A PRIMATE MODEL FOR THE ASSESSMENT OF ANXIOLYTIC DRUG ACTION

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The behavioural repertoire of the common marmoset (Callithrix jacchus) has been studied in full detail, and a complete ethogram for the animal has been published (Stevenson & Poole, 1976). With reference to the observations made by these workers, we now put forward an ethologically relevant, primate model for the assessment of anxiolytic drug action. Marmosets, when confronted with a potentially threatening situation, respond with a series of 'body movements' and 'facial expressions' which have been loosely termed 'postures'. Most of these 'postures' are directed towards the threatening stimulus (i.e. a human observer standing in close proximity to the home cage) and include 'tail postures' (elevation of the tail to expose the genital region), slit-stares (confronting the observer with flattened ear tufts and partial eye closure), scent marking of the cage surfaces, and arching of the back with associated piloerection.

The behaviour of single sex pairs of adult marmosets (290-390g) was assessed over a 2 minute period, during which the number and duration of postures exhibited by each animal were recorded using an electronic key pad connected to a BBC microcomputer. Three additional parameters were also recorded; the time the animals spent at the cage front in confrontation with the observer, the time spent in the nest box, and the number of jumps from the back of the cage to the cage front which provides an index of locomotor activity.

The typical effect of an anxiolytic compound in the test was to decrease (without sedation), the number of 'postures' exhibited by the animal, to increase the time spent at the front of the cage and to decrease the amount of time spent in the nest box. For example, postures were decreased by diazepam (25μg/kg s.c. 40 min. pretreatment) from 11.5$\pm$0.95 to 3.5$\pm$0.5 ($P<0.01$), by pentobarbitone (0.5mg/kg s.c. 40 min pretreatment) from 10.8$\pm$1.5 to 2.5$\pm$0.5 ($P<0.01$) and by buspirone (0.1mg/kg s.c. 40 min pretreatment) from 9.8$\pm$1.6 to 3.3$\pm$1.3 ($P<0.001$). At the above doses, diazepam, pentobarbitone and buspirone did not cause sedation as could be detected in the general activity of the animals or in the number of jumps performed by the animals.

A similar profile of action was seen for 8OH-DPAT (0.01 - 1 mg/kg), tiapride (0.6 - 2.5 mg/kg), and the 5-HT_3 antagonists GR38032F (0.01 - 1 μg/kg) and ICS 205-930 (0.01 - 1 μg/kg). Compounds which are centrally acting but which are without anxiolytic activity, such as haloperidol and amitriptyline, have also been tested in the model and do not alter the parameters recorded except at sedative doses.

Thus the 'human threat model of anxiety' in the marmoset may provide a novel primate test procedure for the assessment of anxiolytic drug action.

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AUTORADIOGRAPHIC MEASUREMENT OF $GABA_A$ AND $GABA_B$ BINDING SITES IN RAT CAUDATE PUTAMEN AFTER DENERVATION OF NEURONAL INPUTS

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Neuronal projections to the caudate putamen are well documented and include the dopamine input from the substantia nigra pars compacta and the corticostriatal excitatory pathway which may release glutamate or aspartate from its terminals. It seems likely that $GABA_B$ receptors are present on the terminals of these projections since $GABA_B$ receptor activation can reduce the stimulated release of dopamine and glutamate from striatal slices (Bowery et al 1980; Potashner & Gerard, 1983) and chronic lesion of the cortical-striatal pathway reduces $GABA_B$ binding on striatal membranes (Kilpatrick et al 1983). We have now examined the effects of lesioning these two major inputs on the density of $GABA_B$ and $GABA_A$ sites in the caudate putamen using quantitative receptor autoradiography.

The nigrostriatal pathway was lesioned by unilateral injection of 6-hydroxydopamine (8 ug) stereotaxically into the nigra of rats under equithesin anaesthesia. Cortical ablation was performed unilaterally in another group of rats in which a piece of skull from the right hemisphere was removed. Cerebral cortex was aspirated taking care not to damage the underlying striatum. 14, 30 and 60 days after surgery, lesioned rats were perfused-fixed with 0.1% paraformaldehyde and transverse sections (10 um) prepared for autoradiography. [3H] GABA was used to label $GABA_A$ and $GABA_B$ receptors as described by Wilkin et al (1981).

Nigral lesion produced an ipsilateral decrease in $GABA_B$ binding sites within the caudate putamen. The mean decrease was 15% (n=30 sections) compared to the normal side at all times studied. In contrast $GABA_A$ sites remained unchanged. Decorticated animals showed an increase in $GABA_A$ binding sites in the caudate putamen on the lesioned side. The mean increases were 18%, 29% and 23% at 14, 30 and 60 days after surgery respectively (n=60 sections in each case from 4 animals). In sections from the same animals a decrease in $GABA_B$ sites was observed on the lesioned side (33%, 34% and 25% at 14, 30 and 60 days after surgery respectively (n=60 sections in each case from 4 animals).

In conclusion, nigral lesions reduced the density of $GABA_B$ but not $GABA_A$ binding sites whereas cortical ablation increased $GABA_A$ but reduced $GABA_B$ binding density in the caudate putamen. These data support the view that a proportion of $GABA_B$ sites are present on dopamine and glutamate/aspartate terminals in the striatum. The reason for the apparent increase in $GABA_A$ sites after cortical ablation is at present unknown.

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RO 19-4603: A BENZODIAZEPINE RECEPTOR PARTIAL INVERSE AGONIST WITH PROLONGED PROCONVULSANT ACTION IN RODENTS

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Ro 19-4603 (Figure 1) is an imidazothienodiazepinone derivative structurally related to the imidazobenzodiazepinone Ro 15-3505 (Pieri et al., 1985). Ro 19-4603 exhibits a specific high-affinity binding to the central benzodiazepine receptor (BZR), which is comparable to that of the BZR antagonist flumazenil or the BZR partial agonist Ro 16-6028 (Martin et al., 1988).

Ro 19-4603 was found to antagonize the protective effect of a supramaximal dose of diazepam against pentylenetetrazol-induced (PTZ: 120 mg/kg i.p.) tonic convulsions in mice (ED_{50}= 80 and ED_{90}= 120 µg/kg p.o. or ED_{50}= 10 and ED_{90}= 21 µg/kg i.v.) and in rats (ED_{50}= 0.6 and ED_{90}= 1.4 mg/kg p.o.).

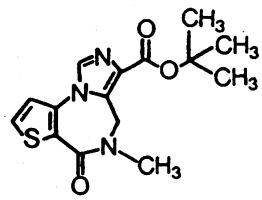

Figure 1 Ro 19-4603

Given p.o. (15 min) or i.v. (5 min) prior to a low dose of PTZ (60 mg/kg i.p.) which typically induced tonic convulsions (TC) in 5-20 % of mice and rats, Ro 19-4603 exhibited a clear-cut proconvulsant effect. In mice, the minimal effective proconvulsant dose (MED) was 100 µg/kg p.o. (11 from 20 mice showed TC) or 10 µg/kg i.v. (12/20 mice with TC). In rats, the MED was 30 µg/kg p.o. (12 from 20 rats showed TC). This effect was dose-dependent, with a tendency to plateau at the highest doses. This proconvulsant effect lasted 4-5 h after 1 mg and longer than 6 h after 3 mg/kg Ro 19-4603 p.o. in rats. Flumazenil (3-10 mg/kg p.o. or i.p.) dose-dependently antagonized this proconvulsant effect of Ro 19-4603 in mice and rats. A similar proconvulsant effect of Ro 19-4603 (0.1-0.5 mg/kg p.o.) was seen in rats using a threshold electroshock, which was also dose-dependently antagonized by flumazenil.

Only at 30 mg/kg p.o. or more did Ro 19-4603 by itself induce weak clonic convulsions in mice and rats.

Tonic convulsions induced with acoustic stimulation (AS: 14 KHz, 110 dB, 60 s) in the DBA/2J mouse strain (genetically and age-dependently susceptible to AS) can be prevented with diazepam. This protective effect of diazepam was antagonized by Ro 19-4603 given orally 15 min prior to AS with ED_{50}= 112 and ED_{90}= 140 µg/kg. Furthermore, oral Ro 19-4603 given 15 min prior to a threshold AS (83 dB) was proconvulsant with ED_{50}= 34 and ED_{90}= 58 µg/kg. The proconvulsant effect of Ro 19-4603 in this paradigm could be dose-dependently prevented or reversed by 3-30 mg/kg flumazenil (p.o. or i.p.) given before or after Ro 19-4603.

Ro 19-4603 given p.o. (0.7-5 mg/kg) 45 min after phenobarbitone (60 mg/kg i.p.) or meprobamate (200 mg/kg i.p.) and 15 min before PTZ (120 mg/kg i.p.) to mice did partially counteract the anticonvulsant action of phenobarbitone or meprobamate.

Ro 19-4603 by itself, in doses slightly higher than those eliciting a proconvulsant effect, increased startle response, locomotion, sniffing and grooming in rodents. The presence of increased sniffing and grooming might indicate an indirect dopaminergic activation through a reduced GABAergic inhibition of dopaminergic neurons.

In conclusion, Ro 19-4603 is the most potent partial BZR inverse agonist found to date in the chemical class of imidazodiazepinone derivatives. Although of no immediately apparent therapeutic value, it could offer a fruitful pharmacological tool for further functional research on BZR.

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INTERACTION OF CHLORMETHIAZOLE WITH LIGAND BINDING SITES ASSOCIATED WITH THE $GABA_A$ RECEPTOR COMPLEX

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Chlormethiazole (CMZ) is used clinically as a sedative hypnotic and anticonvulsant agent (Evans et al 1986). Several classes of compound with this profile of action, including barbiturates, potentiate the effects of GABA by acting at the $GABA_A$ receptor complex (Simmonds & Turner, 1987). In the present study we have used ligand binding techniques to study the interaction of CMZ with the $GABA_A$ receptor complex. [^{3}H]-muscimol was used to label the GABA binding site, [^{3}H]-flunitrazepam to label the benzodiazepine site and the [^{3}H-t-butylbicyclophosphorothionate ([^{35}S]-TBPS) to label the chloride channel of the receptor complex.

Male Lister Hooded rats (~200g) were used throughout. Membranes were prepared from cerebral cortex by homogenisation in 50mM Tris citrate pH 7.4 using an Ultra-Turrax, and centrifugation at 20,000g for 20 min. The pellet was resuspended in buffer containing 100mM NaCl and washed 4 times by centrifugation and resuspension. Membranes were incubated with 5nM [^{35}S]-TBPS and displacing agents in 50mM Tris-citrate containing 100mM NaCl for 90 min at room temperature. Bound ligand was separated by filtration over glass fibre filters. Non-specific binding was defined as that not displaced by 10μM picrotoxin. [^{3}H]-muscimol binding and [^{3}H]-flunitrazepam binding were performed as described previously (Cross et al 1980) in the presence of 100mM chloride.

[^{35}S]-TBPS binding was fully displaced by CMZ and by the sedative barbiturate pentobarbitone (Table 1). Both CMZ and pentobarbitone stimulated [^{3}H]-muscimol binding. In contrast, whilst pentobarbitone stimulated [^{3}H]-flunitrazepam binding, CMZ was ineffective (Table 1). When added in combination, CMZ (10^{-3}M) inhibited the enhancement of [^{3}H]-flunitrazepam binding induced by 10^{-3}M pentobarbitone (110 ± 7% control).

Table 1. Interaction of CMZ and pentobarbitone with ligand binding to the $GABA_A$ receptor.

	Inhibition of [^{35}S]-TBPS (IC_{50}μM)	Stimulation of [^{3}H]-muscimol (% control)(a)	Stimulation of [^{3}H]-flunitrazepam (% control)(a)
Chlormethiazole	140 ± 30	137 ± 3	93 ± 5
Pentobarbitone	95 ± 24	138 ± 3	145 ± 7

Mean ± SEM of at least 3 determinations. (a) 10^{-3}M drug

CMZ interacts with the $GABA_A$ receptor complex at concentrations similar to those of pentobarbitone. It has been shown by others that CMZ potentiates electrophysiological responses to GABA in a manner similar to the barbiturates (Simmonds & Turner 1987, Hedlund & Ogren 1987). However, unlike pentobarbitone, CMZ did not enhance [^{3}H]-flunitrazepam binding suggesting that it does not interact with $GABA_A$ receptor in an identical manner.

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EFFECTIVENESS OF CONCURRENTLY ADMINISTERED DIHYDROPYRIDINES IN PREVENTING THE WITHDRAWAL SYNDROME FROM BARBITAL

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We have recently obtained considerable evidence that dihydropyridine-sensitive calcium channels are involved in dependence on ethanol. Chronic ethanol treatment increased the number of dihydropyridine binding sites in the cerebral cortex and the neuronal actions of dihydropyridines (Dolin et al, 1987). Administration of dihydropyridine calcium channel antagonists during chronic ethanol treatment prevented the development of tolerance to ethanol (Little & Dolin, 1988) and the withdrawal syndrome (Whittington & Little, 1988). The present study was undertaken to determine whether or not dihydropyridine calcium channel antagonists can prevent the development of barbiturate dependence. The dihydropyridines were given chronically with barbital, until 24h before the withdrawal, so that effects on the development of changes responsible for the withdrawal syndrome could be studied, rather than any acute actions. Both the methods used of calcium channel antagonist administration prevented the ethanol withdrawal syndrome (Whittington & Little, 1988).

Male mice, TO strain (30 - 35g) and C57 strain (25 - 30g), were given barbital in powdered food; 3 mg/g for 2 days, 4 mg/g for 2 days and 5 mg/g for 2 days. In the first study i.p. injections of nitrendipine, 50 mg/kg, twice daily were given. Separate groups of animals received barbital plus injections of vehicle (Tween 80, 0.05%) and vehicle and nitrendipine injections without barbital. In the second study, nicardipine was added to the food at 1 and 2 mg/g. Separate groups of animals were given barbital alone, barbital plus nicardipine, nicardipine alone or normal powdered food. Dihydropyridine administration was stopped 12h before removal of barbital. Ratings of convulsive behaviour on handling (Littleton & Little, 1987) were made hourly from 12h after removal of barbital (ie. 24h after cessation of dihydropyridine treatment). All ratings were carried out by observers who did not know the prior drug treatment. Barbital intake was monitored throughout the treatments; the changes observed could not be explained by alterations in the amount of barbital consumed.

Table 1. Ratings of convulsive behaviour: (medians; times = from barbital withdrawal)

Time:	13h	14h	15h	16h	17h	18h	19h	20h	21h	22h
C57s: con:	0.5	1	1	1	1	1.5	1.5	1	1	1
barb:	2	2	2	3	2	2	2	2.5	2.5	2.5
barb+nit*:	1	2	1.5	1	2	1	1.5	1	2	2
TOs: con:	0	0	0	0	0	0	0	0	0	0
barb:	1	1	3	2.5	2	1.5	1.5	1	0.5	0.5
barb+nit*:	1	0	2	2	1	1	1	0	0	0

N = 7-15; *P<0.001 cf.barbital alone, nonparametric analysis of variance (Meddis, 1984).

