INVESTIGATIONS INTO THE MOTOR INHIBITORY PROPERTIES OF APOMORPHINE AND 5HT USING AN INTRACEREBRAL INJECTION TECHNIQUE IN THE MOUSE

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Low doses of apomorphine and other dopamine (DA) agonists cause a reduction in spontaneous locomotor activity (SLA) of mice, which is neuroleptic sensitive (Costall et al, 1981). This motor inhibitory property of low doses of DA agonists has been attributed to their ability to preferentially stimulate DA 'autoreceptors' (Di Chiara et al, 1976; Costall et al, 1981). Although a correlation between locomotor depression and decreased mesolimbic DA utilisation has been shown in the rat (Hjorth et al, 1981; Costall et al, 1980), there is no direct evidence as to the cerebral site mediating motor inhibitory effects of DA agonists in the mouse. In the present study, we have investigated the effects of intra-accumbens administration of apomorphine and 5HT on locomotor activity in the mouse.

Male B.K.W. mice (35-40 g) were stereotaxically implanted with guide cannulae for drug administration into the nucleus accumbens using the modified technique of Costall and Naylor (1976) (implantation technique described in detail elsewhere). Intra-accumbens administration of DA (0.75-6.25 µg) to mice, 7 days post-operatively, caused a dose-related increase in locomotor activity, which was antagonised by haloperidol (0.025-0.1 mg/kg i.p.) and (±)sulpiride (2.5-10 mg/kg i.p.). In contrast, intra-accumbens administration of 5HT (5-20 µg) caused a dose-related decrease in SLA of mice. This decrease in SLA by 5HT was potentiated by pre-treatment with fluoxetine (5.0 mg/kg i.p.) and antagonised by methysergide (1.0 mg/kg i.p.). Also, intra-accumbens administration of 5HT (5 µg) caused a significant reduction in DA-induced locomotor hyperactivity. This reduction was antagonised by methysergide (1.0 mg/kg i.p.) but not by haloperidol (0.0125 mg/kg i.p.). Similarly to 5HT, intra-accumbens apomorphine (0.125-1.0 µg) caused a reduction in DA-induced locomotor hyperactivity, which, in contrast to the reduction by 5HT, was antagonised by haloperidol (0.0125 mg/kg i.p.) but not methysergide (1.0 mg/kg i.p.)

We conclude, firstly, that the intra-accumbens administration of DA in the mouse can cuase a locomotor hyperactivity response which is attenuated by intra-accumbens administration of apomorphine and 5HT. Secondly, apomorphine reduction of the DA hyperactivity response in the mouse is mediated through a neuroleptic sensitive mechanism within the area of the nucleus-accumbens.

This work was supported by the Parkinson's Disease Society.

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THE BIOCHEMISTRY OF INDOLEAMINE-INDUCED MYOCLONUS IN GUINEA-PIGS

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L-5-Hydroxytryptophan (5HTP) (with or without carbidopa), L-tryptophan (plus pargyline) and tryptamine (plus pargyline) all evoke myoclonus in guinea pigs through brain stem indoleamine mechanisms (Chadwick et al, 1978; Luscombe et al, 1981). 5-Hydroxytryptamine (5HT) antagonists differently inhibit the myoclonic jerking induced by 5HTP (plus carbidopa) compared to that evoked by tryptamine (plus pargyline); this may indicate distinct 5HT and tryptamine receptors (Jenner et al, 1981; Luscombe et al, 1981). From studies of biochemical changes produced by indoleamines in rats Marsden & Curzon (1979) suggested tryptamine may contribute to 5HT dependent behaviour. We now report a biochemical study of indoleamine-induced myoclonus in guinea pigs.

L-Tryptophan (25-500 mg/kg ip; 90 min previously) caused a dose-dependent elevation in brain tryptophan, 5HT and 5HIAA (5-hydroxyindoleacetic acid) concentrations, and tryptophan (200 mg/kg ip) induced a marked increase in whole brain tryptamine content, but no myoclonus resulted. L-tryptophan (200 mg/kg ip 90 min prior to death) plus pargyline (75 mg/kg ip; 30 min previously) increased brain tryptamine content less than tryptophan alone, but cerebral 5HT levels were more elevated and jerking now was observed.

5HTP (50-200 mg/kg sc; 60 min previously) produced a dose-dependent rise in brain 5HT and 5HIAA levels. 5HTP (200 mg/kg sc) depressed the brain tryptamine content, but caused a five-fold increase in cerebral 5HT concentrations, and induced pronounced myoclonus. 5HTP (20-80 mg/kg sc; 60 min prior to death) plus carbidopa (25 mg/kg ip; 60 min previously) also induced dose-related elevations of 5HT and 5HIAA concentrations, and evoked dose-dependent myoclonus. 5HTP (80 mg/kg sc) plus carbidopa did not alter the cerebral tryptamine content but markedly elevated brain 5HT levels and induced pronounced jerking.

Tryptamine (10 mg/kg) plus pargyline (75 mg/kg ip; 60 min previously) caused a greater elevation of tryptamine content than tryptophan (200 mg/kg) plus pargyline (75 mg/kg), but did not evoke myoclonus. Tryptamine (40 mg/kg ip; 30 min prior to death) plus pargyline (75 mg/kg ip; 60 min previously) caused only an average 60% increase in regional 5HT levels, but cerebral tryptamine content increased one-hundred-fold and myoclonus was observed.

The biochemistry of myoclonus induced by tryptophan, 5HTP and tryptamine indicates that 5HT mediates 5HTP-induced jerking and probably is the dominant amine in tryptophan plus pargyline-evoked myoclonus. Tryptamine, however, may play a role in myoclonus induced by tryptamine plus pargyline in guinea pigs.

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CATION DEPENDENCY OF (^{3}H)-SULPIRIDE BINDING IS DUE TO ALTERATION IN THE NUMBER OF STRIATAL BINDING SITES

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The specific binding of ^{3}H-sulpiride to rat striatal preparations in vitro is critically dependent on the presence of sodium ions (Theodorou et al,1980). In contrast specific ^{3}H-spiperone binding is only slightly altered by variation of the cation content of the incubation buffer. In the present study we have examined the specificity of this phenomena by attempting to replace sodium by other cations from the same group of the periodic table. In addition we have examined whether a change in Bmax or K_D is associated with the cation effects.

Washed striatal preparations from female Wistar rats (150 $\pm$ 10 g) were incubated with either ^{3}H-sulpiride (26.2 Ci/mmole;2.5-40 nM; specific binding defined by 5 x 10^{-6}M (+)-butaclamol) or ^{3}H-spiperone (15.5 Ci/mmole; 0.125-4.0 nM;specific binding defined by 5 x 10^{-6}M (+)-butaclamol) utilising a 50 mM tris HCl buffer pH 7.7 to which the various cations were added.

Incubation of ^{3}H-sulpiride (10 nM) in a cation free buffer resulted in minimal specific binding. Incorporation of sodium chloride (120 mM) restored specific binding to maximal levels (7.2 $\pm$ 0.5 pmoles/g wet weight of tissue). Replacement of sodium ions by caesium chloride (1-100 mM) or rubidium chloride (1-100 mM) did not restore specific binding of ^{3}H-sulpiride (10 nM). However, incorporation of lithium chloride (1-100 mM) caused a partial restoration of specific binding to 39% of that observed in the presence of sodium chloride (120 mM).

Specific binding of ^{3}H-spiperone (0.2 nM) did not differ whether carried out in a cation free buffer or in the presence of sodium chloride (120 mM), lithium chloride (1-100 mM), caesium chloride (1-100 mM) or rubidium chloride (1-100 mM).

Scatchard analysis of specific ^{3}H-sulpiride (2.5-40 nM) binding carried out in the presence of an increasing concentration of sodium chloride (5-120 mM) showed a progressive increase in the number of binding sites (Bmax) but no change in the dissociation constant (K_D). Incorporation of lithium chloride (5-120 mM) had an identical effect but the Bmax did not reach the level observed in the presence of sodium chloride. Similar analysis of specific ^{3}H-spiperone (0.125-4.0 nM) binding in the presence of sodium chloride (5-120 mM) or lithium chloride (5-120 mM) did not alter Bmax but tended to increase K_D.

The cation dependency of specific ^{3}H-sulpiride binding would appear highly specific to sodium ions, although lithium ions can substitute partially. The alteration in specific binding appears to be due to changes in the number of available binding sites rather than to alteration in the affinity of the ligand for this site. The failure to observe similar changes with ^{3}H-spiperone suggests that the interaction of ^{3}H-sulpiride with its binding site may differ from that of other neuroleptic ligands.

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DIFFERENTIATION OF THE BINDING OF (^{3}H)-PIFLUTIXOL TO D-1 AND D-2 RECEPTORS USING SULPIRIDE

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Both ^{3}H-flupenthixol and ^{3}H-piflutixol selectively label D-1 adenylate cyclase linked dopamine receptors in rat striatal preparations (Hyttel, 1978,1981). However, analysis of displacement by butyrophenone compounds reveals both ^{3}H-flupenthixol and ^{3}H-piflutixol to identify D-1 and D-2 (about 20% of total) receptors in calf and rat striatal preparations respectively (Cross & Owen,1980; Hyttel, 1981). We find that the two binding sites in rat striatal preparations labelled by ^{3}H-piflutixol can be differentiated by the use of sulpiride into D-1 and D-2 receptors.

Scatchard analysis of specific ^{3}H-piflutixol (11.7 Ci/mole; 0.06-1.6 nM) binding defined using 1 uM cis-flupenthixol gave a Bmax of 122±5 pmoles/g tissue and K_D 0.21±0.02 nM. In the presence of sulpiride (100 uM) Bmax was reduced to 97±3 pmoles/g tissue but K_D was unaltered at 0.29 ± 0.05 nM. The difference in Bmax was 25 pmoles/g tissue, which was approximately equal to the Bmax for specific ^{3}H-spiperone (0.04-1.0 nM) to the same tissue preparation as defined by 1 uM (+)-butaclamol, of 27.7±0.5 pmoles/g tissue with K_D 0.10±0.01 nM.

Cis-flupenthixol (IC50 8 - 9 nM), or cis-piflutixol (IC50 0.8-0.9 nM), displaced the specific binding of ^{3}H-piflutixol (0.3 nM) and that of ^{3}H-spiperone (0.2 nM) to the same degree, with a Hill slope close to 1.0 in each case. In contrast, haloperidol (10^{-10}-10^{-4} M) was more potent in displacing ^{3}H-spiperone (IC50 5 nM) than ^{3}H-piflutixol (IC50 1000 nM); Hill slope for displacement of ^{3}H-spiperone was 0.91 but for ^{3}H-piflutixol a biphasic plot was obtained with slopes of 0.40 for the high affinity component and 0.87 for the low affinity component.

Close analysis of the displacement of ^{3}H-piflutixol (0.8 nM) by haloperidol (10^{-10}-10^{-4} M) in the presence and absence of sulpiride (100 uM) (defining the extent of specific binding by incorporation of 1 uM cis-flupenthixol) revealed two components. A high affinity component comprising 21% of total specific binding, that was removed by the addition of 100 uM sulpiride, and a low affinity component, insensitive to sulpiride. In the presence of sulpiride (100 uM), haloperidol displaced ^{3}H-piflutixol with an IC50 value of 1700 nM and gave a Hill slope of 0.85. Analysis of haloperidol displacement of the sulpiride sensitive component of ^{3}H-piflutixol binding (obtained by subtraction of displacement produced in the presence and absence of sulpiride) gave an IC50 value of 6.5 nM and a biphasic Hill plot of slopes of 0.70 for the high affinity component and 0.40 for the low affinity component.

The data is consistent with ^{3}H-piflutixol binding with equal affinity to a large number of D-1 and a smaller number of D-2 dopamine receptors in rat striatal preparation. The D-2 selective butyrophenone haloperidol displaced ^{3}H-piflutixol from D-2 receptors with high affinity, but from D-1 receptors with low affinity. The 20% bound to D-2 receptors can be specifically removed by the addition of a high concentration (100 uM) of sulpiride, which does not displace the ligand from D-1 receptors.

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KAINIC ACID-INDUCED CIRCLING IS MEDIATED VIA THE ANGULAR COMPLEX AND ROSTRAL SUPERIOR COLLICULUS

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Rotation induced in the rat by unilateral injection of kainic acid into the striatum is mediated via the strio-nigral pathway (Jenner et al, 1980). Outflow pathways from zona reticulata of substantia nigra to the angular complex (peri-aqueductal grey and adjacent mesencephalic reticular formation) and the deep layers of the superior colliculus have been implicated in the expression of circling behaviour induced by dopamine agonists in unilateral 6-hydroxydopamine nigro-striatal lesioned animals (Reavill et al, 1981), or in animals stimulated to rotate by intranigral injection of muscimol (Collingridge et al, 1981). We now investigate the involvement of these areas in circling caused by intra-striatal administration of kainic acid.

Unilateral intrastriatal injection of kainic acid (10 nmol in 1 ul 0.9% saline adjusted to pH 6.9 with 2N sodium hydroxide) into female Wistar rats (180-220 g) caused circling behaviour characterised by initial weak ipsiversive rotation lasting up to 2 h followed by marked contraversive rotation lasting in excess of 10 h. Unilateral electrolytic (3 mA for 8 sec) or kainic acid (2.5 nmol/0.5 ul 0.9% saline) lesions of the angular complex (A 0.6; L 1.0; V -1.4, De Groot, 1959) 4 days previously caused no spontaneous circling. However, marked 'head-to-tail" ipsiversive rotation was induced by administration of apomorphine hydrochloride (MacFarlan Smith Ltd., 0.5 mg/kg s.c., 15 min previously) in rats with such lesions, whether electrolytic (12.8 ± 3.0 turns per min; n = 12) or produced by kainic acid (13.5 ± 3.4 turns per min; n = 8). The duration of the initial ipsi-versive rotation induced by the unilateral intrastriatal administration of kainic acid (10 nmol) was not modified by unilateral electrolytic lesions or kainic acid lesions of the angular complex 11 to 13 days previously. However, the subsequent contraversive rotation was markedly reduced ($P < 0.001$; n = 10) in animals with electrolytic lesions and was abolished ($P < 0.001$; n = 7) in animals with kainic acid lesions of this area. Bilateral electrolytic lesions of the angular complex did not modify the duration of ipsiversive rotation but attenuated ($P < 0.001$; n = 13) the subsequent contraversive rotation induced in control animals (n = 9) by unilateral injection of kainic acid into the striatum.

