REPEATED ECS ENHANCES DOPAMINE D-1 BUT NOT D-2 AGONIST-INDUCED BEHAVIOURAL RESPONSES IN RATS

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It is well documented that repeated electroconvulsive shock (ECS), which is antidepressant in man, enhances the behavioural responses in rodents induced by dopaminergic agents such as apomorphine and amphetamine (see Green & Nutt, 1987). However, it is unknown whether this effect of ECS can be attributed to a particular dopamine receptor subtype. Currently it is believed that there are two central dopamine receptor subtypes, D-1 and D-2, and that there are selective pharmacological agents for each (Stoof & Kebabian, 1984). The present study tested the effect of acute and repeated ECS on the behavioural responses in rats induced by the D-1 agonist SKF 38393, the D-2 agonist RU 24213 and the D-1/D-2 agonist apomorphine.

Male Sprague Dawley rats were administered either ECS (150 v, 50 Hz, 1 sec) under halothane anaesthesia or halothane alone, once (acute) or 5 times over 10 days (repeated). Drug-induced behavioural responses of individual rats were measured in a single blind manner 24 h after the last treatment. The behavioural effects of SKF 38393 (7.5 mg/kg s.c.) and RU 24213 (0.75 mg/kg s.c.) were assessed using a modified version of the behavioural check list method of Molloy and Waddington (1987). Briefly, rats were scored for the presence of 5 behavioural components during 20 sec observation every min for 5 min. This cycle was repeated at 10 min intervals. Apomorphine (0.5 mg/kg s.c.)-induced stereotypy was assessed using a 0-6 rating scale every 10 min. Drug-induced behaviours were observed for 70 min and accumulated behavioural scores were calculated.

Table 1. Effect of repeated ECS on dopamine agonist-induced behaviours

	Sniffing	Loco-motion	Rearing	Grooming	Intense grooming
SK&F 38393					
Repeated ECS (n=12)	21.8±1.4*	5.2±0.8	12.3±2.6	12.2±1.0**	10.5±1.0*
Control (n=12)	17.6±0.8	4.0±0.6	8.3±1.6	8.0±0.9	7.3±0.9
% change	+23.9%	+30.0%	+48.0%	+52.5%	+43.8%
RU 24213					
Repeated ECS (n=6)	21.3±2.6	5.8±2.6	1.3±1.8	-	-
Control (n=6)	26.2±3.2	9.5±1.9	3.2±2.4	-	-
% change	-18.7%	-38.9%	-59.4%	-	-

Mean ± SEM accumulated scores (max=35) * $p<0.05$ ** $p<0.01$ vs control (Mann-Whitney U test)

Table 1 shows that in comparison to halothane-treated controls, repeated ECS increased certain behaviours, in particular grooming, induced by SKF 38393 but not those produced by RU 24213. Behaviours induced by SKF 38393 and RU 24213 were not affected by acute ECS (data not shown). Repeated but not acute ECS increased apomorphine-induced stereotypy (+26.3%, n=6, $p<0,05$).

These results suggest that the increase of dopamine-mediated behaviour in rats seen after repeated ECS is due to a selective increase in central dopamine D-1 function. The possible involvement of the dopamine D-1 receptor in the antidepressant effects of electroconvulsive treatment deserves further investigation.

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STUDIES ON THE ROLE OF BRAIN DOPAMINE SYSYEMS IN THE PSYCHO-STIMULANT RESPONSE TO NICOTINE

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Previous studies suggest that the psychostimulant properties of nicotine may be associated with its ability to stimulate dopamine (DA) secretion in the brain (Imperato et al 1986). Increased DA secretion has also been observed in animals exposed to anxiogenic stimuli (D'Angio et al 1987). In this study drugs which enhance or antagonise neurotransmission at brain DA synapses have been used to examine further the possible role of brain DA systems in the effects of nicotine and aversive environmental stimuli on rat locomotor activity.

Male Sprague-Dawley rats (N=7 per gp) were given subcutaneous injections of saline or nicotine (0.1 or 0.4 mg/kg) 3 minutes before daily test sessions on either an elevated open platform (Benwell & Balfour 1982) or a less aversive enclosed platform of the same dimensions, different groups of rats being tested on each platform. The locomotor activity of the rats was monitored using infrared photobeams mounted along adjacent sides of the platform (each beam interruption = 1 activity unit). Fifteen daily trials (10 min) were used to habituate the rats to the platforms. The activity of the rats was then studied at 3 day intervals in rats pretreated subcutaneously with saline, cocaine (5 or 15 mg/kg), haloperidol (0.05, 0.1 or 0.4 mg/kg) or α-flupenthixol (5 mg/kg) 30 (cocaine) or 60 minutes (neuroleptics) prior to testing using a counter-balanced design.

Analysis of the data from the experiments with cocaine showed that nicotine increased ($F(2,36) = 39$; $P<0.001$) and the open platform decreased ($F(2,72) = 4.7$; $P<0.05$) activity. Cocaine increased the activity of the rats ($F(2,72) = 20$; $P<0.001$). The 1st injection x 2nd injection x platform interaction was also significant ($F(4,72) = 3.4$; $P<0.05$). On the open platform, cocaine (15 mg/kg) increased ($P<0.01$) locomotor activity in rats subsequently given saline or nicotine (0.1 mg/kg) from 56 ± 19 to 177 ± 42 and 158 ± 18 to 268 ± 22 activity units respectively. Cocaine (5 mg/kg) also increased ($P<0.05$) the activity of the rats given a second injection of saline to 121 ± 25 units. In the enclosed platform, cocaine (15 mg/kg) increased the activity of the rats which received a second injection of saline ($P<0.01$) or nicotine (0.4 mg/kg; $P<0.05$) from 140 ± 10 to 270 ± 34 and from 264 ± 22 to 409 ± 50 units respectively. The lower dose of cocaine also increased the activity ($P<0.05$) of the nicotine (0.4 mg/kg)-treated rats to 385 ± 46 units. Haloperidol decreased activity ($F(3,105) = 41$; $P<0.001$) although this effect was influenced by the subsequent treatment (1st injection x 2nd injection $F(6,105) = 4.0$; $P<0.05$). Subsequent analysis showed that the highest haloperidol dose tested (0.4 mg/kg) depressed activity ($P<0.001$) in all treatment groups. The lowest dose (0.05 mg/kg) also decreased the activity ($P<0.05$) of the rats given 0.4 mg/kg nicotine from 269 ± 13 to 225 ± 14 activity units. α-flupenthixol had no significant effects on activity. The data obtained are complex although they appear consistent with the hypothesis that exposure to the open platform is associated with decreased brain DA secretion and that the stimulant properties of nicotine may, in part at least, be mediated by increased DA secretion.

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THE INFLUENCE OF ENVIRONMENTAL STIMULI ON THE PSYCHOPHARMACOLOGICAL RESPONSE TO DIAZEPAM

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The administration of diazepam to rats tested in a novel environment can result in increased locomotor activity and a diminished plasma corticosterone response (File 1985). In this study the aversive properties of the test environment used have been examined for their effects on these responses to diazepam.

Groups of rats (N=6 per gp) were given vehicle (40% propylene glycol in water) or diazepam (5 mg/kg) orogastrically. Thirty minutes later they were placed in one of four test environments, an elevated open platform (Benwell & Balfour 1982), an enclosed platform of the same dimensions, an elevated X-maze composed entirely of open-runways or an enclosed X-maze of the same dimensions (Copland & Balfour 1987), different groups being used for each environment. The activity was then measured in 5 minute increments for 20 minutes using infra-red photobeams mounted along adjacent sides of the platforms or in the arms of the mazes. Plasma corticosterone was measured in blood samples collected at the end of the trial using the method of Mattingly (1962).

The mean activity of the rats tested in the open maze (29±2 entries per trial) was lower ($F(1,20) = 17$; $P<0.01$) than that of rats tested in the enclosed maze (46±4 entries per trial). Diazepam increased activity ($F(1,20) = 6.2$; $P<0.05$) from 27±2 to 32±5 entries per trial in the open maze and from 39±5 to 54±5 entries per trial in the enclosed maze. The apparent increase in the magnitude of the response in the enclosed maze was not statistically significant. Diazepam had no significant effects on the plasma corticosterone response to either of the mazes. Rats tested on the open platform were less active ($F(1,20)= 13.9$; $P<0.01$) than those tested on the enclosed platform. The platform x drug interaction was also significant ($F(1,20) = 5.7$; $P<0.05$). Subsequent analysis showed that the activity of the vehicle-treated rats was reduced ($F(3,60) = 18$: $P<0.01$) from 378±46 to 158±25 units per trial. This was not the case for diazepam-treated rats. The effects of diazepam were influenced by the duration of the trial (drug x subtrial $F(3,60) = 7.0$; $P<0.01$). Diazepam increased the activity of the rats tested on the open platform during subtrials 1 and 4 whereas it decreased the activity ($P<0.05$) of rats tested on the enclosed platform during subtrials 2,3 and 4. Diazepam had no effect on the plasma corticosterone respone to the open platform but decreased (drug x platform $F(1,20) = 5.3$; $P<0.05$; t-test $P<0.01$) the response to the enclosed platform from 25±2 to 17±1 µg/100 ml. It is concluded that diazepam attenuates responses to novel environmental stimuli and enhances behavioural habituation to a simple open field. It appears to have little effect on responses to the aversive properties of an elevated open environment.

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DELTA OPIOID SITES IN THE SPINAL CORDS OF VARIOUS RODENTS

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Opioids are effective analgesic agents after intrathecal injection in man and animal models by an action mediated at the spinal level. The role of opioid delta receptors in this anti-nociceptive response has been a matter for recent debate. Several authors have found evidence for delta-mediated spinal analgesia (Porreca et al., 1984; Rodrigues et al., 1986) while others have suggested an action of delta agonists at mu receptors (Birch et al., 1987). The difficulties of interpretation of data have led one group to postulate an action of mu and delta agonists at a common site (Porreca et al., 1987). Part of the problems may arise from the use of different species and different delta agonists. We have therefore studied the binding properties of the selective delta agonist [^{3}H]-[D-Pen^2,D-Pen^5]enkephalin ([^{3}H]DPDPE) to spinal cord homogenates from different rodent spinal cords.

Binding of [^{3}H]DPDPE to homogenates prepared from whole spinal cord tissue was carried out as described by Cotton et al., (1985) using a buffer of Hepes (pH7.4) containing 10mM Mg++ to enhance delta binding. The specific binding of [^{3}H]DPDPE was determined as the difference in counts obtained in the presence and absence of unlabelled naloxone (10μM).

The results show (table 1) that high affinity binding sites are present in all species studied. Lowest levels were observed in the rat and hamster and highest levels in young mice normally used for intrathecal studies. Highest binding affinity was seen in the guinea-pig.