Nitrendipine injections significantly decreased the behavioural ratings on withdrawal from barbital, but did not completely prevent the withdrawal hyperexcitability, in contrast to our previous results with ethanol. Nicardipine had no effect, at either dose. The dihydropyridines alone did not have any consistent effect on behaviour. The results indicate that dihydropyridine-sensitive calcium channels may play some part in barbiturate dependence, but this may be a smaller component than that suggested for ethanol dependence.

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PRELIMINARY SCREENING OF A SINGLE-DOSE NATIVE ANTIEPILEPTIC PREPARATION USED IN NIGERIA

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Many traditional medical practitioners in Nigeria claim to have preparations which suppress convulsive disorders but only a few of these natural products have been investigated (Atalabi, 1964; Oppe, 1978; Onuaguluchi, 1986). Recently, we came across a powder prepared from four plant products* which is claimed to cause **permanent** suppression of epileptic seizures after a **single** transdermal application. We report here the results of our initial evaluation of the effects of this native powder on electroshock convulsions in rats.

Male Wistar rats weighing 250 - 350 g were randomly allocated to six treatment groups (N = 8 per group) namely: 0.9% saline (control), diazepam 8 mg/kg, phenobarbitone 8 mg/kg and 16 mg/kg, native powder (a suspension in water for injection) 30 mg/kg and 50 mg/kg. All were injected i.p. A single electroconvulsive shock (ECS: 120V, 50Hz, 50msec, Havard Stimulator) was delivered via ear-ring electrodes to each animal 2.5h, 6h, 1d, 2d, 3d & 4d after treatment. Inhibition of tonic or tonic-clonic hind limb extension was taken as protective (anticonvulsant) effect.

Table 1 Percentage of rats protected against ECS (N = 8/group)

Time	Control	Diazepam 8mg/kg	Phenobarbitone 8mg/kg	Phenobarbitone 16mg/kg	Native powder 30mg/kg	Native powder 50mg/kg
2.5h	0.0§	75.0	50.0	0.0	25.0	0.0
6h	0.0	12.5	25.0	25.0	62.5	25.0
1day	0.0	12.5	71.4§	37.5	62.5	12.5
2days	0.0	12.5	42.9	25.0	57.1§	37.5
3days	0.0	0.0	28.6	25.0	57.1	50.0
4days	0.0	0.0§	28.6	0.0	14.3	12.5

§ N = 7 due to 1 death.

The above data were subjected to 2-way ANOVA by treatment and time, after arcsine transformation. Newman-Keuls testing then showed that phenobarbitone and the native powder, but not diazepam, caused significant anticonvulsant effect, compared with control ($p < 0.01$). The anticonvulsant effects of phenobarbitone and the native powder were not significantly different from each other ($p > 0.05$). The effect of the native powder appeared to build up more slowly than those of diazepam and phenobarbitone.

These results, and its claimed effectiveness in man, support the necessity for more extensive investigation of this native medicinal preparation.

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EFFECTS OF FORSKOLIN ON EPILEPTIFORM ACTIVITY IN RAT CORTICAL SLICES

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Cyclic 3,5' adenosine monophosphate (cyclic AMP) may play an important role in the pathophysiology of seizure disorders (Purpura & Shofer, 1972). In this study we have investigated the ability of forskolin, a potent activator of adenylate cyclase, to influence epileptiform activity in vitro using rat cerebral cortex slices. Removal of magnesium ions from the superfusing medium relieves a voltage-dependant block of the ion channel linked to the N-methyl-D-aspartate (NMDA) receptor. This produces spontaneous epileptiform discharges which are susceptible to antagonism by NMDA antagonists and clinically effective anticonvulsants (Aram & Lodge, 1987).

Cortical wedges (500μm) comprising cerebral cortex and corpus callosum were prepared and placed in a tissue bath such that a grease seal barrier separated grey from white matter (Harrison & Simmonds, 1984). The tissue was perfused with oxygenated Krebs solution. Magnesium was removed from the grey matter side and DC potentials across the barrier recorded using silver/silver chloride electrodes. Under these conditions, large negative-going potentials appeared spontaneously with up to 15 afterpotentials superimposed on the decay phase.

Superfusion of 0.01μM forskolin for 60 min had no effect on the frequency of spontaneous bursts or the number of afterpotentials in each burst. Higher doses of forskolin (0.1-31.6μM) reduced the number of afterpotentials per min and afterpotentials per burst by 20-30% after 15, 30 and 60 min of superfusion ($n=5-6$; $P<0.05$ paired t-test). Burst frequency was increased by 20.8±6.2% after 30 min in the presence of 0.1μM forskolin ($n=7$; $P<0.05$), but higher concentrations of drug had no effect on burst frequency. Complete recovery from all doses of forskolin was observed within 15-30 min of drug washout.

These results suggest that forskolin may have a dual action on epileptiform activity in vitro. The effective concentrations implicate adenylate cyclase activation in these mechanisms of action and we are currently trying to elucidate contributions from pre- and post-synaptic elements.

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A COMPARISON OF THE EFFECTS OF TWO THROTROPHIN-RELEASING HORMONE (TRH) ANALOGUES ON EPILEPTIFORM ACTIVITY IN VITRO

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TRH may play an important role in a variety of functions of the central nervous system (Metcalf, 1982). It has been suggested that stabilised analogues of TRH may be of use in the management of cerebral ischaemia and epilepsy (Latham et al., 1985; Inanaga & Inoue, 1981). However, two such analogues, RX77368 (pGlu-His(3,3'dimethyl)ProNH_2, Reckitt & Colman) and RGH2202 (pyro-2-aminodipyl-Leu-ProNH_2, G. Richter Ltd.) appear to have opposite effects in an in vivo model of cerebral ischaemia in the rat (O'Shaughnessy et al., 1987). We have therefore compared the effects of these particular TRH analogues as potential anticonvulsants using an in vitro cortical slice model.

500 μm cortical slices were mounted in tissue baths using greaseseal techniques to separate grey from white matter (Harrison & Simmonds, 1984). Oxygenated Krebs solution (magnesium free) was perfused at a rate of 2ml/min for two hours before the introduction of drugs into the side containing grey matter. Spontaneous activity was detected using silver/silver chloride electrodes. This comprised large negative-going potentials with a number of afterpotentials superimposed on the decay phase.

Superfusion of 10nM RX77368 and 10nM RGH2202 for 60 min had no effect upon the frequency of spontaneous bursts. After 15 min of superfusion, RGH2202 had reduced the number of afterpotentials per burst by 21% ($n=4$; $P<0.05$ paired t-test) whereas RX77368 showed no effect. After 30 min of superfusion, both afterpotentials per burst and afterpotentials per min were significantly reduced by both RGH2202 and RX77368. RGH2202 inhibited activity by 25%, RX77368 by 15-20% ($n=5$; $P<0.05$). The effects of RX77368 but not RGH2202 were maintained for 60 min. Complete recovery from both drugs was observed on washout although an increase in burst frequency was noted after recovery from RGH2202 ($n=4$; $P<0.01$).

These results indicate that the two TRH analogues RX77368 and RGH2202 have a similar anticonvulsant profile in this in vitro cortical slice preparation.

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SOLUBILISATION OF HIGH AFFINITY FORSKOLIN BINDING SITES FROM RAT CNS TISSUE

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The diterpene forskolin has been shown to label high affinity binding sites with a unique distribution within the CNS (Gehlert et al, 1985). These sites are present in particular abundance in the striatum. The striatal sites are modulated by guanine nucleotides such that the density of the sites is increased upon addition of GppNHp (Poat et al, 1988). The present report describes the solubilisation of [^{3}H]-forskolin binding sites from the striatum and cerebellum and the effect of GppNHp upon these sites.

Solubilisation was achieved by the incubation of membranes with 1% CHAPS (3-[3-chlolamidopropyl)-dimethylammonio]-1-propane sulfonate) at 0°C for 10 min at a protein/detergent ratio of 1/1 in 50mM Tris 10mM $MgCl_2$ buffer, pH 7.4, containing 10µg/ml bacitracin, soya bean trypsin inhibitor and 1mM DTT. The soluble protein was collected by centrifugation at 200,000g for 30 min and assayed for specific [^{3}H]-forskolin binding. The assay consisted of protein (50-100µg), 0.6-60nM [^{3}H]-forskolin and 1µM forskolin to define non-specific binding in a total volume of 300µl buffer. Following a 2h incubation, free and bound ligand were separated by filtration through GF/C filters presoaked overnight in 1% polyethyleneimine (Bruns et al, 1983). Membranes were pretreated for 10 min with GppNHp (50µM) and collected by centrifugation at 80,000g for 10 min prior to solubilisation.

In the absence of guanine nucleotides solubilisation of striatal [^{3}H]-forskolin sites was low (16 ± 1.4% activity, n = 4), although 50% of the protein was solubilised. Preincubation with GppNHp increased the amount of specific binding solubilised to 28.5 ± 2.0% (n = 4 $p < 001$), protein 52.7 ± 1.8%. Preincubation with the guanine nucleotide increased the binding in the residual membranes also. GppNHp however, had no effect upon cerebellar membranes.

Table 1

	Soluble (fmol/mg)		Pellet (fmol/mg)	
	-GppNHp	+GppNHp	-GppNHp	+GppNHp
Striatum	50 ± 15	322 ± 82*	249 ± 84	823 ± 176*
Cerebellum	21 ± 6	32 ± 10	129 ± 5	106 ± 33

[^{3}H]-forskolin 20nM * $p < 0.05$ n = 4

Preincubation with GppNHp yielded a solubilised striatal preparation with a Kd 21.6 ± 5.4nM and Bmax 430 ± 59 fmol/mg for [^{3}H]-forskolin. The results suggest that guanine nucleotide binding sites on the high affinity [^{3}H]-forskolin sites are retained following detergent solubilisation, from dopamine-rich regions of the brain.

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CHANGES IN [3H]D-ASPARTATE AND [3H]KAINATE BINDING IN SCHIZOPHRENIC POST MORTEM BRAINS

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Schizophrenia may involve disturbances in inter-hemispheric communication via association fibres (Reynolds, 1983). Glutamate is the transmitter of many association fibres in cerebral cortex. We have measured the binding of [^{3}H]D-aspartate, a marker for glutamate uptake sites in human brain (Cross et al., 1986) and [^{3}H)kainate, a glutamate receptor ligand, in normal and schizophrenic brains.

Dissected areas from 14 control and 14 schizophrenic brains were homogenized in Tris-acetate buffer (50 mM, pH 7.4, 4°C), centrifuged, resuspended in buffer and centrifuged again. The pellet was suspended in Tris buffer. Aliquots were incubated for 60 min with 100 nM [^{3}H]D-aspartate plus 300 mM NaCl, or 4nM [^{3}H]kainate. Bound ligand was separated by filtration.

There was a significant reduction in [^{3}H]D-aspartate binding in the left amygdala and a bilateral increase in orbital frontal cortex in schizophrenic samples (Table 1).

Table 1 [^{3}H]D-Aspartate binding in post-mortem brains fmol/mg prot.

	Controls		Schizophrenics	
Brain area	Left	Right	Left	Right
Medial temporal areas:				
Polar cortex (BA 38)	1185±102	1084±83	1063±194	1271±136
Amygdala	824±79	858±69	757±82*	892±68
Hippocampus	233±30	274±28	235±40	195±35
Lateral temporal areas:				
Superior gyrus (BA 21)	1457±190	1292±178	1639±183	1364±181
Middle gyrus (BA 22)	1380±133	1429±107	1152±70	1269±125
Anterior cortex (BA 10)	1149±14	876±118	1167±148	1163±193
Orbital cortex (BA 11)	1152±142	1119±72	1709±131**	1466±116**

*P <0.05 compared to right side; **P<0.05 compared to controls

In orbital frontal cortex, [^{3}H]kainate binding was bilaterally increased (P = 0.02) in schizophrenic samples (control: 52.6±3.0 - left, 49.9±3.1 - right; schizophrenic: 59.5±3.5 - left, 61.4±3.0 - right [fmol/mg protein]). There were no other changes in [^{3}H]kainate binding in the brain areas measured. These findings are similar to those of Nishikawa et al., (1983) who found increased [^{3}H]kainate binding in medial and dorsolateral prefrontal cortex.

Our findings point to regional abnormalities of glutamate system in schizophrenia. It is suggested that schizophrenia involves abnormalities of cortical glutamate neurones in orbital frontal cortex and left temporal lobe.

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IN VIVO [^{3}H]-MK-801 BINDING IN MOUSE BRAIN

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MK-801 is a potent, orally active anti-convulsant and neuroprotective agent (Clineschmidt et al, 1982; Gill et al, 1987). Both properties are thought to reside in the compound's ability to non-competitively antagonise NMDA responses (Wong et al, 1986). High affinity MK-801 binding sites have been demonstrated in the mammalian CNS, associated with the NMDA receptor complex (Wong et al, 1988). We have studied [^{3}H]-MK-801 binding *in vivo* in mouse brain using a filtration assay technique and autoradiography (ARG) in order to estimate the occupancy of this binding site under pharmacological doses of MK-801 and related compounds.

Male Swiss Webster mice (20-25g) were injected i.v. with a bolus injection of 10μCi [^{3}H]-MK-801 (22.5 Ci/mmol), 15 min prior to which animals received i.p. injections of displacing drug or saline. Non-specific binding was determined by a pre-injection of MK-801 (3mg/kg). Animals were sacrificed after 10 min and brains rapidly removed. For filtration assays, brains were homogenised in 50 vol ice-cold Tris buffer (5mM, pH 7.4). 500μl aliquots were then filtered through Whatman GF/B filters and washed briefly with 2 x 5ml buffer. Filters and homogenate aliquots were counted by scintillation spectrometry. For ARG, brains were frozen, sectioned on a cryostat and thaw mounted onto glass slides. The sections were then briefly rinsed (2 x 20 s) in Tris buffer, dried in a stream of cold air and apposed to Amersham Hyperfilm with appropriate standards for up to 6 weeks.

In the filtration assay specific binding constituted 72% ± 4% (n = 10) of total binding. [^{3}H]-MK-801 binding was displaced stereoselectively by the isomeric forms of MK-801 (ED_{50}: MK-801 = 0.17 ± 0.04mg/kg, n = 5; (-) MK-801 = 1.04 ± 0.24mg/kg, n = 3) and was also displaced by other non-competitive NMDA receptor antagonists such as thienylcyclohexylpiperidine (TCP, ED_{50} = 1.82 ± 0.88mg/kg, n = 3) and etoxadrol (ED_{50} = 5.08 ± 0.85mg/kg, n = 3). In ARG sections, [^{3}H]-MK-801 binding (displaceable binding = 66% ± 4%, n = 4), exhibited clear regional selectivity with the highest density in the hippocampus, cortex and thalamus and with virtually no specific binding in the cerebellum.