Histological examination of brains from animals with either kainic acid or electrolytic lesions of the reticular formation revealed extensive cell loss and demyelination of the dorsal reticular formation and some damage to lateral aspects of the periaqueductal grey. In addition, intermediate and deep layers of superior colliculus were damaged in the majority of animals at rostral but not at mid- or caudal levels.

The results suggest that part or the whole of the area comprising the dorsal mesencephalic reticular formation and lateral aspects of the periaqueductal grey (angular complex) and the intermediate and deep layers of the superior colliculus plays a role in the maintenance of postural symmetry and in the mediation of circling behaviour induced by unilateral injection of kainic acid into the striatum.

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MODULATION OF GABA FUNCTION IN THE ANGULAR COMPLEX MIMICS DOPAMINE-MEDIATED CIRCLING BEHAVIOUR

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Unilateral electrolytic or kainic lesions of the lateral periaqueductal grey and adjacent mesencephalic reticular formation (the angular complex) reduce or reverse contraversive circling caused by administration of apomorphine to animals with a unilateral 6-hydroxydopamine lesion of the medial forebrain bundle (Jenner et al,1981). We have ascribed this effect to the existence of an outflow pathway from zona reticulata of substantia nigra to the angular complex that is responsible for mediating dopamine-dependent circling behaviour. We report now the effect of manipulation of GABA function in the angular complex on rotational behaviour induced either by apomorphine in 6-OHDA lesioned animals or by muscimol injected into zona reticulata of substantia nigra.

Muscimol (100 ng/0.1 ul 0.9% saline) injected into the region of the left angular complex (A 0.6; L 1.0; V -0.5; Konig & Klippel, 1963) of naive rats caused ipsiversive rotation (9.4 $\pm$ 0.5 turns per min). Picrotoxin (100 ng/0.1 ul 0.9% saline) injected into the same area caused contraversive rotation (7.6 $\pm$ 1.1 turns per min). Unilateral 6-OHDA hydrobromide (8 ug in 3 ul 0.9% saline containing 2 ug ascorbic acid) lesions of the medial forebrain bundle at the level of the left lateral hypothalamus (A 4.6; L 1.9; V -3.0; de Groot,1959) caused contraversive circling (16.3 $\pm$ 1.4 turns per min) to apomorphine hydrochloride (0.5 mg/kg s.c. 15 min previously). Four days later, muscimol (100 ng/0.1 ul 0.9% saline) injected into the left angular complex caused ipsiversive rotation (4.4 $\pm$ 0.7 turns per min) 10 min later. Injection of apomorphine (0.5 mg/kg s.c.) 30 minutes after muscimol into the same animals again resulted in ipsiversive rotation (8.0 $\pm$ 1.8 turns/min).

Focal injection of muscimol (100 ng in 0.1 ul 0.9% saline 30 min previously) into the left zona reticulara of substantia nigra (A 1.6; L 2.0; V -2.6; Konig & Klippel,1963) caused contraversive rotation (25.6 $\pm$ 4.2 turns per min). Picrotoxin (100 ng in 0.1 ul 0.9% saline) injected into the same site caused ipsiversive rotation (6.8 $\pm$ 1.1 turns/min). A prior injection of muscimol (100 ng in 0.1 ul 0.9% saline 30 min earlier) into the angular complex prevented this contraversive rotation; such animals rotated ipsiversively (1.5 $\pm$ 1.0 turns per min).

The data suggest that manipulation of GABA function in the angular complex can mimic the behavioural effects produced by dopamine agonist in 6-OHDA lesioned animals. That the angular complex is involved in the outflow pathway from the basal ganglia responsible for the modulation of this behaviour is suggested by the dominance of rotation induced from the angular complex over rotation induced from striatum or substantia nigra. The precise anatomical area involved remains to be defined since Collingridge et al (1981) and Pope et al (1980) have attributed a similar role to the adjacent deep layers of the superior colliculus. Our histological evidence favours the critical site to be in the mesencephalic reticular formation.

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GUANYL NUCLEOTIDES DECREASE THE BINDING AFFINITY OF $GABA_B$ BUT NOT $GABA_A$ RECEPTORS IN THE MAMMALIAN CNS

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^{3}H-GABA binds with high affinity and in a saturable manner to two pharmacologically distinct populations of GABA receptor in the mammalian CNS. Binding to $GABA_A$ receptors is independent of the presence of ions and is depressed by isoguvacine and bicuculline (Enna & Snyder, 1977; Krogsgaard-Larsen, 1978). By contrast, saturable binding of ^{3}H-GABA to baclofen-sensitive $GABA_B$ receptors is dependent upon the presence of divalent cations and is not influenced by high concentrations of bicuculline or isoguvacine (Hill & Bowery, 1981).

Recently a number of hormone binding sites have been shown to be influenced by the guanine nucleotides guanosine triphosphate (GTP) and guanosine diphosphate (GDP) but not by guanosine monophosphate (GMP) or adenine nucleotides such as adenosine triphosphate (ATP) (Tsai & Lefkowitz, 1978; Creese et al. 1979; Pert & Taylor,1980) We now present evidence from radioligand binding studies to show that $GABA_B$ but not $GABA_A$ receptors are also sensitive to guanine nucleotides.

Rat crude synaptic membranes were prepared according to Zukin et al (1974) and stored at -15°C before assay. Binding of ^{3}H-GABA (50 Ci/mmol, 5-10 nM) to four times washed membranes was assayed (10 min, 20°C) in tris-HCl buffer solution (50 mM pH 7.4) containing 1.2 mM $MgSO_4$ or 2.5 mM $CaCl_2$ in the presence of either 40 µM unlabelled isoguvacine or 100 µM (±)baclofen to saturate $GABA_A$ or $GABA_B$ receptors respectively. ^{3}H-baclofen (8.8 Ci/mmol, 20 nM) binding was determined in Krebs'-Henseleit solution or tris-HCl buffer plus 2.5 mM $CaCl_2$. Non-specific binding was obtained in the presence of 100 µM isoguvacine (for $GABA_A$ sites) or (±)baclofen (for $GABA_B$ sites).

Binding of ^{3}H-GABA to bicuculline-sensitive $GABA_A$ receptors was not altered by GTP (100 µM) whereas binding to $GABA_B$ sites was suppressed in a dose-dependent manner by GTP (IC_{50} 15.7 ± 4.8 µM, n=3) and GDP (IC_{50} 48.2 ± 8.1 µM, n=3) but not by GMP (up to 300 µM). This effect was observed only with the guanine nucleotides, 300 µM ATP producing less than 20% inhibition of specific binding. Specific ^{3}H-baclofen binding to the $GABA_B$ receptor in Krebs'-Henseleit solution was also suppressed by GTP (IC_{50} 1.0 ± 0.49 µM, n=4) and GDP (IC_{50} 24.0 ± 1.8 µM, n=3). Both GMP and ATP were only weakly active (IC_{50} > 300 µM). The IC_{50} value for inhibition of ^{3}H-baclofen binding in tris-buffer plus $CaCl_2$ (11.3 ± 1.2 µM, n=3) was significantly greater ($p < 0.02$) than that obtained in Krebs'-Henseleit solution but similar to the value obtained using ^{3}H-GABA in the same medium. Analysis of the binding of ^{3}H-GABA to $GABA_B$ sites in the presence of GTP (30 µM) showed that the reduction in saturable binding resulted from a decrease in the affinity (increased K_d) of the receptors for GABA. There was no change in receptor binding capacity (B_{max}). The K_d value was increased from 78.0 ± 7.1 nM (control n=6) to 125 ± 11.1 nM (plus GTP n=3) whilst the B_{max} values were 1.17 ± 0.06 pmol/mg protein and 1.18 ± 0.17 pmol/mg protein respectively. We conclude that $GABA_B$ but not $GABA_A$ receptors are sensitive to modulation by guanine nucleotides.

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FUNCTIONAL INTERACTIONS OF BENZODIAZEPINE ANTAGONISTS

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Recently two specific antagonists of benzodiazepine (BDZ) drugs have been described. One, ethyl-β-carboline-3-carboxylate (β-CCE), demonstrates intrinsic activity by lowering seizure thresholds when administered alone (Cowen et al, 1981). The other, Ro 15-1788, blocks the anticonvulsant effects of BDZs but has itself no effect on seizure threshold (Hunkeler et al, 1981). We have studied the interactions between the effects of these drugs on seizure threshold in vivo and on responses to GABA in vitro.

Seizure thresholds were determined in rats by infusing a 10 mg ml^{-1} solution of pentylenetetrazol (PTZ) (Nutt et al, 1981). Ro 15-1788 (10 mg kg^{-1}) blocked both the anticonvulsant action of diazepam (5 mg kg^{-1} i.p.) and the proconvulsant effect of β-CCE (1 mg kg^{-1} i.v.) (Table 1). It had no effect on the anticonvulsant action of phenobarbitone (40 mg kg^{-1} i.p.), and at this dose no anticonvulsant effect alone. At higher doses Ro 15-1788 (50 mg kg^{-1}) did exert intrinsic anticonvulsant activity (seizure thresholds to PTZ: vehicle 30 ± 4 mg kg^{-1}(5); Ro 15-1788 36 ± 5 mg kg^{-1}(6) $P < 0.05$).

Table 1 Seizure thresholds to PTZ (mg kg^{-1})

Treatment	Vehicle (Tween)	Ro 15-1788 10 mg kg^{-1}	P Value
Saline	34 ± 2(5)	35 ± 2(6)	N.S.
β-CCE	26 ± 3(5)	35 ± 3(6)	<0.001
Diazepam	62 ± 14(7)	39 ± 4(5)	<0.001
Phenobarbitone	52 ± 5(6)	56 ± 9(6)	N.S.

Numbers are mean ± S.D. with number of rats in brackets. Ro 15-1788 or Tween given i.p. 15 min prior to infusion. β-CCE given 10 min after Ro 15-1788. Diazepam and phenobarbitone given 15 min before Ro 15-1788.

The same interactions were noted when the drugs were tested in vitro on the rat superior cervical ganglion using the method of Brown & Marsh (1974). BDZs increase the responses to GABA in the presence of bicuculline (Bowery & Dray, 1978). Ro 15-1788 (835 nM) antagonised this facilitation of GABA responses (38.8 μM) by chlordiazepoxide (14.9 μM). There was no effect of Ro 15-1788 on the facilitation of GABA produced by phenobarbitone (393 μM - 1.57 mM). β-CCE (1 μM) caused small reductions in the responses to GABA under the same conditions and Ro 15-1788 (835 nM) antagonised this effect. High concentrations of Ro 15-1788 (167-334 μM) increased the responses to GABA.

These results show that high affinity ligands for the BDZ receptor may produce opposite pharmacological effects and yet have a common antagonist, Ro 15-1788.

We thank Glaxo (β-CCE) and Roche (benzodiazepines and Ro 15-1788).

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GABA AND BENZODIAZEPINE BINDING IN MICE SUSCEPTIBLE AND RESISTANT TO AUDIOGENIC SEIZURES (AS)

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DBA/2 mice show a high susceptibility to AS, which is maximal between 21-28 days of age. The reason for the susceptibility to AS is uncertain but drugs which block the breakdown or reuptake of GABA or GABA agonists prevent or reduce the severity of AS in DBA/2 mice (Anlezark et al, 1976; Horton et al, 1979; Meldrum & Horton, 1980). These findings prompted us to study age-related (8-40 days) (^{3}H) GABA binding in well-washed, frozen and thawed, Triton X-100 treated crude synaptic membranes prepared from whole brains of DBA/2 mice and T/O mice, a strain less susceptible to AS. High and low affinity binding sites were apparent in both strains of mice at all ages studied. DBA/2 mice had fewer high affinity binding sites for (^{3}H)GABA than T/O mice (10-43%) throughout the age range studied but binding was of higher affinity (K_D; DBA/2 3-11.8nM, TO 11.8-30nM). The number of low affinity binding sites was significantly lower in DBA/2 mice at 8 and 40 days (45% and 76% respectively) but there were no significant differences between strains from 17 to 28 days of age. The K_D of the low affinity site was significantly higher at 28 days and lower at 40 days in DBA/2 mice.

Benzodiazepine (BZ) binding sites are proposed to constitute part of the GABA receptor complex. BZs potentiate GABA-mediated inhibition and GABA stimulates (^{3}H) BZ binding. (Haefely, 1978; Martin & Candy, 1978). In view of the interaction between GABA and BZ binding sites and of the demonstrated differences in GABA binding sites in these two strains of mice, we have proceeded to study (^{3}H) BZ binding. Specific (^{3}H)flunitrazepam binding to well-washed, frozen and thawed membranes prepared from whole brain was determined by a filtration technique. The results are shown in Table 1.

Table 1 Binding of ^{3}H Flunitrazepam in DBA/2 and T/O mice

	DBA/2		T/O	
AGE (days)	K_D	R_T	K_D	R_T
8-9	1.49±0.07	1.08±0.03	1.71±0.07	1.05±0.03
17-18	2.40±0.04	1.52±0.02	2.39±0.17	1.45±0.07
22-23	2.25±0.17	1.36±0.07*	2.23±0.19	1.29±0.07
28-29	2.83±0.13	1.50±0.05*	3.09±0.08	1.65±0.03
40-43	2.61±0.08	1.40±0.03	2.80±0.10	1.45±0.04

K_D = equilibrium dissociation constant (nM), R_T = number of binding sites (pmoles/mg protein). Values are means ± s.e. mean derived from linear regression analysis of Scatchard plots, using 8 concentrations of (^{3}H) flunitrazepam (0.07-10nM) and repeated on three occasions for each age. Significant differences between strains are denoted by * $p < 0.05$, ** $p < 0.01$ (students t test).

The marked differences in GABA binding are clearly not reflected in BZ binding. Possible differences in the degree of coupling between GABA and BZ binding sites are currently under investigation.