Table 1. Binding characteristics of [^{3}H]DPDPE in homogenates of rodent cords

Species	K_D (nM)	Bmax (pmols.g^{-1})
Guinea-pig	1.83 ± 0.11	2.92 ± 0.15
Hamster	2.39 ± 0.19	1.93 ± 0.11
Mouse - adult	2.62 ± 0.52	3.60 ± 0.61
- 18-20g	4.33 ± 0.30	5.42 ± 0.46
Rat	3.76 ± 0.21	1.96 ± 0.15

The binding of [^{3}H]DPDPE in cords from 18-20g mice was displaced by the mu-ligand [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin in a biphasic manner. A high affinity site with a Ki of 1.97 ± 0.69nM and a low affinity site with a Ki of 207 ± 40nM were discerned. The high affinity site accounted for 16.9 ± 4.5% of the binding of 3.4nM [^{3}H]DPDPE.

The results confirm the presence of delta sites in the cords of all species studied and thus support a role for the delta receptor in spinally-mediated analgesia. It can only be conjectured at this time as to whether the bi-phasic displacement of [^{3}H]DPDPE by [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin represents some common high affinity site for mu- and delta-ligands.

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ADENOSINE INHIBITION OF RAT HIPPOCAMPAL POPULATION POTENTIALS IS MAGNESIUM DEPENDENT

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Adenosine inhibits epileptiform activity induced by magnesium free media or bicuculline (Aram et al 1987, Ault and Wang 1986) but with a marked variation in potency. Using the rat hippocampal slice we have looked at the effect of varying magnesium concentrations on the synaptic inhibitory response to adenosine.

Hippocampal slices were prepared 450μM thick from male Wistar rats and superfused with ACSF of composition (mM): NaCl 115, KCl 2, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 2.2, $NaHCO_3$ 25, $CaCl_2.2H_2O$ 2.5, glucose 10 gassed with 95%/5% O_2/CO_2. A bipolar stimulating electrode was placed on the stratum radiatum. Orthodromic evoked population potentials (stimulation 0.1 Hz, 0.1ms, 200-350μA) were recorded from the CA_1 pyramidal cell layer, displayed on a digital storage oscilloscope and chart recorder. Changes in potential size were expressed as a percentage change from the control.

In 1.2mM magnesium, adenosine depressed the synaptically evoked population spike with an IC50 of 37 ± 3.4μM (n=30). In 0.4mM magnesium the IC50 increased to 56 ± 7.5μM (n=3), while in magnesium free medium the effect of adenosine was abolished at concentrations below 10μM. In some cases a small excitatory effect was observed at the low concentrations. Above 10μM adenosine, inhibition was greatly reduced with 100μM adenosine giving only a 21% ± 11.7 (s.e.m.) decrease compared to 76% ± 5.9 in normal ACSF. In 4mM Mg^{2+} the adenosine IC50 was 11.7 ± 2μM (n=4). 2-chloroadenosine showed similar changes in sensitivity indicating that an increased uptake and metabolism of adenosine was not the reason for this loss of effect.

The increase in population spike size due to removal of magnesium, (12% ± 1.6, n=13) did not account for the decrease in adenosine potency. In poorer slices, zero Mg^{2+} gave no increase in response size, but the adenosine response was decreased. An NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (50μM) gave no reduction in excitation, or enhancement of adenosine inhibition. Reducing the potential size by reducing calcium concentration also failed to improve the inhibition by adenosine, though the addition of cobalt and manganese restored the inhibitory potency of adenosine.

These experiments suggest that adenosine may require the presence of divalent cations for its interaction with the A_1 adenosine receptor. The weak excitatory effect of adenosine seen at low concentrations of adenosine in zero Mg^{2+} may involve an A_2 receptor.

J.T.B. is an S.E.R.C., C.A.S.E. research student.

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REGIONAL BRAIN CONCENTRATIONS OF PUTATIVE AMINO ACID NEUROTRANSMITTERS DURING CATECHOL-INDUCED SEIZURES

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The convulsant agent catechol has been shown to selectively increase the K^+ evoked release of endogenous aspartate from rat olfactory tract nerve terminals (Collins & Dewhurst, 1986). A similar action at other sites in the CNS could explain the mechanism of action of catechol and be expected to result in elevated levels of excitatory amino acids in certain brain regions. This work investigates changes in regional brain concentrations of aspartate, glutamate, GABA, glycine and taurine during catechol-induced convulsions.

Female albino rats were anaesthetised with urethane (1.4 g/kg i.p.). Half of these received catechol (65 mg/kg i.p.), while controls were treated with 0.9% NaCl. Animals were killed 3 min after injection (at the peak of the catechol convulsion), by decapitation, the head being immediately immersed in liquid nitrogen. After partial thawing the brains were removed and carefully dissected on ice into four regions: cerebral cortex, brainstem, cerebellum and midbrain. These were weighed, homogenised in perchloric acid, centrifuged and analysed using a Chromaspek Amino Acid analyser with ninhydrin detection (570nm and 440nm; norleucine internal standard).

The results showed that levels of glutamate, aspartate and glycine measured at the peak of the catechol convulsion (PC) were not significantly different from controls (C) in all brain regions. In two animals, whose brains were removed 20 min after catechol (post-convulsion), concentrations of these amino acids were not significantly different from controls or from brains removed at peak convulsion, indicating that there is no delayed change. GABA and taurine levels were found to be significantly increased ($P < 0.05$, Student's t-test, $n = 4$) in cerebellum and brainstem at the peak of the convulsion (GABA, µmol/g wet wt., cerebellum (C) 2.9 ± 0.6, (PC) 4.5 ± 0.9; brainstem (C) 2.6 ± 0.3, (PC) 4.2 ± 0.14; Taurine; cerebellum (C) 3.7 ± 0.6, (PC) 5.2 ± 0.8; brainstem (C) 1.9 ± 0.3, (PC) 2.6 ± 0.7). These levels were still elevated post-convulsion. Control values for all amino acids measured correlate well with those found by other workers (Huxtable et al., 1982).

The results indicate that excitatory amino acids are not involved in the mechanism of action of catechol, although it is possible that changes in release are too small to be detected in such large amounts of tissue. The observed rise in GABA and taurine levels may well be a result of seizure activity, rather than of catechol itself.

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INHIBITION OF *IN VITRO* [^{3}H]-MONOAMINE UPTAKE BY PLASMA TAKEN FROM VOLUNTEERS DOSED WITH THE PUTATIVE ANTIDEPRESSANT SIBUTRAMINE HCL

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Sibutramine hydrochloride (BTS 54 524) has a pharmacological profile indicative of potential antidepressant activity (Buckett *et al*, 1987a,b). For example, sibutramine HCl rapidly and potently down-regulates cortical β-adrenoceptors in the rat, an effect at least partly due to potent monoamine uptake inhibition by its metabolites (Luscombe *et al*, 1987). We have now studied whether sibutramine HCl is also a monoamine uptake inhibitor in man by analysing the ability of plasma obtained from drug-treated, healthy male volunteers to inhibit the *in vitro* uptake of [3H]-noradrenaline (NA) and [3H]-5-hydroxytryptamine (5HT).

Plasma was derived from venous blood taken at regular intervals during repeated treatment of healthy male volunteers with sibutramine HCl at either (i) 5, 10 or 20 mg/day for 14 days, or (ii) 15 mg twice daily for 6 days. Plasma was then incubated with either rat cortical synaptosomes to study inhibition of [3H]-NA uptake or with human platelets obtained from untreated male volunteers to investigate inhibition of [3H]-5HT uptake (Wynne *et al*, 1986).

Plasma from volunteers administered repeated oral doses of sibutramine HCl preferentially inhibited [3H]-NA uptake *in vitro* with a weaker effect on *in vitro* [3H]-5HT uptake. These inhibitory effects were dose-dependent (5 - 20 mg) and increased with the duration of treatment, reaching a plateau of 50% inhibition of [3H]-NA uptake and 20-30% inhibition of [3H]-5HT uptake after sibutramine HCl (20 mg/day) (Table 1). Higher plateaux of approximately 60% [3H]-NA and 40% [3H]-5HT uptake inhibition were attained more rapidly by twice daily treatment with sibutramine HCl (15 mg).

Table 1 Effect on [3H]-NA and [3H]-5HT uptake in vitro of plasma obtained during repeated administration of sibutramine HCl (20 mg/day) to volunteers

Monoamine	Uptake inhibition (% ± s.e.mean) by plasma taken 2h after dosing on					
	day 1	day 3	day 6	day 9	day 12	day 14
[3H]-NA	36.4 ± 5.8	38.6 ± 4.1	49.8 ± 7.7	51.2 ± 5.8	50.8 ± 6.7	53.0 ± 7.7
[3H]-5HT	1.7 ± 2.4	5.5 ± 3.7	22.8 ± 2.9	22.2 ± 3.5	30.0 ± 3.1	30.3 ± 2.5

n=5-6 volunteers. NA and 5HT uptake assayed concurrently on same plasma sample.

The preferential inhibition of NA uptake over 5HT uptake by plasma removed from healthy male volunteers repeatedly administered sibutramine HCl is in agreement with its relative potency against these monoamines after a single oral dose (Wynne *et al*, 1986). These results are also in general agreement with the relative activity of sibutramine HCl as a monoamine uptake inhibitor in rodents suggesting that these assays may provide an indirect measure of drug activity in man. In addition, the activity of sibutramine HCl as a monoamine uptake inhibitor in man further supports the preclinical evidence (Buckett *et al*, 1987a,b) that it will have an antidepressant effect.

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ADMINISTRATION OF SIBUTRAMINE HCl AND OTHER ANTIDEPRESSANTS INCLUDING ECS DECREASES CORTICAL β_1- BUT NOT β_2-ADRENOCEPTORS

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CGP 20712A (1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-2-propanol methanesulphonate) is a highly specific β_1-adrenoceptor antagonist which can be used as a tool to differentially quantify β-$_1$ and β_2-adrenoceptors in ligand-receptor binding studies (Dooley & Bittiger, 1987). We have used CGP 20712A to determine the effects of sibutramine HCl (BTS 54 524) and various other antidepressant treatments on β_1- and β_2-adrenoceptors in rat cortex.

Adult male CD rats (Charles River) weighing 75-175 g were used. BTS 54 524 (3 mg/kg), amitriptyline (10 mg/kg), desipramine (DMI; 10 mg/kg) and zimeldine (10 mg/kg) were given orally once daily. Controls received distilled water. Five electroconvulsive shocks (ECS; 200 V, 2s) were administered to anaesthetised rats over 10 days. Controls received halothane. Rats were killed 24 h after the final treatments and saturation binding analysis was performed on cortical membranes using [^{3}H]-dihydroalprenolol (0.14-2.8 nM). Specific binding to β_1- and (β_1 + β_2)-adrenoceptors was defined with 10 μM CGP 20712A and 200 μM isoprenaline, respectively. β_2-Adrenoceptor parameters were then calculated by difference. Significance was determined using Student's t-test.

