	16.16 (0.50)	17.16 (0.83)

* 10 PTU-treated, 10 control, figures in parentheses, standard errors; † pmol/g tissue, upper figures specific binding, lower figures non-specific; ‡ µmol/h/g tissue; ** P < 0.001, Student's two tailed t-test, variances homogenous.

Nigro-striatal dopaminergic nerve terminals form synapses largely with dendrites, and their spines, of intrinsic striatal neurones (Bak et al, 1975) widely accepted as cholinergic neurones. Striatal TOH activity (Table 1) suggests these terminals are unaffected by thyroid deficiency induced in adult rats. The specific spiroperidol binding reflects reduced DA-receptor binding capacity. The formation of dendritic spines after 6 weeks of age may be impaired or final dendritic morphology altered, both effects observed in the visual cortex (Ruiz-Marcos et al, 1980). However ChAT activity (Table 1) suggests axonal arborization of the striatal cholinergic neurones remains unimpaired.

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AN INVESTIGATION INTO DOPAMINERGIC INFLUENCES ON SEPTAL DRIVING OF HIPPOCAMPAL THETA RHYTHM

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Stimulation of the medial septal nucleus induces rhythmic slow wave activity in the hippocampus (hippocampal theta; RSA) (Stumpf, 1965). There is a characteristic relationship between the threshold current required for driving theta and the frequency of stimulation; the curve thus generated over the range of 5-10 Hz has been shown to have a minimum threshold at 7.7 Hz (James et al., 1977). Recently, evidence has been provided indicating that there are dopaminergic afferents to the hippocampus originating from the A9 and A10 areas (Scatton et al., 1980). In this study we have investigated the possibility of a dopaminergic influence on medial septal theta driving.

The majority of experiments were conducted under urethane anaesthesia (1.2 - 1.5 g/kg i.p.). However, 5 animals were anaesthetized with chloral hydrate (400 mg/kg i.p.) and a further 5 were prepared as encéphale isolé under halothane anaesthesia. These latter animals were allowed a period of at least one hour for dispersal of the anaesthetic prior to experimentation. All wound edges and pressure points were routinely infiltrated with 1% lignocaine. Drugs were administered i.v. via a femoral cannula. For each drug treatment, data was collected from five animals. Threshold values for theta driving by septal stimulation were determined at frequencies of 5.5, 6.5, 7.7, 8.5 and 9.5 Hz. Five determinations were made at each frequency in random order.

The curve of threshold current for septal driving of hippocampal theta rhythm as a function of frequency exhibits a characteristic 'vee' shape, with its minimum at 7.7 Hz. Apomorphine was seen to have a biphasic effect on this curve. At low doses (0.05 and 0.1 mg/kg) the threshold current at 7.7 Hz was raised, thereby abolishing the 'dip'. However, at higher doses (0.5 or 1.0 mg/kg) the septal driving curve remained unchanged. The same biphasic effect was observed when apomorphine (0.1 and 1.0 mg/kg) was tested on either animals under chloral hydrate anaesthesia or on encéphale isolé subjects, suggesting that the anaesthetic agent does not interfere with the drug effect. Haloperidol (0.2 mg/kg) raised the driving threshold at 7.7 Hz again abolishing the dip, but a lower dose (0.1 mg/kg) had no effect. Trans-flupenthixol (0.25 and 2.5 mg/kg) had no effect on theta driving thresholds. The active isomer, cis-flupenthixol, on the other hand, at a low dose (0.25 mg/kg) tended to lower the thresholds at 6.5 and 8.5 Hz, while the higher dose (2.5 mg/kg) had no effect.

These preliminary results show that drugs acting on dopaminergic systems affect the thresholds for hippocampal theta driving. This therefore supports the concept of a dopaminergic influence on the mechanism involved. However the nature of such an influence requires further investigation.

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INTRALAMINAR THALAMIC MODULATION OF DOPAMINERGIC FUNCTION IN THE RAT CAUDATE-PUTAMEN COMPLEX

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Aside from the major inputs from the cerebral cortex, raphe nuclei and mesencephalic dopamine (DA) neurones, the rat caudate-putamen complex (CP) also receives fibres from thalamic intralaminar nuclear groups, including the parafascicular nucleus (PF; Veening et al, 1980). Whilst lesions of the PF have been reported to enhance haloperidol-induced catalepsy in rats (Ahlenius, 1978), the role of this nucleus in the control of motor behaviour is not established. This investigation has studied the time-dependence of changes in spontaneous locomotor activity arising from bilateral PF lesions in the rat and has further assessed lesion-induced alterations in DA receptor function in the CP using (^{3}H)-spiperone binding techniques.

Male Porton rats (180-200 g) were anaesthetised with halothane (1.5% in O_2) and sham or temperature-controlled radiofrequency lesions (51^{o}C, 1 min) stereotaxically placed in both PF nuclei. More rostral intralaminar cell groups (e.g. central lateral nucleus) were also lesioned in an attempt to gain maximum destruction of thalamic input to the CP (Veening et al, 1980). Rats were killed at 1,2,4 and 10 week intervals and CP tissue lying rostral to the anterior commissure taken for receptor binding assay. Care was taken to exclude cortical, septal and accumbens tissue from the samples. Specific (^{3}H)-spiperone binding (0.5-2000 pM) was defined as that displaced by 1μM (+)-butaclamol. The remainder of the brain was taken for histological verification of the lesion sites. One day prior to sacrifice, spontaneous locomotor activity was assessed by 10 min summations of line crossings in an open field.

Sham-operated controls exhibited saturable (^{3}H)-spiperone binding to CP membranes (K_d~80 pM; B_{max} 474 $\pm$ 30 fmol mg^{-1} protein). At 1 and 2 weeks, PF-lesioned rats exhibited significantly enhanced locomotor activity compared with sham-operated controls. At these times, increases in specific (^{3}H)-spiperone binding capacity were apparent (+20% at both intervals). At 4 weeks, however, lesioned animals did not differ in their activity from controls and displayed a decreased capacity for (^{3}H)-spiperone binding (-13.0%, $p<0.05$). At 10 weeks, B_{max} values were again significantly increased in PF-lesioned rats (+15.6%, $p<0.05$). At no time in these studies was a state of catalepsy detected in the lesioned rats (cf. Ahlenius, 1978).

The results pose an interesting relationship regarding the role of the PF nucleus in the modulation of CP DA receptor function and its possible importance in the control of motor activity.

I.C.K. is supported by the Wellcome Trust.

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EFFECT OF THYROID STATUS ON CENTRAL ADRENOCEPTOR FUNCTION IN THE ADULT RAT

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Besides having marked effects on central dopaminergic and serotonergic neurones in the adult rat CNS (Atterwill, 1981) thyroid hormone also appears to play an important role in regulating central noradrenergic function. Thus, there alterations in noradrenaline (NA) turnover in hyperthyroid animals (see Strömbom et al, 1977) and changes in postsynaptic function have also been demonstrated. Hyperthyroid animals show increased behavioural responses to receptor activation by clonidine or NA (Strombom et al, 1977; Emlen et al, 1972) and recently Gross et al (1980, 1981) have reported altered densities of certain adrenoceptors in cortical membranes from hypothyroid adult rats. We now wished to extend our knowledge of central adrenoceptor function in both hyper- and hypothyroid rats by monitoring clonidine-induced hypoactivity (presynaptic α2 receptor function: see Heal et al, 1981) and [^{3}H]Dihydroalprenolol (DHA) binding (postsynaptic β receptors).

Male rats (140-160g) were made hyperthyroid with 14 x daily s.c. injections of T_3 (100μg/kg: L-triiodothyronine). Hypothyroidism was induced orally (14 days) with 6-propyl-2-thiouracil (PTU: 50mg/rat). 24 hours after the last treatment, clonidine-induced hypoactivity (0.5mg/kg clonidine i.p) was measured as described by Heal et al (1981). Specific [^{3}H]DHA binding was measured essentially as described by Gross et al (1980) but using isoprenaline (100μM) to displace specific bound ligand.

In hyperthyroid adult rats the clonidine-induced sedative effect was significantly enhanced whereas in the hypothyroid animals it was attenuated at most time points up to 60 min following clonidine administration. Since low doses of clonidine appear to cause sedation by stimulating presynaptic α2 adrenoceptors and decreasing NA release (see Heal et al, 1981) these results suggest an increased central, presynaptic α2 receptor function in adult hyperthyroidism and a decreased function in hypothyroidism, although the precise brain region(s) mediating these responses is unknown. Gross et al (1981) have recently demonstrated decreased [^{3}H]clonidine binding in cerebral cortex from hypothyroid rats, with no change in hyperthyroid rat brain, in contrast to the presynaptic α2 receptors. However, [^{3}H]clonidine binding primarily measures postsynaptic α2 sites (U'Prichard et al, 1980).