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REGIONAL RAT BRAIN GABA CONCENTRATIONS FOLLOWING REPEATED SEIZURES

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Following a series of electroconvulsive shocks (ECS) given once daily for 10 days, rats display enhanced behavioural responses to 5-hydroxytryptamine (5-HT) and dopamine agonists (see Green, 1980). Twenty-four hours after the final of a series of ECS given once daily for 10 days it has been reported that GABA concentrations rise in both the corpus striatum and nucleus accumbens. The turnover rate of this transmitter is also decreased and it was suggested that this change might be associated with the enhanced monoamine-mediated responses (Green et al, 1978). However, Deakin et al (1981) did not observe an increased striatal GABA concentration at this time when the ECS had been given to rats whilst anaesthetised and suggested that the anaesthetic in some way prevented the change in GABA biochemistry occurring in the striatum. Nevertheless, enhanced monoamine-mediated behaviours occur following repeated ECS irrespective of whether the animal is or is not anaesthetised during the convulsion. We have therefore investigated regional brain GABA concentration following repeated ECS given both with and without anaesthesia to clarify the possible involvement of GABA in the changed monoamine function.

Male Sprague-Dawley derived rats (final weight 120 g) were given an ECS (125 v, 1 s) once daily for 10 days. They were killed by exposure of the head for 4 s to a focussed high intensity microwave beam (Guidotti et al, 1974), the brain dissected and GABA measured by an enzymatic-fluorimetric assay. A significant rise in striatal GABA concentration was observed both 30 min and 24 h after the final ECS (handled: 6.35 ± 0.36 (9); 30 min: 8.03 ± 0.79 (4); 24 h: 7.49 ± 0.31 (11); both significant $p < 0.05$, results as μmol/g brain and show mean ± S.E.M.). No change in GABA concentration was seen in cortex, hypothalamus or hippocampus 30 min after the last ECS, but a significant increase in hypothalamus ($p < 0.02$) 24 h later. The increase in striatal GABA concentration was still present 72 h after the final ECS.

The increase in striatal GABA concentration was also seen 24 h after the last of a series of ECS given once daily for 10 days to rats anaesthetised with halothane, compared with an anaesthetised only control group (anaesthetic x 10: 7.95 ± 0.26 (5); ECS x 10: 10.30 ± 0.58 (4), $p < 0.01$). Similarly, the rise was apparent in the striatum following administration to anaesthetised rats, of five ECS spread over 10 days (Days 1, 3, 5, 8 and 10), a protocol similar to the administration of electroconvulsive therapy (ECT) and which also results in enhanced monoamine-mediated responses in rats (Green & Deakin, 1980).

Daily exposure to the inhalant convulsant, flurothyl, for 10 days results in enhanced monoamine-mediated behaviours (Green, 1978) and was observed to increase the striatal (handled x 10: 5.73 ± 0.10 (4); flurothyl x 10: 6.95 ± 0.23 (8), $p < 0.01$) and hypothalamic GABA concentration 24 h after the last seizure.

Like Deakin et al (1981) we observed no change in benzodiazepine receptor binding characteristics in the striatum following repeated ECS.

Julie M. Bowdler holds an MRC Studentship.

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EFFECT OF REPEATED ELECTROCONVULSIVE SHOCKS ON GROWTH HORMONE SECRETION AND GROWTH HORMONE RESPONSES TO CLONIDINE IN INTACT RATS

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The effect of repeated electroconvulsive shocks (ECS) on growth hormone (GH) secretion was studied. Male Sprague Dawley rats were housed 6-9 to a cage in a temperature controlled room with a twelve hour light cycle (0700-1900 hours) and were fed and watered ad libitum. ECS were administered under halothane anaesthesia daily for 10 days. A Theratronix electroplexy unit was used to deliver 50-100 mA for 1 second to the head via ear clips. A convulsion was always observed. Control animals were anaesthetized only. Twenty four to 48 hours before GH studies, a right atrial indwelling cannula was inserted under ether anaesthesia. Animals were then caged separately.

GH studies were performed at 1000 hours 24 hours after the last ECS or sham procedure. Rats weighed 210-260 g at the time of study. One hour prior to the commencement of the study, a long piece of Teflon tubing was connected to the atrial cannula and led to the outside of the cage. Animals were not disturbed after this. Blood samples were taken for GH estimation at -30, -15, 0, +5, +15, +30, +45, +60, +75 and +90 minutes in relation to clonidine administration at 0 minutes. Clonidine 0.01, 0.025, 0.05, or 0.1 mg/kg was given intravenously dissolved in normal saline in a volume of 1 ml/kg. Plasma GH was measured by standard radioimmunoassay techniques using the NIAMDD kit.

All rats became sedated following clonidine administration. Areas under the curve (AUC) for GH response to clonidine and for GH secretion during the entire study were calculated by the trapezoidal rule method. There was good correlation between AUC for response and maximal rise in plasma GH level ($r=0.8-1.0$). The AUC for GH response was subtracted from the AUC for the entire study to give the background GH secretion. For each level of clonidine dose and for the combined data the background GH secretion was greater in ECS than in sham treated rats (unpaired $t=3.80$, 50df, $p<0.001$). Dose response curves for GH response to clonidine calculated either as AUC or as maximal rise im plasma GH concentration were not significantly different between the two groups when compared by 2-way analysis of variance ($F=0.205$, p NS, 1,51 df). However, the effect of different doses of clonidine was highly significant ($F=48.33$, $p<0.001$, 3,41 df).

There was no relationship between background GH secretion and GH response to clonidine.

It is concluded that repeated ECS causes an increase in non-specific GH secretion which may be related to increased neuronal activity. Stimulation of GH secretion with clonidine does not demonstrate this difference and may relate to the development of compensatory inhibitory mechanisms to clonidine-stimulated GH release in ECS treated rats.

EFFECT OF GABA-ERGIC DRUGS ON PLASMA GROWTH HORMONE LEVELS IN THE RAT

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Pentobarbitone is known to elevate plasma growth hormone levels (Haward and Martin, 1971). Since the barbiturates are thought to facilitate inhibition at central GABA synapses (Haefely et al, 1979) we were interested to investigate the effect on growth hormone secretion of other agents affecting the GABA system including baclofen, a ligand for a novel type of GABA receptor (Bowery et al, 1979; 1980).

Male rats of the Alderley Park strain and weighing ≃100g were used throughout. The drugs used, pretreatment times, doses and routes of administration are shown in Table 1. Blood was collected from the peri-orbital sinus of anaesthetised rats, centrifuged at 10,000 rpm and plasma aliquots stored at -20°C until assay by a modification of the double antibody radioimmunoassay technique described by Hérvas et al (1974).

Table 1 Effect of pentobarbitone and drugs affecting the GABA system on plasma growth hormone levels

Pretreatment	Plasma growth hormone ng/ml ± s.e.m.(n)
Saline s.c.(20 min) + Saline i.p. (10 min)	11.0 ± 2.1 (5)
Saline s.c.(20 min) + Pentobarb. 30mg/kg i.p. (10 min)	418.4 ± 164.0 (5)
Bicuculline 0.25mg/kg s.c.(20 min) + Pentobarb.30mg/kg i.p. (10 min)	294.0 ± 169.0 (4)
Picrotoxin 1mg/kg s.c. (20 min) + Pentobarb. 30mg/kg i.p. (10 min)	509.0 ± 200.0 (6)
Saline 0.5ml/100g s.c. (30 min)	14.1 ± 3.6 (6)
Muscimol 1mg/kg s.c. (30 min)	25.5 ± 4.8 (6)
Nipecotic acid 20mg/kg s.c. (30 min)	13.7 ± 3.4 (6)
Saline 0.5ml/100g s.c. (4 hrs)	21.4 ± 6.4 (6)
Aminooxyacetic acid 50mg/kg s.c. (4 hrs)	23.4 ± 7.6 (6)
Saline 0.5ml/100g s.c. (20 min)	12.9 ± 4.8 (6)
Baclofen 10mg/kg s.c. (20 min)	2.57 ± 2.35 (6)

Pentobarbitone produced a large increase in plasma growth hormone level (Table 1). This was not prevented by prior injection of either bicuculline or picrotoxin. Muscimol, nipecotic acid and aminooxyacetic acid were without effect whilst baclofen appeared to lower growth hormone levels. These results do not lend support for a role for GABA in the basal release of growth hormone in the rat nor do they suggest that pentobarbitone is acting via the GABA system.

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FAILURE OF MPF TO INTERACT WITH THE DOPAMINE SYSTEM IN RODENT BRAIN

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α-Melanocyte stimulating hormone(α-MSH)produces pigmentation in the skin of the lizard Anolis carolinensis(Carter & Shuster,1978 (a)).β-endorphin,which alone has very low pigmentary activity,is synergistic with α-MSH(Carter et al.1979)and using this assay system the terminal tetrapeptide of β-endorphin(Lys-Lys-Gly-Glu -melanocyte potentiating factor(MPF))was identified as being the active factor. Neuroleptics also produce pigmentation in this assay(Carter & Shuster,1978(b)) which can be potentiated by MPF(Carter & Shuster,1981). We have therefore investigated the possible interaction between MPF and neuroleptics on several dopamine systems in rodents.

Our studies were performed with ICI M149,463(Ac-Lys-D-Lys-Sar-Glu),a stable analogue of MPF(Morley et al.1981)and its activity was assessed in the following systems which were sensitive to both neuroleptics and dopaminergic agonists:

1. Apomorphine-induced climbing and hypothermia;groups of 5 male mice(20-22g)were treated with M149,463(100mg/kg i.v.) or saline 35 min prior to receiving apomorphine hydrochloride(0.5,1.0,2.5 or 5.0mg/kg i.p.).Temperature and climbing activity were recorded at 5 min intervals for 30 min.The effect of M149,463 on spontaneous climbing was also observed.
2. Amphetamine hyperactivity;groups of 6 mice were treated with M149,463 (100mg/kg i.p.)or saline 20 min prior to receiving a subthreshold dose of amphetamine sulphate(1mg/kg s.c.).Vertical and horizontal movements were recorded in locomotor cages over a period of 2h.
3. Haloperidol catalepsy;groups of 6 mice were treated with a subcataleptic dose (1mg/kg i.p.)or cataleptic dose (10mg/kg i.p.)of haloperidol.M149,463 (100mg/kg i.v.)or saline was injected 30 min later and catalepsy assessed after 2,5,15,30, 60 and 90 mins.
4. Binding studies;the ability was assessed of M149,463(10μmol-100nmol)to displace(±)-sulpiride from its binding sites on rat striatal synaptic membranes (for method see Freedman & Woodruff,1981).
5. Electrophysiological studies;M149,463 was iontophoretically applied(20-60nA) to dopamine sensitive cells in the rat substantia nigra and its effects on firing rate recorded.
6. Angiotensin-induced drinking;the drinking induced in rats by angiotensin (400pmol)ICV in 1-2μl 0.9%NaCl)can be inhibited by fluphenazine(ED_{50}=50nmol ICV). M149,463(200nmol ICV)was microinjected either in combination with these compounds, or alone,and its effects on drinking were recorded.

M149,463 was inactive in all the above tests.We have therefore been unable to obtain evidence for an interaction of M149,463 with mammalian dopamine systems either in vitro or in vivo.

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THE BINDING OF (^{3}H)-OXOTREMORINE-M TO MEMBRANES PREPARED FROM RAT BRAIN

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The binding of antagonists to the muscarinic receptor in rat brain membrane preparations is consistent with first order mass action kinetics. Agonist binding, on the other hand, is complex but can be rationalised in terms of multiple binding sites having different affinities for agonists (Birdsall et al. 1978). It has been suggested that the different classes of muscarinic agonist binding sites, termed low, high and super high affinity sites, represent independant states of the same receptor molecule (Birdsall et al., 1980). These experiments have relied, however, on the indirect determination of agonist-receptor affinities. In the experiments reported here, we have investigated agonist-receptor binding directly using the radioligand ^{3}H-oxotremorine-M (^{3}H-oxo-M).

Membranes isolated from rat forebrain were used in these experiments. Briefly, a P2 Fraction was isolated and homogenised, using a Polytron, in Tris-HCl buffer, pH 7.4, containing 0,1 % ascorbic acid, 2 mM Ca^{++}, 1 mM Mg^{++}, 5 mM K^+ and 120 mM Na^+. Membranes ($\simeq$ 1,5 mg/ml) were incubated in plastic centrifuge tubes containing ^{3}H-oxo-M (83,6 Ci/mmole; NEN) for 30 min at 25 °C. The incubation was stopped by centrifugation, and the pellets dissolved in scintillation fluid (Lumagel). Non-specific binding was determined in parallel experiments in which the tubes contained 10 mM carbachol.

The isotherm for the specific binding of ^{3}H-oxo-M to rat fore-brain membranes can be resolved to reveal a high affinity, low capacity (K_D 2,9 nM; Bmax 79,9 fmol/mg protein) and a low affinity high capacity (K_D 29,8 nM; Bmax 573,3 fmol/mg protein) binding site.

An attempt was made to examine the pharmacology of the high and low affinity binding sites independently. Two concentrations of ^{3}H-oxo-M were employed: 0,1 nM which labels almost exclusively the high site; and 10 nM, which almost saturates the high site and labels the low site.

On this basis, the two binding sites could be differentiated pharmacologically using muscarinic agonists as competing ligands. The potency order for the high site was oxotremorine > acetylcholine > oxotremorine-M >> arecoline = carbachol = muscarine >> pilocarpine and for the low site oxotremorine >> oxotremorine-M > acetylcholine > muscarine = carbachol >> arecoline > pilocarpine.

The antagonists atropine, scopolamine and pirenzepine also had a high affinity for both sites.

These results suggest that, on membranes prepared from rat fore-brain, ^{3}H-oxo-M labels two populations of binding sites which may represent different receptor populations rather than different states of the same receptor.

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THE UPTAKE AND CALCIUM-DEPENDENT RELEASE OF ETHYLENEDIAMINE IN RAT BRAIN SLICES

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We have previously reported the GABA-like properties of ethylenediamine (EDA) causing neuronal depression and ganglionic depolarisation (Perkins et al., 1981) as well as its ability to reduce GABA uptake and induce GABA release (Forster et al., 1981). The present experiments were designed to examine the uptake of (^{14}C)-EDA itself and to determine whether it could be released from slices of rat brain.