Initial experiments revealed a single population of β_2-adrenoceptors in rat cerebellum, but β-adrenoceptors in the cortex consisted of β_1 (66%) and β_2 (34%) subtypes. The values ± s.e. mean for cortical β_1- and β_2-adrenoceptors were respectively: Bmax (fmols/mg protein) 77 ± 7, 40 ± 5; Kd (nM) 1.1 ± 0.4, 0.78 ± 0.1, n = 10 and are in agreement with data published by Dooley et al (1986). After 10 days administration, β_1-, but not β_2-adrenoceptors, were reduced in rat cortex by all treatments: BTS 54 524 (47%, $P<0.01$); amitriptyline (28%, $P<0.01$); DMI (45%, $P<0.01$); zimeldine (27%, $P<0.05$) and ECS (31%, $P<0.01$). After 3 days administration, BTS 54 524 and DMI significantly reduced β_1-adrenoceptors by (23%, $P<0.05$) and (40%, $P<0.01$), respectively, while amitriptyline and zimeldine were without effect. Cortical β_1-adrenoceptors were also unaltered after rats were given a single ECS.

Viewed overall, the data show that antidepressant drugs which are reuptake inhibitors of 5-HT and noradrenaline (amitriptyline), noradrenaline (DMI) and 5-HT (zimeldine) and ECS, which does not inhibit monoamine reuptake (Minchin et al, 1983), all induce β-adrenoceptor desensitization by reducing β_1-, but not β_2-adrenoceptors. Furthermore, BTS 54 524, previously reported to rapidly decrease cortical β-adrenoceptors (Buckett et al, 1987) effects this solely by reduction of the β_1-adrenoceptor population, the β-adrenoceptor subtype which was diminished by all of the other antidepressant treatments tested.

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[^{3}H]UK14,304 BINDING TO RAT BRAIN MEMBRANES IN PHYSIOLOGICAL SALT SOLUTION

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Extensive use has been made of Tris-based incubation media in ligand-binding studies on alpha-adrenoceptors. In binding studies involving the selective alpha$_2$-agonist (^{3}H)UK14,304 the addition of certain divalent cations has been shown to more than double the ligand's specific binding capacity (Loftus et al, 1984; Gibson et al, 1985) though the concentrations of those cations far exceeds those found in vivo. Doxey et al (1983) reported that the inhibition constants (Ki) of a group of alpha$_2$-antagonists against (^{3}H)Idazoxan in physiological salt solution (PSS) correlated well with pharmacological evaluation of antagonist potency. In the present study a series of antagonists were evaluated for inhibition of binding of (^{3}H)UK14,304 to rat cortical membranes incubated in PSS.

Cortical membranes from rat were prepared as previously described (Doxey et al, 1983). Membranes were suspended in physiological salt solution containing (mM): NaCl 118; KCl 4.8; CaCl2 1.3; KH2PO4 1.2; MgSO4 1.2; and NaHCO3 25, equilibriated at 25°C with 95% O2 / 5% CO2 at pH7.4 before use. They were incubated at 25°C for 30 mins in the presence of 8 or 10nM (^{3}H)UK14,304 alone and in the presence of various concentrations of competing drug.

(^{3}H)UK14,304 bound saturably and specifically to a single population of receptor sites with an equilibrium dissociation constant (Kd) of 11.2 +/- 2.5 nM and a Bmax of 63.7 +/- 2.5 fmoles/mg protein. A set of structurally diverse adrenergic antagonists, with a wide range of potencies at the alpha$_2$-adrenoceptor, were assessed for inhibition of (^{3}H)UK14,304 binding. The Ki values obtained were compared with the IC50 values found by Loftus et al (1984) using (^{3}H)UK14,304 binding to rat cortical membranes in Tris buffer containing $MnCl_2$. The two sets of results did not completely correlate using linear regression ($r = 0.863$ $n = 8$) though the order of potency for the antagonists was the same. The Ki values found in PSS did correlate well, however, with an extended set of Ki values obtained by Doxey et al (1983) and Lane (1987) against (^{3}H)Idazoxan binding to rat cortical membranes in PSS ($r = 0.994$, $n = 12$). The regression equation, Ki (^{3}H)UK14,304 = 499 + 0.470 Ki (^{3}H)Idazoxan obtained from these two sets of data indicates that twice the concentration of antagonists were required to displace (^{3}H)UK14,304 as (^{3}H)Idazoxan. Results show that (^{3}H)UK14,304 binds to high affinity sites on alpha$_2$-adrenoceptors in rat cortical membranes. Further, results provide evidence that binding studies can be successfully performed in physiological salt solution thereby more closely reflecting in vivo conditions.

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LOSS OF SCOPOLAMINE-INDUCED UP-REGULATION OF MUSCARINIC RECEPTORS BY MEYNERT LESIONS IN RAT

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Scopolamine, a selective muscarinic receptor antagonist has been shown, after repeated administration, to increase the density of muscarinic receptors (Russell et al., 1983). In this investigation our objective was to determine if chronic scopolamine treatment has a similar effect on muscarinic receptors of the frontoparietal cortex in rat lesioned at the level of nucleus basalis of Meynert (nBM).

Anaesthetized male Sprague Dawley rats (280-300 g) were stereotaxically (A 0.5 ; L ± 2.8 and H 6.8 as defined by König and Klippel (1967)) injected in the nBM (mono or bilateral) with 0.5 µl ibotenic acid 25 nmoles in Na phosphate 50 mM, pH 7.4 buffer. Sham-operated rats were similarly treated except that no neurotoxin was injected. The lesions resulted in a reproducible depletion of choline acetyl transferase (ChAT) in the frontoparietal cortex (Fonnum, 1975) of 35-50 %. Some animals were administered scopolamine bromide (SCOP) (10 mg/kg/day i.p.) for 21 days starting three weeks after the lesion. The animals were sacrified 24 h after the last injection. Muscarinic cholinergic receptor binding was measured using [^{3}H]-quinuclidinylbenzylate ([^{3}H-QNB]) (Watson et al., 1985).

	n	Bmax (fmoles/mg tissue)	ChAT activity (% of control)
Control	16	121 ± 4	100
SCOP-treated control	16	155 ± 6***	100
Sham-operated	22	104 ± 4	100
SCOP-treated Sham-operated	20	126 ± 7**	100
Bilaterally lesioned	21	108 ± 4	51 ± 2
SCOP-treated bilaterally lesioned	19	110 ± 7	54 ± 2
Monolaterally lesioned			
.contralateral side	12	125 ± 7	100
.SCOP-treated contralat.side	12	153 ± 7*	100
.ipsilateral side	12	130 ± 9	63 ± 3
.SCOP-treated ipsilat.side	12	140 ± 10	65 ± 4

Values are mean ± sem ; * p = 0.02 ; ** p < 0.01 ; *** p < 0.001, Student t-test

In intact animals scopolamine treatment resulted in a significant up-regulation. This up-regulation was abolished in rats with lesions of the nBM. In all cases, no significant changes in the affinity (Kd) of [^{3}H]-QNB for the muscarinic receptor were observed. These results suggested that a functional presynaptic cholinergic terminal is necessary for receptor plasticity.

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ANTAGONISM OF SCOPOLAMINE-INDUCED IMPAIRMENT OF SWIMMING MAZE LEARNING IN MICE

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Treatment with cholinergic drugs such as scopolamine impairs learning and memory in animals and man (Anderson and Haubrich, 1981). We therefore determined the effects of scopolamine in mice a) on spatial learning using a spatial learning test, namely a swimming maze based on that described for rats by Morris (1984) and b) on locomotor activity. The ability of selected cholinergic drugs to antagonise the scopolamine-induced effects in both tests was then assessed.

The swimming maze consisted of a white plastic circular pool, 90 cm diameter and 30 cm high, filled to a depth of 14 cm with water (temperature 19-20°C) made opaque with milk powder. The task consisted of learning to escape from the water onto a 6 x 6 cm platform hidden 1 cm below the surface of the water. The latency to locate the platform was recorded, allowing a maximum time of 120 s after which the mouse was placed on the platform. Locomotor activity was monitored in a transparent plastic enclosure (35 x 24 x 35 cm) with infrared photocells positioned 1 cm above the grid floor and spaced 6 cm apart. Distance moved per min over 10 min was measured.

Young, adult, female NMRI mice (24-27 g), housed in groups of 8-10 animals under a 12 h light-dark cycle with lights on at 6.00 h, were injected intraperitoneally (10 ml/kg) with drugs or vehicle each day and subjected to water maze training (3 consecutive trials per day) for at least 3 days followed the next day by measurement of activity.

Scopolamine bromide, 20 min pre-test, increased the escape latencies in the swimming maze, at a dose of 3.0 mg/kg but not 1.0 mg/kg. Both doses caused hyperactivity. However, d-amphetamine sulphate, 3.0 mg/kg, which also caused hyperactivity, did not impair swimming maze learning, nor did methyl scopolamine (3.0 mg/kg), a drug which penetrates poorly into the brain. Physostigmine salicylate, 0.1 and 0.2 mg/kg, in "cocktail" with scopolamine 3.0 mg/kg, caused dose-related antagonism of scopolamine-induced deficits in the swimming maze but did not affect scopolamine-hyperactivity despite an hypoactive effect per se. Oxotremorine sesquifumarate, 0.02 mg/kg but not 0.01 mg/kg, 10 min prior to scopolamine, antagonised the deficits in the swimming maze but not the hyperactivity despite hypoactivity per se. In contrast, the peripherally acting cholinergic drug, neostigmine, was inactive against scopolamine in either test at 0.1 mg/kg.

These results show that scopolamine impairs spatial learning in a swimming test in mice. This effect appears to be mediated centrally and can be dissociated from drug-induced hyperactivity. Moreover, the scopolamine-induced deficits in the swimming maze test can be reduced by pretreatment with cholinergic drugs.

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THE EFFECTS OF 4-AMINOPYRIDINE ON ACETYLCHOLINE RELEASE IN VIVO AND IN VITRO

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4-aminopyridine (4-AP) has been shown to block voltage dependent potassium channels and to enhance acetylcholine (ACh) release in vitro (Dolezal and Tucek, 1983; Thesleff, 1980). However Casamenti et al (1982), using the cortical cup technique in conscious rats, did not find that 4-AP increased ACh release in vivo. We have compared the effects of 4-AP and potassium on ACh release from cortical slices in vitro and on in vivo ACh release as measured by intrastriatal dialysis in conscious rats.