Neither hypo- or hyperthyroid states caused a change in cortical [^{3}H]DHA binding (control B_{max} = 190.6 ± 25 fmols/mg protein; Hyperthyroid = 184.8 ± 40; Hypothyroid = 186.6 ± 11; Kd = approx 1.0nM). These results are in partial disagreement with Gross et al (1980) who, using a longer PTU-treatment protocol, found a reduction in β receptor density in hypothyroid rat cerebral cortex. Furthermore, it appears that the thyroid hormone-induced changes in adrenoceptors may be brain-region specific since preliminary results indicate that hyperthyroidism increases the density of striatal β receptors whereas hypothalamic and cortical β receptors remain unaltered.

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INTERACTIONS OF β-ADRENOCEPTOR ANTAGONISTS WITH 5-HYDROXYTRYPTAMINE RECEPTOR SUBTYPES IN RAT CEREBRAL CORTEX

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It has been proposed that multiple receptors for 5-hydroxytryptamine (5HT) can be identified in cerebral membranes using ligand binding techniques (Peroutka & Snyder, 1979). ^{3}H-5HT labels pharmacologically different sites ($5HT_1$ receptors) to those identified by ^{3}H-spiperone ($5HT_2$ receptors) and it has been further suggested that it is only the latter sites through which agents with central anti-5HT properties exert their action when assessed in a behavioural model using 5HT (Peroutka et al, 1981). In this respect it would be anticipated that the stereo-specific antagonism by propranolol of 5HT-induced behaviour (Green & Grahame-Smith, 1976) would be reflected by stereospecific inhibition of ^{3}H-spiperone binding even though Middlemiss et al (1977) have previously reported a stereospecific interaction of propranolol with $5HT_1$ sites.

In the present communication we report the affinity of a number of beta-adrenoceptor antagonists in these 5HT receptor models. Membranes were prepared and preincubated as described by Peroutka & Snyder (1979) and binding assays with ^{3}H-5HT and ^{3}H-spiperone were performed using filtration techniques. Initial characterisation of these binding sites revealed pharmacological profiles in good agreement with Peroutka & Snyder (1979). ^{3}H-5HT - K_D = 6.6 ± 0.29 nM, B_{max} = 244 ± 9 fmoles/mg protein; ^{3}H-spiperone - K_D = 0.47 ± 0.02 nM, B_{max} = 204 ± 14 fmoles/mg protein.

Effects of beta-antagonists on $5HT_1$ and $5HT_2$ receptors in rat cerebral cortex

Beta-adrenoceptor antagonists	IC_{50} ± SEM nM $5HT_1$ ^{3}H-5HT	$5HT_2$ ^{3}H-Spiperone	$\frac{\bar{x}\ IC_{50}\ 5HT_2}{\bar{x}\ IC_{50}\ 5HT_1}$
(-)-Propranolol	295 ± 83	5,896 ± 3,451	20
(+)-Propranolol	13,300 ± 4,397	16,083 ± 3,585	1.2
(-)-Alprenolol	259 ± 85	11,867 ± 3,165	46
(+)-Alprenolol	21,066 ± 2,757	24,456 ± 11,134	1.2
(-)-Pindolol	99 ± 41	205,466 ± 33,076	2,075
(+)-Pindolol	29,566 ± 8,358	82,620 ± 16,254	2.8

The results are the means of at least three experiments performed in duplicate with at least six concentrations of each agent competing against ^{3}H-5HT (1-3 nM) or ^{3}H-spiperone (0.5-1 nM). The results show that the isomers of propranolol, alprenolol and pindolol are markedly stereoselective at $5HT_1$ but not at $5HT_2$ sites and further that the (+)-isomers, unlike the (-)-isomers, do not discriminate between the two receptor models (see ratio column). In addition, examination of a large number of other beta-antagonists revealed that selective $beta_1$ antagonists (atenolol, p-oxprenolol and betaxolol) or the selective $beta_2$ blocker ICI 118.551, possess very low affinity and do not discriminate between the receptor systems. In conclusion, the non-stereoselectivity of beta-adrenoceptor antagonists at $5HT_2$ receptors in binding assays is not consistent with the mediation of behavioural effects entirely through this receptor system.

This study was supported in part by S.E.R.C.

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α_1-ADRENOCEPTOR AND MUSCARINIC RECEPTOR-STIMULATED BREAKDOWN OF INOSITOL PHOSPHOLIPIDS IN RAT CEREBRAL CORTEX

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Activation of a number of cell-surface receptors has been shown to stimulate the hydrolysis and turnover of phosphatidylinositol (PI) and/or polyphosphoinositides and it has been suggested that this response may be closely related to Ca++ gating mechanisms (Michell et al, 1981; Berridge, 1981). However, until recently the 'PI response' has not been extensively characterised in the central nervous system in view of its relative insensitivity. However, Berridge et al (1982) have recently exploited the ability of lithium to inhibit the breakdown of myo-inositol-1-phosphate (IP) and have demonstrated greatly amplified agonist-dependent PI responses in brain and salivary gland with this ion. In the present study we confirm this effect of lithium and pharmacologically characterise the response induced by noradrenaline and carbachol in rat cerebral cortex slices.

Rat cerebral cortex slices (350 x 350 µM) were preincubated at 37^{o} in Krebs-Ringer bicarbonate buffer for 30 min. Slices were then transferred to tubes containing lithium (5 mM) and ^{3}H-myo-inositol (0.18 - 0.25 µM) and incubated for a further 30 min. The slices were then exposed to appropriate agonists and antagonists for a further 45 min. ^{3}H-IP was extracted and separated from ^{3}H-inositol by ion-exchange chromatography.

100 µM carbachol, noradrenaline or 5-hydroxytryptamine stimulated the accumulation of ^{3}H-IP by 150%, 190% and 60% respectively in the presence of 5 mM lithium, whereas in its absence, stimulation was less than 12% with all three agonists. The responses to carbachol and noradrenaline were dose-related (carbachol EC_{50} 100 µM, maximal stimulation 300-400%; noradrenaline EC_{50} 8 µM, maximal stimulation 150-200%) and dose-response curves to carbachol were selectively shifted to the right by the muscarinic antagonist atropine with a K_1 value of 6 x 10^{-10} M. This value agrees well with that obtained for atropine (8 x 10^{-10} M) obtained in muscarinic ligand binding assays with membranes of the same tissue. Likewise, the dose-response curve to noradrenaline was shifted to the right by the alpha$_1$ adrenoceptor antagonist prazosin with a K_1 of 1.3 x 10^{-9} M, again in close agreement with the K_D for ^{3}H-prazosin (4 x 10^{-10} M) obtained in binding assays. The accumulation of ^{3}H-IP by 5HT was resistant to both atropine and prazosin and, indeed, was also unaffected by the 5HT antagonists methysergide (10^{-7} M) and ketanserin (10^{-7} M).

These data provide clear evidence for the potentiating effect of lithium on neurotransmitter PI breakdown in cortical slices. This approach has now allowed responses of those receptors not positively linked to adenylate cyclase to be easily assessed and suggests that typical muscarinic and alpha$_1$ adrenoceptors are linked to PI breakdown in rat cerebral cortex.

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(^{3}H)-RX 781094, A PREFERENTIAL α_2-ADRENOCEPTOR ANTAGONIST RADIOLIGAND, LABELS α_2-ADRENOCEPTORS IN THE RAT BRAIN CORTEX