Uptake of EDA was studied using slices of rat cerebral cortex prepared by a McIlwain tissue chopper and preincubated in oxygenated physiological medium at 37°C. After 10 min (^{14}C)-EDA (s.a. 25 mCi mmol^{-1}; Amersham International) was added and incubation continued for 10 min. Slices were separated from the medium by filtration, washed and placed overnight in trichloracetic acid (TCA) after which an aliquot of the TCA was dispersed into scintillant and counted.

For release experiments slices were pre-incubated for 15 min, followed by 15 min in the presence of (^{14}C)-EDA and then transferred to small chambers where they were perfused at approx. 0.5 ml min^{-1}. Fractions were collected every 2 min and aliquots counted.

EDA uptake at 37°C was reduced by 63% and 89% at room temperature and 0-4°C respectively, suggesting the involvement of an active uptake process. Uptake at 37°C was partly dependent on sodium concentration, being reduced by 40% in sodium free medium. Preliminary kinetic analysis indicated a low affinity uptake system for EDA having a Km of 1.36 mM and a Vmax of 1.0 mol min^{-1} g^{-1} x 10^{-6}.

The efflux of EDA was increased by 318% in the presence of 30 mM K^+ and by 134% on electrical stimulation. Release by both forms of depolarisation was substantially and significantly reduced when external Ca^{++} was lowered to 0.1 mM.

It is concluded that mechanisms exist for the active accumulation and depolarisation-related release of EDA from brain slices. It remains unclear how these relate to the corresponding mechanisms for GABA but it would be of interest to determine whether EDA occurs endogenously in brain.

Supported by the Wellcome Trust.

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ETHYLENEDIAMINE POTENTIATES IN VITRO (^{3}H)-DIAZEPAM BINDING TO RAT CEREBRAL CORTEX

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Ethylenediamine (EDA) has been reported (Forster et al., 1981; Perkins et al., 1981) to be qualitatively similar to GABA when applied iontophoretically. This may well be due, at least in part, to an indirect action of EDA in that EDA can release GABA from brain slices and inhibit GABA uptake (Forster et al., 1981). Since GABA and GABA-mimetics have been reported to have a modulatory effect on benzodiazepine receptor binding (Tallman et al., 1978) this system was selected to investigate possible direct GABA-receptor linked effects of EDA.

Binding of ^{3}H-diazepam was carried out according to a modified method of Möhler and Okada (1977) in a 50 mM TRIS-HCl or Krebs medium, pH 7.4 at 20°C using a 4 times washed and once frozen crude synaptosomal membrane preparation of rat cerebral cortex. Non-specific binding was estimated using an excess of flurazepam and constituted less than 10% of total binding.

Specific binding of ^{3}H-diazepam at a free concentration of 2 nM was found to be potentiated about 30% by EDA, but only at relatively high concentrations of EDA (1-10mM). Scatchard analysis indicated this to be due to a change in receptor number and not receptor affinity and further studies showed that EDA-induced potentiations appeared to be particularly susceptible to bicuculline. Results obtained in Krebs were similar to those obtained in Tris buffer.

It is concluded that EDA can act directly at membrane GABA-related sites, but probably not in the same manner as GABA, in view of the different reported (Tallman et al., 1978) mode of action of GABA (viz. by an increase in receptor affinity and not receptor number).

Supported by Action Research, Wellcome Trust, S.K.F. Foundation and London University C.R.F. P.F.M. is an M.R.C. Student.

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IONTOPHORETIC STUDIES WITH MEPTAZINOL IN THE RAT CNS

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Maptazinol is a potent analgesic with opiate antagonist characteristics (Stephens et al., 1978). Its mechanism of action is not understood, though binding sites are concentrated in the cerebral cortex and spinal cord (Bill et al., 1981). The present experiments were designed to test the effects of meptazinol on neuronal activity in these areas, when applied by microiontophoresis.

Male rats were anaesthetised with urethane (1.3g/Kg i.p.) and prepared for extracellular unit recording. Drugs were applied by microiontophoresis from 7-barrelled micropipettes, and unit activity recorded through a single electrode fixed alongside.

On 10 cortical units meptazinol (9-40 nA for 1-4 min) reduced excitatory responses to both glutamate and N-methyl-D-aspartate (NMDA), with little preference for either agonist, but reduced glutamate only on 1 unit (mean reductions of 67% of glutamate and 56% of NMDA).

On 4 cortical cells meptazinol reduced quisqualic acid excitation by 57% and NMDA by 77%, and on 7 cells it reduced glutamate and acetylcholine responses equally. GABA responses were unchanged on 6 cells.

In the spinal cord meptazinol also reduced excitatory responses of dorsal horn cells, though with no apparent differentiation between glutamate, NMDA, quisqualate and acetylcholine.

In all the above cases, doses of meptazinol were used which had no apparent effect on spontaneous firing. At higher doses meptazinol itself did cause a depression of firing, sometimes associated with a reduction of spike height, reminiscent of local anaesthetic action.

Finally we have found that the two isomeric forms of (±) meptazinol are approximately equi-active on cell firing and evoked excitation. It is of interest that the two isomers also show very similar affinities for the binding sites.

In conclusion, we suggest that meptazinol can block in a non-selective fashion neuronal excitation induced by aminoacids or acetylcholine, and that the same site may be involved in these effects as can be demonstrated in binding experiments.

Supported by Action Research.

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THE ANTIDEPRESSANT DOTHIEPIN REDUCES CORTICAL β-ADRENOCEPTOR BINDING AFTER SUBCHRONIC ORAL ADMINISTRATION TO RATS

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Development of β-adrenoceptor subsensitivity following prolonged administration of a number of antidepressants has been established (Banerjee et al, 1977; Clements-Jewery, 1978) and it has been suggested that the time of onset of this subsensitivity is related to the commencement of clinical improvement observed with these drugs in depressed patients (Sulser, 1978). As dothiepin (Prothiaden, Dosulepin) is a widely-used clinically effective antidepressant, it was of interest to investigate it in this regard. This communication presents evidence to show that dothiepin can induce rapid desensitization of β-adrenoceptors in the rat after oral administration.

Male Wistar (Charles River) rats (180-240 g) in groups of eight received dothiepin HCl (10 or 100 mg/kg orally in 0.25% hydroxyethylcellulose suspension) once daily at 09.00 h for 1, 3, 7 or 14 days. Control rats received vehicle alone. For receptor binding assays the cortex was rapidly dissected out 24 h after the last drug treatment, homogenized in 30 vol Tris HCl (50 mM) and centrifuged three times (30,000 g x 10 min) at 4°C with intermediate resuspension in 50 vol buffer (pH 7.8 at 25°C). 0.8 ml of homogenate containing approx. 1 mg protein was added in duplicate to tubes containing ^{3}H-dihydroalprenolol (0.1-3 nM) with or without (-)-isoprenaline HCl at 25°C for 25 min. Reactions were terminated by rapid filtration (GF/F filters) and washing three times with 4 ml ice-cold buffer. The filters were placed in vials with 10 ml Ria/Lipo-luma (50 : 50) for counting. In vitro displacement studies with dothiepin were carried out with cortical β-receptors. As a behavioural correlate, nocturnal motor activity of rats was measured between 20.00 h and 06.00 h on selected days.

After a single dose of dothiepin (10 or 100 mg/kg) no significant change in β-adrenoceptor binding was observed. Treatment with dothiepin (100 mg/kg/day) led to significant reductions in B_{max}, with no change in K_D, after 3 days (15.6%; $p<0.01$), 7 days (24.7%: $p<0.001$) and 14 days (25.2%; $p<0.025$) when determined by Scatchard plots and analyses of variance. The lower dose of dothiepin (10 mg/kg/day) was ineffective. Nocturnal activity of rats treated with dothiepin (100 mg/kg/day) was reduced by 38% (day 1), 34% (day 3) and 39% (day 8) compared with controls and the differences between these reductions were not significant. As with other antidepressants (Tang & Seeman, 1980) dothiepin did not displace ^{3}H-dihydroalprenolol from cortical β-receptors up to 3×10^{-5} M.

These results indicate that subchronic oral administration of dothiepin can induce β-adrenoceptor desensitization like most other typical and atypical antidepressants (Woolfe et al, 1978), although it appears to be somewhat less potent in this respect since 100 mg/kg/day was necessary to demonstrate the effect. This may be due to the short plasma half-life of dothiepin in the rat, in contrast to man. The rapid adaptive change in β-adrenoceptor binding which is apparent after the third day of medication with dothiepin is not reflected in the effect on motility which remains constant suggesting that this behaviour does not involve β-receptors.

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EFFECTS OF SOME ANTIDEPRESSANTS ON β-ADRENOCEPTOR BINDING AND ON NORADRENALINE-STIMULATED ADENYLATE CYCLASE IN RAT FOREBRAIN

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Various antidepressant therapies eg. chronic administration of antidepressants, including the monoamine oxidase inhibitors and repeated electroconvulsive shocks induce β-adrenoceptor subsensitivity in rats as determined from ligand binding techniques and noradrenaline-stimulated adenylate cyclase preparations (Sulser, Vetulani & Mobley, 1978 for references).

This study attempts to directly relate the changes revealed by β-adrenoceptor binding studies to functional changes by simultaneously determining both ^{3}H-dihydroalprenolol binding (Bylund & Snyder, 1976) and stimulation of noradrenaline sensitive adenylate cyclase (Horn & Phillipson, 1976) in homogenates prepared from the same forebrains of rats which had received daily intraperitoneal injections of antidepressants for 4 weeks. The drugs studied included desipramine, the tetracyclic mianserin and the atypical antidepressant nomifensine. They were given at 2 dose levels, a low dose (calculated from a maintenance clinical dose) and a larger dose (for comparison with other animal studies).

Desipramine produced β-adrenoceptor subsensitivity, as shown by a decrease in Bmax, and diminished noradrenaline-induced stimulation of adenylate cyclase when given in daily doses of 2 and 10mg/kg; rats receiving the larger dose exhibited signs of toxicity (failure to thrive). The results with desipramine confirm those reported by others (Sulser et al. 1978). In contrast similar doses of mianserin lacked effect on both measures; larger amounts of mianserin (30mg/kg daily) reduced the cyclic AMP response without affecting β-adrenoceptor binding (Mishra, Janowsky & Sulser, 1980. Nomifensine (10mg/kg) significantly decreased induced stimulation of adenylate cyclase but both this dose and a dose of 2mg/kg were without effect on β-adrenoceptor binding. Crews & Smith (1978) have shown that onset of β-adrenoceptor subsensitivity to desipramine may be hastened by simultaneous administration of phenoxybenzamine (7.5mg/kg), the effect now occurring within 3 days (Crews & Smith, 1978). We have not been able to confirm this observation finding that neither β-adrenoceptor binding nor noradrenaline-induced activation of cyclic AMP were significantly affected by this treatment.

The above preliminary observations are being extended and antidepressants with no known action on the noradrenergic system (e.g. citalopram) are also being studied. We have no explanation for our failure to replicate findings of Crews & Smith (1978) but this is to be examined in greater detail.

We wish to thank the Wellcome Trust and the Medical Research Council for financial support.

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(-)-PROPRANOLOL IS NOT AN ANTAGONIST OF 5-HT IN VIVO

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Receptor binding studies (Middlemiss, Blakeborough & Leather, 1977) have shown that (-)-propranolol binds stereospecifically to 5-hydroxytryptamine (5-HT) binding sites within the CNS. Evidence from behavioural studies involving 5-HT precursor loading (Green and Grahame-Smith, 1976), and experiments on isolated tissue preparations (Schechter and Weinstock, 1974) also suggest that propranolol is a 5-HT antagonist. However, no behavioural evidence has been presented to show that propranolol can antagonise 5-HT when the 5-HT is injected directly into the CNS.

We have therefore attempted to obtain direct evidence for an action of propranolol on 5-HT induced rat body shake behaviour (Drust, Sloviter & Connor, 1979). Antagonism of 5-HT induced rat body shake was assessed in adult male rats of the Alderley Park SPF strain (180-200g) on automated microcomputer controlled activity monitors. The rats were pretreated with desmethylimipramine (25mg/kg i.p.) before an intracerebroventricular (icv) injection of 5,7-dihydroxytryptamine (5,7-DHT) dissolved in 20µl of artificial cerebrospinal fluid (CSF) 160µg/20µl. The neurotoxin was injected through a previously implanted icv guide cannula over 5 minutes under sodium pentobarbitone (40mg/kg i.p.) anaesthesia, to prevent neurotoxin induced seizures on recovery.

After a period of seven days, lesioned rats received an approximate ED_{50} dose of 5-HT (45µg/10µl) and only those rats which responded in the expected manner, i.e. rapid rotational movements of head, neck and trunk ($>$200/75min observation period), were used in the 5-HT antagonist studies. Following a 4 day recovery period, 5-HT antagonists were administered icv in 10µl CSF 15 minutes prior to the standard dose of 5-HT.

Standard 5-HT antagonists effectively reduced the body shake response in the following relative order of potency; $>$ methiothepin $>$ methergoline $>$ cyproheptadine $>$ cinanserin $>$ methysergide, based on ID_{50} estimations obtained by regression analysis the log-dose antagonist v 5-HT response plot. However, (-)-propranolol ($>$386n moles) was inactive in this model. These data confirm results from recent microiontophoretic (Bradley & Gladman, 1980) and neurophysiological studies (Cox, Lee & Martin, 1980) and do not support the contention that (-)-propranolol is a postsynaptic 5-HT antagonist. We have however, found that (-)-propranolol can antagonise fenfluramine hyperthermia, a test which relies on release of 5-HT from neuronal stores (Sulpizo, Fowler & Macko, 1978). This further evidence suggests propranolol's action may be on as yet unclassified 5-HT receptor involved in release mechanisms.