The release of endogenous ACh from cortical slices was measured using a procedure similar to that described by Auerbach and Lipton (1985). Slices were pretreated with 1mM dichlorvos to inhibit cholinesterase, and incubated in the presence of K^+ either 1mM (basal) or 35mM (stimulated). ACh concentrations were measured using a radioreceptor assay with [^{3}H]-oxotremorine as ligand. For in vivo experiments male Lister Hooded rats weighing 180-220 g were implanted in the right striatum with a guide cannula under Sagatal anaesthesia (co-ordinates: IB-0.33; AP 5.9; L 3.0; V 3.0). Rats were allowed at least 24 hours to recover from the anaesthesia before perfusion began. The dialysis probe was inserted such that the dialysis surface was exposed approximately 3 mm past the tip of the guide. The probe was attached to a Watson Marlow 202U pump with the perfusion rate set to 2µl.min^{-1}. Samples were collected every 20 minutes and frozen on solid CO_2 for later analysis. The perfusion solution consisted of NaCl 120mM; KCl 2.56mM; $MgCl_2$ 1.2mM and $CaCl_2$ 1.2mM plus 2µM of neostigmine bromide. Under these conditions recovery of ACh from the probe was 12±1.4% (n=4). High K^+ (125mM) or 4-AP (100µM) were perfused for 20 minutes and the results are shown in the table. 4-AP (100µM) produced a four-fold increase of ACh in the extracellular fluid and a similar effect was seen with K^+. This stimulation was abolished when neostigmine was removed from the perfusate.

The Effects of K^+ and 4-AP on In vivo and In vitro ACh release

	Ach release (pmol)		
	Basal	K^+	4-AP (100µM)
In vitro (2min)	1.1 ± 2.4 (4)	12.7 ± 1.8 (3)	5.8 ± 6.4 (3)
In vivo (20min)	4.9 ± 0.9 (5)	15.7 ± 1.1 (3)	18.6 ± 3.9 (4)

(n) = number of experiments. Data are mean ± s.e.m.

4-AP (in the presence of 1mM K^+) stimulated ACh release from cortical slices although this was not as effective as high K^+ (table). The present results suggest that 4-AP can stimulate ACh release both in vivo and in vitro. Intracerebral dialysis combined with a sensitive radioreceptor assay may be useful for studying the interactions of centrally acting drugs with cholinergic systems.

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WY 27587: A NOVEL AND HIGHLY SELECTIVE INHIBITOR OF 5-HT UPTAKE IN VITRO AND IN VIVO

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Wy 27587 (N-[[[1-[(6-fluoro-2-naphthalenyl)methyl]-4-piperidinyl]amino]carbonyl]-3 pyridine carboxamide) is a novel analogue of the previously described 5-HT uptake inhibitor, panuramine (Blurton et al., 1984). In this report we compare the potency and selectivity of Wy 27587, both in vitro and in vivo, with several of the most selective 5-HT uptake inhibitors currently available.

Male Sprague-Dawley rats (300-400g) and female T/O mice (22-30g) were used. The effects of inhibitors on the active uptake of ^{14}C-5-HT and ^{3}H-noradrenaline (NA) were examined in rat brain cortical synaptosomes (Wood and Wyllie, 1981). The attenuation of p-chloroamphetamine (pCA)-induced '5-HT syndrome' (Fletcher and Forster, 1985) and potentiation of 5-hydroxy-L-tryptophan (5-HTP)-induced syndrome in the rat were employed as indices of 5-HT uptake inhibition in vivo. Reversal of reserpine-induced hypothermia and tetrabenazine-induced ptosis in the mouse were used to assess catecholamine uptake inhibition in vivo. The effects of drugs on amphetamine-induced stereotypy in the rat (Quinton and Halliwell, 1963) were also examined. Antagonism of oxotremorine-induced tremor, salivation and hypothermia in the mouse was used to assess anticholinergic actions in vivo. The overt effects of high doses (up to 400mg/kg p.o.) of uptake inhibitors in mice and rats were also examined.

Wy 27587 potently and selectively inhibited synaptosomal 5-HT uptake with IC_{50} values (mean ± s.e.m.) of 17.5 ± 3.9 nM (5-HT) and 5620 ± 1200 nM (NA). In vivo, Wy 27587 was the most potent uptake inhibitor examined, possessing ED_{50} values (with 95% conf. limits) of 2.8 (0.9-6.1) and 5.0 (2.2-11.0) mg/kg p.o. for pCA-inhibition and 5-HTP potentiation respectively. At doses up to 100 mg/kg p.o. Wy 27587 did not reverse reserpine-induced hypothermia or tetrabenazine-induced ptosis. Amphetamine-induced stereotypy in the rat was not facilitated by Wy 27587 up to a dose of 50 mg/kg p.o. Wy 27587 slightly attenuated oxotremorine-induced tremor and salivation (by 18% and 48% respectively) at a lowest effective dose of 100 mg/kg p.o. At high doses the major overt effects of Wy 27587 were sedation and cutaneous vasodilatation which occurred at doses (>50 mg/kg p.o.) in excess of those required for maximal 5-HT uptake inhibition in vivo. In contrast, zimelidine, fluvoxamine, citalopram, femoxetine, fluoxetine and paroxetine all significantly antagonised tetrabenazine-induced ptosis at doses within the range required for 5-HT uptake inhibition; although the effect of citalopram was non-dose related and was significant only at 10 mg/kg p.o. Paroxetine and femoxetine also significantly reversed reserpine-induced hypothermia. With the exception of Wy 27587, all 5-HT uptake inhibitors significantly potentiated and prolonged amphetamine-induced stereotypy in the rat. In addition, zimelidine, fluoxetine, femoxetine, and paroxetine markedly reduced oxotremorine-induced behaviours at doses similar to those required for inhibition of 5-HT uptake.

We conclude that Wy 27587 is a highly potent and selective inhibitor of 5-HT uptake both in vitro and in vivo; suggesting that the compound may be effective in clinical conditions responding favourably to 5-HT uptake inhibitor therapy (Asberg et al. 1986).

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NITRENDIPINE PREVENTS THE ETHANOL WITHDRAWAL SYNDROME, WHEN ADMINISTERED CHRONICALLY WITH ETHANOL PRIOR TO WITHDRAWAL

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Chronic administration of ethanol increased the B_{max} of dihydropyridine-sensitive binding sites and the actions of the dihydropyridines (eg. Dolin *et al.*, 1987) and acute administration of calcium channel antagonists prevented the ethanol withdrawal syndrome (Little *et al.*, 1986), suggesting a causal relationship between the binding site changes and dependence on ethanol. This was supported by the fact that the dihydropyridine calcium channel antagonist, nitrendipine, given concurrently with ethanol in chronic treatment, prevented the development of tolerance to ethanol (Little & Dolin, 1988) and the increase in dihydropyridine binding sites. We have now studied the ethanol withdrawal syndrome, when nitrendipine was given during the chronic ethanol treatment. It was injected until 24h before withdrawal, so it would not be present in the brain during study of the withdrawal syndrome.

Male mice, TO strain, 30 - 35g, were exposed to ethanol vapour, 15 mg L^{-1}, in an inhalational chamber for two weeks (Gp 1). I.p. injections of nitrendipine, 50 mg/kg or its tween vehicle (0.05%), were given every 12h, except during the 24h before withdrawal. A second group of TO mice (Gp 2) were exposed to ethanol vapour in the same way, with injections given only for the last two days (4 injections), until 24h before withdrawal from ethanol. In the second method (Gp 3), male mice, C57 strain, 25 - 30g, were given ethanol, 24% v/v, as sole fluid, for ten weeks. This ethanol adminstration was continued for another two weeks, with injections, i.p., of nitrendipine, 50 mg/kg, or tween vehicle, given every 12h, until 24h before withdrawal. The ethanol withdrawal syndrome was measured by ratings of convulsive behaviour on handling, expressed as median values, every hour for 12h (Littleton & Little, 1987). The observer was not aware of which prior treatment had been given to the mice. Controls (con) were given vehicle injections alone (i.e. no ethanol). N = 6-8; * $P < 0.01$, cf. ethanol alone, nonparametric analysis of variance (Meddis, 1984).

Table 1. Ratings of convulsive behaviour on handling:

Time:	3h	4h	5h	6h	7h	8h	9h	10h	11h	12h
Gp 1 con:	1	0.5	1	0.5	0.5	1	1	0.5	1	1
eth:	2	2.5	3	2	2.5	2	2	2	1.5	1
eth+nit*:	1	1	1	1	1	1	0	0	1	0
Gp 3 con:	1.5	1	1	1	1	0.5	0.5	0.5	0.5	1
eth:	3	3	3	3	3	4	4	3.5	3	3
eth+nit*:	1	0	0	1	0	1	0	0	0	1

Animals receiving ethanol alone (eth), or ethanol plus 2 days nitrendipine, showed significant convulsive behaviour on handling during ethanol withdrawal. In neither the TO nor the C57 strain was significant convulsive behaviour seen in mice given nitrendipine for two weeks (eth + nit). It is unlikely that nitrendipine was present in the brain during the withdrawal (Little & Dolin, 1987), although this is currently being measured. We suggest that nitrendipine, when given chronically during ethanol administration, can prevent, or reverse, the development of the changes responsible for the ethanol withdrawal syndrome. If this is the case, the effect may be of considerable value in treatment of alcohol dependence.

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AUTORADIOGRAPHIC LOCALISATION OF 5-HT$_{1A}$ BINDING SITES IN THE BRAINSTEM OF THE CAT

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In the cat vagal preganglionic neurones innervating the heart are found predominantly in the nucleus ambiguus (Spyer & Jordan,1987). Recent investigations in anaesthetised cats of the hypotensive action of the 5-HT$_{1A}$ agonists 8-OH-DPAT, ipsapirone and flesinoxan have shown that such drugs can cause an increase in central vagal tone whereas other drugs with central hypotensive actions such as clonidine have no such effect (Bevan et al., 1986; Ramage & Fozard 1987). It is therefore suggested that 5-HT neurones, acting on 5-HT$_{1A}$ receptors provide one of the excitatory drives to cardiac vagal motoneurones. The present experiments were carried out to determine whether 5-HT$_{1A}$ and also if 5-HT$_2$ binding sites are present in the nucleus ambiguus and in other areas of the cat brain stem.

Frozen 20μm serial sections of the cat brainstem were prepared for autoradiography as described previously (Dashwood et al., 1985), and incubated in [^{3}H]-8-OH-DPAT or [^{3}H]ketanserin (2nM) in order to identify 5-HT$_{1A}$ and 5-HT$_2$ binding sites. The degree of binding to non-specific sites was established by incubating in the presence of excess concentrations (1μM) of the above unlabelled ligands as well as prazosin and 5-HT. Our analysis of this material revealed that there was a marked [^{3}H]-8-OH-DPAT binding to a number of brainstem regions, notably the nucleus of the tractus solitarius (NTS), dorsal vagal nucleus (DVM), nucleus ambiguus (NA) and the raphe (fig. 1). Ketanserin also showed dense binding to the NTS and DVM, and weak binding to the raphe. However, ketanserin binding differed from that of 8-OH-DPAT in that it was not observed in the nucleus ambiguus but did show strong binding to the olivary nuclei.