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The subclassification of α-adrenoceptors into α-1 and α-2 categories is based on their pharmacological selectivity for agonists and antagonists irrespective of their anatomical localization (Langer, 1980). Radioligand binding studies have supported the existence of distinct α-1 and α-2-adrenoceptor sites. We report here the use of a new α-2-antagonist ligand (^{3}H)-RX 781094 [(Imidazolinyl-2)-2 benzodioxane-1,4 ^{3}H-3 chlorhydrate, 40 Ci/mmol, C.E.A. Saclay, France prepared by Dr. L. Pichat] for the labelling of α-2-adrenoceptors in the rat brain cortex. RX-781094 is an antagonist with a higher ratio of selectivity α-2/α-1-adrenoceptors than yohimbine or rauwolscine (Chapleo et al., 1981). Rat cortical membranes were prepared by homogenization and centrifugation and resuspended in potassium sodium phosphate buffer 50 mM, pH = 7.4. (^{3}H)-RX 781094 binding was measured by the filtration technique. Specific binding was defined as the binding inhibited in the presence of 10 µM phentolamine and it represented 70 % of the total binding at 4 nM (^{3}H)-RX 781094. (^{3}H)-RX 781094 binding is saturable and reversible. Association, dissociation and saturation studies revealed the presence of a single population of non-interacting sites. The apparent dissociation constant (Kd) was 3.9 ± 0.4 nM and the maximal number of binding sites (Bmax) was 188.9 ± 12.9 fmol/mg protein (n=6). Competition curves with agonists and antagonists indicated that (^{3}H)-RX 781094 labelled receptor sites with the characteristics of the α-2-adrenoceptor subtype. The IC_{50} of inhibition of (^{3}H)-RX 781094 binding for rauwolscine was 63 nM while for prazosin it was 380 nM. The IC_{50} of inhibition of (^{3}H)-RX 781094 by agonists was 80 nM for clonidine and 7000 nM for phenylephrine. DSP4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) produces a selective degeneration of central noradrenergic nerve terminals in the rat and mouse (Jonsson et al., 1981). Treatment with DSP4 (50 mg/kg, i.p.) produced a 93% decrease of noradrenaline (NA) levels. [NA control : 248 ± 25 ng/g tissue (n=10) ; DSP4 : 15 ± 2 ng/g tissue (n=8) P<0.001] as measured at 10 days post-injection. In these animals, the (^{3}H)-RX 781094 binding characteristics were unchanged. [Control : Kd = 3.9 ± 0.1 nM ; Bmax = 220 ± 9 fmol/mg prot. (n=4) ; DSP4 : Kd = 3.3 ± 0.4 nM ; Bmax = 224 ± 28 fmol/mg prot. (n=4)]. Local injection of 6-hydroxydopamine (2 µg/µl) in the superior cerebellar peduncle induces a degeneration of noradrenergic pathways which was reflected in a 93% decrease of NA levels [Control : 255 ± 35 ng/g tissue (n=10) ; 6OHDA : 16 ± 2 ng/g tissue (n=5) P<0.001]. Three weeks after 6OHDA lesions, the (^{3}H)-RX 781094 binding characteristics were not changed [control : Kd = 5.3 ± 0.8 nM ; Bmax = 165 ± 25 fmol/mg prot. (n=5) ; 6OHDA lesions 3 weeks : Kd = 6.6 ± 0.8 nM ; Bmax = 135 ± 9 fmol/mg prot. (n=5)]. In conclusion, (^{3}H)-RX 781094 labels with high affinity a single population of binding sites which have the pharmacological characteristics of the α-2-adrenoceptor subtype. The treatment with DSP4, or lesions with 6OHDA, which produce degeneration of noradrenergic nerve terminals failed to modify the parameters of (^{3}H)-RX 781094 binding, indicating that the major proportion of the α-2-adrenoceptors in the rat cerebral cortex are not located on noradrenergic terminals.

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EFFECTS OF DRUGS ACTING ON α_2-ADRENOCEPTORS IN AN ANIMAL MODEL OF ANXIETY

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Previous studies have indicated the possibility that noradrenergic systems are involved in fear and anxiety (Redmond & Huang, 1978; Hoehnsaric et al., 1981). The present study was undertaken to compare the anxiolytic and anxiogenic profiles of α_2-adrenoceptor agonists and antagonists respectively, in an animal approach - avoidance behavioural system.

Exploratory activity was assessed in male hooded (150-170 g) rats in an apparatus similar to that described by Montgomery (1955). 30 minutes after drug injection (i.p. in 1 ml kg^{-1} 0.9% NaCl) rats were placed in the centre of an elevated (70 cm) X maze with two open and two (opposite) enclosed 45 cm arms. The enclosed arms had wooden sides 10 cm high and no lid. The number of entries into each type of arm was noted over a 10 minute period; an entry being defined as a whole body length excluding tail. Total exploratory activity was measured as the total number of entries. The relative number of open to enclosed arm entries was taken as a measure of the strength of fear-induced inhibition of open arm entry.

As expected, ACTH which has apparent anxiogenic actions (File and Vellucci, 1978), significantly reduced the open/enclosed arm entry ratio. Total number of entries were also reduced. Diazepam (1-2 mg kg^{-1}) on the other hand increased the open/enclosed ratio. The α_2-adrenoceptor blocking agents yohimbine (1.25 mg kg^{-1}), piperoxane (5-10 mg kg^{-1}) and RS21361 (5-10 mg kg^{-1}), like ACTH significantly reduced the open/enclosed ratio. Higher doses of yohimbine (2.5-5 mg kg^{-1}) also reduced total entries. The α_2-adrenoceptor agonists azepexole (1 mg kg^{-1}), clonidine (0.01 mg kg^{-1}) and guanabenz (0.1 mg kg^{-1}) however significantly increased the open/enclosed ratio; these drugs tended to increase the total number of entries.

These findings demonstrate that this test situation may be a valid model for examining anxiolytic and anxiogenic effects of drugs and confirms the likelihood of the involvement of noradrenergic systems in fear and anxiety.

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ACCUMULATION AND DEPOLARIZATION-INDUCED RELEASE OF PROPRANOLOL IN SYNAPTOSOMES FROM RAT CEREBRAL CORTEX

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It has been reported that propranolol (PR) may be accumulated by peripheral noradrenergic neurons after chronic oral administration in the dog, and that the drug may be released subsequently in association with norepinephrine (NE) during nerve stimulation (Daniell et al., 1979). PR readily traverses the blood-brain barrier after peripheral administration, and the occurrence of centrally-mediated effects is well documented (for review see Middlemiss et al., 1981). We therefore investigated the possibility that PR may interact with central neurons in a manner similar to that observed in the periphery.

Synaptosome-enriched (P_2B) fractions were prepared from rat cerebral cortex (Gray & Whittaker, 1962). After resuspension in oxygenated, buffered Krebs solution (pH 7.4), synaptosomes were incubated at 30° C with (-)-[^{3}H]-PR or (-)-[^{3}H]-NE (0.1 μM). Incubations were terminated by filtration, and the incorporation of radioactivity was estimated by liquid scintillation spectrometry. In experiments on the release of PR and NE, synaptosomes were pre-loaded with the radiolabelled drug, collected on glass-microfibre filters, and perfused (1 ml/min) with control medium, or media containing elevated K^+ or the depolarizing agent veratridine (VER). Radioactivity released into the medium was estimated along with that remaining in the tissue at the end of the experiment.

During a 10 min period, synaptosomes accumulated 24.2 ± 0.8 pmol PR/mg protein (mean ± s.e. mean; n=3), as compared with 20.0 ± 1.7 pmol/mg for NE. Sub-synaptosomal fractionation (Whittaker et al., 1964) revealed that, of the total radioactivity taken up after incubation with PR, 37% appeared to be associated with synaptosomal plasma membrane fractions, 20% with incompletely-lysed synaptosomes, 16% with synaptic vesicle and microsomal fractions and 6% with the mitochrondrial fraction. The remainder of the radioactivity was recovered in the supernatant.

After synaptosomal loading, and following a 5 min period of washing with normal Krebs, the basal release of [^{3}H]-PR during a further 1 min exposure to control buffer was 2.47 ± 0.30 pmol/mg protein (n=12). In preparations exposed to elevated K^+ (75 mM) or VER (75 μM), PR release during the corresponding time period was increased to 3.08 ± 0.29 ($P < 0.01$) and 3.46 ± 0.41 ($P < 0.01$) pmol/mg respectively. In comparison, basal release of [^{3}H]-NE was 1.99 ± 0.11 pmol/mg protein (n=12), release being increased to 2.82 ± 0.14 ($P < 0.01$) and 3.47 ± 0.47 ($P < 0.01$) pmol/mg in the presence of 75 mM K^+ and 75 μM VER. The effects of these agents on both PR and NE release showed concentration dependence. PR release induced by 75 mM K^+ was reduced 37% ($P < 0.05$) by removal of Ca^{2+} and addition of 2mM EGTA to the medium. This manipulation almost totally abolished K^+-induced NE release.

These data suggest that PR may be accumulated by central nerve terminals, and that stored drug may be released by depolarizing stimuli. Although K^+-induced PR release appears to be partly Ca^{2+}-dependent, the bulk of release seems to occur by a Ca^{2+}-independent mechanism.

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AN ATROPINE-SENSITIVE EFFECT OF ACETYLCHOLINE (Ach) ON THE RAT OLFACTORY CORTEX SLICE

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Neuroanatomical, biochemical and histochemical studies (Macrides et al, 1981) show the presence of cholinergic neurones in the primary olfactory cortex (OC) whilst other studies demonstrate high concentrations of both muscarinic (Rotter et al, 1979) and nicotinic (Morley & Kemp, 1981) cholinoceptors in this brain region. The present experiments were to study the possible effect of Ach on synaptic transmission in the rat OC slice.