These studies indicate that the [^{3}H]-MK-801 recognition site can be labelled using in vivo binding techniques. The rank order of potency, in the filtration assay of MK-801 > (-) MK-801 = TCP > etoxadrol corresponds to the expected order of potency of these drugs as anticonvulsants (Clineschmidt et al, 1982; Hayes and Balster, 1985). The coincidence of the distribution of [^{3}H]-MK-801 binding by *in vivo* and *in vitro* (Bowery et al, 1988) methods further endorses the ability to label a pharmacologically relevant site by this *in vivo* approach.

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COMPARATIVE AUTORADIOGRAPHY OF [^{3}H]-(+)-MK-801 AND [^{3}H]-GLYCINE IN THE MAMMALIAN CNS

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MK-801 is a potent antagonist of N-methyl-D-aspartate (NMDA) receptors acting at the associated ion channel (Kemp, et al 1986). Glycine potentiates responses to NMDA in a strychnine-insensitive manner (Johnson and Ascher, 1987). This finding is of interest in view of our previous studies showing that the distribution of strychnine-insensitive glycine binding sites in rat brain (Bristow et al, 1986) is similar to that of sites labelled with [^{3}H]-MK-801 (Bowery et al 1988). In the present study in vitro autoradiography has been used to study further the distribution of binding sites for [^{3}H]-(+)-MK-801 and [^{3}H]-glycine. Cryostat cut sections of rat (12μm) and monkey brain (20μm) were used for autoradiography as described previously (Bristow, et al 1986; Bowery, et al 1988). Sections were incubated in either 50mM Tris-HCl (pH7.4, 20°C, 20min) containing 10nM [^{3}H]-(+)-MK-801 (NEN) using 10μM (+)-MK-801 or Thienylcyclohexylpiperidine (TCP) to define non-specific binding, or 50 mM Tris-Citrate (pH 7.2, 4°C, 20 min) containing 100nM [^{3}H]-glycine, (Amersham International) strychnine 10μM, and either glycine (1mM) or D-Serine (1mM) to define non-specific binding. After rinsing in fresh buffer sections were rapidly dried in a stream of cool air before contact with [^{3}H]-sensitive film for 4 to 6 weeks.

Fig 1 : Binding sites for [^{3}H]-glycine (left) and [^{3}H]-(+) MK-801 (right) in transverse sections of rat brain. (Scale bar = 4mm)

The distribution of binding sites for [^{3}H]-(+)-MK-801 and [^{3}H]-glycine are similar in both rat and monkey CNS with high densities in hippocampal CA1 neurones. The overall distribution for both ligands is comparable to that described for NMDA receptors labelled with ^{3}H-L-glutamate (Monaghan, Yao and Cotman, 1985) and supports the notion that strychnine-insensitive glycine sites may be located on the NMDA receptor complex.

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FPL 63547 - A POTENT AND LONG-ACTING INHIBITOR OF ANGIOTENSIN-CONVERTING ENZYME

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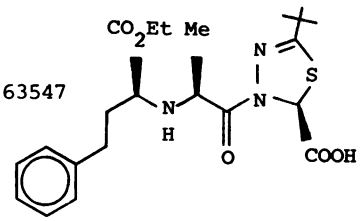
FPL 63547



FPL 63547 is the ester prodrug of a novel thiadiazoline inhibitor of angiotensin-converting enzyme (ACE). The active form, FPL 63547 diacid, has been compared with some standard ACE inhibitors for potency to inhibit rabbit lung ACE in vitro. FPL 63547 diacid demonstrated potent activity in this system, IC_{50} 0.51 nM (mean, n=5). Other values - lisinopril 0.63 nM, enalapril diacid 0.95 nM and captopril 2.27 nM (all n=6). In conscious normotensive dogs FPL 63547 (0.01 - 0.3 mg kg^{-1} i.v., n=4) inhibited pressor responses to angiotensin I in a dose-related fashion.

The antihypertensive properties of FPL 63547 were investigated in conscious spontaneously hypertensive rats (SHR) pretreated with hydrochlorothiazide (HCTZ, $2x10^{-5}$ mol kg^{-1} p.o.) to stimulate indirectly the renin-angiotensin system. Systolic blood pressure (SBP) was measured using the tail-cuff method at various times after dosing. Rats were dosed either in the morning (1-5 h readings) or in the evening (15-24 h readings) to produce a composite picture of the overall time course of the SBP response. A control group was used in each experiment. Comparative antihypertensive activity is shown in Table 1.

Table 1 HCTZ-SHR Mean percentage fall in SBP at various times after dosing

mol kg^{-1} p.o.		1	3	5	15	18	21	24	h
FPL 63547	10^{-5}	13%	25%	32%	19%	19%	20%	26%	
Enalapril	10^{-5}	NS	17%	26%	NS	NS	NS	NS	
Lisinopril	10^{-5}	NS	28%	24%	21%	19%	17%	18%	
Captopril	10^{-4}	NS	19%	21%	NS	NS	NS	NS	

Values shown (n=5-6) were significant ($p < 0.05$, unpaired t-test) against both pre-dose reading and time-matched control group reading. NS - not significant.

As an antihypertensive in the SHR, FPL 63547 was both potent and long-acting and, on this single dose comparison, more effective than captopril or enalapril. In multi-dose studies FPL 63547 ($3x10^{-7}$ - 10^{-5} mol kg^{-1} p.o.) produced significant falls in SBP at some or all of the readings between 15-24 h after treatment. By comparison, minimum active doses (15-24 h) for lisinopril, enalapril and captopril were 10^{-6}, $3x10^{-5}$ and $>10^{-4}$ mol kg^{-1} p.o. respectively. Chronic treatment of HCTZ-SHR with FPL 63547 (0.5 mg kg^{-1} day^{-1} p.o.) resulted in a marked lowering of SBP 5 h post-dose on each day of the 23 day treatment period. From day 11 onwards SBP was also lowered at the pre-dose reading (23 h).

Thus, FPL 63547 is a potent ACE inhibitor possessing antihypertensive properties in the SHR which compare favourably with lisinopril, enalapril and captopril. Its duration of action appears sufficient for once daily oral dosing. In an accompanying communication (Carr et al, 1988) we report that FPL 63547 is unusual amongst ACE inhibitors in being preferentially eliminated by the biliary route.

Carr, R.D. et al (1988) This meeting

ORG10325, A NEW CARDIOTONIC AGENT WITH POSITIVE INOTROPIC AND VASODILATOR PROPERTIES

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Org 10325 (N-hydroxy-5,6-dimethoxy-(IH)-indene-2-carboximidamide-HCl) has been developed as an agent with potential use in the treatment of congestive heart failure. In preliminary experiments the pharmacological actions were characterised in isolated cardiac and vascular tissue preparations. Aortic strips and right ventricular papillary muscles from male NZW rabbits were suspended in Krebs solution containing (mmoles/l): NaCl 118, KCl 4.7, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11.7 and allowed to stabilise for 60 min before start of experiment. The effects of Org10325 on electrically evoked (0.4 Hz) papillary muscle contractions or on aortic strips pre-contracted with phenylephrine (10^{-5}M) were examined by cumulative additions of the compound to the organ bath. Mechanical responses of the tissues were recorded by conventional methods. The effects of Org10325 on tissue cyclic nucleotide levels were examined according to Rodger and Shahid (1984).

Org10325 produced concentration-dependent positive inotropic (EC_{50}: 360μM±90μM) and relaxant (EC_{50}: 220μM±22μM) responses in isolated papillary muscles and aortic strips respectively. The maximal positive inotropic response was not significantly different from isoprenaline. At a subthreshold inotropic concentration (74μM) Org10325 potentiated the effects of isoprenaline producing a 14 fold parallel shift to the left in the isoprenaline dose-response curve, but did not significantly influence response to calcium. The cardiac effects of Org10325 were not significantly affected by propranolol (10^{-7} M) or by depletion of catecholamine stores by reserpinization (5 mg/kg). The data for the effects of Org10325 on tension responses and cyclic nucleotide levels in rabbit papillary muscles are shown in Table 1. It is clear that the positive inotropic effects of Org10325 were associated with significant increases in cAMP levels. Carbachol (22μM) partially reversed the positive inotropic effect of Org10325 (1.9 mM) producing a 38% decrease in the size of the response.

Table 1 Effects of Org10325 on tension responses and cyclic nucleotide levels in rabbit papillary muscles (mean S.E. mean; n=4-6) **P<0.01 (Student t-test compared to control

Treatment	Conc. (M)	Tension (mg)	cAMP (pmol/mg tissues)	cGMP
Control	vehicle	537±64	0.60±0.06	0.021±0.002
Org10325	9.3×10^{-5}	585±94	1.02±0.05**	0.02 ±0.003
	3.7 10^{-4}	811±119	1.26±0.05**	0.033±0.006
	1.9×10^{-3}	1638±165	1.46±0.06**	0.025±0.003
Isoprenaline	2×10^{-6}	1894±176	1.45±0.02**	

The vascular relaxant effects of Org10325 were also associated with a 2 fold increase in cAMP levels in aortic strips.

The results indicate that Org10325 possesses both positive inotropic and vasodilator properties and that these effects may be mediated by an increase in intracellular cAMP level. Further experiments have shown that Org10325 produces inhibition of cAMP hydrolysis by purified cardiac phosphodiesterases.

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ORG10325 A NEW CARDIOTONIC AGENT: CARDIOVASCULAR EFFECTS IN ANAESTHETISED ANIMALS

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Org10325 (N-Hydroxy-5,6-dimethoxy-(1H)-indene-2-carboximidamide HCl) is a new cardiotonic agent with interesting positive inotropic and vasodilator activities in isolated tissues [Shahid et al, (1988) this meeting]. These pharmacological effects of Org10325 were further characterized by examining its cardiovascular actions in anaesthetized animals.

Beagle dogs (10-15 kg) were anaesthetized with pentobarbitone (30 mg/kg) and instrumented for iv drug administration and measurement of blood pressure (BP), left ventricular pressure (LVP) and its derivatives (LV dP/dt and dP/dt/P). Samples of arterial and coronary venous blood were taken and analysed for oxygen content for the determination of myocardial oxygen consumption (MVO_2). Stroke volume (SV) and coronary blood flow (CBF) was also monitored. Following a stabilization period of 30 min Org10325 (0.5 mg/kg/min) was administered by slow iv infusion over a 20 min period. Measurement of haemodynamic parameters, CBF and MVO_2 were taken immediately prior to and at 5 min intervals during drug infusion. The cardiovascular actions of Org10325 were also examined in pentobarbitone-anaesthetized cats in which myocardial contractility had been depressed by administration of propranolol (2 mg/kg), 15 min prior to a large bolus injection of pentobarbitone (10-20 mg/kg). Stable depression of cardiac function was achieved by constant infusion of pentobarbitone (15 mg/kg/h). A stabilization period of 60-90 min was allowed before administration of drug.

The results from the anaesthetized dog experiments are shown in Fig. 1. It is clear that Org10325 produced marked increases in cardiac contractility which continued to rise throughout the infusion period and which were accompanied by a small fall in BP. There was an initial slight increase in HR which returned to control values and by one hour post infusion was 20-30 b/min lower than pre-drug level. Org10325 also produced a transient increase in coronary flow and a 20% rise in MVO_2. In the 'depressed' cat Org10325 (1-10 mg/kg) produced dose-related increases in contractility and decreases in BP (eg at 10 mg/kg the peak % changes were: SV 27±12; dP/dt/P, 98±16; BP -40±6). Heart rate was marginally increased immediately after drug administration but declined to 20 b/min below control levels 30 mins after administration.

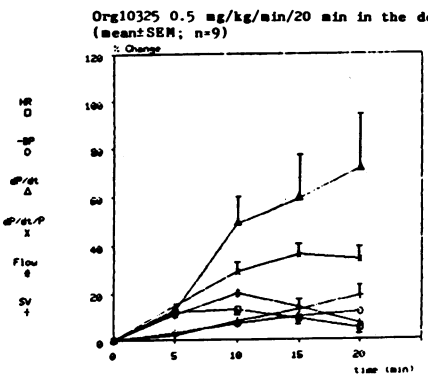

Fig. 1

In conclusion, the above results indicate that Org10325 produced marked increases in contractile force accompanied by small decreases in blood pressure. This represents an unusual and useful profile for the treatment of congestive heart failure.

SATERINONE (BDF 8634): A POSITIVE INOTROPIC VASODILATOR WITH POTENT PDE TYPE III AND α_1-ADRENOCEPTOR INHIBITORY ACTIVITIES

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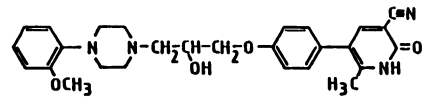
The search for orally active non-glycoside, non ß-adrenergic cardiotonic agents was remarkably stimulated by the discovery of amrinone (Farah & Alousi, 1978). In contrast to digoxin, amrinone and related drugs possess a vasodilatatory activity in addition to their inotropic effects. Agents with vasodilating activitiy have become increasingly important in the treatment of chronic heart failure (chf) even though they may lack direct inotropic activity. Prazosin, and more recently the angiotensin converting enzyme inhibitors, have been used to treat patients with chf, albeit with varying degrees of success (Packer, 1983). We report here



Saterinone (BDF 8634)

the pharmacological actions of saterinone, a cardiotonic agent that combines the pharmacological properties of cAMP phosphodiesterase (PDE) inhibition with selective alpha-1-receptor blockade in a single molecule. Saterinone (inset) was characterized in isolated organs and in intact guinea pigs. In the isolated, electrically driven right papillary muscle (1 Hz), force of contraction was increased by saterinone in a concentration dependent fashion, the EC50 value of 0.3µmol/l was 100-fold more potent than milrinone (30µmol/l). The chronotropic action of saterinone in the isolated right atria was moderate (about 40% in reference to isoprenaline) and evident at same EC50 as the inotropic effect. In guinea pig right ventricular homogenates, saterinone inhibited crude cAMP and cGMP phosphodiesterase activities in a concentration dependent manner: IC50 values being 14 and 23µmol/l, respectively. In fractionated preparations, using the method described by Weishaar et al (1986) to separate the PDE isoenzymes, saterinone exerted a greater activity in inhibiting the cAMP dependent isoenzyme (type III). The IC50 for inhibiting specific PDE-III activity was 0.06µmol/l, compared to 2.6 and 1.3µmol/l for inhibiting PDE-I and II, respectively.
Specific PDE inhibition may also have contributed to saterinone's inhibition of human platelet aggregation induced by ADP, collagen or arachidonate. Saterinone (IC50 = 0.17µmol/l) was about 40-fold more potent than acetylsalicylic acid in preventing arachidonate induced platelet aggregation.
Saterinone was a competitive antagonist to phenylephrine in the isolated guinea pig thoracic aorta, giving rise to a pA_2-value of 8.46 (prazosin: 8.87). Alpha-1-receptor activity was further confirmed by receptor ligand binding studies using (^{3}H)-prazosin (33 Ci/mol) and rat cortex membranes. The Ki-values for saterinone and prazosin being 17.8 and 1.9 nmol/l, respectively. In pithed guinea pigs, saterinone (i.v.) exerted a dose-dependent elevation of left ventricular dp/dt max; the EC50-value was 0.3 mg/kg. In the same preparation 0.3 mg/kg saterinone shifted the ED50 of phenylephrine to the right by a factor of 11. Thus inotropic and alpha-receptor blocking activities are manifest in a comparable dose range in vivo. Similar effects were found in further in vivo studies in conscious rabbits and cats. Saterinone thus exhibits a pharmacological profile hitherto uncommon among inotropic agents. The therapeutic value of the agent would be dependent on the maintenance of these dual receptor actions in the human situation.