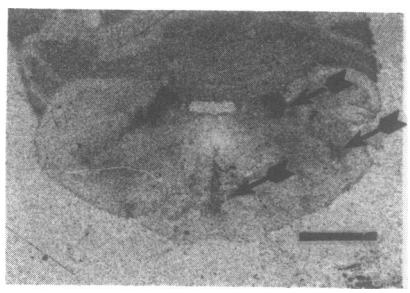

Fig.1. Section of cat brainstem showing binding[^{3}H]-8-OH-DPAT at the NTS and DMV (top arrow), the NA (middle arrow) and the raphe (bottom arrow). Scale bar = 2mm.

These results suggest that both 5-HT$_2$ and 5-HT$_{1A}$ binding sites can be identified in the medial nucleus of the tractus solitarius and the dorsal vagal nucleus whereas those in the nucleus ambiguus would seem to be exclusively 5-HT$_{1A}$. These results would support the views that activation of central 5-HT$_{1A}$ receptors can alter vagal drive and that 5-HT-containing neurones may provide one of the excitatory drives to cardiac vagal motoneurones.

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DECREASE OF HIPPOCAMPAL 5-HT RELEASE FOLLOWING INFUSION OF 8-OH-DPAT INTO THE DORSAL RAPHE

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Evidence from autoradiographic (Verge et al 1985), electrophysiological (Sprouse and Aghajanian 1987) and behavioural (Hutson et al 1986) studies indicate that 5-HT_{1A} receptors are located on 5-HT cell bodies and/or dendrites in the raphe nuclei. However, while systemic administration of 5-HT_{1A} agonists decreases 5-HT release in the hippocampus (Sharp et al 1988) it has not yet been shown that this effect is mediated by the raphe nuclei. We now present evidence that infusing 8-OH-DPAT, a 5-HT_{1A} agonist, into the dorsal raphe nuclei decreases extracellular 5-HT concentration in the hippocampus. Male Sprague-Dawley rats (260-300 g) (Charles River U.K. Ltd.) were anaesthetised with chloral hydrate (400mg/kg i.p.) and implanted stereotaxically with a dialysis probe in the ventral hippocampus (co-ordinates: A 0.32 mm from the intra-aural line; L 4.6 mm; V 8 mm below dura). The probe was perfused with artificial CSF containing 0.2 µM citalopram (a 5-HT reuptake blocker), at a flow rate of 0.8 µl/min. Samples were collected every 20 min and immediately analysed for 5-HT and 5-HIAA using HPLC with electrochemical detection essentially as described by Sharp et al (1987). Approximately 3 h later values for 5-HT and 5-HIAA were essentially stable (see Table 1) at which point an infusion cannula was lowered into the dorsal raphe (co-ordinates A 0.15 mm; L 0.0 mm; V 6.5 mm) and infused with either 1 µl 0.9% NaCl or 1 µl 0.9% NaCl containing 1 µg 8-OH-DPAT over a period of one minute.

Values for 5-HT and 5-HIAA were essentially unchanged following 0.9% NaCl infusion. However, 1 µg 8-OH-DPAT significantly decreased 5-HT concentration within the first 20 min period when compared with pre-injection values (see Table 1). 5-HT values remained low for at least 1.7 h after infusion. 5-HIAA concentration following 8-OH-DPAT was also lower than pre-injection values although this effect was small and only statistically significant at 60-80 min (Table 1). Thus, infusing 8-OH-DPAT into the dorsal raphe rapidly leads to a prolonged decrease of extracellular 5-HT in the hippocampus, an effect presumably mediated by 5-HT_{1A} somatodendritic autoreceptors.

TABLE 1: Effect of 8-OH-DPAT (1µg) infusion in the dorsal raphe on 5-HT and 5-HIAA concentration in hippocampal extracellular fluid

Time after 8-OH-DPAT (1µg) (min)	5-HT	5-HIAA
	ng/ml	
pre infusion	0.55 ± 0.06	50.4 ± 8.01
0 - 20	0.34 ± 0.06*	44.6 ± 12.5
20 - 40	0.37 ± 0.07	45.2 ± 15.0
40 - 60	0.40 ± 0.11	48.1 ± 17.2
60 - 80	0.33 ± 0.06*	38.8 ± 9.0*
80 - 100	0.33 ± 0.06*	40.1 ± 11.7

Values are means ± SEM (n=4), *$P < 0.05$ compared with pre-infusion values using Dunnetts test following ANOVA with repeated measures.

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BEHAVIOURAL PROFILE OF 1-(2,5-DIMETHOXY-4-IODOPHENYL)-2-AMINOPROPANE (DOI), A SELECTIVE 5-HT_2 AGONIST

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Until recently there has been no agonist with a high selectivity for 5HT2 as opposed to 5HT1 receptors. Glennon et al (1986) reported that (±)DOI is such an agent. We have therefore started to investigate its behavioural properties. Male MF1 mice (20-28g) were tested according to the behavioural profiles of Irwin (1968), revealing dose dependent (0.2-10 mg/kg ip) increases in the following variables:- startle and touch responses, pinna reflex, fearfulness, scratching with self and mutual grooming, sniffing, restlessness, reactivity and twitches (head-shakes). Cyanosis was noted at 10 mg/kg. Subsequent experiments showed head-twitch frequency to be dose dependent from 0.1 mg/kg (7.4 ± 1.0 per mouse counted over alternate minutes from 4th to 16th minute after ip injection) to 1.0 mg/kg (29.3 ± 4.2 twitches per mouse). Higher doses up to 10 mg/kg produced slightly smaller responses. In rats, wet-dog shakes (WDS) were measured from 2 to 32 min; WDS occurred throughought the dose range 0.5-5.0 mg/kg, being maximal (13 ± 1.1) at 5.0 mg/kg. In these rats, flat body posture occurred intermittently at 5 mg/kg only (score intensity 2 over scale 0-3), together with forepaw treading (score 1). There was no other sign of 5HT1 receptor stimulation. Chewing was also seen at 5 mg/kg (score 1) and intermittent tremor of forepaws throughout the dose range.

Since apparent signs of fear were seen in mice, rats were tested in the elevated X-maze test of anxiety (Handley & Mithani, 1984), 20 min after .05-1.0 mg/kg ip. A significant anxiolytic-like increase in entry ratio was seen after 0.1mg/kg (0.44 ± 0.01 cf saline 0.35 ± 0.02) and 1.0mg/kg (0.44 ± 0.03 cf saline 0.37 ± 0.01) but was not dose-dependent. There were no consistent changes in total activity. It was striking that, while in the X-maze, rats showed WDS in the enclosed arms and centre square at 0.5-1.0 mg/kg but no WDS occurred in the open arms at any dose.

The behavioural profile of DOI is consistent with selective 5-HT2 receptor stimulation at doses below 5 mg/kg in both rats and mice. Signs of probable 5-HT1 receptor stimulation occurred at higher doses and the chewing behaviour in rats may be dopaminergic. Studies with selective antagonists will be required to confirm these findings. The anxiolytic-like effects will also require further investigation, especially since we have reported the specific 5HT2 antagonist ritanserin also to be anxiolytic in the X-maze (Critchley & Handley, 1987).

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PREVENTION OF 8-OH-DPAT ANXIOGENIC EFFECT BY IPSAPIRONE AND BY 5-HT_1 ANTAGONIST β-ADRENOCEPTOR ANTAGONISTS

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We have previously reported that the 5-HT1A agonist 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT) causes an anxiogenic-like fall in the entry ratio in the elevated X-maze. Pindolol,on the other hand, raised the entry ratio and prevented the effect of 8-OHDPAT (Critchley & Handley, 1987). Pindolol is an antagonist at both beta adrenoceptors and 5-HT1 receptors (Nahorski & Willcocks 1983) Since beta-blockers are anxiolytic in man, the above profile of pindolol could be due to effects at either 5-HT1 or beta receptors. We have therefore investigated several other beta adrenoceptor antagonists for their effects in the elevated X-maze. Male hooded PVG rats (200-260g) were tested as described by Handley & Mithani (1984). Drugs were injected ip 20 min before 8-OH-DPAT 0.05-0.1 mg/kg or saline (ip) and exposed to the maze 10 min later.

As shown in Table 1, only pindolol among the beta adrenoceptor antagonists showed any anxiolytic effect of its own. Despite this, (-) alprenolol, (±)timolol as well as (±) pindolol, mixed beta-adrenoceptor and 5-HT1 antagonists Nahorski & Willcocks, 1983), antagonised 8-OHDPAT anxiogenesis as did the 5-HT1A partial agonist ipsapirone. On the other hand metoprolol and ICI 118,551, highly selective antagonists of beta1-and beta2-adrenoceptors only, were inactive although the doses used have been shown to have central effects (Handley & Singh, 1986). The dose range of buspirone, another 5-HT1A partial agonist was severely limited by sedative-like falls in total entries above .05 mg/kg.

Table 1. Effects on open/total ratio Mean (s.e.m.)

drug/ dose(mg/kg	A: vehicle B: vehicle	vehicle drug	8-OHDPAT vehicle	8-OHDPAT drug	F(AxB) - ANOVA----	df	p
pindolol .1	.33(.01)	.44(.02)**	.13(.02)**	.28(.03)	1.2	1/44	<.01
alprenolol.5	.31(.03)	.33(.01)	.06(.02)**	.14(.02)*	2.2	1/16	<.05
timolol 40	.37(.01)	.35(.03)	.23(.04)**	.34(.02)	6.9	1/16	<.01
metoprolol 3	.30(.01)	.31(.02)	.11(.03)**	.17(.05)*	.65	1/16	ns
ICI 118551 1	.31(.01)	.31(.01)	.25(.01)*	.25(.01)*	.06	1/16	ns
ipsapirone 1	.33(.02)	.47(.02)**	.21(.02)**	.36(.02)	8.3	1/10	<.01
buspirone .05	.33(.01)	.33(.02)	.11(.01)**	.06(.03)**	.86	1/10	ns

*p<.05 **p<.01 vs vehicle/vehicle control (from ANOVA)

These results suggest that the ability of the mixed beta1/beta2 antagonists to prevent the anxiogenic effect of 8-OH-DPAT is due to their effects at 5-HT1A receptors and not to their actions at beta adrenoceptors. Ipsapirone was anxiolytic in the X-maze and appears to act as a 5-HT1A antagonist in this test.