When slices of OC are perfused at ambient temperature, stimulation of the lateral olfactory tract (LOT) evokes a series of surface field potentials (Pickles & Simmonds, 1976, 1978) comprising a monosynaptically evoked massed epsp (N-wave) and two longer latency polysynaptic massed field potentials (late N- and I-waves). In slices preincubated and perfused with solution containing physostigmine (10μM) and in which the recording electrode was located on the piriform cortex, application of Ach caused a concentration-dependent depression of the N-wave amplitude which possessed the following characteristics (values are means $\pm$ s.e.);

1. The reduction in N-wave amplitude was linear between Ach concentrations of 5 to 80μM but thereafter reached a plateau percentage reduction of 44.0 $\pm$ 3.7 (n = 5) at an Ach concentration of 5mM. The Ach concentration causing a 25% reduction in amplitude (IC_{25}) was 24.6 $\pm$ 3.7μM (n = 5).
2. Atropine caused a parallel shift in the Ach log dose-response curve to the right (IC_{25} value plus atropine (0.05μM), 1.02 $\pm$ 0.17mM; plus atropine (0.2μM), 8.17 $\pm$ 1.0mM, n between 3 and 7).
3. The amplitude of the late N-wave was less susceptible to Ach (IC_{25} = > 5mM, n = 5) than the N-wave (see 1) whereas the amplitude of the I wave was variably affected.
4. In recordings made from the olfactory tubercle, the maximum depression of N-wave amplitude by 5mM Ach (10.1 $\pm$ 2.6%, n = 5) was significantly less ($P < 0.001$) than that recorded from the piriform cortex (see 1).
5. In the absence of physostigmine, bethanecol (a relatively specific muscarinic agonist) also depressed the N-wave amplitude (IC_{25} = 1.06 $\pm$ 0.1mM, n = 3) with a plateau effect at 10mM of 39.7 $\pm$ 2.9% reduction (n = 3) whereas sufficient Ach (80mM) almost entirely abolished all field potentials.

These experiments suggest that Ach inhibits transmission at the LOT-superficial pyramidal cell synapse by an atropine-sensitive mechanism. Preliminary experiments failed to show any depressant effect of Ach on the K^+ evoked release of aspartate, the presumed transmitter of the LOT (Collins & Probett, 1981), implying a postsynaptic site of action for Ach.

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A COMPARISON OF THE RELATIVE ANTICONVULSANT POTENCIES OF 2APV AND 2APH

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Neuronal excitation due to dicarboxylic amino acids can be antagonised by ω-phosphonic α-carboxylic amino acids with carbon chain lengths of 5-8 (Perkins et al, 1981; Evans et al, 1982). These compounds preferentially block excitation induced by N-methyl-D-aspartate (NMDA) rather than that induced by quisqualate or kainate (Watkins & Evans, 1981). We have recently reported that 2 members of this series, 2-amino-5-phosphonovaleric acid (2APV) and 2-amino-7-phosphonoheptanoic acid (2APH) prevent audiogenic seizures in mice following i.c.v. administration (Croucher et al, 1982). The greater anticonvulsant potency of 2APH in this test system is consistent with the relative potencies of these agents as antagonists of NMDA-induced excitation of rat cortical neurones following iontophoretic application. However, 2APV is more potent than 2APH at blocking depolarization induced by NMDA in the isolated frog spinal cord (Evans et al, 1982). We report here further experiments to clarify the relationship between the anticonvulsant activity of these compounds and their ability to block excitation induced by NMDA or aspartate.

In the rat cerebral cortex single cell firing rates were recorded to evaluate blockade by 2APV and 2APH of excitation due to NMDA, L-aspartate or L-glutamate. Relative anticonvulsant potencies of the compounds were estimated against sound-induced seizures in DBA/2 mice after both i.c.v. and i.p. administration and against seizures induced by NMDA or by 3-mercaptopropionic acid in Swiss mice (i.p. drug administration). 2APV and 2APH were essentially equipotent as antagonists of glutamate-induced excitation of rat cortical neurones. However, against NMDA and aspartate, 2APH showed greater activity than 2APV (potency ratios approximately 1.5). Following either i.c.v. or i.p. administration, the heptanoate derivative was approximately 10 times as potent as 2APV at suppressing the individual phases of the audiogenic seizure response. Additionally the (-) isomer of each antagonist showed greater anticonvulsant activity than the (+) isomer. Against the clonic phases of NMDA- and 3MPA-induced seizures 2APH was more than twice as potent as 2APV. The potency ratio for suppression of tonic seizures after 3MPA was far greater but could not be precisely determined.

These results support a relationship between the relative anticonvulsant potencies of 2APV and 2APH and their relative activities as antagonists of NMDA- and aspartate-induced excitation in the mammalian cerebral cortex. The apparently different relative activities in the frog spinal cord may be due to methodological differences, species differences, or regional differences in excitatory amino acid receptors.

This work is supported by the Medical Research Council and the Science Research Council. We thank J.F. Collins for the gift of 2APV and 2APH.

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REPEATED ELECTROCONVULSIVE SHOCK ALTERS DIAZEPAM PHARMACOKINETICS IN RATS

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In a previous communication to the Society the effect of electroconvulsive shock (ECS) on the anticonvulsant potency of diazepam and flurazepam was reported (Cowen & Nutt, 1982). It was found that 24 hours following the last of 10 once-daily ECS, the increase in seizure threshold produced by both of these benzodiazepines was greater in ECS-treated animals as compared with handled controls. Since pharmacokinetic factors could account for this difference, we investigated whether a course of ECS affects concentrations of diazepam (DZM) and its major metabolite, desmethyldiazepam (DMD) in rat brain following i.p. administration.

Male Sprague-Dawley rats weighing 100-125 g at the start of the experiment were given ECS (125 v, 1 s, 50 Hz) via ear-clip electrodes once daily for 10 days. Control rats were handled. All rats received DZM (2 mg kg^{-1} i.p. in commercial vehicle) 24 h after the last treatment. Animals were killed 20 min later (the time when seizure thresholds were previously shown to be significantly elevated) and the brain rapidly removed. Brain DZM and DMD levels (21 animals, mean brain weight 1.74 ± 0.07 g) were determined by high performance liquid chromatography.

As can be seen from Table 1, DZM levels were significantly greater in ECS-treated rats. DMD levels were not significantly altered.

Table 1 Concentrations of DZM and DMD in whole rat brain

	Control	ECS	
DZM	0.23 ± 0.12 (11)	0.46 ± 0.14 (10)	P<0.005
DMD	0.07 ± 0.02 (11)	0.12 ± 0.12 (10)	N.S.

Figures represent mean ± S.D. concentration in μg/g wet wt. brain. Numbers in brackets represent number of animals studied. P values show the significance between control and ECS-treated animals by Student's 't' test. N.S. = not significant.

Repeated ECS significantly increases brain concentrations of DZM. Such increases of about two-fold are similar in magnitude to those seen in the seizure threshold experiments (Cowen & Nutt, 1982) and it seems likely that the seizure threshold elevation is a direct consequence of the raised brain anticonvulsant levels. Interestingly, increased threshold were not found to other anticonvulsants such as sodium valproate and phenobarbitone, and further work is underway to determine whether ECS affects brain concentrations of these drugs.

These findings reveal altered pharmacokinetics of DZM in ECS-treated animals. They are consistent with earlier observations of an increased blood-brain barrier permeability following ECS (Bolwig et al, 1977; Preskorn et al, 1981) but might also result from differences in uptake, distribution and metabolism of DZM.

Similar pharmacokinetic phenomena should always be excluded as explanations of altered drug sensitivity with 'in vivo' experiments.

We thank Roche for the gift of diazepam and desmethyldiazepam.

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VARIATIONS OF NEURONAL SENSITIVITY TO QUINOLINIC ACID WITHIN THE RAT CENTRAL NERVOUS SYSTEM

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We have recently described the excitatory action of quinolinic acid, an endogenous metabolite of tryptophan, on cortical neurones, and have presented evidence that this compound preferentially activates the N-Methyl-D-aspartate receptor (Stone & Perkins 1981). The present work is a study of neuronal sensitivity to this compound in different regions of the neuraxis.

Male Wistar rats were anaesthetized (urethane 1.5g/Kg i.p.), mounted in a stereotaxic frame and rectal temperature maintained at 37°C. The spinal cord and/or the cortex above the areas of interest were then exposed (Pellegrino et al., 1981).

L-glutamic and quinolinic acid (50 mM, pH5) were ejected by conventional iontophoretic methods from 7-barrelled micropipettes. Recordings were made through a single electrode glued alongside the micropipette.

Relative sensitivity was assessed using the currents required to give comparable plateau responses where possible or, if not, total charge in nanocoulombs required for comparable peak responses; both were then used to derive a Glu : Quin potency ratio.

Six electrodes were tested in the sequence spinal cord - cerebral cortex or cortex-cord-cortex. Quinolinate was excitatory in cortex but not in spinal cord. The mean Glu : Quin ratio in cortex was 0.71 $\pm$ 0.05 ($\pm$ s.e.m.) for all electrodes. In the dorsal horn of the cord, using the same electrodes only 4/17 cells responded to quinolinate (mean Glu : Quin ratio 0.14 $\pm$ 0.014).