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CENTRAL AND PERIPHERAL CHANGES IN α-ADRENOCEPTORS IN THE RAT AFTER CHRONIC TRICYCLIC ANTIDEPRESSANTS

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Chronic administration of some antidepressants is associated with central changes in α-adrenoceptors. This can be shown physiologically e.g. the DMI facilitated escape from a clonidine induced surpression of MHPG-SO_4, or biochemically using receptor binding techniques(Smith et al 1981,Sugrue et al 1981, Johnson et al 1980) In light of the latter observation it was of interest to know in which subtypes of the α-adrenoceptor these changes occured and if they were extended to the periphery Adult Wistar rats were treated with imipramine (10mg/Kg/day,21days) or clorgyline (1mg/Kg/day,21 days i.p) prior to anaesthesia with pentobarbitone. Blood was taken from the inferior vena cava into heparin and platelet membranes were isolated. Liver and brain (cortex and brainstem subsequently dissected) were removed & stored at -80°C until assayed for α-adrenoceptor binding. Assays employing the antagonist ligands ^{3}H-dihydroergocryptine (total α-population), ^{3}H-Yohimbine (α_2) and ^{3}H-prazosin(α_1) were performed in 50mM tris, 0.5mM EDTA, pP 7.5, specific binding was defined by 10^{-5}M phentolamine. Assays employing the agonist ligand ^{3}H-clonidine (high affinity α_2 sites) were performed also in the presence of 10 mM Mg^{2+} and the specific binding was defined by 10^{-5}M adrenaline. B_{max} & K_d values were obtained by Scatchard analysis.

In cortex and brainstem there was no difference between the ^{3}H-DHEC binding from control and antidepressant treated rats (cortex B_{max} 349±28, brainstem B_{max} 138±28 fmol/mg protein). There was no change in the ^{3}H-yohimbine binding, which labels both high and low affinity forms of the α_2-adrenoceptor.(Daiguji et al 1981, Hoffman et al 1980). However cortices from rats after 3 weeks imipramine or clorgyline showed a significant decrease in ^{3}H-clonidine binding over control values (50-60%,$p<.001$). This wes reflected as a change in B_{max} not in K_d. In brainstem the reduction was only significant after imipramine. In both brain areas there was a small but significant increase in ^{3}H-prazosin binding (15-20%,$p<.05$).

The same pattern of results was not seen in the periphery.In the liver there was no change in ^{3}H-clonidine binding or in ^{3}H-prazosin binding after antidepressant treatment. In the platelet small amounts of tissue prevented the use of full Scatchard analysis, but preliminary results with high concentrations of ^{3}H-DHEC and ^{3}H-clonidine showed no change in total α-adrenoceptor population but a downward trend in ^{3}H-clonidine binding, significant only after imipramine.These results indicate that chronic administration of clorgyline and imipramine is associated with a decrease in α_2-adrenoceptors and an increase in α_1-adrenoceptors. The change in α_2-adrenoceptors occurs only in the high affinity state, without changing the overall number of sites. While it is not possible to draw any physiological conclusions it can be hypothesised that as the α_1-adrenoceptors are probably post-synaptic and at least some of the $\alpha_2$adrenoceptors are likely to be presynaptic, the effect of these reciprocal changes will be to facilitate transmission in systems containing these receptors. This action may be related to the therapeutic properties of these drugs.

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LEAD EXPOSURE AND ITS EFFECT ON CATECHOLAMINE METABOLISM IN DISCRETE BRAIN NUCLEI

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In previous studies (Meredith et al, 1980) we have attempted to clarify the earlier studies of the neurochemical and behavioural effects of lead in animals. This involved the measurement not only of steady state catecholamine levels but also the measurement of the more dynamic indices, the activity of catecholamine synthesising enzymes in lead exposed rats. These studies demonstrated that in the adult rat acute and chronic lead exposure was associated with pronounced changes in catecholamine levels and a decrease in tyrosine hydroxylase (TH) activity in both the anterior and posterior hypothalamus.

In the present study male Wistar rats (100g) were treated either chronically for 26 weeks with lead in their drinking water at a concentration of 2 m mol/l or acutely for 14 days with intraperitoneal injections of lead (as acetate) at a dose of 20 μmol/kg. Animals were killed by decapitation their brains excised and frozen on dry ice. The forebrain nuclei were removed with stainless steel punches from 300 μm thick serial sections according to the method of Palkovits (1973). The nuclei selected were the periventricular, paraventricular, median eminence, posterior and anterior hypothalamic, caudate putamen and globus pallidus. Catecholamine concentrations, TH and phenyl ethanolamine N methyl transferase (PNMT) activities were all assayed as previously described (Meredith et al, 1980).

In the acute study no significant changes in catecholamines and catecholamine synthesising enzymes were observed but lead levels in the nuclei were increased.

In the chronic study significant elevations in lead levels were associated with a significant fall in TH activity in the periventricular and anterior hypothalamic nuclei and in the median eminence (table 1). In the median eminence and anterior hypothalamic nucleus the decrease in TH activity was associated with a significant fall in noradrenaline levels. Whilst in the periventricular nucleus both dopamine and noradrenaline concentrations were significantly increased (table 1).

Table 1

	Periventricular	Median Eminence	Posterior Hypothalamic
Noradrenaline			
control	26.3 ± 7.0	26.2 ± 10.5	48.2 ± 16.4
chronic	51.4 ± 16.3*	9.2 ± 3.6*	30.7 ± 9.2*
Dopamine			
control	10.3 ± 4.2	10.4 ± 6.2	8.2 ± 3.7
chronic	19.4 ± 4.3*	7.0 ± 3.6	8.0 ± 2.7
Tyrosine Hydroxylase			
control	7.1 ± 0.9	16.8 ± 3.4	2.9 ± 0.5
chronic	5.2 ± 0.9*	10.8 ± 3.6*	1.8 ± 0.6*

* $p<0.01$

Therefore lead ingestion at levels known to result in behavioural effects are associated with significant decreases in 'in vitro' noradrenaline synthesis in specific hypothalamic nuclei. The changes may contribute to the central psychomotor effects of lead intoxication.

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MONOAMINE METABOLITES IN CSF AND THEIR RELATIONSHIP TO BRAIN CONCENTRATIONS

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Numerous studies have suggested that changes in CSF monoamine metabolites reflect variations in concentrations or turnover of monoamines in the CNS (see Garelis et al, 1974 for review).

We have used a variety of pharmacological manipulations to further examine the relationship between brain and CSF monoamine metabolites. Thus, groups of rats (5 per group, male Sprague-Dawley 300-350 g) were treated with α-monofluoromethyldopa (MFMD) at 250 mg/kg p.o. to deplete brain monoamines (Bey et al, 1980); MFMD (1 mg/kg p.o.) plus L-DOPA (50 mg/kg); L-DOPA alone; MFMD plus L-5HTP (50 mg/kg) or L-5HTP alone, to elevate brain catecholamines and indoleamines respectively (Palfreyman et al, 1979); probenecid (200 mg/kg i.p.); haloperidol (1 mg/kg s.c.) or probenecid + haloperidol to alter metabolite transport and/or turnover.

At various times after treatment, CSF was withdrawn from the cisterna magna under pentobarbitone anaesthesia and then the brain was removed and plunged into liquid nitrogen. Catechols and indoles were determined by HPLC with electrochemical detection (Wagner et al, 1981). Brain and CSF concentrations were analysed by linear regression and correlation coefficients, r, calculated. There were impressive overall correlations between the concentrations of dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindole acetic acid (5HIAA) and O-methyldopa in the CSF and those in the brain ($r > 0.93$; $p < 0.001$).

Analysis of individual experiments contributing to the overall correlation show similar significant r values but markedly different slopes. Table 1 shows these values for HVA and 5HIAA concentrations. Similar differences in slopes were seen with DOPAC and O-methyldopa.

Table 1 Correlation between brain and CSF metabolite concentrations

Metabolite		Overall correlation	Experimental conditions A	B	C	D
HVA	r	0.95	0.84	0.94	0.90	0.97
(n=90)	s	0.65	0.24	0.66	0.1	0.38
		(0.6-0.7)	(0.2-0.3)	(0.6-0.7)	(0.06-0.14)	(0.3-0.4)
5HIAA	r	0.93	0.96	0.97		0.96
(n=85)	s	0.63	0.80	0.64		2.27
		(0.6-0.7)	(0.7-0.9)	(0.6-0.7)		(1.9-2.6)

Experimental conditions: A=MFMD 250 mg/kg p.o.; B=MFMD plus L-DOPA or L-5HTP; C=Haloperidol; D=Probenecid. s=slope; 5% confidence limits in brackets.

These data suggest that the various pharmacological manipulations, acting by different mechanisms, produce changes in different pools of monoamine neurotransmitters and that measurement of CSF metabolites to assess changes in brain amine function should be interpreted cautiously.

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EVIDENCE THAT D145 IS LIMBIC SPECIFIC

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1,3 dimethyl-5-aminoadamantane (D145) increases spontaneous locomotor activity in rodents, when injected peripherally. This can be specifically antagonised by GABA-mimetic agents (Menon and Clark, 1978/9). These authors implicated disinhibition of GABA feedback systems as the mechanism by which D145 produced its locomotor stimulation. D145 is unusual among GABA antagonists in that it does not induce convulsions, this implies an element of anatomical specificity in its actions; moreover, the D145 behavioural syndrome is qualitatively similar to that seen when GABA antagonists, or dopamine, are injected directly into the nucleus accumbens (Pycock and Horton, 1979). The aim of this study was to investigate further the action of D145 especially with a view to any interaction with dopaminergic mechanisms.

In biochemical experiments D145 hydrochloride (30mg/kg, mice; 40mg/kg, rat) was given subcutaneously 1 hour before sacrifice. Homovanillic acid (HVA) levels were assayed in mouse whole brain, rat striatum and nucleus accumbens according to an adaption of Westerink and Korf (1975), using male CD rats (200-210g) and male CD1 mice (20-30g). Mouse whole brain levels of HVA were unaffected by D145; rat striatal tissue similarly showed no change, whereas nucleus accumbens tissue derived from the same rats showed a significant elevation of HVA levels, 55% over control ($P<0.001$, $n = 12$).

Pharmacological studies were also carried out in which locomotor hyperactivity induced by D145 (20mg/kg i.p.) was examined for 30 minutes in groups of 4 male CD1 mice (20-30g) using a method adapted from Menon and Clark (1979); antagonists were given 15 minutes prior to D145. The GABA mimetics muscimol (1mg/kg) and baclofen (10mg/kg) antagonised D145 induced hyperactivity significantly ($P<0.005$ and $P<0.001$ respectively; $N = 16$ in both cases). These results are in agreement with Menon and Clark (1978/9). The dopamine antagonists haloperidol (0.1mg/kg i.p.) and chlorpromazine hydrochloride (3.0mg/kg i.p.) as well as the "atypical anti-psychotic" clozapine (1mg/kg i.p.) were also found to antagonise D145 hyperactivity significantly ($P<0.02$, <0.01, <0.005 respectively; $N = 16$ in each case).

The results of the two series of experiments provide evidence that D145 can influence dopaminergic mechanisms. The biochemical evidence especially points to the nucleus accumbens being primarily involved and we would suggest this selectivity may be a consequence of the disinhibition of GABA feedback in the mesolimbic system. This would implicate differences in GABA feedback in the limbic and striatal systems. The antagonist activity of clozapine against D145 is also of interest as biochemically it has been suggested to have a preferential effect on dopamine turnover in limbic structures (Bartholini, 1977).

Results were analysed throughout using Students t-test for independent samples.

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ROLE OF MESOLIMBIC DOPAMINE IN MORPHINE REINFORCEMENT

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Selective destruction of mesolimbic dopamine (DA) terminals has been associated with a reduction in the self-administration of both d-amphetamine (Lyness et al, 1979) and cocaine (Roberts et al, 1980). Appetitive reinforcement is produced by microinjections of morphine into the ventral tegmental area (Phillips and LePiane, 1980) and nucleus accumbens (van der Kooy et al, in press), origin and major termination field of this DA system. An investigation into the possible role of mesolimbic DA in mediating morphine's hedonic effects was therefore undertaken. These effects were evaluated using a place conditioning procedure whereby morphine administration is regularly paired with a set of highly discriminable environmental cues and the rat's preference for these cues over those associated with saline injections is assessed.

As is true of several psychoactive drugs, morphine has aversively as well as appetitively reinforcing effects; the pairing of a distinctively flavoured solution with morphine leads to a subsequent avoidance of this flavour. Paradoxically, morphine's aversive effects appear to be temporally correlated with its rewarding actions (van der Kooy and Phillips, 1977). Although an attenuation of morphine-induced taste aversion is observed following 6-OHDA lesions of ascending noradrenergic pathways (Roberts and Fibiger, 1977), the ability of pimozide to produce a similar reduction (Sklar and Amit, 1977) suggests a DA contribution as well. It was therefore of interest to include a measure of conditioned taste aversion (CTA) in evaluating the impact of mesolimbic DA loss.

Subjects (300g male Sprague-Dawley rats) received bilateral infusions of 6-OHDA (4 µg/ µl over two minutes) or vehicle (0.2 µg ascorbate/ µl saline) into the nucleus accumbens and anterior ventral striatum with DMI (20-25 mg/kg) and pargyline (50 mg/kg) ip pretreatment. Rats were divided into two groups; one for morphine place conditioning in a two-sided black and white chamber with visual, tactile and olfactory cues; the other for morphine CTA to a 0.1% saccharin solution.

The efficacy of morphine (10 mg/kg ip) in establishing a decisive preference for the side of the chamber in which it previously had been administered was unaffected by damage to mesolimbic DA terminals. However CTA associated with morphine injections was significantly reduced ($p < .05$). Lithium chloride CTA, in which structures outside the blood-brain barrier are thought to play a major role, was unimpaired by the lesion, suggesting that attenuated morphine CTA is not secondary to hypoactivity or learning deficits.

It is suggested that central DA pathways, particularly the mesolimbic DA projection, are involved in morphine's aversively reinforcing effects. The possibility of DA-NA interactions in this behaviour deserve further study. It is also clear that systemic morphine can exert appetitively reinforcing effects despite substantial depletion of mesolimbic DA terminals.

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FAILURE OF THE ENKEPHALINASE INHIBITOR THIORPHAN TO PRODUCE THE NARCOTIC CUE

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Morphine and related opiates are capable of inducing a dose-related, highly specific, naloxone-reversible stimulus which may be used as a 'cue' for differential responding in operant procedures (Colpaert, 1978). β-endorphin and several synthetic opioid peptides have also been reported to produce stimulus generalisation to the opiate cue when administered intracerebroventricularly (ICV) (Browne & Fondren, 1978; Chipkin & Stewart, 1978; Woods et al, 1980). Since thiorphan has been reported to inhibit enkephalin breakdown *in vitro* and to be antinociceptive when administered systemically (Roques et al, 1980), we have investigated the ability of thiorphan to produce stimulus generalisation to morphine.