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THE β_2-ADRENOCEPTOR-MEDIATED POSITIVE INOTROPIC EFFECT OF DOPEXAMINE IN THE FAILING HUMAN HEART

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(introduced by J.B.Farmer)

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Dopexamine-HCL (Dopex) is a novel cardiotonic agent with agonist properties at β_2-adrenoceptors (AR) and peripheral dopamine receptors but only minor activity at β_1- and α-AR. In the failing human heart, there is a reduction of β_1-AR whereas the number of β_2-AR remains greatly unaffected (Bristow et al., 1986). As a consequence there may be a rationale in using a β_2-AR agonist to provide inotropic support in the treatment of heart failure. In fact, beneficial effects have been observed recently in patients with haemodynamic heart failure (Tan et al., 1987). However, in studies on patients or whole animals, drug effects on the heart can hardly be distinguished from effects on peripheral circulation such as pre- or afterload reduction. - In order to study whether Dopex produces a direct positive inotropic effect (PIE) on the human heart, the PIE of the compound was investigated on isolated, electrically driven (1Hz) left ventricular papillary muscle strips from patients with moderate (NYHA II-III) or severe (NYHA IV) heart failure and on right atrial trabeculae (HAT) from patients with coronary heart disease. The PIE of Ca^{2+} was studied to measure maximal response in each individual preparation. - In HAT, the PIE of Dopex (30.2±7.5 % of the PIE of Ca^{2+}) was more pronounced than in ventricular heart muscle . Dopex produced a weak PIE in NYHA II-III amounting to 8±3.2% of the PIE of Ca^{2+}. In NYHA IV, Dopex failed to increase force of contraction. In the presence of the phophodiesterase inhibitor milrinone (100µM), Dopex increased the force of contraction in all preparations to 47.0±9.8% of the PIE of Ca^{2+} in NYHA II-III (n=9) and to 25.5±6.5% of the PIE of Ca^{2+} in NYHA IV (n=8). The PIE in both HAT and ventricular myocardium was mediated by β_2-AR since the concentration-response-curves produced by Dopex were shifted to the right by the β_2-AR antagonist ICI 118.551 (0.05µM, n=8) but unaffected by the β_1-AR antagonist CGP 207.12A (0. 3µM, n=7). It is concluded that Dopex is capable of producing positive inotropic responses in the failing human heart in the presence of phosphodiesterase inhibitors. With Dopex alone, only minor effects can be observed. Hence, the beneficial effects of Dopex in patients with heart failure might greatly be due to extracardiac effects such as afterload or preload reduction.

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DO CARDIAC α-ADRENOCEPTORS PLAY A ROLE IN THE INOTROPIC ACTIONS OF (-)-DOBUTAMINE?

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Dobutamine exists as two isomers; both are potent ß-adrenoceptor stimulants, but they have opposing actions at α_1-adrenoceptors - the (-)-isomer is a partial agonist whereas the (+)-isomer is an antagonist (Ruffolo et al., 1981). The positive inotropic effect of dobutamine is mediated largely via stimulation of $ß_1$-adrenoceptors, although it has recently been suggested that cardiac α-adrenoceptor stimulation might also be involved in this response in the rat (Ruffolo and Messick, 1985) and cat (Kenakin and Johnson, 1985). As the (-)-isomer of dobutamine is the most likely candidate for such an effect we have investigated its actions in more detail.

Strips of rat left ventricle were paced at 1 Hz (5 msec pulse width, 50% above threshold voltage) in modified Krebs solution at 32°C containing cocaine ($3x10^{-5}$M), corticosterone ($4x10^{-5}$M) and atropine ($1x10^{-6}$M). Cumulative administration of phenylephrine ($1x10^{-6}$-$1x10^{-4}$M) in the presence of sotalol ($1x10^{-4}$M) produced concentration-related positive inotropic responses; this effect was competitively antagonised by prazosin (pA_2=8.98±0.08; slope=1.04±0.02; n=4), which confirms that α_1-adrenoceptors are present on rat ventricle. (-)-Dobutamine ($1x10^{-7}$-$1x10^{-4}$M) also increased the force of contraction of rat left ventricular strips. Prazosin ($1x10^{-7}$M) reduced responses to low (<$3x10^{-6}$M) but not to high ($1x10^{-5}$-$1x10^{-4}$M) concentrations of (-)-dobutamine. Sotalol ($1x10^{-4}$M) reduced responses to (-)-dobutamine ($1x10^{-6}$-$1x10^{-4}$M) by 50-75%. In the presence of sotalol ($1x10^{-4}$M), prazosin ($1x10^{-7}$M) displaced the residual concentration-effect curve to (-)-dobutamine 100 fold to the right. In further experiments the affinity of (-)-dobutamine for cardiac α_1-adrenoceptors was confirmed. In the presence of sotalol ($1x10^{-4}$M) the positive inotropic effect of phenylephrine was antagonised by (-)-dobutamine (pA_2=6.05±0.05; slope=1.08±0.04; n=5). Thus, in rat ventricular muscle, a small component of the positive inotropic response to (-)-dobutamine, particularly at low concentrations, seems to be mediated via α_1-adrenoceptors; however, this effect amounted to only 12% of that produced by phenylephrine.

Infusion of (-)-dobutamine ($1x10^{-9}$-$3x10^{-8}$ moles/kg/min i.v.) into chloralose -anaesthetised cats produced phentolamine-sensitive increases in diastolic blood pressure (DBP) and left ventricular (LV) dP/dt max with little increase in heart rate. Infusion of angiotensin II ($1x10^{-12}$-$3x10^{-11}$ moles/kg/min i.v.) or graded inflation of a balloon catheter located in the descending aorta produced equivalent pressor responses to (-)-dobutamine, smaller increases in LV dP/dt max and no effect on heart rate. Pretreatment with propranolol ($1x10^{-6}$ moles/kg i.v.) did not affect the responses to angiotensin II or mechanical occlusion of the aorta. The pressor response to (-)-dobutamine was also unaffected by propranolol, whereas the chronotropic response was abolished, and the inotropic response was reduced to the same level as that produced by equipressor doses of angiotensin II or aortic occlusion. This implies that the residual inotropic response to (-)-dobutamine is attributable to an increase in afterload which is known to increase LV dP/dt max (Furnival et al., 1970). These experiments suggest that if α_1-adrenoceptor stimulation plays any role in the actions of (-)-dobutamine in anaesthetised cats, it is likely to occur at the level of the vasculature, rather than the heart.

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POTENTIAL ROLE OF 5-HT$_1$-LIKE RECEPTORS IN MYOCARDIAL INFARCTION AND BLOOD FLOW IN A BEAGLE DOG MODEL

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There is evidence that 5-HT$_1$-like receptors play a role in modulation of coronary vascular tone (Cohen, 1986). This present study was undertaken to investigate the role of 5-HT$_1$-like receptors in myocardial ischaemia using the specific 5-HT$_1$-like agonist 3-[2-(dimethylamino)ethyl]-N-methyl-1H-indole-5-methanesulphonamide (synthesized by Wellcome but first described as GR 43175 by Humphrey et al., 1987) in a beagle dog model of coronary artery occlusion/reperfusion.

Anaesthetised beagle dogs (n = 20, weight 11.7 ± 0.3 kg) instrumented for blood pressure, blood gas monitoring and lead II electrocardiogram were used. A left thoracotomy was performed and the left anterior descending coronary artery isolated. An occlusive snare was placed around the vessel distal to the first major diagonal branch and occluded for 90 min with subsequent reperfusion (120 min). The area of myocardium at risk and infarcted was delineated and measured as previously described (Hughes et al., 1987). Regional myocardial blood flow (RMBF) was assessed with radio-labelled microspheres in control animals (n = 11) prior to occlusion, at the end of occlusion and after 2hrs reperfusion. In those animals treated with GR 43175 (1 mgkg^{-1} slow i.v. bolus, n = 9) RMBF was assessed before GR 43175, 15 mins post GR 43175, after 90 minutes occlusion and 2hrs reperfusion.

GR 43175 elicited a significant decrease in heart rate (151 ± 5 to 127 ± 6 beats min^{-1}, $p<0.001$) with transient changes in blood pressure. There was a significant reduction in endocardial RMBF (1.30 ± 0.12 to 1.19 ± 0.13 mlg^{-1} min^{-1}, $p<0.05$) but epicardial RMBF remained unaltered (1.24 ± 0.08 to 1.18 ± 0.09 mlg^{-1} min^{-1}). There was an increase in infarct/risk ratio in these animals (47.3 ± 5.6%) compared to the control group (34.0 ± 7.2%) although risk zones in both groups were similar (control: 31.4 ± 7.1% GR43175: 34.5 ± 2.3%). In the GR 43175 group, collateral flow to the ischaemic bed, a major determinant of tissue injury was 11.9 ± 2.7% of flow to the non-ischaemic region, whereas in the control group collateral flow to the ischaemic region was 22.7 ± 5.3% of normal. These effects were not associated with an increased arrhythmogenesis during either the occlusion or reperfusion period.

Thus, treatment with GR 43175 reduced RMBF and enhanced myocardial infarct size in this beagle dog model suggesting that activation of 5-HT$_1$-like receptors may play a role the pathophysiology of acute myocardial infarction.

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SYSTEMIC AND REGIONAL HAEMODYNAMIC EFFECTS OF THE PUTATIVE 5-HT_{1A} AGONISTS 8-OH-DPAT AND FLESINOXAN

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8-OH-DPAT (Ramage and Fozard, 1987) and flesinoxan (Wouters et al., 1988) lower mean arterial blood pressure (MAP) and heart rate (HR) by an action within the central nervous system on putative 5-HT_{1A} receptors. In the present communication we report on the effects of these drugs on cardiac output (CO), total peripheral conductance (TPC) and regional blood flows (RBF) in cats anaesthetized with pentobarbital sodium (35 mg.kg^{-1}, ip). CO was measured with a pre-calibrated electromagnetic flow probe placed on the ascending aorta, while RBF were measured using radioactive microspheres (Saxena et al., 1980).

TABLE 1. Systemic and regional haemodynamic effects of 8-OH-DPAT and flesinoxan.

	8-OH-DPAT (μg.kg^{-1}, iv), n=6				Flesinoxan (μg.kg^{-1}, iv), n=6			
	BSLN	10	30	100	BSLN	10	30	100
		% Change from BSLN				% Change from BSLN		
MAP (mmHg)	124±4	-31±6*θ	-38±6*θ	-44±5*θ	117±7	-8±4θ	-22±3*θ	-37±4*θ
HR (Beats min^{-1})	186±13	-5±10	-18±9*θ	-27±10*θ	177±19	-4±3	-10±5*θ	-21±7*θ
CO (ml min^{-1})	577±60	-16±4*	-24±6*θ	-34±3*θ	440±32	4±4	-6±3	-10±4*
TPC (Units)	469±50	24±6*θ	24±4*θ	21±8*θ	380±28	15±6*θ	21±6*θ	44±9*θ
RBF (ml min^{-1}.100g^{-1})								
Heart	154±23	-34±10*	-52±7*θ	-51±7*θ	180±38	-23±8*	-42±7*	-49±7*θ
Kidneys	254±29	22±16	13±21	14±27	366±80	4±13	-3±13	-7±10
Brain	36±6	-8±10	-13±10	0±11	39±7	-4±6	-12±9	-8±8
GI Tract	45±8	-36±7*	-39±9*	-27±11*	61±4	-25±8*	-44±5*	-46±8*
Skeletal Muscles	4±1	23±16	10±13	12±19	8±3	15±34	0±30	-15±19
Liver	48±7	53±14	36±55	5±29	67±10	12±19	2±17	-12±9*
Skin	9±3	-39±10*	-52±10*	-46±13*	12±4	-14±18	-36±11*	-48±10*
Eyes	57±11	-29±9*	-47±10*	-53±8*θ	70±13	-21±11*	-41±11*	-56±9*θ

Means±s.e. means; *, $p < 0.05$ vs. baseline (BSLN); θ, $p < 0.05$ vs. corresponding changes from BSLN in saline-treated cats (data not shown); TPC units = 100 x ml min^{-1} mmHg^{-1}; GI Tract, gastrointestinal tract.

As shown in Table 1 both 8-OH DPAT and flesinoxan decreased MAP and HR dose-dependently to similar extents but the reductions in CO and the maximum increase in TPC were somewhat more with, respectively, 8-OH-DPAT and flesinoxan. Regionally, both drugs decreased blood flows to the heart, GI tract, skin and eyes but no appreciable changes were observed in the kidneys, brain, skeletal muscles and liver indicating increases in the vascular conductances in these tissues.

It is concluded that the two putative 5-HT_{1A} agonists, 8-OH-DPAT and flesinoxan have similar systemic and regional haemodynamic profile in the anaesthetized cats though the magnitude of some changes differ. Of particular interest is the preservation of renal and cerebral blood flows; the decrease in myocardial flow may be secondary to reduced O_2 consumption secondary to hypotension and bradycardia.

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CARDIOVASCULAR STUDIES WITH THE 5-HT_{1A} AGONIST FLESINOXAN IN RATS

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Strong evidence has been obtained which indicates that flesinoxan lowers blood pressure and heart rate in anaesthetized cats via agonism at central 5-HT_{1A} receptors (Wouters et al., 1988). In the present study we have investigated the cardiovascular profile of flesinoxan in conscious freely moving spontaneously hypertensive (SH) rats after both acute and chronic administration. In addition, antagonism experiments were done using 8-Methoxy -2-(N-2-chloroethyl-N, n-propyl) aminotetralin (8-MeO-ClEPAT) as a putative 5-HT_{1A} antagonist.