In the hippocampus, 23 cells responded to both glutamate and quinolinate. Comparable glutamate responses were produced with mean currents of 6.9 nA in hippocampus and with 24 nA in cortex. For quinolinate the mean currents were 15 nA and 36.5 nA respectively. The Glu : Quin currents ratio was significantly lower at 0.47 $\pm$ 0.06.

Only 12/30 cells in the cerebellum responded to quinolinate, all being excited by glutamate. The Glu : Quin ratio for these cells was 0.15 $\pm$ 0.03, compared to 0.86 $\pm$ 0.08 in the cerebral cortex using the same electrodes.

All 60 neurones tested in the neostriatum were excited by quinolinate and glutamate. In the anterior striatum (approx. 0.5 mm anterior to bregma) the Glu : Quin ratio was 0.75 $\pm$ 0.06 and in the posterior striatum it was 0.33 $\pm$ 0.04 (P 0.001).

We suggest that this degree of variation in neuronal sensitivity indicates a specificity in the localization of receptors for quinolinate which might reflect a neurotransmitter function.

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AGING IS ASSOCIATED WITH A DECREASE IN SENSITIVITY OF CORTICAL NEURONES TO NORADRENALINE IN THE RAT

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The cerebellum of the rat exhibits a decrease in β-adrenergic binding sites, a reduction of stimulation of adenylate cyclase by noradrenaline (NA) and a decrease in neuronal sensitivity to iontophoretically applied NA with advancing age (Greenberg & Weiss, 1978; Schimdt & Thornberry, 1978; Marwaha et al, 1981). In the cerebral cortex, however, the former two biochemical parameters appear to be little altered in old compared to young rats while the latter parameter has not been studied. In the present study we compared the responsiveness of neurones in the rostral cortex to iontophoretically applied NA in young (3-4 months) and old (22-24 months) rats.

Rats were anaesthetized with urethane. A single iontophoresis electrode was used to determine the responsiveness of four spontaneously active cortical neurones in one old and one young rat on the same day. Seven such experimental pairs were used. Neuronal responses were compared by measuring the percentage decrease in firing rate and also the duration of depression. In addition to the effects of NA, the depressant effects of GABA were also compared on the same neurones. The results of the NA comparison are shown in Table 1.

Table 1

	% dep^n		duration (s)	
current (nA)	Young	Old	Young	Old
5	37±5	24±5***	91±13	47±9**
10	54±5	45±5	153±14	110±12*
20	71±3	65±4	212±14	169±12*
40	80±3	79±4	284±16	246±17*
80	90±2	89±2	332±25	290±16

Data are presented as mean ± SEM (n=28) *P 0.05, **P 0.025, ***P 0.01 (Student 't' test)

The duration of the NA responses was significantly less in the older rats at all ejecting currents tested except 80 nA. The maximum percentage decrease in firing rate was also reduced at the lower ejecting currents but this was only significant at 5 nA. In contrast to the situation with NA there was no detectable difference in either response parameter for GABA.

Thus, the data indicate that despite biochemical indications to the contrary (Greenberg & Weiss, 1978; Schmidt & Thornberry, 1978) the cerebral cortex, like the cerebellum exhibits a decrease in postsynaptic neuronal sensitivity to NA with advancing age in the rat.

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LOCALIZATION AND PROPERTIES OF NICOTINE BINDING SITES IN SELECTED REGIONS OF RAT BRAIN

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Relatively few studies have utilized radiolabelled nicotine to characterise central nicotine binding sites, and the results of those which have been reported are equivocal (Abood et al 1978; Romano and Goldstein 1980; Martin and Aceto 1981; Sershen et al 1981). The present studies were designed to clarify the characteristics of these binding sites in brain tissue, and to ascertain their subcellular and regional distribution.

^{3}H-Nicotine binding was measured in crude homogenates of rat hippocampus, hypothalamus and cerebral cortex or in subfractions of these brain regions obtained using the procedure of Gray and Whittaker (1962). Samples of the tissue suspensions were incubated at 25°C in Krebs Ringer solution buffered to pH 7.4 with phosphate buffer, containing approximately 4 nM ($\pm$) ^{3}H-nicotine (61.2 Ci/m mole; New England Nuclear) for 30 minutes. Bound ligand was separated from free by filtration through Whatman GF/C filters, and counted by liquid scintillation spectrometry. Non-displaceable nicotine binding was determined in the presence of 100 μM unlabelled (-) nicotine. Other unlabelled drugs were added to some of the incubations to characterise the binding sites.

Displaceable nicotine binding was present in all subfractions from all the brain regions studied. The highest concentrations of nicotine binding sites (approximately 18 x 10^{-15} moles/mg protein) were found in the synaptosome fraction of all brain regions studied, mitochondrial fraction of hippocampus and hypothalamus, myelin fraction of hippocampus and the microsomal fraction of hypothalamus and cerebral cortex.

Computer analysis of the binding data obtained using crude homogenates indicated that there were two binding sites present in all three brain regions. The Kd and B max values for the high affinity sites were 1.7, 3.6 and 4.5 nM and 8.0, 15.0 and 16.0 x 10^{-15} moles/mg protein respectively for the hippocampus, hypothalamus and cerebral cortex. The equivalent values for the low affinity sites were 1.8, 5.8 and 2.2 μM and 1.7, 5.3 and 1.9 x 10^{-12} moles/mg protein. Competition studies suggested that agonists at nicotinic receptors (e.g. Lobeline, DMPP and carbachol) have higher affinities for the two binding sites than nicotinic antagonists (e.g. mecamylamine, hexamethonium, tubocurarine, pancuronium), while α-bungarotoxin did not compete with nicotine for the binding sites over the concentration range tested (1 nM to 10 μM).

The characteristics of the nicotine binding sites described here are very similar to those reported previously for whole brain membranes by Romano and Goldstein (1980). The sites appear to be cholinergic, but they are not necessarily identical to either of the nicotinic receptors present in peripheral tissues. They are not located exclusively in the synaptosome fraction as previously suggested by Yoshida and Imura (1979).

The authors are grateful to the Scottish Home and Health Department for financial support.

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CHRONIC ETHANOL ADMINISTRATION CAUSES INCREASED PHOSPHOLIPASE ACTIVITY IN RAT BRAIN

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The development of ethanol tolerance has been suggested to be a consequence of altered synaptic membrane lipid composition (Littleton & John, 1977; Chin, Parsons & Goldstein, 1978) but the mechanism for the observed alterations is unknown. Evidence that brain membrane phospholipid turnover is increased in rats receiving ethanol chronically has been reported previously (Sun et al, 1977; Johnson et al, 1979; Nhamburo et al, 1982). Here we report that breakdown by synaptosomal preparations of exogenous phosphatidylcholine labelled with ^{3}H-oleic acid at the second position is increased in rats receiving ethanol in vivo.

Radiolabelled 1-acyl-2[^{3}H]oleoyl-phosphatidylcholine was prepared by the method of Lands (1960) using ^{3}H-oleic acid (Amersham) and 1-acyl lysophosphatidylcholine (Sigma) as substrates and a rat liver microsomal preparations as a source of acyl transferase. Synaptosomes were prepared from whole rat brain homogenates using Cotman's method (1974) and a suspension of synaptosomes was then incubated with sonicated radiolabelled phosphatidylcholine for 15 minutes at 37^{o}C. The reaction was stopped by the addition of ice-cold chloroform:methanol:HCL (2:1:0.02, v/v/v) and the extracted lipids separated by thin-layer chromatography using the solvent system chloroform:methanol:water and concentrated ammonia (65:25:4:0.4, v/v/v/v). Spots corresponding to oleic acid, phosphatidylcholine and lysophosphatidylcholine were scraped from the glass plates and placed in vials. 10 mls of scintillant (PPO and 2-ethoxyethanol) were added to these vials and samples were assayed for ^{3}H by scintillation counting. Phospholipase activity was assessed by measuring the fraction of total radioactivity associated with free oleic acid compared to that associated with phosphatidylcholine or as a percentage of [^{3}H] in oleic acid to the total recovered in three fractions (oleic, PC and LPC).

Synaptosomal fractions from brains of control rats (CD Sprague Dawley, 200-250 g) degraded 1-acyl-2[^{3}H]oleoyl-phosphatidylcholine at a much greater rate than its spontaneous breakdown (10 times more). The addition of ethanol to the reaction mixture caused a dose-related inhibition of this activity, significant inhibition being obtained at 50 mM ethanol. Synaptosomal fractions from rats which received ethanol either acutely (3 g kg^{-1} I.P. for 30 minutes) or chronically (intoxication by inhalation for 6-8 days) both showed a significantly increased rate of phosphatidylcholine breakdown compared to sham-treated controls (controls, 100 ± 4.3%; acute ethanol, 128.8 ± 5.8%; chronic ethanol, 151.0 ± 14.2%). The addition of 50 mM ethanol to synaptosomal preparations from rats which had received ethanol in vivo produced less inhibition of phospholipase activity than in control preparations.