Six male Alderley Park rats were trained in standard, 2-lever, operant chambers to respond on one lever following morphine (10mg/kg i.p. 45 mins pretreatment) and on the opposite lever following saline (1 ml/kg i.p.). Correct responses were reinforced (on a fixed ratio (FR) 10 schedule) with sweetened condensed milk. Incorrect responses were of no consequence to the animal. The criterion for reliable discrimination was the completion of 10 consecutive sessions in which the correct lever was selected within the first 12 responses (i.e. pre FR$\leq$12). Generalisation to morphine was said to have occurred when a novel drug induced responding on the lever associated with morphine during training.

The morphine cue was significantly antagonised by the narcotic antagonist naloxone (1mg/kg i.p.). Thiorphan failed to generalise to morphine at 100mg/kg i.v., 30mg/kg i.p. and 75mg/kg i.p., 10 mins prior to testing and 75mg/kg i.p. 45 mins prior to testing. Thiorphan also failed to potentiate a threshold dose of morphine (3mg/kg i.p.) which by itself induced drug responding in only 1 out of 6 animals. Synthetic D-Ala^2-Leu^5 enkephalin, which is partially aminopeptidase resistant but enkephalinase susceptible, did not induce morphine-appropriate responding when administered alone (75mg/kg i.p.) or in combination with thiorphan (75mg/kg i.p.).

The results suggest that either thiorphan may not be significantly inhibiting enkephalinase at the doses used or enkephalinase may not be the most important enzyme involved in opioid peptide degradation *in vivo*. Alternatively, endogenous enkephalin may not form part of the neuronal substrate involved in the opiate stimulus complex. If this were the case, exogenous opiates may act on receptors which are not innervated by endogenous enkephalin.

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BEHAVIOUR AFTER INTRA-ACCUMBENS AND CAUDATE AMPHETAMINE IN A PRIMATE SPECIES

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Although the behavioural effects of peripherally administered amphetamine have been studied extensively in primates (Ellinwood, 1971; Randrup and Munkvad, 1967), little work has been done to try to localize these effects within specific brain areas. From studies using intracerebral injections in rodents it has been suggested that the accumbens and caudate nuclei may be involved in different aspects of amphetamine induced behaviour (See Iversen, 1980). In this experiment we injected amphetamine into the accumbens and caudate nuclei of the small, New World primate, the common marmoset (callithrix jacchus) to see which components of the behavioural response to peripherally administered amphetamine (Scraggs and Ridley, 1978) might be mediated by these two brain areas.

Guide cannulae were implanted bilaterally at an angle so that in each animal drug could be delivered either to the nucleus accumbens or caudate depending on the length of the injection cannulae. Each marmoset served as its own control, receiving amphetamine and saline injections into these two nuclei in a balanced design. Readings were taken every 10 minutes during the 90 minutes following injection. For each reading behaviour was classified every second over 50 seconds into five mutually exclusive categories: 1. Inactivity, 2. checking (small head movements), 3. locomotion, 4. physical contact with another animal, 5. activities (e.g. eating, drinking, self-grooming) (see Scraggs and Ridley, 1978). Histological examination revealed all the cannulae placements to be in or close to the intended sites.

20 µg of amphetamine injected into the accumbens increased checking and locomotion (compared with saline, matched pair t-test, 5 d.f., $t = 4.0$, $p < 0.01$ and $t = 2.58$, $p < 0.05$, respectively) and decreased inactivity and contact with other animals ($t = 3.0$, $p < 0.05$ and $t = 2.7$, $p < 0.05$, respectively). These effects were greatest 10 minutes after injection and behaviour gradually returned to normal over an hour and a half. In contrast 20 µg amphetamine injected into the caudate produced only slight, none significant, changes in these behaviours.

The response following intra-accumbens amphetamine was qualitatively different from that after peripherally injected (i.m.) amphetamine. Increased locomotion was only seen after intracerebral injection and checking, although increased, never reached such high levels and did not seem as stereotyped as has been observed after i.m. amphetamine. In these aspects the behaviour after injections of amphetamine into the nucleus accumbens resembled more that seen after low doses of i.m. apomorphine (Scraggs et al, 1979) than i.m. amphetamine. A different distribution of drug within the tissue of the relevant area or the involvement of additional brain areas after i.m. injections may account for the differing behavioural responses to i.m. and intracerebral injections of amphetamine in the marmoset.

L.E.A. is an MRC student.

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THE STEREOISOMERS OF NICOTINE SHOW SIMILARITIES IN THEIR EFFECTS ON RAT BRAIN IN VITRO AND ON RAT BEHAVIOUR

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Evidence is accumulating that the effects of (-)-nicotine on the brain are not solely attributable to interaction with classical nicotinic receptors (e.g. Abood et al). In peripheral tissues, where effects do result from interaction with classical nicotinic receptors, the naturally occurring (-)-isomer of nicotine is many times more potent than the (+)-isomer (e.g. Barlow & Hamilton). Our experiments suggest that the stereoisomers are equipotent in producing some effects on the rat c.n.s. in vitro and in vivo.

Rat brain slices were incubated with tritiated amine (^{3}H-dopamine, ^{3}H-noradrenaline or ^{3}H-5-hydroxytryptamine) and then superfused with Kreb's solution. Superfusate was collected sequentially and taken for scintillation counting throughout two periods of K^+-induced (40 mM) amine release. The effects of adding (-)-nicotine or (+)-nicotine at 5 x 10^{-6}M to the second period of K^+ depolarisation were studied. In hypothalamus, corpus striatum and hippocampus both isomers increased fractional release of tritiated amines from depolarised slices by 10-40% as compared with a second period of depolarisation containing K^+ alone. The increased efflux caused by (-)-nicotine was significant ($p < 0.05$ in Student's t-test) in hypothalamus (all amines) corpus striatum (dopamine) and hippocampus (noradrenaline). The increased efflux of 5-hydroxytryptamine and dopamine produced by (+)-nicotine from hypothalamus was also significant, and the magnitude of the effect in these instances was similar to that of (-)-nicotine. Qualitatively similar but smaller effects were seen by nicotine on the spontaneous efflux of tritiated amines.

In experiments on rat behaviour nicotine hydrogen tartrate was administered by aerosol to grouped Sprague Dawley rats in an inhalation chamber (Littleton & Umney, 1977) and behaviour monitored automatically. A control group received an aerosol of sodium tartrate or distilled water. Administration of (-)-nicotine (5 min aerosol every 15 min to produce a peak concentration of (-)-nicotine of c100 ng/ml in plasma during the aerosol period) for 21h per day for 15 days produced increased locomotor activity and rearing behaviour in the "light" period and reduced food and water intake. Substitution with a control aerosol now reversed these trends, the "withdrawn" group becoming more lethargic during the light period and increasing food and water intake. Substitution with (+)-nicotine in the same concentration however did not cause the behavioural change in the light period although food and water intake still increased.

These results support the concept that many of the effects of nicotine on brain and behaviour are not mediated by classical nicotinic receptors. They also suggest that (+)-nicotine may partially substitute for the (-)-isomer in dependent subjects without producing the peripheral effects of l-nicotine.

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THE EFFECTS OF TRAZODONE ON THE ELECTRICALLY STIMULATED RAT BRAIN SLICES PRELOADED WITH (^{3}H) DOPAMINE

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Trazodone has an unusual spectrum of pharmacological activity, and there appear to be discrepancies in published data with respect to brain dopamine levels (Stefanini et al, 1976; Boissier et al, 1974). However, it is agreed that, in common with neuroleptics, it eliminates the conditioned response, leaving the escape response intact in the classical avoidance escape paradigm (Gatti, 1973) which suggests that dopaminergic tracts are involved.

The release of tritium from brain slices pre-incubated with ^{3}H dopamine by electrical stimulation has been studied by several investigators, e.g. (Dismukes & Mulder, 1977). To study the effect of trazodone on electrically mobilisable radiolabel from rat striatal slices preloaded with ^{3}H dopamine, an improved system was recently developed principally for studying the effects of neuroleptics on endogenous dopamine release in conditioned rats (unpublished material). A modified version of a brain slicer (Brown & Halliwell, 1981) was used to obtain reproducible coronal slices from striatal regions. Each test slice in a micro electrode chamber (0.2ml) received 2 min. periods of stimulation via gold electrodes by square impulses of alternating polarity (10/s, 2ms, 20mA). The volume of eluate in each superfusate fraction was monitored using standard ^{99}Tc^m. Experiments were carried out with and without Ca^{2+} in the superfusing media to test the dependency of electrically induced ^{3}H dopamine release on Ca^{2+}, generally regarded as essential for depolarisation induced release. Results are calculated on the basis of the total tissue radioactivity at the onset of stimulation. The percentage release of tritium from slices superfused with bicarbonate saline containing Ca^{2+} was 0.9129 $\pm$ 0.3386 (n=9) first stimulation and 0.7006 $\pm$ 0.4553 (n=5), second stimulation. For slices superfused with calcium-free solution the figures were 0.0012 $\pm$ 0.0015 (n=4) first stimulation, and 0.0007 $\pm$ 0.0017 (n=5), second stimulation. The results strongly suggest that the release is Ca^{2+} dependent. To measure the effect of trazodone on slices preloaded with ^{3}H dopamine, electrically evoked release in the absence of the drug established control release levels. Subsequently the drug was added to the superfusing medium (0.024mM) prior to the second electrical stimulation. Control release levels were 0.8855 $\pm$ 0.4586 (n=4). In the presence of trazodone the release levels were 6.024 $\pm$ 3.3945 (n=4).

Preliminary results show that in our modified system, significant release of radiolabel was calcium ion dependent and trazodone apparently increased the proportion of electrically mobilisable ^{3}H dopamine or its metabolites. These results contribute to the puzzle of the pharmacological actions of trazodone on brain dopamine, but do not clarify the issue. The influence of drugs such as trazodone on releasable endogenous dopamine in naive and conditioned animals is currently under test.

Thanks to K. Jeyasingh for advice and help with ^{99}Tc^m measurements (Department of Nuclear Medicine).

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DAPSONE INHIBITS DRUG METABOLISM IN THE RAT

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Dapsone (4,4-diaminodiphenylsulfone) is the parent compound of the sulfone drugs. An estimated 2 million people who suffer from leprosy are treated with dapsone, although in the West the main application is in the treatment of dermatitis herpetiformis. Dapsone exhibits a Type II spectral change in microsomal preparations (Franklin, 1976) but overall little is known about the effects on drug metabolizing enzymes.

In vivo, groups of 5 male Wistar rats were given dapsone (10 or 50 mg/kg) or saline i.p. 30 min before determination of pentobarbitone sleeping time (40 mg/kg). Also the effect of dapsone (50 mg/kg) on zoxazolamine paralysis time (60 mg/kg) and antipyrine clearance (5 µCi/kg; 15 mg/kg 14C-antipyrine) was investigated. In vitro, the demethylation of aminopyrine (2.5 mM) was studied in the presence of dapsone (0.001, 0.01, 0.1, 1 and 10 mM) and deethylation of ethoxyresorufin (ERR, 250 nM) with dapsone (250 nM). Finally, Lineweaver-Burk plots were constructed for aminopyrine n-demethylation kinetics using substrate concentrations of 0.25, 0.75, 1.5 and 2.5 mM and dapsone concentrations of 0.2 and 2.0 mM.

Dapsone (10 mg/kg) increased pentobarbitone sleeping time from 116.0 $\pm$ 16.8 to 191.0 $\pm$ 23.6 min (mean $\pm$ s.e.mean; $P < 0.05$) and caused a further increase at 50 mg/kg to 300.0 $\pm$ 10.5 min ($P < 0.01$). The same dose (50 mg/kg),increased zoxazolamine paralysis time from 140 .0 $\pm$ 10.0 to 319.5 $\pm$ 29.3 min ($P < 0.001$). The addition of 1.0 mM dapsone to rat liver microsomes resulted in a 40% inhibition in the formation of formaldehyde (1.40 $\pm$ 0.18 to 0.81 $\pm$ 0.08 nmoles/min/mg protein). With 10 mM dapsone there was a further decrease to 0.66 $\pm$ 0.09 nmoles/min/mg. ERR deethylase activity decreased by 28.5% in the presence of 250 nM dapsone. Lineweaver-Burk plots showed dapsone to inhibit aminopyrine demethylase non-competively at both concentrations.

We conclude that dapsone inhibits drug metabolism in the rat, affecting both cytochrome P-448 and P-450 enzymes, and that this inhibition may lead to pharmacokinetic drug interactions in vivo.

C.S. was a Visiting Research Fellow supported by the Deutsche Forschungsgemeinschaft.

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THE EFFECT OF ACUTE RENAL FAILURE ON THE PHARMACOKINETICS OF BROMOSULPHOPHTHALEIN IN THE RAT

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We have previously shown that glycerol-induced acute renal failure (ARF) in the rat produces a marked impairment of the hepatic uptake of indocyanine green (Bowmer & Yates, 1981). This effect has been investigated further with bromosulphophthalein (BSP); a dye commonly used to study liver function.

ARF was induced in male Wistar rats (Bowmer & Yates 1981) and 48 h later the plasma disappearance and biliary excretion of BSP (25 mg kg^{-1} i.v.) were determined in anaesthetised animals. Plasma concentration-time data were analysed using the model devised by Richards et al., (1959). Significant decreases in k_{12} and k_{el} were observed in uraemic rats (Table 1). However, Vc and Vd were significantly increased in the uraemic animals which may explain why there was no significant change in plasma clearance. In uraemic rats the percentage recovery of BSP in bile over 3 h and bile flow rate were not significantly different from control values but the rate of biliary excretion during the first 10 min after administration of the dye in uraemic rats (175 ± 115 μg min^{-1} kg^{-1}) was significantly slower than in control animals (527 ± 118 μg min^{-1} kg^{-1}; $P < 0.001$). At other time periods there was no significant difference in biliary excretion rates.