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THE ANTIEMETIC ACTIONS OF 5-HT_3 RECEPTOR ANTAGONISTS ARE ENHANCED BY FENFLURAMINE

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Agents which disrupt 5-hydroxytryptamine (5-HT) function by acting as 5-HT_3 receptor antagonists or to inhibit the synthesis or release of 5-HT can antagonise the emesis induced by cytotoxic agents (Miner and Sanger, 1986; Barnes et al., 1987, 1988). In the present study we investigate whether a combined treatment of such agents to reduce 5-HT function and antagonise cisplatin induced emesis in the ferret is more effective than either treatment given alone.

Male ferrets (1.5-2.0kg) were injected with dl-fenfluramine (F, 1.0mg/kg i.p.) or vehicle and subsequently anaesthetised with halothane (N_2O/O_2 carrier). A cannula was inserted into the jugular vein and, 1h after the injection of dl-fenfluramine, i.v. injections of vehicle (1ml/kg) or metoclopramide (M, 1.0mg/kg) or ICS205-930 (ICS, 0.01mg/kg) were followed immediately by the i.v. injection of cisplatin (10mg/kg). On recovery from the anaesthetic (15-20 min) the time to onset of emesis and the number of episodes, vomits and retches were recorded over a 4h period.

Table 1. The interaction between dl-fenfluramine, metoclopramide and ICS205-930 to antagonise cisplatin-induced emesis.

Treatment	Onset (min)	Episodes	Retches	Vomits
Cisplatin (C)	73± 5	13±2	59±20	13±2
F + C	81±10	15±4	85±18	16±5
ICS + C	93± 9	8±1	42±13	7±1*
F + ICS + C	155±33*	3±1*	11± 6*	2±1*
M + C	93±10	14±5	55±19	13±5
F + M + C	177±37*	2±1*	7±4*	1±1*

Each value is the mean ±S.E.M. of 5 determinations. Significant differences compared to control values are indicated *P<0.05 (Mann-Whitney) (onset taken as 240 min if animal completely protected against emesis).

The doses of dl-fenfluramine, metoclopramide and ICS205-930 were selected on the basis of preliminary experiments as either failing or having little action in their own right to antagonise cisplatin-induced emesis. dl-fenfluramine (1.0mg/kg i.p.) failed to antagonise cisplatin-emesis with a trend to delay the onset of emesis and increase the number of episodes, retches and vomits. Metoclopramide (1.0mg/kg i.v.) also failed to antagonise cisplatin-induced emesis. It was found more difficult to regulate the dose of ICS205-930 but whilst the use of 0.01mg/kg i.v. caused some reduction in emesis, only the reduction in the number of vomits achieved significance. In combination with ICS205-930 or metoclopramide, dl-fenfluramine delayed the onset of emesis and reduced the number of emetic episodes, retches, and vomits, the significance of the changes being shown on Table 1.

It is concluded that dl-fenfluramine can enhance the actions of metoclopramide and ICS205-930 to antagonise cisplatin-emesis and that such effects reflect a disruption of 5-HT function.

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[^{3}H]-ZACOPRIDE IDENTIFIES 5-HT$_3$ BINDING SITES IN RAT ENTORHINAL CORTEX

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The identification and distribution of 5-HT$_3$ receptors in rat brain has been determined in radioligand binding studies using ^{3}H.GR65630, an indole derivative with potent antagonist action on 5-HT$_3$ receptors in peripheral tissues (Kilpatrick et al., 1987). A particularly high level of binding of ^{3}H.GR65630 was found in the entorhinal cortex and in the present studies we further investigate the nature of 5-HT$_3$ binding sites in this brain area using ^{3}H.Zacopride, a potent 5-HT$_3$ receptor antagonist from the substituted benzamide series (Smith et al., 1988).

Tissues from the entorhinal cortex of male Hooded Lister rats (200-225g) were pooled (70 mg/rat) and homogenised (Polytron, setting 7 for 10 sec) in 20 vol HEPES buffer (50 mM) pH 7.4 also containing the constituents of Krebs' buffer and centrifuged (48000 x g) at 4°C for 10 min. The supernatant was discarded and the process repeated for the pellet. The pellet was suspended in the above buffer and for binding, assay tubes contained 500 µl of membrane suspension (0.1-0.15 mg protein), 50 µl ^{3}H.Zacopride (54.9 Ci/mmol) and 50 µl of displacing agent (used in a range of 15 concentrations, 10^{-10}-10^{-4}M) or buffer to a final volume of 600 µl. Tubes were incubated for 15 or 20 min at 37°C and the incubation terminated by rapid filtration through Skatron glass fibre filters which were washed immediately with 3.5 ml buffer solution. All individual assays were carried out in replicates of three and results are the means ± S.E.M. of at least three separate experiments.

Using the 5-HT$_3$ receptor antagonist BRL 43694 (10 µM) to define specific binding, Scatchard analysis of ^{3}H.Zacopride (0.05 - 5.0 nM) binding revealed a single saturable site of high affinity (K_D of 0.76 ± 0.08 nM) and a B_{max} of 77.5 ± 6.5 fmol/mg protein with Hill slopes close to unity (0.97 ± 0.02). Displacements of the binding of 0.25 nM ^{3}H.zacopride were recorded using zacopride and other 5-HT$_3$ receptor antagonists, GR38032F, ICS 205-930, metoclopramide and cocaine, with K_i values of and 1.98 ± 0.56, 4.77 ± 0.32, 2.01 ± 1.92, 326 ± 66 and 3336 ± 826 nM respectively, the displacement curves indicating a single binding site. ^{3}H.zacopride was also displaced by the 5-HT$_3$ receptor agonists 5-HT and 2-methyl-5-HT with K_i values of 642 ± 188 and 1128 ± 458 but not by the 5-HT$_1$/5-HT$_2$ receptor agonists (+)S-α-methyl-5-HT and 5-carboxamidotryptamine. The 5-HT$_1$/5-HT$_2$ receptor antagonists ritanserin, methysergide, mesulergine and methiothepin, and other compounds mepyramine, ranitidine, idazoxan, prazosin, propranolol, fluphenazine, sulpiride, SCH23390, atropine, hexamethonium, fenfluramine, naloxone, chlordiazepoxide, GABA, glycine, dopamine, noradrenaline and histamine had Ki values greater than 10^{-5}M.

The data indicates that ^{3}H.zacopride labels with high affinity a single population of binding sites in the rat entorhinal cortex which recognise 5-HT$_3$ receptor agonist and antagonist compounds. The data supports the findings of Kilpatrick et al. (1987) and indicates that ^{3}H.zacopride may prove a useful tool in a study of 5-HT$_3$ recognition sites.

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FURTHER EVIDENCE THAT NAFION COATED CARBON FIBRE ELECTRODES MEASURE EXTRACELLULAR 5-HT IN VIVO

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We have recently reported that a new chemically and electrically pretreated Nafion coated carbon fibre electrode (Nafion CFE) can monitor basal extracellular levels of serotonin (5HT, Peak B at 250 mV) in the frontal cortex (FC) and the dorsal raphe nucleus (DRN) of anaesthetised rats (Crespi et al 1987). This study compares the in vitro and in vivo characteristics of the original 12 μm CFEs, which detect 5-hydroxyindoleacetic acid (5-HIAA) in vivo (Peak 3 at +300 mV) (Crespi et al 1984) with the new Nafion CFEs to further confirm the chemical identity of Peak B in vivo.

Single carbon fibres (30 μm or 12 μm in diameter) were used to prepare CFEs as previously described (Crespi et al 1984, 1987). The 30 μm carbon fibres were first treated with chromic acid; then both types of CFEs were electrically pretreated in phosphate buffer saline (PBS) 0.1 M pH 7.4 (Crespi et al 1987). Finally the 30 μm CFEs were electrically coated 4 times with Nafion, a sulphonate polymer which repels anions and is selectively permeable to cations (Nagy et al 1985).

As previously reported with 12 μm CFEs 3 peaks corresponding to the oxidation of ascorbate (Peak 1), DOPAC (Peak 2) and 5HIAA (Peak 3) were observed in vitro but only two peaks were observed with the Nafion CFEs due to the oxidation of dopamine (Peak A) and 5-HT (Peak B) (Crespi et al 1984, 1987).

Anaesthetised rats (chloral hydrate 500 mg/Kg ip) were held in a stereotaxic frame and prepared for differential pulse voltammetric (DPV) recordings in the FC. Injection of the MAO inhibitor pargyline (100 mg/Kg ip) increased Peak B by 65 ± 20% (n=5). However Peak 3 was decreased by 70% (n=5) within 70 min of injection. Local infusion of p-chloroamphetamine (4μg in 2μl) into the FC, close to the working electrode, increased Peak B height to a maximum of 200 ± 30% (n=5), but did not significantly change the size of Peak 3 recorded in a separate group of 5 rats. In a second series of experiments, Nafion CFEs were implanted into the left FC and intracerebral dialysis loops into the contralateral FC of six rats pretreated 4 weeks previously with 5,7-dihydroxytryptamine (5,7-DHT, 2 x 200μg in 5 μl infused bilaterally into lateral ventricles). In these 5,7-DHT lesioned rats we found that both Peak B and 5-HT levels in the dialysate, (measured by HPLC-ECD) increased by between 70 and 80% of controls in the 60 min after administration of fenfluramine (10 mg/Kg ip), whereas fenfluramine increased extracellular 5HT by 200% (measured by dialysis) in non-lesioned rats.

These results further support the view (Crespi et al 1987) that Peak B recorded in vivo at +250 mV with a Nafion CFE is the result of the oxidation of 5HT.

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5-HT-INDUCED CURRENTS IN NCB-20 NEUROBLASTOMA HYBRID CELLS AND THEIR BLOCKADE BY A 5-HT_3 RECEPTOR ANTAGONIST

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Depolarising responses to 5-HT occur in various neurones in the peripheral nervous system. Several neuronal clonal cell lines also express a depolarising response to 5-HT which appears to be mediated by the 5-HT_3 receptor subtype (Neijt et al 1987). Here we report some properties of 5-HT-induced currents recorded from NCB-20 neuroblastoma x Chinese hamster brain cell hybrids maintained in cell culture (MacDermott et al 1979).

NCB-20 cells were grown in Dulbecco's modified Eagle's Medium supplemented with foetal calf serum (5% vol/vol), penicillin/streptomycin (50 iu ml^{-1} - 50 µg ml^{-1}) and dibutyryl cyclic-AMP (1 mM) for 3-8 days before use in experiments. Constant current and voltage clamp recordings were performed using the patch clamp technique (Hamill et al 1981). 5-HT was applied to whole cells and isolated outside-out patches by either pressure ejection from modified patch pipettes containing 10 µM 5-HT creatinine sulphate, or by ionophoresis from pipettes filled with 20 mM 5-HT in double distilled water (pH 3.5-4).