SH rats were prepared for recording of mean arterial pressure (MAP) and heart rate (HR) with an indwelling carotid or femoral cannula at least two days before use. Cardiac output was measured in SH rats which had a chronically implanted magnetic flowprobe around the ascending aorta. Flesinoxan was administered to SH rats either acutely (p.o.; s.c.; i.v.) or chronically via subcutaneously implanted osmotic minipumps. In antagonism experiments Wistar rats were pretreated with 8-MeO-ClEPAT or saline and subsequently anaesthetized with pentobarbital (70 mg/kg i.p.). Flesinoxan (100 µg/kg i.v.) was given 60 min after the pretreatment.

In the acute experiments, flesinoxan induced a rapid decrease in MAP after administration via the different routes (ED_{20}: 2 mg/kg p.o.; 1 mg/kg s.c.; 1 mg/kg i.v.), and maximal decreases of up to 35 percent were seen at the highest doses tested. This fall in MAP persisted for more than 4 hrs after a medium oral dose. Increases in blood pressure were never observed. In all cases, HR decreased only moderately up to about 15 percent. In SH rats instrumented for cardiac output measurements, flesinoxan (1 and 3 mg/kg s.c.) did not affect cardiac output whereas MAP decreased by 13 and 28 percent respectively. This indicates that flesinoxan lowers MAP via a reduction in total peripheral resistance. When flesinoxan (20 mg/kg/day) was given continuously via subcutaneously implanted minipumps (Alzet) over 10 days, the decrease in MAP (30 mmHg) was well maintained. HR decreased by only 30 bpm. After removal of the minipumps both MAP and HR returned to baseline values, without evidence of rebound effects. In anaesthetised Wistars, pretreated with saline, flesinoxan (100 µg/kg i.v.) decreased MAP and HR by 29 and 26 percent respectively. After pretreatment with 0.3 and 1.0 mg/kg s.c. 8-MeO-ClEPAT, flesinoxan (100 µg/kg i.v.) reduced MAP by 26 and 7 percent resp. and HR by 17 and 3 percent resp. In all cases, baseline values taken just before administration of flesinoxan, were not different.

It is concluded that the putative 5-HT_{1A} agonist flesinoxan lowers MAP potently by a decrease in total peripheral resistance. HR is only moderately decreased. Based on the sensitivity of these effects to the antagonism by 8-MeO-ClEPAT, the involvement of 5-HT_{1A} receptors in the cardiovascular effects of flesinoxan is suggested.

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BENOXAPROFEN: EFFECT ON REPERFUSION-INDUCED TISSUE NECROSIS AND MYELOPEROXIDASE ACTIVITY IN ANAESTHETISED RATS

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Inhibition of activated polymorphonuclear leukocytes (PMN) accumulation has been suggested to reduce reperfusion-induced tissue injury (Jolly et al, 1983). In the present study, we have developed a small animal preparation with temporary coronary artery occlusion (CAO) and reperfusion and have studied; (a) the time course of PMN accumulation as measured by myeloperoxidase (MPO) activity, a PMN specific enzyme and (b) the effects of benoxaprofen on PMN accumulation and ultimate infarct size.

For the initial study hearts from anaesthetised rats underwent either 30 or 60 min CAO followed by 30, 60 or 120 min reperfusion. At the end of the reperfusion period, the coronary artery was re-occluded and methylene blue dye was injected intravenously to delineate ischaemic tissue. Hearts were frozen and later tissue was dissected from both regions and assayed for MPO activity spectrophotometrically as described previously (Bradley et al, 1982). A large time-dependent increase in MPO activity within the previously ischaemic zone was shown to occur, with the largest increases occurring after the longest durations of both CAO and reperfusion. For studies with benoxaprofen, a time course of 60 min CAO followed by 60 min reperfusion was used. Benoxaprofen was given orally for 3 days, with the final dosage given 1 h prior to CAO. Infarct size was assessed using a single blind procedure where hearts were sliced, stained with Nitro blue tetrazolium and the necrotic area dissected, weighed and expressed as percentage of ventricular tissue.

Table 1. Effect of benoxaprofen on reperfusion-induced MPO activity and infarct size in the anaesthetised rat

	MPO activity (Umg^{-1} tissue) x 10^{-4}		Infarct size (% ventricular tissue)
	Normal tissue	Reperfused tissue	
Vehicle	0.26 ± 0.2	2.7 ± 0.4	37.4 ± 3.8
3 $mgkg^{-1}day^{-1}$	0.40 ± 0.2	1.9 ± 0.2	30.9 ± 3.8
10 $mgkg^{-1}day^{-1}$	0.02 ± 0.007	0.6±0.2***	19.7 ± 4.9**
30 $mgkg^{-1}day^{-1}$	0.40 ± 0.2	0.90±0.2***	15.8 ± 3.2***

** $p<0.01$; *** $p<0.001$ compared to vehicle group ($n>8$ in each group)

Table 1 shows a significant reduction in infarct size by benoxaprofen pre-treatment at both 10 and 30 $mgkg^{-1}day^{-1}$. This reduction in infarct size was associated with a simultaneous reduction in the accumulation of MPO activity within the infarcted zone. This suggests that benoxaprofen may be limiting reperfusion injury by preventing the accumulation of activated PMN within the reperfused tissue.

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DEFIBROTIDE REDUCES INFARCT SIZE IN A RABBIT MODEL OF ACUTE MYOCARDIAL ISCHAEMIA AND REPERFUSION

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Defibrotide (DEF; Crinos, Italy), a single stranded polydeoxyribonucleotide obtained from bovine lungs, has significant anti-thrombotic, profibrinolytic, prostacyclin-stimulating and anti-ischaemic properties (Thiemermann et al., 1985). We have investigated the actions of DEF on infarct size, regional myocardial blood flow (RMBF) and late diastolic coronary vascular resistance (LDCR) in a rabbit model of acute myocardial ischaemia and reperfusion.

Adult rabbits (2.4-3.2 kg) were anaesthetized with pentobarbitone sodium (30mg/kg i.v.) and subjected to 1 h occlusion of the first antero-lateral branch of the circumflex coronary artery (LAL), followed by 3 h of reperfusion. Left ventricular pressure (LVP), mean arterial pressure (MAP), contractile force (dp/dt), heart rate (HR) and lead II limb ECG were continuously recorded. Rabbits were treated with DEF (32mg/kg bolus + 32mg/kg/h i.v.) or saline solution (VEH) starting 5 min after LAL-occlusion. At the end of the experiment, the LAL was reoccluded and 150,000 ^{113}Sn-labelled radioactive microspheres followed by 4ml of 1% Evans blue dye solution were injected into the left ventricle to determine RMBF, LDCR and area at risk respectively. To distinguish between non-ischaemic (NMI), ischaemic (MI) and infarcted (IN) myocardium, the area at risk was sliced and incubated for 20 min in nitro-blue tetrazolium (NBT; 0.5mg/ml).

Infusion of DEF did not result in any significant changes in LVP, MAP, HR and dp/dt. Thus, the pressure-rate index remained unchanged by DEF. In the VEH group, LAL-occlusion caused an ST-segment elevation of 0.28 ± 0.02mV at 1 h, which was largely attenuated by DEF-treatment (0.12 ± 0.02 mV at 1 h; $p<0.01$). In comparison to VEH, DEF completely prevented the development of a pathological Q-wave during reperfusion (VEH: 0.25 ± 0.05 mV; DEF: 0.05 ± 0.02 mV at 4 h; $p<0.01$). In VEH and DEF-treated animals, the area at risk was 41 ± 3% and 38 ± 2% of the left ventricle, respectively. DEF reduced the infarct size from 65 ± 2% (VEH) to 33 ± 2% of the area at risk ($p<0.01$) or from 26 ± 3% (VEH) to 13 ± 2% of the left ventricle, respectively ($p<0.01$).

TABLE 1 RMBF (ml/min/100g) and LDCR (mmHg/ml min 100g)

group	(n)	NMI	MI/AR	IN/AR
RMBF-VEH	7	214 ± 34	35 ± 6	7 ± 2
RMBF-DEF	6	352 ± 52*	102 ± 23*	7 ± 2
LDCR-VEH	7	0.49 ± 0.09	2.48 ± 0.47	30.71 ± 9.69
LDCR-DEF	6	0.25 ± 0.03*	1.02 ± 0.26*	19.61 ± 5.54

*- $p<0.05$

The data show a protective effect of defibrotide on the reperfused ischaemic rabbit myocardium evidenced by the suppression of ST-segment elevation, inhibition of Q-wave development and a 50% reduction of infarct size. The increase in RMBF as well as a reduction in LDCR under DEF infusion may underly the mechanism of the anti-ischaemic action of the compound. We have not determined whether these effects are due to prostacyclin release.

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BLOCKADE OF APOMORPHINE-INDUCED YAWNING IN RATS BY THE SELECTIVE DOPAMINE AUTORECEPTOR ANTAGONIST (+)-AJ 76

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The role of dopamine autoreceptors in mediating yawning induced by dopamine agonists is controversial. Blockade of apomorphine-induced yawning by bilateral 6-hydroxydopamine lesions of the substantia nigra suggested dopamine autoreceptor involvement (Stoessl et al, 1987). In contrast, reserpine-induced dopamine depletion has been claimed to shift the apomorphine-induced yawning dose response curve to the left, suggesting mediation of the response by post-synaptic dopamine receptors (Morelli et al, 1986). In the present study we examined the effects of the novel, selective dopamine autoreceptor antagonist (+)-AJ 76 (cis-(+)-5-methoxy-1-methyl-2(n-propylamino)tetralin) (Johansson et al, 1987) on apomorphine-induced yawning.

Male Sprague-Dawley rats were habituated for 1h to perspex observation boxes prior to s.c. injection of saline or (+)-AJ 76 at doses of 0.21, 0.86 or 3.5mg/kg. 30 min later the rats were injected s.c. with saline or 0.025, 0.05 or 0.1mg/kg apomorphine HCl and their behaviour recorded by an observer using a keyboard interfaced to a BBC microcomputer system. The computer generated latency, frequency and duration data for each behaviour of interest. Yawning induced by 0.05mg/kg apomorphine was dose dependently blocked by (+)-AJ 76, (ANOVA interaction: $F(3,45)=17.02$, $P < 0.001$), significant decreases being observed at 0.86 and 3.5mg/kg of the drug ($P < 0.01$ in both cases). Furthermore, a dose of 0.86mg/kg (+)-AJ 76 produced a shift to the right in the apomorphine dose response curve for yawning (see Table).

	Yawns (40 min)	
Apomorphine (mg/kg)	Saline	(+)-AJ 76 (0.86mg/kg)
Saline	0.7 ± 0.3	0.5 ± 0.2
0.025	3.3 ± 1.9	1.2 ± 0.5
0.05	16.4 ± 2.1	3.8 ± 1.3**
0.1	23.9 ± 3.7	15.0 ± 2.2*

* $P < 0.05$ ** $P < 0.01$ Compared to saline pretreatment

(+)-AJ 76 had no effect on penile grooming and chewing mouth movements which are also elicited by low doses of apomorphine. This suggests that the blockade of yawning by (+)-AJ 76 is unlikely to be due to response competition. The pattern of results in the present study is similar to that observed following bilateral 6-hydroxydopamine lesions of the substantia nigra which also blocked apomorphine-induced yawning but spared penile grooming and chewing mouth movements (Stoessl et al, 1987). Previous studies have suggested that (+)-AJ 76 is a selective dopamine autoreceptor antagonist that has little or no effect on behaviour mediated by post-synaptic dopamine receptors (Svensson et al, 1986). Therefore, the present data together with previous evidence (Stoessl et al, 1987) provide strong support for the hypothesis that apomorphine-induced yawning is mediated by dopamine autoreceptors.

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LOW DOSES OF THE 5-HT_1 AGONIST RU 24969 INDUCE SEDATION IN THE MOUSE: EVIDENCE FOR MEDIATION BY 5-HT_{1B} AUTORECEPTORS

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There is persuasive evidence from in vitro studies in rodents that autoreceptors on the terminals of 5-HT neurones are of the 5-HT_{1B} subtype (Engel et al, 1986; Middlemiss, 1987). Thus the 5-HT_{1A}/5-HT_{1B} agonist RU 24969 decreases stimulation-evoked 5-HT release in tissue slices, whereas the selective 5-HT_{1A} agonist 8-OH-DPAT has no effect (Engel et al, 1986; Middlemiss, 1987). To date however, there is no in vivo evidence for the existence of 5-HT_{1B} autoreceptors. The present study describes hypolocomotion induced by low doses of RU 24969 and provides evidence that this response is mediated by 5-HT_{1B} terminal autoreceptors. Male BKTO mice (28-34g) were habituated for 2 h to individual photocell cages which measured spontaneous activity. Initially a dose response curve to RU 24969 (0.03-30.0 mg/kg) was constructed; subsequently the effects of pretreatment (30 min prior to RU 24969) with (±)-cyanopindolol, methiothepin, ketanserin, ICS 205-930 and citalopram on the RU 24969 response were examined. The effects of RU 24969 on mouse locomotor activity were biphasic, such that low doses (0.3, 1.0 mg/kg) decreased activity whereas high doses (3.0-30.0 mg/kg) increased locomotion. The hypolocomotion was blocked by (±)-cyanopindolol, methiothepin and citalopram but not by ketanserin (0.1-3.0 mg/kg) or ICS 205-930 (0.01/0.1 mg/kg).

Drug Treatment (mg/kg i.p.)		Photocell Counts (0-30 min post-RU 24969)
Vehicle	/Vehicle	274 ± 31
Citalopram 5.0	/Vehicle	248 ± 37
Vehicle	/RU 24969 1.0	118 ± 15**
Citalopram 5.0	/RU 24969 1.0	224 ± 36††
Vehicle	/Vehicle	229 ± 20
Methiothepin 0.025	/Vehicle	241 ± 50
Vehicle	/RU 24969 1.0	136 ± 15**
Methiothepin 0.025	/RU 24969 1.0	234 ± 38†
Vehicle	/Vehicle	260 ± 41
(±)-cyanopindolol 2.0	/Vehicle	235 ± 42
Vehicle	/RU 24969 1.0	128 ± 48*
(±)-cyanopindolol 2.0	/RU 24969 1.0	226 ± 57†

Data are mean ± S.E.M. ($n \geq 10$). Significant differences from respective vehicle/vehicle groups. * $p < 0.05$, ** $p < 0.01$ or respective vehicle/RU 24969 groups: † $P < 0.05$, †† $P < 0.01$.

Although hyperactivity induced by RU 24969 has been observed in previous studies (Oberlander, 1983) this is the first demonstration that low doses of RU 24969 induce sedation. The suppression of activity appears to be due to decreased 5-HT release, since it is attenuated by the selective 5-HT uptake inhibitor citalopram. Furthermore the response is probably mediated by 5-HT_{1B} autoreceptors as it is abolished by the 5-HT_1 antagonists cyanopindolol and methiothepin but not by the 5-HT_2 antagonist ketanserin or the selective 5-HT_3-antagonist ICS 205-930.