These results suggest that one of the reasons for an increased membrane phospholipid turnover associated with ethanol administration is increased phospholipase (probably phospholipase A_2) activity. It is possible that this plays a part in the changes in the composition of membrane lipids which accompany the development of ethanol tolerance.

Acknowledgements. This work was supported by grants from the British Council and the Medical Research Council.

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A CENTRAL SITE OF ACTION FOR METOCLOPRAMIDE TO FACILITATE GASTRIC EMPTYING IN THE GUINEA-PIG

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Metoclopramide's ability to enhance upper gastrointestinal motility is well accepted, and has been applied in the clinic for almost two decades. Yet, the basic site(s) of action remain obscure, the decisive issue being whether the mechanisms are located peripherally and/or centrally. Thus, we have assessed the possibility of a central site for metoclopramide's action, using both peripheral and intracerebroventricular (i.c.v.) routes of administration.

The studies used Dunkin-Hartley guinea-pigs, 500 $\pm$ 50 g, and gastric emptying was measured a.m. using the non-invasive X-ray fluoroscopic method of Cox & Ennis (1980). Polystyrene-coated barium sulphate spheroids (approximately 1.0 mm in diameter) placed at the back of the mouth with a small amount of glycerin were promptly and voluntarily swallowed. Conditions of minimal stress were essential for consistency of gastric emptying which was determined from the number of spheroids remaining in the stomach after 1, 2, 3 and 4 h. X-ray parameters of 50 KV, 40 mA, 0.5-0.8 s. using Kodak plates NS-2T 13 x 18 cm, allowed a ready localisation of the spheroids. Experiments were carried out using normal guinea-pigs and those with chronically indwelling intracerebral guide cannulae stereotaxically located at Ant. 7.9, Lat. -2.2 and 2.0 mm below the dura to allow injection into the ventricular system 3.0 mm below the guide tip.

In normal guinea-pigs 60-70%, 30-40%, 15-20% and <10% of the spheroids remained in the stomach after 1, 2, 3 and 4 h. respectively. Pretreatment for 30 min. with atropine (0.5 mg/kg i.p.) or apomorphine (0.5 mg/kg s.c.) markedly reduced gastric emptying over the entire 4 h. period ($P < 0.01$, $n = 5$). Metoclopramide (30 min. pretreatment) failed to consistently modify gastric emptying at 0.1, 1 and 10 mg/kg i.p. although 100 mg/kg caused intense behavioural excitation and markedly delayed gastric emptying in a similar manner to stress. However, pretreatment with 10 mg/kg i.p. metoclopramide significantly antagonised the reduced gastric emptying caused by 0.5 mg/kg s.c. apomorphine at the 1, 2 and 3 h. assessment times ($P < 0.01$). Further, i.c.v. metoclopramide enhanced gastric emptying (significant to $P < 0.01$ at 2-4 h. at 40 µg, and throughout the entire period at 100 µg when only 30% of the spheroids remained in the stomach at 1 h.). Additionally, the reduced gastric emptying caused by peripherally administered atropine (0.5 mg/kg i.p.) was antagonised (1-4 h.) by i.c.v. metoclopramide (100 µg, $P < 0.01$).

Thus, it would appear that the effectiveness of peripherally administered metoclopramide to facilitate gastric emptying in the guinea-pig can only be revealed when emptying is slowed as by apomorphine. In contrast, centrally administered metoclopramide caused such a powerful facilitation of gastric emptying that even the efficiency of basal emptying could be increased. An interaction with atropine suggests that this central action of metoclopramide to facilitate gastric emptying involves a central and/or peripheral cholinergic mechanism.

This work was supported by the Medical Research Council.

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DIFFERENTIAL EFFECTS OF ISOPRENALINE, DOPAMINE, DIGOXIN AND AMRINONE ON THE GUINEA-PIG ISOLATED, PERFUSED, WORKING HEART

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Compared with isolated heart muscle and Langendorff preparations, the isolated, working heart preparation allows more complete analysis of cardiac function and metabolism, and study of the responses of the heart at fixed preload and afterload (Neely et al. 1967, Zannad et al. 1982.).

We have studied the effects of inotropic drugs with different mechanisms of action : Isoprenaline, dopamine, digoxin and amrinone, on the isolated perfused working guinea-pig heart. We have measured : Heart rate (HR), mean systolic aortic pressure (SP), aortic flow (AF), coronary flow, (CF), total cardiac outpout (CO), and calculated the stroke volume (SV), and the rate of external work (W). The rates of oxygen consumption (VO2) and lactate production (Lact) were also measured and the external efficiency (Eff) calculated.

Basal values of the various measurements at the start of work were as follows : (n=37) : HR = 234 ± 25 beats/min ; AF = 198 ± 43 ml/min/g of dry heart weight (DHW) ; CF = 64 ± 18 ml/min/g DHW ; CO = 266 ± 72 ml/min/g DHW ; SV = 1.14 ± 0.22 ml/beat/g DHW ; W = 22.1 ± 5.1 Kgm/min/g DHW ; VO2 = 2.59 ± 0.65 mmol/h/g DHW ; Lact = 317 ± 111 µmol/h/g DHW and Eff = 19.4 ± 4.4 %.

Hearts were allowed to fail spontaneously after work for 90 minutes. The studied drug was then added at required concentrations to the perfusion medium.

Isoprenaline ($5X10^{-11}$ M to $5X10^{-7}$ M) produced a dose related increase in CF, CO, and W, with a substantial rise in HR, VO2 and Lact. SV was slightly decreased and Eff. significantly lowered.

Dopamine (10^{-5} M) exhibited similar effects to isoprenaline, but SV was significantly increased, and Eff. unaltered.

Digoxin (10^{-7} Mand $2X10^{-7}$ M), significantly increased AF, CO, SV, W, and Eff., without change in HR, VO2 and Lact..

Amrinone (10^{-4} M), increased AF, CF, CO, SV, W and VO2. HR was slightly but significantly increased. lact. and Eff. were not altered. Amrinone (10^{-3}M) substantially increased CF and VO2, but it increased slightly and not significantly AF, CO, SV and W, suggesting the predominance of the coronary vasodilating effect of amrinone at higher doses. Lact. and Eff. remained unchanged.

No arrhythmias occurred with any concentration tested of any drug.

These results demonstrate the differential effects of positive inotropic drugs with different mechanimes of action, and further underlines the suitability of the isolated, perfused, working guinea-pig heart prepration for the analysis of the pharmacological properties of cardioactive drugs.

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ANTIARRHYTHMIC AND ELECTROPHYSIOLOGICAL EFFECTS OF MEPTAZINOL

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Recent studies have demonstrated that the opiate antagonist, naloxone, reduced the severity of the arrhythmias that result from coronary artery ligation in both conscious and anaesthetised rats (Fagbemi et al., 1982). The aim of the present work was to examine whether meptazinol, a partial agonist at opiate receptors, afforded similar protection to that of naloxone against ligation induced arrhythmias and to investigate if any such protection could be related to a direct electrophysiological effect on the cardiac muscle action potential.

Coronary artery ligation was carried out in either anaesthetised (sodium pentobarbitone, 60 mg kg^{-1}) or conscious male Sprague-Dawley rats (Clark et al., 1980 and Lepran et al., 1979). Table 1 compares the severity of the arrhythmias in rats administered either saline (controls) or meptazinol, i.v. fifteen minutes prior to coronary artery occlusion.

Table 1. The incidence of ventricular fibrillation (VF) within 30 min of coronary artery ligation in anaesthetised and conscious rats and the survival at the end of this period

	% Survival				% VF	
	anaes.	(n)	consc.	(n)	anaes.	consc.
Control	70	(10)	31	(26)	50	88
Meptazinol, 1 mg kg^{-1}	100	(10)	75*	(12)	10*	25**
Meptazinol, 2 mg kg^{-1}	100	(10)	83*	(12)	0*	33**

*$P < 0.05$ **$P < 0.001$. n is the number of animals in each group

Meptazinol improved survival in both conscious and anaesthetised rats, this effect being associated with a reduced incidence of VF, which is usually the cause of death in these animals. In conscious rats, at 16h post ligation, survival was significantly increased only by the higher drug concentration.