Table 1. Pharmacokinetic parameters of BSP in control and uraemic rats

Parameter	control (n=7)	uraemic (n=7)	% change
$T_{\frac{1}{2}}$ α (min)	1.4±0.2	2.6±0.6***	+86
$T_{\frac{1}{2}}$ β (min)	27.0±9.6	54.5±32.1	+102
k_{12} (min^{-1})	0.49±0.08	0.27±0.07***	-45
k_{21} (min^{-1})	0.007±0.003	0.005±0.002	-29
k_{el} (min^{-1})	0.029±0.009	0.017±0.009*	-41
Vc (ml)	11.7±1.2	20.5±9.9*	+75
Vd (ml)	182±48	295±114*	+62
Plasma clearance (ml min^{-1} 100gbw^{-1})	1.5±0.2	1.7±0.5	+13
Plasma urea (mg 100 ml^{-1})	37±15	353±205**	+854

mean ± s.d. *P < 0.05 **P < 0.01 ***P < 0.001 by two tailed Student's t-test
Vc = apparent volume of central compartment Vd = apparent volume of distribution
k_{12}, k_{21}, k_{el} = rate constants for a two compartment model

The decrease in k_{12} and the initial decrease in the rate of biliary excretion suggests that the rate of hepatic uptake of BSP was decreased in the uraemic rats. These results are similar to those obtained by Wernze and Spech (1971) for the clearance of BSP in patients with chronic renal failure and suggest that liver function may be perturbed in renal failure.

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THE UNDERLYING MECHANISM OF CICLAZINDOL-INDUCED WEIGHT LOSS IN RATS

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Ciclazindol has previously been shown to produce an energy-wasting effect in rodents without causing overt CNS stimulation (Latham et al, 1981; Rothwell et al, 1981). Resting oxygen consumption was increased by up to 20% by a single oral or parenteral dose of ciclazindol (1-30 mg kg^{-1}).

In the present study we describe the effects of chronic (28 day) ciclazindol treatment on male Sprague-Dawley rats. All animals were fed on a standard pelleted diet and were at least 550g prior to use. Ciclazindol (equivalent to 21.5 and 43 mg kg^{-1} day^{-1}) was introduced into the water supply. Body weight, food and water intake and behaviour were monitored over the next four weeks. All analyses were as described elsewhere (Rothwell et al, 1981).

Alterations in body weight are shown in Figure 1. Both doses of drug produced a significant weight loss relative to control animals. This was equivalent to a 7-10% reduction in initial body weight. There was no evidence of anorexia or overt behavioural effects but time to sleep onset and arousal scores were increased at both dose levels.

In both groups of ciclazindol-treated animals, brown adipose tissue weight and Na^+K^+-ATPase (EC 3.6.1.3) activity were significantly increased. These findings, taken in conjunction with the lack of effect on food intake, point to a thermogenic action for ciclazindol.

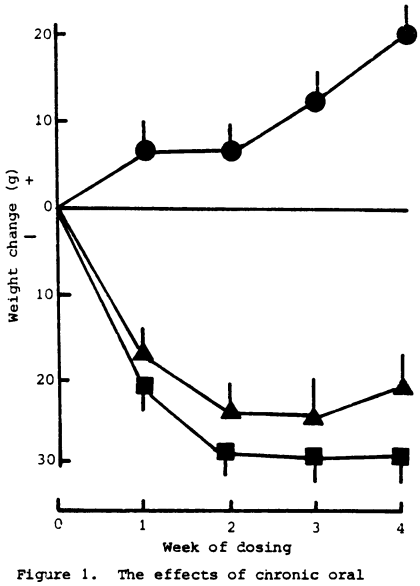

Figure 1. The effects of chronic oral ciclazindol treatment on body weight of rats. All points represent the mean ± s.e. mean of six rats. The drug was introduced into the water supply.
● = no drug. ▲ = ciclazindol 21.5mg kg^{-1} day^{-1}. ■ = ciclazindol 43mg kg^{-1} day^{-1}.

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DIFFERING RANK ORDERS OF POTENCY OF SUBSTANCE P ANALOGUES SUGGEST THE EXISTENCE OF MULTIPLE RECEPTOR SUB-TYPES

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Substance P belongs to a group of naturally occurring peptides known as the tachykinins which possess similar biological actions and share a common C-terminal amino acid sequence, Phe-X-Gly-Leu-Met-NH_2; full agonist activity resides in these last 5 amino acids (Hanley and Iversen, 1980). The tachykinins exhibit differing rank orders of potency on various test systems (Falconieri Erspamer et al, 1980), and two distinct patterns have emerged, which may reflect the existence of 2 sub-classes of substance P receptor. Type I, termed 'SP-P' and typified by the guinea-pig ileum and guinea-pig vas deferens, have the rank order physalaemin > substance P ≃ eledoisin > kassinin, while type II, termed 'SP-E' and typified by the rat duodenum and rat vas deferens, have the rank order eledoisin ≃ kassinin≫physalaemin ≃ substance P. In the present study we have screened a number of substance P analogues on the above in vitro systems.

Correlation coefficients for the potencies of the analogues between the two 'SP-P' systems and between the two 'SP-E' systems were highly significant ($p<0.001$), $r=0.97$ and 0.90 respectively ($n=16$ analogues); furthermore, the slopes and intercepts were not significantly different from 1.0 ($p<0.05$) and 0.0 ($p<0.05$) respectively. In contrast, there was little or no correlation when results from 'SP-P' and 'SP-E' systems were compared.

Eledoisin and kassinin exhibited the greatest selectivity to 'SP-E' systems, while the methyl ester of substance P exhibited the greatest selectivity to 'SP-P' systems (see Table I).

Table 1 Potencies of some substance P analogues

(all potencies are expressed relative to substance P)

	spasmogenic action on guinea-pig ileum (in presence of 1 μM atropine)	potentiation of electrically stimulated contractions		spasmogenic action on rat duodenum
		guinea-pig vas deferens	rat vas deferens	
Substance P	1.00	1.00	1.00	1.00
Physalaemin	2.43	0.75	0.60	1.82
Eledoisin	0.90	0.63	80.00	13.90
Kassinin	0.20	0.26	160.00	11.10
Substance P methyl ester	0.95	0.59	0.015	0.0084

S.P.W. is an MRC Scholar

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AGONIST PROPERTIES OF (D-Pro^2, D-Phe^7, D-Tryp^9) SUBSTANCE P IN VITRO - EVIDENCE FOR MORE THAN ONE TYPE OF SUBSTANCE P RECEPTOR

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There has been considerable effort in recent years to identify an antagonist of substance P (SP) by synthesizing various analogues and fragments of the peptide molecule. One such compound, (D-Pro^2, D-Phe^7, D-Tryp^9)substance P has weak antagonist activity in vitro, in guinea-pig ileum (GPI), and also in vivo (Folkers et al., 1981; Rosell et al., 1981). We have confirmed weak antagonist activity for this compound on SP receptors in GPI smooth muscle, but have also detected a weak agonist action on SP receptors in certain in vitro tissues.

Pieces of GPI, approx. 1-2cm, were taken at least 15cm from the junction with the caecum. They were suspended in 1ml organ baths containing Krebs' solution gassed with 95% O_2/5% CO_2. Contractions of the ileum were recorded via an isometric transducer coupled to a chart recorder. (D-Pro^2, D-Phe^7, D-Tryp^9)SP, obtained from Peninsula Laboratories Inc., consistently produced dose-related contractions of GPI in concentrations varying from $1x10^{-5}$ to $1x10^{-4}$M. The agonist response to (D-Pro^2, D-Phe^7, D-Tryp^9)SP, $1x10^{-4}$M, was completely abolished in 6/6 tissues by prior administration of atropine, $1x10^{-7}$M. In contrast, contractions of the ileum produced by SP, $1x10^{-10}$ to $3x10^{-7}$M, were either unaffected by this concentration of atropine, or were only slightly (< 25%) reduced. This suggests that the bulk of the contractile response to SP in GPI is via smooth muscle receptors, although a small part of the response may be mediated via release of acetylcholine from postganglionic parasympathetic neurones. A similar conclusion for SP was reached by Holzer & Lembeck (1980). In contrast, it appears that the contractile response to (D-Pro^2, D-Phe^7, D-Tryp^9)SP in GPI is mediated entirely via release of acetylcholine from cholinergic neurones.

A direct effect of (D-Pro^2, D-Phe^7, D-Tryp^9)SP on muscarinic receptors in ileum can be discounted as contractile responses to both the analogue and SP were abolished by prior administration of a desensitizing dose of SP, $1x10^{-7}$M, confirming that the analogue is producing its contractile effect via a SP receptor.

The contractile responses to both (D-Pro^2, D-Phe^7, D-Tryp^9)SP and SP were unaffected by hexamethonium, $1x10^{-4}$M, a concentration sufficient to completely abolish matched responses to the nicotinic agonist, 1,1-dimethyl-4-phenyl-piperazine (DMPP). This suggests that both (D-Pro^2, D-Phe^7, D-Tryp^9)SP and SP are affecting SP receptors located somewhere on the postganglionic neurone.

In the rat isolated superior cervical ganglion preparation (Brown & Marsh, 1978) both SP, $1x10^{-8}$ to $1x10^{-6}$M, and (D-Pro^2, D-Phe^7, D-Tryp^9)SP, $1x10^{-5}$ to $1x10^{-4}$M, produced dose-related depolarisations of the ganglion cell bodies. It seems likely, therefore, that these compounds might also depolarise the parasympathetic ganglion cell bodies in the GPI, thereby producing a release of acetylcholine.

(D-Pro^2, D-Phe^7, D-Tryp^9)SP has weak agonist activity on the ganglionic SP receptor and SP antagonist activity on the smooth muscle receptor. This might suggest that the SP receptors in the two locations are different.

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DIFFERENCES IN SENSITIVITY TO SUBSTANCE P AND ANALOGUES OF OUTER AND INNER MUSCLE LAYERS FROM RAT COLON AND GUINEA-PIG ILEUM

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Several authors have noted that the longitudinal muscle of the guinea-pig ileum is very much more sensitive to substance P (sP) than is the circular muscle (see Nieber et al. 1981). The contractile response of the whole ileum to sP may thus be largely attributed to an action on the longitudinal muscle.

During the course of experiments on the rat colon in vitro, we found that sP and some of its analogues failed to produce classical sigmoid log dose-response curves. A possible explanation was that the peptides were acting on more than one cell type and so we attempted to obtain simpler preparations by separating the outer mainly longitudinal and inner mainly circular muscle layers (Lindström et al., 1979). The colon was removed from male Sprague-Dawley rats (200-220 g) and the outer muscle layer peeled off with the aid of a moist cotton wool pellet (Rang, 1964). This yielded a tubular-shaped preparation presumed to contain the longitudinal muscle and a second one presumed to contain the circular muscle. Longitudinal muscle preparations were obtained in a similar manner from the ileum of guinea-pigs (male, Hartley strain, 400-500 g), but the circular muscle was subsequently cut to produce a strip (Brownlee & Harry, 1963). Recordings of isotonic contractions were obtained from all of the preparations. The conditions and dosing schedule adopted for the ileum were as previously reported (Jordan, 1980). Preparations from rat colon were treated similarly but the bathing medium had the following composition: (mM) NaCl 154, KCl 5.6, $NaHCO_3$ 6.0, $CaCl_2$ 0.27, glucose 2.8.

In agreement with previous reports, circular muscle from guinea-pig ileum was far less sensitive to sP than was the longitudinal muscle (approximately 1,000 fold difference).Sensitivity to carbachol was also much lower (approximately 100 fold). In marked contrast to this, the inner muscle layer was the more sensitive component of the rat colon to sP (EC_{50} for sP on circular muscle 70 nM: threshold concentration for outer muscle layer 1 μM). The relative sensitivities to carbachol and 5-hydroxytryptamine (5HT) were, however, in the reverse order (outer muscle layer responded to concentrations of carbachol and 5HT in the range 10-100 nM; inner muscle layer failed to respond to 5HT at concentrations up to 10 μM and was always less sensitive than outer layer to carbachol.

Differences in the sensitivity of the muscle layers to carbachol and 5HT may be due, at least in part, to the presence or absence of nerve plexuses. However, the responses to the peptides are likely to be the result of direct actions on the smooth muscle and seem to reflect genuine differences in sensitivity. Furthermore, these differences extend to the relative activities of sP-like peptides in rat colon and guinea-pig ileum. Thus, the rank order of potencies for four peptides in the whole ileum is: - physalaemin (2.3) > sP_{4-11} (1.1) > sP (1.0) > eledoisin-related peptide (erp) (0.78) > eledoisin (0.64), whilst that for the inner muscle layer of rat colon is - eledoisin (8.4) > erp (2.1) > sP (1.0) ≈ sP_{4-11} > physalaemin (0.26).

Acknowledgements: We are grateful to The Wellcome Trust for financial support. S.J.B. is an M.R.C. Scholar.

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A RELATIVELY INEXPENSIVE COMBINED STIMULUS ISOLATION/CONSTANT CURRENT DEVICE

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The circuit described enables the construction of a combined stimulus isolation (SI) / constant current (CC) device at a small fraction of the cost of comparable commercial units. The complete circuit is shown in Figure 1 and the components in Table 1. SI is achieved by IC1, wherein the stimulus pulse is converted to light. R1 serves to limit the current through IC1 and is based on a 9 Volt input from a dry cell battery. The isolated side of IC1 is connected as a photodiode and light falling on this causes a potential difference (pd) of about 1.2 Volts to appear across RV1. The whole or part of this pd is tapped at pin 3 of IC2, which is a general purpose operational amplifier connected as a voltage follower. Its purpose is to bias TR1 variably, and to maintain a pd across R2 equal to that seen at pin 3 of IC2. If this pd is 0.6 Volt, for example, and R2 is 1.2 k ohm, then a current of 0.5 mA will flow through R2. If a voltage, Vs, is now applied to the collector of TR1, then a current will start to flow through TR1 and R2 towards the 0 Volt line. In so doing, however, the pd across R2 is raised from its initial value (set by RV1) and this in turn causes the output of IC2 to fall. Thus, the base bias of TR1 is reduced and current flow through the transistor is decreased until the pd across R2 is returned to that value seen by pin 3 of IC2. At this point the current in the collector circuit of TR1 is the same as that in R2. This holds true for increasing values of resistance in the collector circuit provided that Vs is sufficiently high. In practice we have found 60 Volts to be suitable for our purposes.