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HYPERPOLARISING AND DEPOLARISING RESPONSES TO 5-HYDROXYTRYPTAMINE IN THE RAT BRAIN STEM *IN VITRO*

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In the hippocampus, *in vitro*, bath applied 5-hydroxytryptamine (5-HT), typically evokes a biphasic response consisting of a hyperpolarisation followed by a long lasting depolarisation (Andrade & Nicoll, 1987). The hyperpolarising response is in all probability associated with a selective increase in K^+ permeability and said to be mediated by a 5-HT_{1A} receptor on the basis that spiperone is the most effective antagonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) is a partial agonist and 5 methoxy-N,N-dimethyl tryptamine (5-MeODMT) an agonist. Unexpectedly methysergide hyperpolarises the cells. The depolarisation, however, which is accompanied by a decrease in resting K^+ conductance, does not seem to be mediated by any of the current class of 5-HT receptors and is unaffected by a wide range of putative antagonists.

We have been looking at the applicability of these findings to other regions of the CNS *in vitro* by studying the hyperpolarising action of 5-HT on dorsal raphe (DR) neurones (Rainnie *et al*, 1987) and the depolarising action on facial nucleus (FN) neurones (Larkman *et al*, 1988) where the responses are mediated by similar changes in K^+ conductance but occur in isolation.

Application of methysergide a non-selective 5-HT_1 and 5-HT_2 antagonist had no effect on resting membrane potentials of neurones in either preparation but caused a dose dependent antagonism of 5-HT induced depolarisations in the FN. 10 µM methysergide caused a 60% reduction in a 200 µM 5-HT evoked depolarisation and reduced the increase in input resistance by 48% (n = 5). Conversely 100 µM methysergide was an ineffective blocker of the hyperpolarising response of DR neurones (n = 2). Methiothepin a non-selective 5-HT_1 antagonist and the 5-HT_2 antagonists ketanserin and ritanserin were ineffective blockers of the depolarising response to 5-HT in the FN. Spiperone (10 µM) applied to the DR for prolonged periods (≥ 2 hrs) failed to antagonise the 5-HT evoked hyperpolarisation completely producing a maximal block of 60% (n = 3). 8-OH-DPAT perfused at 10 µM had no effect on FN neurones but fully mimicked the hyperpolarising response of DR neurones to a 100 µM application of 5-HT. In the DR buspirone perfused at a concentration of 10 µM also produced a long lasting hyperpolarisation accompanied by an appropriate change in membrane resistance.

In summary, our experiments to date do not support the view that the hyperpolarising and depolarising responses to 5-HT in the CNS show a common pharmacology even though there is a substantial amount of work to suggest that the final effect on the ion channels has a greal deal in common.

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IS THE 5-HT_1 RECEPTOR AGONIST-INDUCED ROTATION IN RATS LESIONED UNILATERALLY IN THE DORSAL RAPHE MEDIATED BY 5-HT_{1A} RECEPTORS?

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The 5-HT_1 receptor agonist 8-hydroxy-2-dipropyltetralin (8-OH-DPAT), 5-methoxy-3-[1,2,3,6-tetrahydro-4-pyridinyl]1H-indole (RU 24969) and 5-methoxy-dimethyl-aminotryptamine (5-MeO-DMT) induce contralateral rotational locomotion in rats with a unilateral lesion of the dorsal raphe nucleus (DRN)(Blackburn et al., 1987 and refs. quoted therein). The common feature of the three agonists is their high affinity for 5-HT_{1A} receptors. Paradoxical results with a series of other 5-HT_{1A} agonists and antagonists question whether locomotion is indeed induced by activation of 5-HT_{1A} receptors (Blackburn et al., 1987). The present report deals with a further investigation of the 8-OH-DPAT induced rotation of DRN-lesioned rats and extends the list of agonists and antagonists which have been tested.

Male Sprague Dawley rats (180 - 200 g) were pretreated with pargyline (50 mg/kg i.p., - 1 h), anaesthetised with hexobarbitone (150 mg/kg i.p.) and positioned in a stereotaxic frame. The neurotoxin 5,7-dihydroxytryptamine (16 µg salt/2 µl 0.2% ascorbic acid solution), was injected unilaterally into the DRN over a 6 min. period. The following coordinates (Paxinos and Watson, 1982) were used: bregma - 7.8, 1 mm lateral to the midline and - 7 mm ventral to the surface of the skull, incisor bar - 3.2 mm. On the 7th day after operation the animals were injected with 8-OH-DPAT (0.5 mg/kg s.c.) and placed in automated rotameters. Only rats which made 200 or more contralateral turns within 2 h were retained for further experimentation. After 3 weeks the responses to 8-OH-DPAT (tested not more than once per week) became constant and remained stable for at least 4 months. Antagonists were administered s.c. 30 min. prior to 8-OH-DPAT 0.5 mg/kg s.c.; approximately the ED80%) and rotations counted during 2 hours. The correct placement of the lesion was established histologically.

8-OH-DPAT (0.032 - 2.0 mg/kg s.c.) dose-dependently increased rotation, to reach at the highest dose a maximal count of 240 $\pm$ 10 (n = 15). Reserpine (5 mg/kg i.p., - 12 h) but not p-chlorophenylalanine (pCPA; 150 mg/kg s.c., - 72 and - 48 h) inhibited the response to 8-OH-DPAT (0.5 mg/kg s.c.). Prazosin (1 mg/kg) abolished the response to 8-OH-DPAT, but yohimbine (0.1, 1 and 5 mg/kg) was ineffective. (-)-Pindolol (2 mg/kg) and (-)-propanolol (4 mg/kg) were also inactive. Flesinoxan (4 mg/kg), ($\pm$) 8-[2-[2,3-dihydro-1,4-benzodioxin-2-yl methylamino]ethyl]-8-azaspiro[4,5]decan-7,9-dione methane sulphonate (MDL 72832; 1 mg/kg) and ipsapirone (3 mg/kg) significantly increased contralateral rotation. Indorenate (10 mg/kg), 5-carboxamido-dipropylaminotryptamine (DP-5-CT; 1 mg/kg) and (-)-pindolol (2 mg/kg) were inactive.

The present results bear remarkable similarities to those of Tricklebank et al. (1984) who, in intact rats, observed that 8-OH-DPAT-induced ambulation was inhibited by prazosin and reserpine, but not by (-)-pindolol, (-)-propranolol or pCPA pretreatment. In conclusion, the evidence obtained with agonists would be consistent with a key role for 5-HT_{1A} receptors in the turning response to 8-OH-DPAT in DRN lesioned rats. However, the inactivity of pindolol and propranolol is not in agreement with such an interpretation and remains to be explained.

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ACTIONS OF 8-HYDROXY-2-(N-DIPROPYLAMINO)TETRALIN (8-OH-DPAT) AT α_2-ADRENOCEPTORS

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8-OH-DPAT is a relatively selective ligand for 5-HT-1A binding sites (Middlemiss & Fozard, 1983), but this 5-HT-1 agonist has recently been reported to an alpha$_2$-adrenoceptor antagonist with a pA$_2$ of 7.0 on guinea-pig submucous nerves (Crist & Surprenant, 1987). We have examined the actions of 8-OH-DPAT at alpha$_2$-adrenoceptors in the rat isolated atrium and in human platelets.

Rat isolated atria were pre-incubated with [^{3}-H]-NA, and stimulation-evoked release of tritium was taken as a measure of NA release. Prejunctional effects of drugs were assessed as an EC_{30} (concentration producing 30% potentiation of stimulation-evoked overflow of tritium) value. In human platelets, alpha$_2$-adrenoceptor binding sites were examined using [^{3}-H]-yohimbine as ligand.

In rat atria, 8-OH-DPAT had an EC_{30} of 5.80 (95% confidence limits of 4.93-7.03, -log M) and the alpha$_2$-adrenoceptor antagonist yohimbine had an EC_{30} of 7.89 (7.75-8.039, so that 8-OH-DPAT was approximately 120 times less potent than yohimbine.

In human platelets, competitive inhibition experiments were performed and IC_{50}'s (concentration producing 50% of maximum inhibition of [^{3}H]-yohimbine binding) of 7.6 μM (95% confidence limits of 6.3-9.1 μM) and 64.6 nM (34.7-120 nM) were obtained for 8-OH-DPAT and the alpha$_2$-adrenoceptor antagonist phentolamine, respectively. 8-OH-DPAT was approximately 120 times less potent than phentolamine.

In conclusion, although we are able to confirm that 8-OH-DPAT does have alpha$_2$-adrenoceptor antagonist potency, it is more than 100 times less potent than classical alpha$_2$-adrenoceptor antagonists in our studies. We have no explanation as to why 8-OH-DPAT is more than 10 times more potent on guinea-pig submucous nerves (Crist & Surprenant, 1987) as an alpha$_2$-adrenoceptor antagonist than in our studies.

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COMPARISON OF THE ACUTE AND CHRONIC EFFECTS OF BUSPIRONE ON SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS

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Acute treatment with the novel anxiolytic agent buspirone is known to have inhibitory effects in the rat hippocampus, probably by activation of 5-HT1A receptors, both in vitro (Rowan & Anwyl, 1986; Andrade & Nicoll, 1987) and in vivo (Rowan et al., 1988). Since there is a lag time of about 2 weeks for the development of the therapeutic effect of buspirone in the treatment of generalised anxiety and depressive symptoms (Goa & Ward, 1986) it is of interest to determine if the inhibitory action of buspirone in the hippocampus is altered by long-term treatment. The present study investigated this question using non-anaesthetized rats with chronic indwelling electrodes and cannula.

Male Wistar rats (200-250g) had stimulating wire electrodes and a cannula to which two recording wire electrodes were attached, implanted in the dorsal hippocampus under pentobarbitone anaesthesia (60 mg/kg). Animals were allowed one week for recovery before recordings were taken in a restraining hammock. Stimulation and recording was carried out in the stratum radiatum of the CA1 region. Low frequency stimuli (0.05Hz) of 0.4-1.4 mA were used to evoke small amplitude (0.8-1.4 mV) excitatory post-synaptic potentials (e.p.s.p.). Buspirone and vehicle (distilled water) were applied both via the i.p. route and via the cannula directly into the hippocampus (i.h.). For the chronic study animals received single daily injections of either water or 0.5 mg/kg buspirone for 14 days.

Buspirone produced a transient dose-dependent reduction in the amplitude of the e.p.s.p. when acutely injected either systemically (0.5-3 mg/kg, i.p.) or locally (0.1-1 µg, i.h.). In the case of the i.p. route the effect reached a peak 1 hr after the injection (80±3% control at 0.5 mg/kg, $n = 6$, $P < 0.05$) and full recovery was apparent within 2 hr. In contrast, during long-term treatment with 0.5 mg/kg buspirone there was a maintained, gradual reduction of the baseline e.p.s.p. size which reached a plateau between days 7-14. On day 14, 24 hr after the last injection the baseline e.p.s.p. had reached 57±4% ($n = 6$) of the original amplitude on day 1 ($P < 0.05$ compared to 91±8% in water injected controls, $n = 4$). Full recovery was noted 72 hr after the last injection. During the period of maintained baseline reduction (days 7-14), the administration of the daily dose of 0.5 mg/kg buspirone did not produce any further significant change. Similarly, the direct injection of 1 µg buspirone into the hippocampus during this period was without any additional effect. The chronic effect of 0.5 mg/kg buspirone was equivalent to that produced by acute doses which were at or near maximal (2.5-3 mg/kg). At this level of inhibition the addition of 1 µg buspirone i.h. had no further effect on the e.p.s.p.

The chronic effect of buspirone appeared to be due to an extension of its acute inhibitory action on excitatory synaptic transmission in the hippocampus. Following repeated treatment with a relatively low dose of buspirone an apparently prolonged maximal inhibition occurred which may be relevant to the gradual onset of its therapeutic effects in the clinic.

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5-HT_3 RECEPTORS IN THE AREA POSTREMA MAY MEDIATE THE ANTI-EMETIC EFFECTS OF 5-HT_3 ANTAGONISTS IN THE FERRET

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The selective 5-HT_3 receptor antagonist GR38032F (Brittain et al, 1987) potently inhibits emesis induced by the cancer chemotherapeutic agent cisplatin in the ferret (Costall et al, 1987). At present little is known as to the mechanism involved in this response, although such actions of 5-HT_3 antagonists have been attributed to effects at both central (Smith et al, 1988) and peripheral (Hawthorn et al, 1988) sites. However, since it has been reported that ablation of the area postrema (AP), the locus of the chemoreceptor trigger zone for emesis, can prevent cisplatin-induced emesis (McCarthy and Borison, 1984), this prompted us to investigate the involvement of the AP in the anti-emetic effect of various antagonists selective for the 5-HT_3 receptor. We have examined (a) the specific binding of the 5-HT_3 receptor ligand ^{3}H-GR65630 (Kilpatrick et al, 1987) to homogenates of rat and ferret AP and (b) the ability of GR38032F, GR65630A and MDL72222 injected directly into the AP to inhibit emesis induced by parenterally administered cisplatin in the ferret.

Specific ^{3}H-GR65630 (0.2nM) binding to the 5-HT_3 receptor was assessed as described previously (Kilpatrick et al, 1987). High levels of specific binding were evident in homogenates of both rat (29.2±3.3 fmol/mg protein; mean ± sem, n=3) and ferret (40.1±13.2 fmol/mg protein) AP. In the rat the specific binding of ^{3}H-GR65630 in the AP was higher than in any other brain region (Kilpatrick et al, 1987). Specific ^{3}H-GR65630 (0.05-2nM) binding to homogenates of rat AP was saturable with a Kd of 0.15nM and Bmax of 44.5 fmol/mg protein.

Intraperitoneal injections of cisplatin (9mg/kg) provoked an emetic response starting 55-120min after administration. Injections (dose volume 2µl) were made into the AP via chronic indwelling cannula immediately after the initial emetic episode. The cannula were implanted 24-96hr before testing.

Within 5 minutes of dosing, GR65630A (0.001-0.1µg), GR38032F (0.01-1µg) and MDL72222 (0.1-10µg) all produced dose related decreases in the number of retching and vomiting episodes produced by cisplatin by comparison to vehicle treated controls. This antagonism was particularly marked within the 10-20min time period after drug treatment i.e vehicle 27.8±7.3 retches, GR65630A 0.1µg 2.0±1.1 retches $p<0.05$; vehicle 27.6±7.8 retches, GR38032F 1µg 0 retches $p<0.05$; vehicle 24.1±4.7 retches, MDL72222 10µg 0.2±0.2 retches $p<0.05$. Vomiting showed a similar dose response inhibition. After 20min, the emetic response in drug treated animals began to return to control levels; this may reflect the rate of drug elimination from the AP.

Therefore the injection of either GR65630A, GR38032F or MDL72222 into the AP potently inhibits cisplatin-induced emesis. This result, together with the identification of a high density of 5-HT_3 receptors within the AP suggests their involvement in the anti-emetic action of 5-HT_3 antagonists and thus provides further evidence for the functional importance of central 5-HT_3 receptors.