Intracellular action potentials were measured in papillary muscles (stimulation frequency, 1 Hz) excised from anaesthetised rats and pretreated with either saline (n=5) or meptazinol, 2 mg kg^{-1}, (n=5) i.v. fifteen minutes prior to excision. The action potential duration at 50% (APD_{50}) and 90% (APD_{90}) repolarisation was significantly prolonged in drug pretreated muscle (APD_{50} 31.5±1.1 vs 22.0±1.0 ms; APD_{90} 80.2±2.7 vs 56.5±2.5 ms). Similarly, in vitro administration of meptazinol (4-16 mg l^{-1}) to rat papillary muscle caused a marked dependent prolongation of both APD_{50} and APD_{90}.

In summary, meptazinol decreased the severity of coronary artery ligation induced arrhythmias and in particular prevented VF thereby improving survival. This protective effect may at least in part be explained by the drug's ability to increase cardiac action potential duration but an action mediated via opiate receptors cannot be ruled out. Since meptazinol is an analgesic, without significant effect on the circulation (Rashid and Waterfall, 1979), it may be of considerable value in the treatment of early post-infarction arrhythmias.

This work was supported in part by the British Heart Foundation.

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REPERFUSION INDUCED CARDIAC ARRHYTHMIAS IN THE ANAESTHETIZED RAT AND THEIR SUSCEPTIBILITY TO DRUGS

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There is a growing clinical interest in the view that sudden cardiac death may in some cases be due to coronary spasm and subsequent reperfusion of the ischaemic myocardium (Hellstrom, 1979). This reflow may give rise to ventricular arrhythmias culminating in ventricular fibrillation (VF) which is the frequent cause of sudden cardiac death. Our aims were to develop a small animal model for the production of reperfusion arrhythmias and to assess the antifibrillatory activity of drugs, with different pharmacological profiles, on this type of arrhythmia.

Male rats, anaesthetised with sodium pentobarbitone (60 mg kg^{-1} i.p.) were prepared for coronary artery ligation, using a modification of the technique described by Clark et al. (1980). After placing the ligature around the left anterior descending artery, the threads were passed through a small plastic button which was used to occlude the artery. After 5 min of occlusion, reperfusion was achieved by releasing the tension on the threads. Within 10-30s marked arrhythmas occurred. These differ from those observed upon ligation, in that they usually consisted of ventricular tachycardia leading to VF, which if not fatal, reverted to sinus rhythm after 2-3 min of reperfusion. Sinus rhythm was then maintained for at least 30 min post release.

Table 1 summarises the severity of the reperfusion induced arrhythmias in control and drug pretreated animals. All drugs were administered i.v. 10 min prior to ligation.

Table 1 The percentage incidence of ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality during reperfusion

Drug	mg kg^{-1}	n	VT	VF	Mortality
Control		41	100	76	54
Propranolol	2	9	89	33*	22
Melperone	2	8	87.5	37.5*	37.5
Melperone	10	8	87.5	37.5*	12.5*
Org 6001	2	9	89	22	11
Org 6001	10	8	37.5**	12.5*	0
Prazosin	0.1	8	100	50	37.5
Prazosin	0.5	8	100	37.5	12.5
Timolol	2	9	67**	33	0

n is the number of animals in each group. *$P < 0.05$ **$P < 0.01$. To obtain significance levels drug treated groups were compared with a similar number of control experiments performed over the same time period.

The severity of the arrhythmias was reduced by melperone, which prolongs action potential duration, and Org 6001, a fast Na^+ channel blocker. A slight but not statistically significant reduction in the incidence of VF and of mortality was observed with prazosin, 0.5 mg kg^{-1}, an α antagonist. Similarly, both β antagonists, timolol and propranolol reduced the incidence of VF although this effect was only significant with the latter drug.

In summary, we have developed a model in the anaesthetised rat for the production of reperfusion induced cardiac arrhythmias which appears to be susceptible to a variety of drugs.

This work was supported in part by the British Heart Foundation and by Organon Laboratories.

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CHARACTERISATION OF THE CALCIUM PARADOX IN THE GUINEA-PIG ISOLATED HEART

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Exposure of the myocardium to calcium after calcium-free perfusion causes mechanical, biochemical and structural dysfunction (Zimmerman and Hülsmann, 1966). This phenomenon of experimental cardiac injury, the Calcium Paradox, has been well characterised in the rat isolated heart (Hearse et al., 1978), but not in the guinea-pig isolated heart. This study describes the Calcium Paradox in the guinea-pig.

Hearts of male guinea-pigs (530-580g) were set up as described by Langendorff and were perfused (37.5°C) at 75 cm of water. After 30 minutes equilibration with Krebs-Henseleit bicarbonate perfusion medium containing 2.4 mM calcium, the hearts were exposed to either a 1, 1½, 2, 3, 3½, 4 or 5 minutes calcium-free period, followed by a further 10 minutes perfusion with calcium containing perfusion medium. Throughout the experiment developed isometric tension, coronary flow and epicardial ECG were measured and the coronary effluent was collected and assayed for Creatine Kinase (CK) activity. The epicardial ECG was analysed over minute periods for the number of atrial, ventricular and ectopic ventricular depolarisations.

Ventricular contraction ceased within 30 seconds of calcium-free perfusion and throughout the calcium-free period. There was basal CK release (<12 IU/min/g dry wt) for calcium-free periods of 1, 1½, 2 and 3 minutes with raised levels after the 3rd minute, reaching 78.5±26.9 IU/min/g dry weight (n=5) during the fifth minute.

Table 1 shows the time related effect of calcium withdrawal on the parameters measured 10 minutes after calcium readmission, with maximal injury after 3½ minutes calcium-free perfusion.

Table 1. The effect of the duration of calcium-free perfusion on CK release, coronary flow, isometric tension and water content (n=5)

Calcium free period (min)	Total CK released (IU/g dry wt)	Change in coronary flow (ml/min)	% recovery of isometric tension	Water content of heart (gH_2O/g dry wt)
1	14.7±7.6	0±0.6	91±3	5.54±0.14
1½	578.3±328.1	-0.1±0.9	61±8	5.63±0.08
2	1682.0±968.4	-2.2±1.9	36±3	5.61±0.44
3	3400.0±473.5	-7.5±0.4	2±1	6.02±0.41
3½	4549.9±463.1	-7.4±1.1	0	6.72±0.15
4	4547.6±611.4	-7.3±2.1	0	6.30±0.24
5	4606.7±786.9	-8.4±0.3	0	6.92±0.36

After calcium readmission there was little effect on the number of atrial depolarisations. However, with increasing calcium-free period, there was an increasing incidence of ventricular arrhythmias until calcium-free periods of greater than 2 minutes where complete A-V block was observed.

These results indicate that the degree of damage caused by the Calcium Paradox is related to the duration of the calcium-free period in the guinea-pig isolated heart.

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UK-36,327-INDUCED CARDIAC STIMULATION IN THE CONSCIOUS DOG

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UK-36,327, a novel compound with inotropic activity in vitro and in anaesthetised preparations (Ellis & Joice) was assessed for activity in the conscious dog. Positive inotropic activity was determined non-invasively by measurements of QA interval (Alabaster & Henderson, 1982).

Four conscious male normotensive beagle dogs were used. The arterial pressure pulse from an exteriorised carotid artery and Lead II ECG were recorded and QA interval and heart rate (HR) derived. Measurements were made every 10 min. for 30 min. before and 4 hr. after the oral administration of UK-36,327 (by gavage in 5 ml. tap water) at doses of 1, 2 and 4 mg kg^{-1}. In separate experiments, UK-36,327 was administered i.v. by stepwise, cumulative infusion, doubling the rate of infusion every 15 min., from 5-40 $\mu g\ kg^{-1}\ min^{-1}$. QA interval and HR were determined at control and at the end of each infusion period.

Oral administration of UK-36,327 produced a dose dependent decrease in QA interval which was evident within the first hour after dosing and was maintained over the 4 hr. study period (Figure 1). There was not however, even at the highest dose level, an appreciable alteration in HR. Likewise UK-36,327 infused i.v. at 5, 10, 20 and 40 $\mu g\ kg^{-1}\ min^{-1}$ produced decreases in QA interval of 2, 4, 12 and 19 m.sec. respectively without any concomitant tachycardia. This was in marked contrast to the dose dependent tachycardia observed after equi-inotropic doses of isoprenaline in the same four dogs (Alabaster and Henderson, 1982).

UK-36,327 is therefore a potent, orally active, force-selective cardiac stimulant in the conscious dog.