Under current clamp conditions 5-HT elicited a membrane depolarisation, associated with a conductance increase. When voltage-clamped at -60 mV and dialysed with a pipette solution containing CsCl, cells responded to 5-HT with an inward current which increased with hyperpolarization and reversed in sign at 0mV. Under such conditions, the currents displayed inward rectification and declined in the continued presence of 5-HT. The selective 5-HT_3 antagonist GR38032F (Brittain et al 1987) at a concentration of 1 nM, reversibly reduced whole cell currents evoked by 5-HT to 7.0 ± 3.3% (mean ± s.e., n = 6) of their control value.

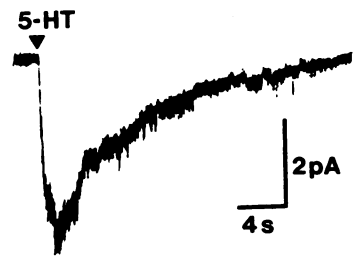

Figure 1. Transmembrane current recorded from an outside-out patch, at a holding potential of -60 mV in response to pressure applied 5-HT (10µM, 100 ms, 1.4 x 10^5 Pa).

On isolated outside out membrane patches voltage-clamped at -60 mV, 5-HT evoked an inward current of -2 to -8 pA which was suppressed by GR38032F (1nM). Despite the high resolution of these recordings discrete single channel events were not discernible during such inward currents (Figure 1). Future experiments will investigate the possibility that 5-HT activates an ion channel of small conductance in NCB-20 cells in a manner analogous to the actions of glutamate on cerebellar neurones (Cull-Candy and Ogden, 1985).

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CHARACTERISATION OF DOPAMINE D_2 RECEPTORS REGULATING TYROSINE HYDROXYLASE IN STRIATAL SLICES

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Although the regulation of striatal tyrosine hydroxylase by dopamine receptors has been disputed for several years, recent studies examining the activity of the enzyme isolated from striatal slices or synaptosomes strongly support the concept of dopamine autoreceptors regulating the synthesis of the catecholamine (El Mestikawy et al. 1986; Strait & Kuczenski, 1986). In attempts to investigate the intracellular mechanisms secondary to dopamine receptor activation we have directly examined tyrosine hydroxylase activity in striatal slices by assaying dihydroxyphenylalanine (DOPA) accumulation following inhibition of DOPA decarboxylase.

Rat striatal slices (350 x 350 µM) were preincubated for 30 min prior to incubation with 30 µM NSD-1055 (p-bromo-benzyl-oxamine) and assay of tissue DOPA using HPLC-electrochemical detection. Under these conditions, production of DOPA (2-3 pmoles/min/mg protein) was linear with time for at least 50 min, was not enhanced by exogenous tyrosine but was suppressed by 80-90% ($P < 0.001$) in the presence of α-methyl-p-tyrosine (50 µM).

Incubation of slices with the D_2 agonist quinpirole maximally reduced the rate of tyrosine hydroxylation by 50-60% ($P < 0.001$) and displayed an EC_{50} of 10 nM. A similar maximal inhibition was observed with pergolide though this ergot displayed greater potency (EC_{50} 2 nM). On the other hand, 7-hydroxy-2-(N,N-dipropyl-amino) tetralin (7-OHDPAT) maximally reduced tyrosine hydroxylation to a greater (80-90%) extent. The D_2 antagonist sulpiride potently and stereo-specifically reversed the inhibition produced by either quinpirole or 7-OHDPAT whereas the D_1 antagonist SCH 23390 ([R]-(+)-8-chloro-2,3,4,5 tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol) was ineffective at concentrations up to 1 µM. In kinetic experiments this D_2-mediated effect was rapid in onset, showed no apparent desensitization over 60 min, but was immediately reversed by sulpiride.

Incubation of slices with exogenous dopamine (10^{-7}-10^{-4} M) dose-dependently and completely suppressed DOPA synthesis but these effects were not reversed by sulpiride. This data is complicated by the uptake and direct effect of this catecholamine on tyrosine hydroxylase since in the presence of the uptake inhibitor GBR 12921 (1 µM), sulpiride (300 nM) suppressed the effects of dopamine (10^{-7}-3×10^{-6}).

These results demonstrate that dopamine D_2 receptors regulate striatal tyrosine hydroxylase in the intact slice and show that this relatively simple assay system can provide a detailed pharmacological characterisation of this response. It should also allow investigation of the potential intracellular mechanisms underlying this D_2 receptor-mediated effect.

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A SELECTIVE ROLE OF MUSCARINIC RECEPTORS IN $K^{\pm}$-EVOKED INOSITOL TETRAKISPHOSPHATE ACCUMULATION IN CEREBRAL CORTEX

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High extracellular K^+ induces phosphoinositide hydrolysis in several tissues including brain though it has been difficult to separate direct and indirect effects of depolarization. We have recently shown however, that K^+ stimulates a different pattern of inositol phosphate production than the muscarinic agonist carbachol (Baird & Nahorski, 1986). The present studies suggest that only accumulated inositol tetrakisphosphate may be secondary to released endogenous acetylcholine.

Inositol phosphate production was assayed in rat cerebral cortical slices as described previously (Baird & Nahorski, 1986). Briefly, after incubation with ^{3}H-inositol, and addition of agonist(s), the reaction was terminated by addition of PCA (10%) and the inositol phosphates extracted and neutralised using freon/ octylamine.

K^+, present at a total concentration of 24 mM, evoked an increased production of all inositol phosphates, as reported previously (Baird & Nahorski, 1986). Maximal effects were observed after 5 to 10 min incubation, with an approx. 2.5-3-fold increase in IP_2 and IP_3, 1.5-fold for IP_1 but over a 5-fold increase in levels of InsP4 occurred. In the presence of the muscarinic antagonist atropine (10 μM), K^+ elicited increases in IP_1, IP_2 and IP_3 which compared to controls were only marginally influenced at 5 min. In contrast, atropine inhibited K^+-stimulated production of inositol tetrakisphosphate (InsP4) by 50%. Thus, K^+-induced production of InsP4 could be 50% accounted for by muscarinic receptor stimulation via endogenous acetylcholine release.

In an attempt to find out whether any other receptor-activating substances were being released by K^+, various antagonists were added. Prazosin, ketanserin, or mepyramine (all 1 μM), unlike atropine, did not affect K^+ stimulated production of the inositol phosphates. Thus, the effects of K^+ were not being produced by α_1, $5HT_2$ or histamine (H_1) receptor interactions. Furthermore, none of these agents affected the atropine-insensitive portion of the K^+-induced inositol phosphate production.

These results provide evidence that in the presence of depolarizing concentrations of K^+, stimulated inositol phosphate production consists of at least two components. Firstly, there is an atropine-sensitive component, which presumably is due to release of endogenous acetylcholine. This is most apparent in the production of InsP4 probably because of the sensitivity of its formation to muscarinic stimuli under depolarizing conditions. Secondly, the major portion of inositol phosphate production in cerebral cortical slices is likely to be due to entry through voltage-sensitive Ca^{2+} channels, directly stimulating phosphoinositide hydrolysis with some preference towards phosphatidylinositol 4-phosphate. Since it is possible that these occur in different cells, the results emphasise the complexity of using a tissue containing heterogenous cell populations.

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BOMBESIN : EFFECTS ON BEHAVIOUR AND FOOD INTAKE IN NEONATAL RATS

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Bombesin is a tetradecapeptide which was originally isolated from the skin of the European frog Bombina bombina. Since its discovery, endogenous bombesin-like peptides have been detected in rat CNS by radioimmunoassay (Brown et al., 1978 ; Moody & Pert, 1979) and specific high affinity binding sites for bombesin have been characterized in rat brain membranes (Moody et al., 1978). Moreover, bombesin has been shown to produce a number of effects in adult rats, for example, it induces excessive scratching/grooming behaviour (Gmerek & Cowan, 1983) and decreases food intake (Gibbs et al., 1979). The pharmacological profile of bombesin in neonatal rats has not been investigated, hence we have examined the behavioural responses to the peptide in 5-, 10- and 20-day old pups and determined its effects on food intake in preweanling rats.

The behaviours of individually-housed Wistar rat pups (male and female) were scored as previously described (Jackson & Kitchen, 1987). Food intake was measured as the change in body weight of 15-day old pups given access to wet mash (powdered rat diet and water) after 3 h separation from the dam. Bombesin (Sigma) was dissolved in 0.9% NaCl and injected i.p. in a dose volume of 0.1 ml/20 g body weight using a blind protocol. Treatment group means (n = 6-8) were statistically compared using analysis of variance and the Mann Whitney U test or Dunnett's test as appropriate.

Bombesin (10 mg/kg) produced a significant increase in scratching and grooming behaviour in 5-, 10- and 20-day old rats. A lower dose of bombesin (1 mg/kg) induced scratching, but not grooming behaviour, in the 5- and 10-day old pups. Locomotion, rearing, wall-climbing, gnawing, sniffing, yawning and stereotyped mouthing behaviours were not significantly altered by 0.1 - 10 mg/kg doses of the peptide. Control 15-day old rats consumed a wet mash diet following 3 h food deprivation (separation from the mother). Low doses of bombesin (0.1, 1 mg/kg i.p.) attenuated food intake and produced a significant drop in body weight which was still evident 3 h after drug administration.

The results of this study show that bombesin produces scratching and grooming behaviour in rat pups as early as 5 days after birth. Furthermore, the peptide is capable of producing satiety in animals which would normally still be suckling from the mother. This pharmacological profile accords with the ontogenesis of receptors for bombesin-like peptides in rat CNS (near maximal levels are reached by day 10) but appears unrelated to peptide development since only low concentrations of bombesin-immunoreactivity may be detected prior to the third postnatal week (Gillati & Moody, 1984).

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BRAIN UPTAKE OF 2-[^{14}C]-DEOXYGLUCOSE AFTER CHRONIC TREATMENT WITH CHLORDIAZEPOXIDE

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As well as being clinically effective anxiolytic drugs, the benzodiazepines (BDZs) also have sedative, muscle relaxant, anticonvulsant and amnesic properties. With daily injections, rapid (within 5 days) tolerance develops to the sedative, muscle relaxant and anticonvulsant properties of the BDZs, whilst tolerance to the anxiolytic effects generally takes 2-3 weeks to develop in the rat (File, 1985). There is also evidence for a withdrawal syndrome that may occur on cessation of this chronic treatment. In this study we have used the 2-[^{14}C]-deoxyglucose (2-DG) autoradiography technique (Meibach et al, 1980) in an attempt to identify the particular brain regions which may be involved in the various behavioural effects of chlordiazepoxide (CDP) in conscious, unrestrained rats.