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MORPHINE ANALGESIA IN THE RAT PAW PRESSURE TEST IS BLOCKED BY CCK BUT POTENTIATED BY THE CCK ANTAGONIST MK 329

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Low doses of cholecystokinin octapeptide (CCK) given i.p. block morphine analgesia in the rat tail flick test (Faris et al, 1983). Furthermore, blockade of CCK receptors by the weak non-selective antagonist proglumide enhances opiate analgesia in the same test (Watkins et al, 1984). Similarly the potent, selective CCK antagonist 1-methyl-3-(2-indoloyl)amino-5-phenyl-3H-1,4-benzodiazepin-2-one (MK-329) (Evans et al, 1986; Chang and Lotti, 1986) has recently been shown to enhance morphine analgesia and prevent morphine tolerance (Dourish et al, 1988). To date, however, studies on CCK/opioid interactions in analgesia have been restricted to thermal pain stimuli. In the present study we examined CCK/opioid interactions in a model of mechanical pain, the rat paw pressure test (Randall & Selitto 1957).

Male Sprague-Dawley rats (250 - 300 g) were used in the experiments which were carried out using an Analgesy Meter (Ugo Basile). Prior to drug administration, baseline pain thresholds (BL) were determined (mean of 3 trials with a 20 min intertrial interval). Animals were injected with drugs and pain thresholds (TL) were determined at 20 min intervals for 120 min. A cut off of 250 g was used to prevent tissue damage. Results were expressed as a % maximal possible effect (%MPE) using the equation:

$$\%MPE = [TL-BL/(250-BL)] \times 100.$$

Initially, a dose response curve for morphine analgesia in the paw pressure test was determined. Morphine (2-16 mg/kg i.p.) induced dose dependent analgesia throughout the 2 h test ($p < 0.001$). A submaximal dose of morphine (8.0 mg/kg) was chosen for the CCK interaction studies. CCK (4,8,16 μg/kg i.p. 10 min prior to morphine treatment) dose dependently blocked morphine analgesia ($p < 0.05$). In contrast, the CCK antagonist MK-329 (0.1, 0.5, 1.0, 2.0, 4.0, 8.0 mg/kg s.c., 20 min prior to morphine treatment) significantly enhanced analgesia induced by 8.0 mg/kg morphine ($p < 0.005$). The dose-response curve for MK-329 enhancement of morphine analgesia was bell-shaped and the peak effect was observed at a dose of 1.0 mg/kg.

The pattern of results in the present study is consistent with data obtained using the tail flick test in which the dose response curve for MK-329 enhancement of morphine analgesia was also bell-shaped (Dourish et al, 1988). The present data demonstrate that CCK modulation of opioid nociceptive mechanisms is not restricted solely to thermal pain stimuli but also occurs in response to mechanical pain.

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A NEW APPROACH TO THE PHARMACOLOGICAL EVALUATION OF OPIOIDS IN VIVO

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A variety of techniques exist for the pharmacological assessment of opioids in vivo. Many of these are relatively insensitive to agents of low efficacy. The technique described here was developed in order to improve the pharmacological evaluation of opioids with particular reference to partial agonists. The technique involves the induction of local irritation by the application of 10 ul allylisothiocyanate (mustard oil) to the dorsal surface of the ear of a guinea-pig. Four guinea-pigs are used for each dose group. Thirty minutes prior to the application of mustard oil the animals were dosed subcutaneously with either saline (1 ml/kg, control group) or test drug (treatment group). Where antagonists were used these were dosed subcutaneously 45 minutes prior to application of mustard oil. Guinea-pigs were then placed individually in large, well ventilated observation boxes. The number of times each animal scratched the affected ear or shook its head were counted for a ten minute period. Animals were then killed by cervical dislocation. The degree of antinociception produced was expressed as the mean of the percentage reduction of nociceptive responses in the treatment group compared to the control group. The results obtained are tabulated below.

Table 1. Effects of opioids in the Guinea-pig mustard oil test.

Agonist	ED50 (ug/kg)	Dose-ratio shift in the presence of: M8008	Naltrexone	NBT
Fentanyl	3.4	90	41	1.2
Bremazocine	3.5	0.5	17	1.6
EKC	6.1	3.3	22	
U69593	66	1.2	18*	
Tifluadom	30	1.4		
Buprenorphine	13	80		
Diprenorphine	5	40		
Nalbuphine	820	1.2		
Nalorphine	230	N.D.		
Xorphanol	10.5	19		
Cyclazocine	275	2.7		

M8008 and NBT (norbinaltorphimine) were dosed at 1 mg/kg. Naltrexone dose was 0.3 mg/kg except asterisk which was 1 mg/kg. N.D. = Not detectable.

The results obtained may be interpreted as effects on receptor subtypes as indicated by the known opioid selectivities of M8008 and naltrexone (Smith, 1987) and of NBT (Portoghese et al., 1987). The failure of NBT, a highly selective kappa antagonist, to reduce the agonist effects of the kappa agonist bremazocine may indicate a failure of NBT to penetrate to the site of action of bremazocine. The test appears to be sensitive to opioid partial agonists of differing selectivity profiles and as such should be useful in the pharmacological evaluation of these agents.

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THE cGMP-PHOSPHODIESTERASE INHIBITOR M&B 22948 POTENTIATES NANC RELAXATIONS OF THE MOUSE ANOCOCCYGEUS

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Non-adrenergic, non-cholinergic (NANC) relaxations of some smooth muscles (bovine retractor penis, opossum lower oesophageal sphincter) may involve activation of guanylate cyclase by the NANC transmitter, with subsequent generation of the second messenger substance cGMP (Bowman & Drummond, 1984 ; Torphy et al., 1986). Recently, we have proposed that the mouse anococcygeus may also belong to this group, since NANC relaxations are reduced in the presence of the guanylate cyclase inhibitor N-methyl-hydroxylamine (Gibson, 1988). In the present study, we have tested this possibility further using the selective cGMP-phosphodiesterase inhibitor M&B 22948 (Bowman & Drummond, 1984).

Anococcygeus muscles were isolated from male mice (LACA strain ; 25 - 35 g) and set up for the recording of isometric tension responses. NANC relaxations to 60 s trains of field stimulation were obtained as described previously (Gibson & Yu, 1983). In all cases, muscle tone was raised by carbachol (50 µM).

By itself, M&B 22948 (1 - 100 µM) produced small, concentration-related reductions of carbachol-tone ; 10 µM M&B 22948 reduced tone by 12 ± 3%, and this concentration was used to study the effects of M&B 22948 on NANC- and drug-induced relaxations.

At lower frequencies of field stimulation (0.5, 1, 2, 5 Hz) NANC relaxations were significantly ($P < 0.05$) potentiated in the presence of 10 µM M&B 22948 ; however, there was no change in the maximum relaxation obtained at 10 Hz. Overall, the log-frequency vs. % relaxation curve was displaced to the left by a factor of 2.

Concentration-related relaxations were produced by sodium nitroprusside (SNP ; 0.01 - 1 µM), hydroxylamine (1 - 100 µM), nitric oxide (3 - 120 µM ; assuming a saturated solution of 3 mM on ice), KCl (10 - 80 mM), 8-Br-cGMP (10 - 400 µM), vasoactive intestinal peptide (VIP ; 0.1 - 2 µM), papaverine (1 - 40 µM), 3-isobutyl-1-methylxanthine (IBMX ; 1 - 40 µM), and adenosine 5'-triphosphate (ATP ; 0.2 - 10 mM). The effect of 10 µM M&B 22948 was studied on concentrations of these relaxants which reduced tone between 15 - 50%. 10 µM M&B 22948 enhanced relaxations to 100 nM SNP (by 91 ± 3%), 10 µM hydroxylamine (by 65 ± 14%), 8 µM nitric oxide (by 57 ± 18%), and 25 mM KCl (by 64 ± 12%) ; relaxations to 100 µM 8-Br-cGMP, 1 µM VIP, 10 µM papaverine, 10 µM IBMX, and 2 mM ATP were unchanged.

In conclusion, several results from this study support the hypothesis that NANC transmission in the mouse anococcygeus involves generation of cGMP : first, NANC relaxations were potentiated in the presence of the cGMP-phosphodiesterase inhibitor M&B 22948 ; second, M&B 22948 also enhanced relaxations to KCl, which releases the NANC transmitter in the anococcygeus (Gibson & James, 1977) ; third, M&B 22948 selectively potentiated the nitrovasodilator drugs, which produce smooth muscle relaxation via activation of guanylate cyclase (Ignarro & Kadowitz, 1985). Finally, the results provide further evidence that neither VIP nor ATP can be considered as candidates for the NANC transmitter.

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EFFECTS OF VIP ANTAGONISTS, AND VIP AND PHI ANTISERA ON NON-ADRENERGIC, NON-CHOLINERGIC TRACHEAL RELAXATION

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The response of guinea-pig isolated trachea to electrical field stimulation (EFS) is biphasic, consisting of a transient contraction followed by a prolonged relaxation. The contractile component is abolished by atropine, indicating its cholinergic nature. A major component of the relaxation is insensitive to β-adrenoceptor blockade indicating that the neurotransmitter is non-adrenergic, non-cholinergic (NANC) in nature. The NANC inhibitory response has been suggested to be mediated either by adenosine or by the neuropeptides VIP and PHI (Karlsson, 1986). The present study examined the effect of adenosine deaminase, the adenosine antagonist NPC205 (1,3-dipropyl-8-(4-hydroxyphenyl)xanthine), the VIP antagonists [Ac-Tyr^1, D-Phe^2]-GRF(1-29)-NH_2 and [4-Cl-D-Phe^6, Leu^{17}]-VIP, and VIP or PHI antiserum on NANC relaxations.

Guinea-pig tracheal strips were suspended between platinum electrodes in tissue baths containing oxygenated Krebs-Henseleit solution at 37°C, to which were added atropine (1μM) and propranolol (1μM). Tissues were stimulated for 30s at 0.1ms and 50V at a frequency range of 0.1-100Hz. In experiments with antisera, tissues were incubated (14-16h, 4°C) either in Krebs (controls) or in VIP or PHI antisera. VIP and PHI antisera were obtained commercially, and exhibited less than 0.001% and 0.04% cross-reactivity respectively.

Neither adenosine deaminase (5U.ml^{-1}), in the presence of dipyridamole (0.5μM), nor NPC205 (1μM) affected the NANC response. The VIP antagonists had no effect on the NANC response or, surprisingly, on relaxations elicited by exogenous VIP (1-100nM) or PHI (10-500nM). VIP antiserum caused a 60% reduction in NANC responses, whereas PHI antiserum caused only a 20% reduction. In the presence of a maximally effective concentration of VIP (3μM), the NANC inhibitory response was reduced by approximately 40%. Neither the P_{2y} antagonist, reactive blue 2 (10μM), nor naloxone (up to 100μM) affected NANC responses, indicating that ATP and opioid peptides respectively are not involved.

The results of the present study suggest that adenosine is not involved in responses of guinea-pig trachea to EFS, since neurogenic relaxation was not affected by adenosine deaminase, or NPC205. Our data are at variance with an earlier report that adenosine deaminase inhibits NANC responses (Satchell, 1984), but is in accord with other studies which demonstrated that theophylline, an adenosine antagonist, had no effect on tracheal NANC responses (Karlsson & Persson, 1984). The marked effect of VIP antiserum strongly suggests that VIP, at least partly, mediates the NANC response. A previous study (Matsuzaki et al, 1980) demonstrated a similar effect using antiserum to VIP, but the specificity of this antiserum was not determined. Moreover, their EFS-induced relaxations were very small (approx. 5mg). Further evidence for the involvement of VIP in the NANC response is provided by the observation that, in the presence of a maximal concentration of the peptide, the neurogenic relaxation was markedly attenuated. That the VIP antagonists did not affect relaxations either to EFS or exogenous VIP, suggests that tracheal VIP receptors are different than those in pancreatic tissue, in which antagonism of VIP by these agents was first demonstrated (Pandol et al, 1986).

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EFFECTS OF PEPTIDASES ON NON-ADRENERGIC, NON-CHOLINERGIC INHIBITORY RESPONSES OF GUINEA-PIG TRACHEAL SMOOTH MUSCLE

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NANC inhibitory responses to electrical field stimulation (EFS) have been demonstrated in airway smooth muscle from a number of species (Barnes, 1986). The NANC neurotransmitter is not known, although a putative candidate in guinea-pig trachealis is VIP (Matsuzaki et al, 1980). In cat trachea, the peptidase α-chymotrypsin abolished the effect of VIP (Altiere & Diamond, 1984), but was without effect on NANC responses (Altiere & Diamond, 1985), suggesting that they are not mediated by VIP in this tissue. Incubation with VIP antiserum, or with a maximally effective concentration of VIP inhibits NANC responses in guinea-pig trachea (Ellis & Farmer, 1988). VIP and peptide histidine isoleucine (PHI) have the same distribution in the airways (Lundberg et al, 1984), and, as putative cotransmitters, may mediate the NANC response (Barnes, 1986). The present study has examined the effects of peptidases on responses to EFS and to VIP and PHI in guinea-pig isolated trachea.

Guinea-pig tracheal strips were suspended between platinum electrodes in tissue baths containing oxygenated Krebs-Henseleit solution at 37°C, to which were added atropine (1μM) and propranolol (1μM). Tissues were stimulated for 30s at 0.1ms, 50V at a frequency range of 0.1-100Hz, and isometric tension recorded. α-Chymotrypsin (2 units.ml^{-1}) or papain (2 units.ml^{-1}) were added to the bath 10 min prior to stimulation and, where appropriate, the peptidase inhibitor aprotinin (1.75 TIU.ml^{-1}) was applied 20 min prior to the peptidases.

The maximum relaxant response to EFS was reduced by 32 ± 5% by α-chymotrypsin (n=8). The peptidase also markedly decreased the duration of NANC responses. For example, at 50 Hz, the time taken to attain 50% prestimulation baseline was decreased from 317 ± 34s (n=10) in controls, to 75 ± 12s (n=8) in the presence of α-chymotrypsin. This peptidase abolished responses, to exogenous VIP or PHI, even at high concentrations. Effects of α-chymotrypsin on NANC responses or responses to peptides were abolished by aprotinin. Papain also reduced the maximum magnitude of NANC responses, by 30 ± 6% (n=6), but did not affect their time-course. Responses to VIP and PHI were abolished by papain, although unlike the case with α-chymotrypsin, aprotinin did not inhibit effects of papain. Aprotinin alone had no effect on tracheal responses. Similarly, the peptidases were without effect on concentration-response curves for methacholine or isoprenaline. Responses to EFS were abolished by TTX (1μM).