To set up, set the stimulator voltage dial to 10 Volts and, with PB1 closed and RV1 wiper set to maximum tapped voltage, increase the stimulator output until a current of 1 mA is seen by a milliammeter placed in series between Vs and the TR1 collector. This stimulator voltage is utilised throughout subsequent use of the SI/CC unit. Currents greater than 1 mA may be obtained by reducing the value of R2. Stimulating current is adjusted by means of RV1.

Figure 1. SI/CC unit circuit. IC pin numbers are shown

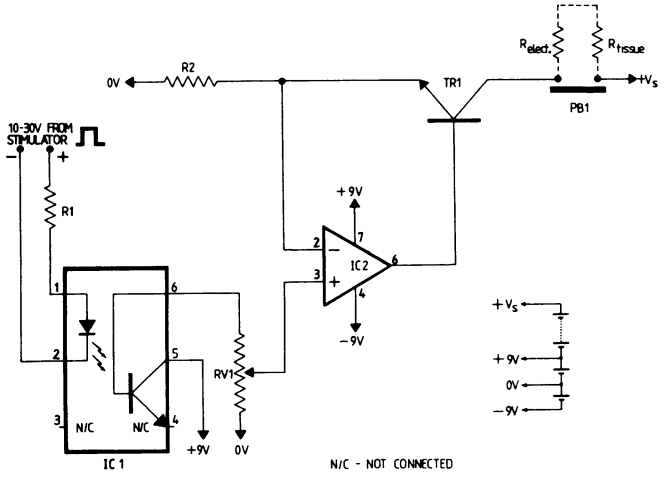

Table 1. Components

R1	400 ohm
R2	1 - 1.5 k ohm
RV1	Linear 50 k ohm
TR1	BFX 85
IC1	OPTO-ISOLATOR *
IC2	µA 741

* This component (order code WL35Q) available from Maplin Electronic Supplies, London, England. (Tel: 01 - 748 0926).

ACTIONS OF AND INTERACTIONS BETWEEN METHYLXANTHINES AND β-ADRENOCEPTOR AGONISTS ON SKELETAL MUSCLE CONTRACTIONS

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Methylxanthines in millimolar concentrations enhance the force of contraction of skeletal muscles, probably by mobilization of intracellular Ca^{2+}. This effect appears to be independent of the inhibitory effect on cyclic AMP phosphodiesterase of these compounds (Kramer & Wells, 1980). β-adrenoceptor agonists decrease the degree of fusion and tension of subtetanic contractions in slow-contracting skeletal muscles such as the soleus. This effect appears to be mediated via cyclic AMP (see Bowman, 1980). In the present study we measured drug-induced effects on twitches and subtetanic contractions of the guinea-pig soleus muscle in vitro (Holmberg & Waldeck, 1980).

IBMX (1-methyl-3-isobutylxanthine) and theophylline in concentrations from 500 μM increased the force of contraction. At 100 μM IBMX caused an isoprenaline-like depression of the subtetanic contractions of the soleus muscle, characterized by a decrease in the ratio between the tension of a subtetanic contraction and the twitch tension. This effect, which was not inhibited by 0.1 μM propranolol is probably due to accumulation of cyclic AMP as a result of phosphodiesterase inhibition. Theophylline which is 100 times less potent than IBMX as an inhibitor of phosphodiesterase did not show the isoprenaline-like effect.

In the presence of 20 μM IBMX the effect of 10 nM terbutaline, a β_2-selective adrenoceptor agonist, on the soleus muscle more than doubled, but the maximum depression by 1 μM terbutaline was if anything diminished. Theophylline, 40 μM, did not potentiate the effect of neither terbutaline nor isoprenaline but tended to diminish the maximum depression of the soleus muscle brought about by isoprenaline (cf Bowman & Nott, 1974). In the presence of 900 μM theophylline the depression by 1 μM terbutaline was reduced by 40 per cent.

In recent experiments on mouse diaphragm Gross & Kelly (1981) showed that high Ca^{2+}-concentrations inhibit isoprenaline-stimulated cyclic AMP accumulation. We suggest that Ca^{2+} mobilized by methylxanthines inhibits the effect of β-adrenoceptor agonists on the contractions of the soleus muscle due to inhibition of cyclic AMP accumulation. This may obscure an expected potentiation of the effects of β-adrenoceptor agonists resulting from inhibition of phosphodiesterase. The outcome of a particular experiment will depend on the properties of the xanthine derivative employed and the experimental conditions used. In support of this view IBMX, which inhibits phosphodiesterase in concentrations not expected to cause Ca^{2+}-release, enhanced the effect of terbutaline whereas theophylline was unable to do so.

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EFFECT OF OESTRIOL AND OESTRADIOL-17β ON PROLACTIN SECRETION IN THE OVARIECTOMIZED RAT

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Oestriol (OE_3) was compared to Oestradiol-17β (OE_2) in respect of it's ability to effect changes in pituitary prolactin secretion. At least 2 weeks post-ovariectomy, wistar rats (150-200g) were injected subcutaneously with varying doses of OE_2 and OE_3, twice daily for 5 days. Thereafter, the rats were anaesthetized (with urethane, 1.4g/kg i.p.), a basal blood sample drawn through a jugular cannula, TRH (50ng) administered intravenously and a further blood sample taken 5 min. after TRH administration. The rats were then sacrificed and the anterior pituitary removed and homogenized in saline. Plasma and pituitary prolactin were determined by radioimmunoassay using rat prolactin (RP-1, National Pituitary Agency, Maryland, USA) as standard.

OE_2(1.0ug) increased basal plasma prolactin from 20.0±2.6ng.ml^{-1}, n=6 (mean ± S.E.M., no. of animals) (saline controls) to 50.7±3.0, n=6 ($p < 0.001$). OE_3(1.0ug) did not increase basal prolactin levels significantly above control values. OE_3(12ug) increased basal plasma prolactin to 36.5±6.1, n=5, which was significantly greater than control values (cited above) ($p < 0.005$), but significantly less than the value obtained with OE_2 (10ug) (71.2±10.5, n=5) ($p < 0.005$). However, with OE_3 (20ug) plasma prolactin levels (87.7± 12.9, n=6) of the same order as the highest levels obtained for OE_2 (e.g. 10ug, values given above)were recorded.

Anterior pituitary prolactin levels were increased maximally by OE_2 (2ug) to 3.0±0.4ug.mg tissue^{-1} (wet wt.) (n=9). This is significantly higher than the level seen after OE_2 (1ug) (1.4±0.2, n=4) ($p < 0.005$) or OE_2 (10ug) (1.4±0.3, n=5) ($p < 0.005$). There was a sharp increase in pituitary prolactin content when animals were treated with OE_3 at doses > 10ug (e.g. OE_3 (10ug): 0.8±0.1, n=5; OE_3 (12ug): 2.3±0.2ug, n=4 and OE_3 (20ug): 2.8±0.3, n=8). Unlike the decrease in content seen at higher OE_2 doses the same prolactin content was maintained up to OE_3 (100ug) (2.53±0.51, n=7), the highest dose tested. The prolactin content after OE_3 doses ≥12ug was of the same order as the maximum content seen after E_2 treatment.

A maximal increase in plasma prolactin over basal levels was obtained in response to intravenous TRH (50ng) in rats pretreated with OE_2 (2ug) (110.0±20ng.ml^{-1}, n=5). No TRH response was detectable for OE_3 doses ≤ 10ug. OE_3 (12ug) showed a TRH response in plasma prolactin of 63.7±8.6, n=3, which is significantly lower than obtained with OE_2 (2ug). Responses to TRH equivalent to maximal for OE_2 (at 2ug) were obtained with all doses of OE_3 ≥ 16ug, tested.

Several authors have shown that OE_3(administered as discreet injections) is only partially active in eliciting many uterotrophic responses when compared to OE_2. Our results would suggest that although OE_3 is less potent than OE_2 in stimulating prolactin biosynthesis and secretion similar maximal responses are obtainable for both oestrogens.

A COMPARISON OF THE EFFECTS OF BUPRENORPHINE AND MORPHINE ON THE BLOOD GASES OF CONSCIOUS RATS

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Previous studies in conscious rats demonstrated that there were differences in the magnitude of the respiratory depression produced by buprenorphine and morphine. Although both agents increased arterial PCO_2, the elevation produced by buprenorphine reached a plateau whereas the response to morphine increased linearly with dose (Cowan et al., 1977). Consequently the maximum elevation of PCO_2 produced by buprenorphine was significantly less than that produced by morphine. These studies have presently been extended to include higher doses of buprenorphine and morphine. In addition the interaction between buprenorphine and either naloxone or morphine was also studied.

All studies were carried out in conscious male Sprague-Dawley rats (225-325g). Drug administration and arterial samples were via an indwelling catheter in the descending aorta. Arterial samples were analysed using an IL 613 blood gas analyser.

In conscious rats intra-arterial injections of buprenorphine at doses of 0.01 - 1.0 mg/kg caused a dose-related increase in PCO_2. At higher concentrations (10 and 30 mg/kg, i.a.) the increase in PCO_2 became progressively less, Figure 1. Morphine (3 - 300 mg/kg, i.a.) caused a dose-related increase in PCO_2, Figure 1.

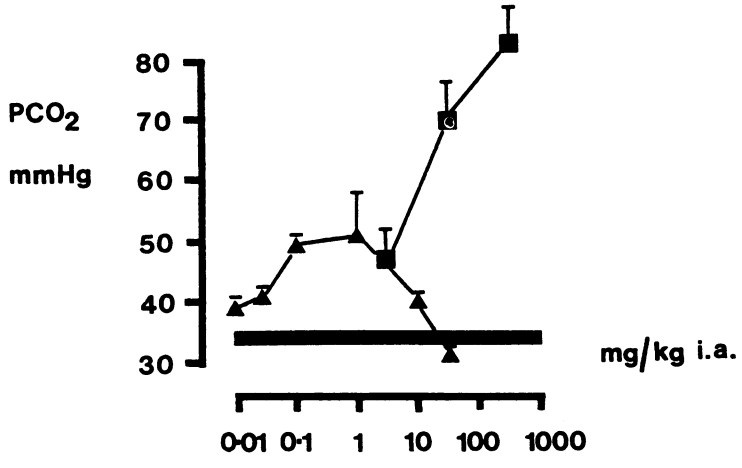

Figure 1. The effects of buprenorphine (▲) and morphine (■) on arterial PCO_2 in conscious rats (30 min post injection). The results are the mean of 5 rats ± s.e.m. Stippled band represents control group.

Pretreatment of rats with naloxone (3mg/kg, i.a.) reduced the elevation of PCO_2 following low doses of buprenorphine (0.01 - 3.0 mg/kg, i.a.) but had little effect on the PCO_2 changes produced by higher doses of buprenorphine. Finally the PCO_2 of rats treated with a combination of buprenorphine (1 mg/kg, i.a.) and morphine (36 mg/kg, i.a.) was 44 ± 3 mmHg whereas the values following these doses of buprenorphine and morphine given alone were 43 ± 2 and 55 ± 4 respectively.

In conclusion therefore the profile of buprenorphine on blood gases reflects the partial agonist character of the compound at opiate receptors.

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DENSENSITIZATION TO CALCITONIN INDUCED ANALGESIA BY WITHDRAWAL OF CHRONIC CALCITONIN TREATMENT

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Calcitonin, by intracerebroventricular (i.c.v.) injection, possesses analgesic properties, in several species, which are not observed when the hormone is given by subcutaneous (s.c.) injection (Pecile et al, 1975; Bates et al, 1981a). Repeated daily i.c.v. injection of calcitonin for 5 days does not induce tolerance (Braga et al, 1978). In mice the withdrawal of chronic treatment with salmon calcitonin (SCT) by s.c. injection induces a prolonged hyperalgesic response (Bates et al, 1981b).

We have investigated the effect of chronic pretreatment with SCT by s.c. injection upon the analgesic action of either SCT, given by i.c.v. injection, or aspirin, given by s.c. injection.

Groups of 10 CFLP mice (♂ ,♀ ,30g) were pretreated with s.c. injections of SCT (10 i.u. kg^{-1}) or vehicle (0.9% NaCl + 1 mg ml^{-1} BSA) given on alternate days for a period of 8 days. The sensitivity to peritoneal irritation by acetic acid was assessed 48 hours after the last s.c. injection of SCT. (Each animal was given 0.3 mls 1% acetic acid in 0.8% NaCl by i.p. injection and the number of abdominal constrictions were counted between the 10th and 14th minute inclusive.) This treatment induces a rate of 3 to 4 constrictions per minute in the control animals and is taken as the baseline response. Substances to be tested for analgesia were administered 10 min prior to acetic acid. SCT (0.04-10 i.u. kg^{-1}) was given by i.c.v. injection as described previously (Bates et al, 1981a). Aspirin (0.125-0.5 mmol kg^{-1}) was given by s.c. injection. Control animals received injections of the appropriate vehicle. In animals, not pretreated with SCT, SCT by i.c.v. injection produced a dose dependent inhibition of abdominal constrictions, with a threshold of 0.04 i.u. kg^{-1} and a maximum response at a dose of 10 i.u. kg^{-1}. The ED_{50} was .36 i.u. kg^{-1}. Aspirin (0.125-1 mmol kg^{-1}) by s.c. injection also produced a dose-dependent inhibition of abdominal constrictions with an ED_{50} of 0.44 mmol kg^{-1}.

In animals, pretreated with SCT, the frequency of abdominal constrictions was increased by 32-48% ($p<0.005$) 48 hours after the last injection of SCT. In these pretreated animals, SCT by i.c.v. injection (0.04-10 i.u. kg^{-1}) was approximately 100 fold less effective in decreasing the frequency of abdominal constrictions (ED_{50} = 66 i.u. kg^{-1}). In contrast, the sensitivity of the pretreated animals to aspirin (0.125-0.5 mmol kg^{-1} was unaffected (ED_{50} = 0.25 mmol kg^{-1}).

In summary; the withdrawal of chronic treatment with SCT induces an increase in the response to peritoneal irritation with acetic acid. Pretreatment with SCT, does not affect the sensitivity to aspirin but desensitizes the mice to the analgesic actions of SCT by i.c.v. injection. It is thus possible that the hyperalgesia associated with withdrawal of chronic treatment with SCT may result from a central effect of the hormone.

The salmon calcitonin was generously donated by Armour Pharmaceuticals Corporation, Eastbourne, U.K.

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