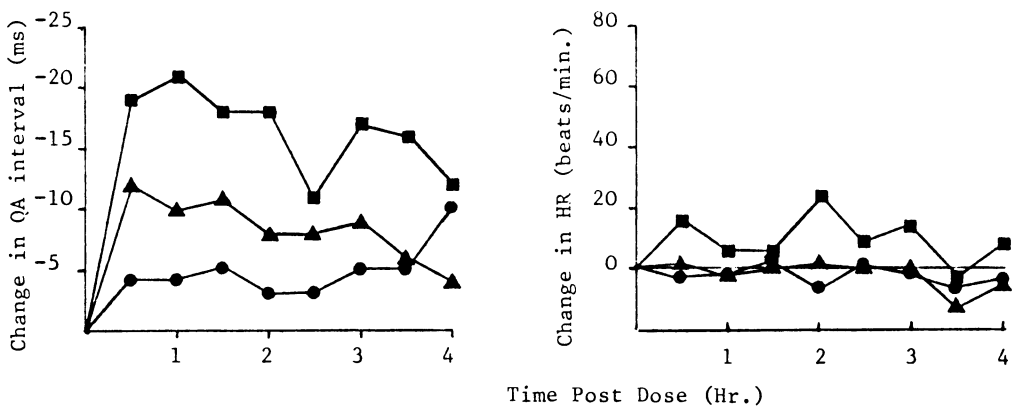

Figure 1. Inotropic and chronotropic responses to oral administration of UK-36,327 1 mg kg^{-1} (●—●), 2 mg kg^{-1} (▲-▲) and 4 mg kg^{-1} (■—■).

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UK-36,327: A NOVEL CARDIAC STIMULANT AGENT

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In spontaneously beating guinea pig atria, over the concentration range $5x10^{-7}$ to $1.28x10^{-4}$M, UK-36,327 (N-{2-[1-(6,7 dimethoxy-4-quinazolinyl)-4-piperidinyl] propyl}-N-methylacetamide produced dose dependent increases in contractile amplitude, whilst eliciting much smaller increases in the frequency of beating (70% and 17% respectively of the maximum increases produced by isoprenaline). UK-36,327 (0.3 to 10 mg) produced dose-related increases in contractility (dp/dt max) in a dog heart/lung preparation, achieving a 130% increase in dp/dt max but only a 30% increase in heart rate over control values at 10 mg. In pentobarbitone anaesthetised dogs, bolus injections of UK-36,327 (25 - 200 µg/kg) produced dose-related increases in dp/dt max, accompanied by transient falls in blood pressure and small increases in heart rate (max changes over control: dp/dt max ↑ 80%, BP ↓ 40 mmHg, HR ↑ 25 beats/min). When administered to anaesthetised dogs close arterially (femoral artery) and close venously (metatarsal vein), UK-36,327 (0.1 to 1000 µg total dose) produced dose-related decreases in both hind limb and venous resistance, achieving 50% of the max reduction in resistance at 20 µg and 10 µg total dose respectively. In addition, UK-36,327 has been shown to be actice both intravenously and orally in the conscious dog (Alabaster and Henderson, this Meeting).

UK-36,327 did not inhibit Na^+K^+ATPase from guinea pig brain *in vitro* over the concentration range $1x10^{-6}$ to $5x10^{-5}$M, and did not stimulate adenyl cyclase activity from guinea pig heart *in vitro* over the range $5x10^{-6}$ to $5x10^{-4}$M. However, UK-36,327 was a potent inhibitor of bovine heart cAMP-phosphodiesterase (PDE) *in vitro* with an ID_{50} (mean ± s.e.mean) of 3.8 ± 0.29 µM (compared with 100 ± 8.5 µM for theophylline), and increased intracellular cAMP levels in guinea pig atria from control levels of 4.02 ± 0.34 pmoles/mg tissue protein to 7.02 ± 0.63 pmoles/mg tissue protein at the peak inotropic response produced by $2x10^{-5}$M UK-36,327.

UK-36,327 therefore displays the properties of a force/rate selective inotropic agent with balanced arteriolar and venous dilator activity, and with PDE inhibition postulated as the mechanism of action.

EFFECTS OF ENCAINIDE AND ITS METABOLITES ON VENTRICULAR ARRHYTHMIAS IN DOGS

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Encainide (E) is a new Class I anti-arrhythmic drug which has been shown to abolish experimental atrial and ventricular arrhythmias and promote the return of sinus rhythm without adverse effects in a variety of animal models (Byrne and Gomoll, 1973; Byrne et al, 1977). Encainide is metabolised after IV and oral administration in man with the formation in the majority of patients of O-demethyl encainide (ODE) and 3 methoxy-O-demethyl encainide (3 MODE). The plasma concentrations of ODE and 3 MODE at times exceed those of encainide (Kates et al., 1982). It has been suggested that these metabolites may contribute to the anti-arrhythmic effect of encainide in man (Roden et al,1980). In the present study we investigated the effects of E, ODE and 3 MODE on ventricular arrhythmias produced in dogs either by cardio-toxic doses of ouabain or coronary artery ligation. Ventricular tachycardia (VT) was induced in anaesthetised dogs by the IV injection of ouabain. In the placebo treated dogs the ventricular tachycardia lasted 108.8 ± 11.2 min. (n=5). Drugs were given by IV infusion. E infused at 0.02 mg/Kg/min converted VT to sinus rhythm (SR) after a mean dose of 0.42 mg/Kg in 3/6 dogs; at 0.1 mg/Kg/min after a mean dose of 0.6 mg/Kg in 3/4 dogs; at 0.2 mg/Kg/min after a mean dose of 1.1 mg/Kg in 2/3 dogs. ODE infused at 0.02 mg/Kg/min converted VT to SR after a mean dose of 0.268 mg/Kg in 5/7; ODE at 0.1 mg/Kg/min did not produce a definite return to SR. 3 MODE infused at 0.01 mg/Kg/min (n=1) and 0.02 mg/Kg/min (n=2) did not produce a return to SR. 3 MODE infused at 0.04 mg/Kg/min (n=2) produced a return to SR with a dose of 0.64 mg/Kg in 1/2 dogs.

In conscious dogs 24 hours after 2 stage ligation of the left anterior descending coronary artery frequent ventricular ectopic beats (VEB) were present. After a control period of 30 min, E. ODE and 3 MODE were administered by bolus injection (0.125, 0.25 mg/Kg etc) every 10 minutes until a return to sinus rhythm for 5 minutes. The ECG was recorded continuously during the control period and throughout drug administration. Less than 10% of the ventricular beats were of sinus origin before drug administration. The VEB were suppressed in all dogs and SR was restored in 4/4 after a mean cumulative dose of 2.375 mg/Kg E, in 4/4 dogs after a mean cumulative dose of 0.625 mg/Kg ODE and in 4/4 dogs after a mean cumulative dose of 1.39 mg/Kg 3 MODE.

We conclude that ODE and 3 MODE may contribute to the anti-arrhythmic effect of encainide in patients and to the variability in response to the drug.

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HAEMODYNAMIC RESPONSES TO IBOPAMINE, AN ORALLY-ACTIVE DOPAMINE ANALOGUE, IN ANAESTHETIZED CATS

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Ibopamine, the di-isobutyric ester of N-methyl-dopamine, increases cardiac contractility, renal blood flow and diuresis (Casagrande et al., 1980; Merlo et al., 1982). Systemic hydrolysis of ibopamine (IB) to epinine (EP) (N-methyl-dopamine) is believed to be required for activity.

We have studied the haemodynamic effects of IB and EP and undertaken some pharmacological analysis of these responses.

Experiments were carried out in cats of either sex; body weight 2.2 - 4.5 kg, anaesthetised with sodium pentobarbitone (50 mg/kg i.p.). Blood pressure (BP) was monitored from a femoral artery and the signal used to trigger a heart rate (HR) meter; aortic blood flow (ABF) was measured by placing an electromagnetic flow probe around the ascending aorta. Stroke volume (SV) and total peripheral resistance (TPR) were derived from the measured variables.

Infusions of EP (5 - 80 μg/kg/min) or IB (10 - 80 μg/kg/min) for 10 minutes produced dose-dependent increases in ABF and SV with no consistent effects on HR. BP increased initially, reflecting the increases in ABF; TPR was initially little changed and tended to decline subsequently. Responses reversed rapidly on completion of each infusion.

Responses to injections of EP (7.5 - 60 μg/kg) and IB (15 - 60 μg/kg) were associated with dose-dependent increases in HR, BP, ABF and SV; TPR fell but this response was not dose-dependent.

Propranolol (1 mg/kg) slightly potentiated pressor responses to both agonists. The initial response under these circumstances was an increase in BP and TPR, bradycardia and a fall in ABF reflecting a decreased SV. Subsequently ABF rose while TPR returned rapidly to control values.

Phentolamine (2 mg/kg) abolished pressor and vasoconstrictor responses to EP and IB, resulting in depressor and vasodilator responses which showed poor dose-dependency. Effects on ABF, SV and HR were significantly attenuated.

EP and IB clearly possess both α- and β-adrenoceptor agonist activity. The increases in ABF and SV reflect the known inotropic actions of these agents. The minimal effects on TPR may be interpreted as reflecting a balance of α-mediated vasoconstriction and vasodilatation due to actions at both β-adrenoceptors and dopamine receptors.

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