These results suggest that neuropeptides, possibly VIP and/or PHI, mediate, in part, NANC responses of guinea-pig trachea, and that they may be inactivated by peptidases. That the peptidase inhibitor aprotinin did not affect responses to exogenous peptides or to EFS indicates that if local enzymatic degradation of peptide transmitters is important, it is probably due to peptidases which are resistant to the inhibitory effect of aprotinin. If peptide degradation products are not responsible for the peptidase-resistant component of the neurogenic response, it is possible that a non-peptide substance, released from airway nerve endings, may also mediate the NANC inhibitory response.

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EFFECTS OF HISTAMINE ON cAMP, cAMP-DEPENDENT PROTEIN KINASE ACTIVITY, cGMP AND INOSITOL TRISPHOSPHATE IN AIRWAY SMOOTH MUSCLE

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Both cyclic nucleotide generating systems and inositol phospholipid metabolites have been suggested to be involved in the regulation of smooth muscle contraction (Andersson et al, 1975; Duncan et al, 1987). The present study examines the effects of histamine on cAMP and cGMP content, cytosolic cAMP-dependent protein kinase (A-kinase) activity and the generation of inositol trisphosphate (IP_3) in guinea-pig isolated trachealis.

Guinea-pig tracheal rings were suspended in Krebs-Henseleit solution, containing flurbiprofen (1μM). Changes in tension were measured isometrically using conventional methods. After addition of the agonist, tissues were freeze-clamped and placed in liquid nitrogen at pre-determined periods, between 0 and 15min for cyclic nucleotide studies and 0 and 60s for IP_3 measurements. The cAMP and cGMP content of the tissue was determined by protein binding and radioimmunoassay. A-kinase activity (expressed as activity ratio) was measured using a modification of the method described by Torphy et al (1986) using the synthetic heptapeptide, kemptide, as the substrate. Levels of IP_3 were determined by the method of Berridge et al (1983). Results are expressed as mean ± s.e.mean.

Histamine (30μM) was without effect on the cAMP content or A-kinase activity ratio over the first 15min of agonist stimulation. Inclusion of mepyramine (1μM) or cimetidine (1μM) in the incubation medium did not affect these data.

Histamine produced an initial significant ($P<0.05$, Mann-Whitney) increase in cGMP content at 1min, from 31.9±2.3 to 55.0±6.4 fmol/mg tissue, returning to basal level after 4min (n=5). This effect was abolished by pretreatment with mepyramine but was unaffected by cimetidine.

IP_3 levels were significantly ($P<0.05$, Analysis of Variance and t-Test) increased by 12.95 ± 2.5CPM/mg tissue from a basal level of 26.5 ± 4.9 CPM/mg, 5s after stimulation with histamine (30μM). Thereafter, IP_3 levels progressively returned to basal values during the following 60s (n=5). Mepyramine, but not cimetidine, blocked this effect.

The results suggest that the initial rapid generation of tension induced by histamine and the increased generation of both IP_3 and cGMP are mediated by H_1 receptors. Histamine does not modify cAMP levels or A-kinase activity in this tissue.

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ANALYSIS OF THE INTERACTION OF HEXAMETHONIUM WITH MUSCARINIC RECEPTORS IN VITRO

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Hexamethonium, in addition to its effects at nicotinic receptors, has been shown to reduce pA_2 values for muscarinic antagonists at atrial (M2) but not ileal (M3) receptors (Leung and Mitchelson, 1982). It has also been reported (Zonta et al., 1987) that hexamethonium alone exhibits a pA_2 value of 4 for atrial muscarinic receptors, but less than 2.8 at bronchial (M3) receptors (Barlow et al., 1972). These data suggest that hexamethonium can act as a selective, albeit weak, muscarinic antagonist at M2 receptors. In the present study we have examined this hypothesis using functional and radioligand binding assays.

Guinea pig (Dunkin-Hartley, 300-350 g) ileum, trachea, urinary bladder, oesophageal muscularis mucosae, paced left atria (threshold voltage + 10%, 4 Hz, 5 ms duration) and rat (Sprague-Dawley, 200-250 g) anococcygeus muscle were prepared according to methods described previously (Eglen et al., 1988). Concentration response curves to carbachol were established in the absence and presence of hexamethonium (10 μM - 3.2 mM), atropine or methoctramine (10 nM - 10 μM), using an equilibration period of 60 min. Antagonist affinities were estimated by the method of Arunlakshana and Schild (1959). Radioligand binding assays were conducted as described previously (Michel & Whiting, 1988) in which M1 receptors in rat (Sprague-Dawley 200-250 g) cortical membranes were labelled using [^{3}H]pirenzepine (0.3 nM) while M2 and M3 receptors present in cardiac and submaxillary gland membranes, respectively, were labelled using [^{3}H]NMS (0.1 nM).

Hexamethonium, at concentrations up to and including 3.2 mM, did not exhibit any significant ($P>0.05$) effect on the potency of carbachol at M3 receptors in the ileum, trachea, urinary bladder, oesophageal muscularis mucosae or anococcygeus muscle. In contrast, at 3.2 mM hexamethonium, a significant ($P<0.05$) dextral shift was observed at atrial M2 receptors, and the pK_B value was 3.3 ± 0.09 (mean ± SEM, n = 8). At atrial M2 receptors, the pA_2 value (mean ± SEM, n = 4) for atropine and methoctramine were significantly ($P<0.05$) reduced in the presence of hexamethonium (3.2 mM); control pA_2 values (and Schild slopes) were 8.9 ± 0.08 (0.9) and 7.8 ± 0.06 (1.0); pA_2 values in hexamethonium were 7.8 ± 0.07 (1.0) and 6.6 ± 0.03 (1.0) respectively.

In binding experiments, hexamethonium displayed higher potency at the M2 (cardiac) receptor (pK_i = 3.6; nH = 0.85) and the M1 (cerebrocortical) receptor (pK_i = 3.4; nH = 0.84) than at the M3 (gland) receptor (pK_i = 2.5; nH = 0.74). The low Hill slopes (nH values) may be due to allosteric effects of hexamethonium since at 10^{-2} M the compound slowed the dissociation of [^{3}H]NMS from cardiac binding sites.

We conclude that firstly hexamethonium selectively antagonises M2 receptors in the atria, but not M3 receptors in smooth muscle. The order of selectivity (15 fold) is comparable to other M2 antagonists. Secondly, in binding studies, the compound clearly differentiates M1 and M2 receptors from M3 receptors.

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PC12 PHEOCHROMOCYTOMA CELLS CONTAIN AN ATYPICAL MUSCARINIC RECEPTOR

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Ligand binding studies (De Jonge et al., 1986; Waelbroeck et al., 1986) have indicated that at least 3 muscarinic receptor subtypes, termed M1, M2 and M3 (De Jonge et al., 1986), exist. These can be classified according to their affinity for the receptor antagonists AF-DX 116, methoctramine and pirenzepine (Delmendo et al., 1988). M1 receptors are present in cerebral cortex and have high affinity for pirenzepine. M2 receptors are present in heart and have low pirenzepine affinity and high affinity for methoctramine and AF-DX 116. The M3 receptor occurs in gland tissue and displays low affinity for AF-DX 116 and methoctramine. In the present study we report on a muscarinic receptor in the PC12 cell line which differs pharmacologically from the M1, M2 and M3 muscarinic receptors.

Muscarinic receptors on intact PC12 cells (1 x 10^6 per assay tube) were labeled with [^{3}H]N-methylscopolamine (0.1 nM) by incubation in 1 ml of Tris-krebs (Michel & Whiting, 1988) for 2 h at 37°C. Incubations were terminated by vacuum filtration using a Brandel cell harvester. Non specific binding was determined using 1 uM atropine. Data were analysed using iterative curve fitting techniques. Affinity estimates for the M1, M2 and M3 receptor were obtained as described in Michel & Whiting (1988)

	MEMBRANE PREPARATIONS			PC12
LIGAND	CORTEX (M1)	HEART (M2)	SUBMAXILLARY (M3)	CELLS
AF-DX 116	6.59 (1.05)	7.02 (0.92)	5.99 (1.04)	6.75 (1.03)
CPPS	8.36 (1.00)	7.56 (0.92)	7.70 (1.11)	8.27 (1.09)
4-DAMP	8.51 (0.89)	7.97 (0.95)	8.74 (1.02)	8.91 (0.99)
HHA	8.64 (0.94)	7.35 (1.04)	7.97 (0.95)	8.11 (1.03)
METHOCTRAMINE	6.86 (0.97)	7.76 (0.91)	6.29 (1.02)	6.83 (0.91)
PIRENZEPINE	7.72 (0.92)	6.38 (0.91)	6.79 (1.11)	6.89 (0.89)

The ligand was [^{3}H]NMS except in the M1 assay where [^{3}H]pir was used. Values are pKi (nH), SEM <5%, n=4. nH values not different ($p<0.05$) to 1.

The results are summarised above. The pharmacology of the PC12 muscarinic receptor was not consistent with that expected of the M1, M2 or M3 receptors. For AF-DX 116, methoctramine and cyclohexylphenyl (2-piperidinoethyl)silanol (CPPS) affinity estimates were consistent with an M1 receptor yet for pirenzepine, 4-DAMP and hexahydroadiphenine (HHA) affinity estimates were more consistent with an M3 receptor. This did not appear to be due to receptor heterogenity since all nH values were close to unity and there was no [^{3}H]pirenzepine binding in the PC12 cells. Whether the receptor corresponds to that expressed by the m4 receptor gene described by Bonner et al., (1987) awaits further study.

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EVIDENCE AGAINST THE HYPOTHESIS THAT β-ADRENOCEPTOR AGONISTS PROMOTE THE TRANSLOCATION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE *IN VIVO*

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Cyclic AMP-dependent protein kinase (A-kinase) is believed to be intimately involved in the regulation of many intracellular processes including glycogenolysis and smooth muscle relaxation. Under *in vitro* conditions we (Giembycz & Diamond, 1988) and others (e.g. Krall *et al*, 1978) have reported that, as a direct consequence of activating A-kinase, β-adrenoceptor agonists promote the physical migration of this enzyme from the cytosol to membrane-associated protein substrates. It was logically hypothesised that intracellular translocation of free catalytic subunits may represent a unique mechanism specifically linked with the regulation of β-adrenoceptor-mediated phenomena. This abstract describes some data which questions whether translocation of A-kinase in guinea-pig lung occurs *in vivo*.

Soluble and particulate A-kinase was prepared essentially as described by Krall *et al* (1978). Enzyme activity was measured using a modification (Cook *et al*, 1982) of the method originally described by Witt and Roskoski (1975) using kemptide as substrate.

In guinea-pig lung, isoprenaline (0 - 10 uM) produced a concentration-dependent reduction (23.2 ± 4.4%; n = 6) in the amount of soluble A-kinase and a corresponding increase (18.4 ± 3.03%; n = 6) in the amount of this enzyme in the Triton X-100-solubilised pellet when tissue was homogenised in a buffer of ionic strength (*I*) 69 mM. Qualitatively identical results were obtained when lung homogenates were treated with cyclic AMP (0 - 10 uM). However, when tissue was homogenised in a high salt buffer (*I* = 229 mM) no translocation of A-kinase was observed at any concentration of isoprenaline or cyclic AMP tested. Moreover, the addition of KCl (0 - 400 mM) to cyclic AMP (10 uM)-containing, low *I* lung homogenates resulted in a concentration-dependent increase in soluble A-kinase activity (from 9.13 ± 1.01 to 21.77 ± 0.58 pmol min-1, n = 6). High salt, therefore, not only prevented cyclic AMP stimulated A-kinase translocation but effectively reversed it.

It is concluded that the interaction of A-kinase with the particulate material is ionic in nature, not hydrophobic. It follows, therefore, that since the intracellular fluid has an *I* = 200 mM translocation of A-kinase in guinea-pig lung probably does not occur *in vivo*. Whilst this conclusion is consistent with the data obtained in cardiac muscle (Keely *et al*, 1975) it nevertheless conflicts with the work of Krall *et al* (1978) who reported β-adrenoceptor-mediated translocation of A-kinase in rat myometrial smooth muscle after homogenisation of this tissue in buffer containing 500 mM NaCl. This raises the interesting possibility that a physiologically relevant translocation of A-kinase may be a tissue-specific phenomenon.

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HOMOLOGOUS DESENSITIZATION OF ADENOSINE A_2 RECEPTORS ON NG108-15 CELLS IS NOT DUE TO LOSS OF [^{3}H]-NECA BINDING SITES

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The NG108-15 neuroblastoma x glioma hybrid cell line (Kenimer & Nirenberg, 1981) provides a useful model system in which to study the mechanisms of homologous and heterologous desensitization of various adenylate cyclase-linked receptor systems. In this study, we have examined the effects of culturing NG108-15 cells in the presence of a stable adenosine A_2 receptor agonist 5'-(N-ethyl)-carboxamidoadenosine (NECA) on subsequent responses to NECA and other stimulators of adenylate cyclase activity, and on the [^{3}H]-NECA binding properties of these cells.

NG108-15 cells (passage 16-25) were grown to confluency in 80cm^2 flasks and then cultured for 24h in the presence or absence of 10μM NECA. Cells were harvested, washed three times to remove the NECA and then frozen at -80°C. Adenylate cyclase activity was measured in homogenates of these cells as described by Edwards et al. (1987). The binding of [^{3}H]-NECA (3-2000nM) was measured in the same homogenates, in 50 mM Tris-HCl, 5mM $MgCl_2$, pH 7.4, using 100μM NECA to define non-specific binding.

In control cell homogenates, the maximal response to NECA was a 3-4 fold stimulation of adenylate cyclase activity over basal levels (~10 pmol cAMP/min/mg protein). In the NECA-desensitized cells this response was decreased by 80-90%. In both control and desensitized cells, the EC_{50} for NECA was 450nM. There was no change in the iloprost, Gpp(NH)p or NaF-mediated activation of adenylate cyclase.

In control cell homogenates, [^{3}H]-NECA bound to an apparently homogeneous population of saturable binding sites, with an affinity of ~1000 nM (which compares favourably with the EC_{50} for NECA in the adenylate cyclase assay) and a relatively high capacity - ~10 pmol/mg protein. Specific [^{3}H]-NECA binding was displaced by a range of A_2 receptor ligands. The IC_{50}'s for NECA, 2-chloro-adenosine, IBMX and theophylline were ~1, 10, 100 and 300 μM respectively.

In contrast to the homologous desensitization of β-adrenoceptor responses in a number of other systems (Harden, 1983), there was apparently no loss of A_2 receptors in NG108-15 cells following prolonged exposure to NECA. The number and pharmacological characteristics of the [^{3}H]-NECA binding sites were unaltered in desensitized cells.

Thus, while A_2 receptors are uncoupled from stimulation of adenylate cyclase activity upon desensitization with NECA, [^{3}H]-NECA binding sites, which have characteristics consistent with A_2 receptors, are not degraded.

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