Male hooded Lister rats received vehicle control, acute CDP, CDP after 5 or 21 days pretreatment, or withdrawal (24h) after 21 days pretreatment. The dose of CDP was 10 mg/kg i.p. in every case (chronically treated rats received once daily injections i.e. 10 mg/kg/day i.p.). To equate handling, all rats received 21 daily injections of CDP or vehicle. On the test-day, rats received 2-DG (125μCi/kg in saline i.v.) 30 mins after CDP or vehicle, and brains were removed 45 mins later.

Acute CDP reduced 2-DG uptake in virtually every brain area examined in agreement with previous studies with diazepam (Ableitner et al, 1985). Five days of chronic treatment produced tolerance to this reduction in: neocortex, nucleus accumbens, caudate, substantia nigra compacta, dorsal tegmental nucleus and dorsal raphe. It seems unlikely, therefore, that these areas are responsible for CDP's anxiolytic properties but may well mediate some of its other actions such as sedation and anticonvulsant effects. In several other brain areas the development of tolerance to the reduction in 2-DG uptake parallelled the development of tolerance to CDPs anxiolytic effects i.e. CDP was still able to reduce 2-DG uptake after 5 days of pretreatment, but tolerance had developed after 21 days. These included areas of the limbic system (septum, amygdala, hippocampus & mammillary body), thalamus & lateral habenula. Thus they may play a role in mediating CDP's anxiolytic effects. Finally, withdrawal from chronic treatment increased 2-DG uptake in many brain areas reaching significance in cingulate cortex, dorsal tegmental nucleus, dorsal and median raphe, globus pallidus, hypothalamus, dorsal geniculate and the interpeduncular nucleus. Thus, the pattern for withdrawal was very different from the pattern for acute effects and tolerance.

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MK-801 IS A POTENT ANTAGONIST OF NMDA-STIMULATED NORADRENALINE RELEASE FROM RAT HIPPOCAMPUS SLICES

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Dissociative anaesthetics such as phencyclidine (PCP) can block N-methyl-D-aspartate (NMDA)-stimulated release of acetylcholine (Snell and Johnson, 1985), dopamine (Jones et al., 1987a) and noradrenaline (Jones et al., 1987b) from brain slices. PCP, however, displays high affinity for both PCP and sigma sites in the CNS (Quirion et al., 1987). The effects of this drug in blocking NMDA-stimulated neurotransmitter release could therefore be due to an action at either of these putative receptors. MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate) is a non-competitive NMDA antagonist which displays high affinity for the PCP site but low potency at sigma sites (Wong et al., 1986). The present study reports the effects of MK-801 on NMDA-stimulated noradrenaline release from rat hippocampal slices.

Slices of the rat hippocampus were incubated with [^{3}H]-noradrenaline, washed and superfused in Mg^{2+}-free Krebs buffer. After 30 min of superfusion, 10 successive 4 min fractions of the superfusate were collected. NMDA was added at the start of fraction number 3 and was present until the end of fraction 6. Antagonists, where present, were added to the superfusion media 8 min before the exposure to NMDA. NMDA-evoked [^{3}H]-noradrenaline release was estimated by liquid scintillation counting and the effects of antagonists expressed as % inhibition of control NMDA-stimulated release.

NMDA (5-100μM) caused a concentration related release of [^{3}H]-noradrenaline from rat hippocampal slices with a maximum release of 16.5 ± 0.9% (mean ± SEM, n = 12) of tissue stores occurring at 100μM NMDA. PCP (100nM-1μM) caused a concentration related inhibition of 100μM NMDA-stimulated release with an IC_{50} (concentration to cause 50% inhibition of release) of 246nM. NMDA-stimulated noradrenaline release was also blocked by MK-801 (10nM-1μM, IC_{50} 16nM), (-)-MK-801 (30nM - 3μM, IC_{50} 67nM) and 3-((±)-2-carboxy-piperazin-4-yl)propyl-1-phosphonic acid (CPP) (300nM - 30μM, IC_{50} 3970nM).

The relative order of potencies of these drugs as NMDA antagonists (MK-801 > (-)-MK-801 > PCP > CPP) together with the high potency of MK-801 indicate that this site controlling noradrenaline release is of the PCP type (Quirion et al., 1987). The results also further support the claim that MK-801 is a potent NMDA antagonist (Wong et al., 1986).

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THE NEUROTOXICITY OF MPTP AND MPP^+ IN MARMOSET CELL CULTURES

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The neurotoxicity of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) in vivo is considered to be dependent on the biotransformation to MPP^+ (1-methyl-4-phenyl-pyridinium): the selectivity of action for the dopamine neurone may reflect a high affinity for the dopamine uptake system (Javitch et al, 1985). In the present study we further investigate the toxic effects of MPTP and MPP^+ on nigral and other neurones taken from the marmoset using the cell culture technique.

Marmoset brains were removed 48h post-gestation and tissue taken from the area of the substantia nigra, raphe nucleus, caudate nucleus and frontal cortex. After cell dissociation, 0.15 ml of cell suspension was placed into each Corning cell well previously treated with lysine and rat's tail collagen and incubated at 37°C, 100% humidity and 5% CO_2 for 24h. The cells were subsequently grown in a medium of Ham's solution F12/Dulbecco's modification of Eagle medium (50:50) containing insulin (5µg/ml), transferrin (100µg/ml), progesterone (20nM) putrescine (100µM) and selenium (30nM), the media being replaced every 48h and specific for neuronal cell growth. After 7 days the media was replenished with one containing MPTP or MPP^+ for a further 48h. Live and dead cells were identified using ethidium bromide/acridine orange staining techniques.

Table 1. The neurotoxic effects of MPTP and MPP^+ on marmoset cell cultures.

Treatment	Substantia Nigra	Raphe Nucleus	Caudate Nucleus	Frontal Cortex
MPTP				
Control	89	96	85	87
10^{-9}	52	65	82	88
10^{-8}	14	52	44	54
10^{-7}	2	33	29	30
10^{-6}	4	2	2	3
	(2.7×10^{-9})	(1.3×10^{-8})	(2.3×10^{-8})	(2.9×10^{-8})
MPP^+				
Control	88	97	84	88
10^{-11}	47	60	83	78
10^{-10}	22	65	54	54
10^{-9}	10	46	35	36
10^{-8}	7	29	23	22
10^{-7}	5	2	5	3
	(1.3×10^{-11})	(4.2×10^{-10})	(5.6×10^{-10})	(4.4×10^{-10})

Values in parentheses indicate the molar concentrations required to cause the death of 50% of cells.

The results indicate that neuronal cells taken from a number of areas of the marmoset brain are very sensitive to the neurotoxic effects of MPTP and MPP^+, MPP^+ being approximately 100 times more potent than MPTP (Table 1). The most important finding is that cells taken from the area of the substantia nigra are an order of magnitude more sensitive to the neurotoxic actions of MPTP and MPP^+ than the other cells. Therefore the 'in vivo' findings of a particular sensitivity of nigral neurones to the neurotoxicity of MPTP/MPP^+ finds a correlation in the 'in vitro' experiments.

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ANTAGONISM BY MAZINDOL OF MPTP INDUCED NEUROTOXICITY IN RAT CELL CULTURES

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The neurotoxic effects of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) on the dopamine neurone may relate to its oxidation to MPP^+ (1-methyl-4-phenyl-pyridinium) which has high affinity for the dopamine uptake mechanism (Javitch et al. 1985). In the present study we use mazindol, a dopamine reuptake inhibitor, and cell cultures from rat embryo to investigate the importance of dopamine uptake processes to the neurotoxicity of MPTP and MPP^+.

Rat foetuses were taken at 17 days gestation and brain nuclei from the mesencephalon containing the substantia nigra or raphe nucleus were removed and 0.15ml of dissociated cell suspension was added to each Corning cell well, previously treated with poly-L-lysine and rat's tail collagen, and incubated at 37°C, 100% humidity and 5% CO_2 for 24h. The media (Ham's solution F12/Dulbecco's modification of Eagle Medium (50:50), foetal calf serum 10%, penicillin 5000u/ml/streptomycin 5mg/ml, 1ml/100ml, L-glutamine 200mM, 1ml/100ml) was then replaced with fresh media containing mazindol, MPTP or MPP^+, and incubated for 48h. The live and dead cells were identified using ethidium bromide/acridine orange staining and fluorescent microscopy.

Table 1. The effect of mazindol to modify the neurotoxicity of MPTP and MPP^+ in rat foetal tissue cultures.

		'Substantia nigra'	'Raphe nucleus'
		% live cells	
Control		92.9±1.0	94.7±1.3
MPTP 100µM		49.8±0.6***	66.2±1.3**
MPTP plus mazindol	1	69.4±8.4	67.5±3.7*
	10	87.1±3.1^{++}	82.8±4.8
	100nM	90.3±3.5^{++}	77.4±4.2
Control		92.9±1.0	94.7±1.3
MPP^+ 10µM		43.5±1.8***	66.4±2.9**
MPP^+ plus mazindol	1	63.9±5.9*	75.1±3.9*
	10	60.9±2.4**$^+$	59.5±2.7**
	100nM	69.4±0.8**$^{++}$	65.6±3.8*

Each value is the mean ±S.E.M. of 3 determinations. Significant reductions compared to control *P<0.05, **P<0.01, ***P<0.001; antagonism of the effect of MPTP and MPP^+ significant to $^+$P<0.05, $^{++}$P<0.01 (Students t test).

Both MPTP and MPP^+ reduced the live cell count in cell cultures obtained from the rat midbrain containing nigral and raphe cells by approximately 50 and 30% respectively. Mazindol (1-100nM) caused a concentration related antagonism of the effects of MPTP in the 'nigral' cell cultures but caused only a partial antagonism of the effects of MPP^+. In contrast mazindol failed to attenuate the toxic effects of either MPTP or MPP^+ in the 'raphe' cell cultures.

The ability of mazindol to prevent the toxic effects of MPTP in 'nigral' cell cultures supports the importance of neuronal uptake to dopamine cell toxicity. A component of action of MPP^+ may also require an active uptake system. In contrast, the neurotoxic effects of MPTP and MPP^+ on 'raphe cells' in culture is mediated independently of dopamine uptake mechanisms.

Javitch, J.A. et al. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 2173-2177

DISTRIBUTION OF CALCITONIN GENE-RELATED PEPTIDE IN RAT AND RABBIT SPINAL CORDS

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Calcitonin gene-related peptide (CGRP) -like immunoreactivity (LI) is concentrated in primary afferents innervating the dorsal horn of the spinal cord and is also present in a lower concentration in the ventral horn of most species so far examined (Gibson et al., 1984). However, the distribution in the rabbit is unknown. In the ventral horn, CGRP appears to be synthesised in motoneurones (Gibson et al., 1986) but its functional role in these cells remains to be established. The present study aimed to develop a sensitive radioimmunoassay to accurately measure CGRP in the ventral horn of rat and rabbit spinal cords as a means to examine the role of this peptide in motoneurones in future studies.

Male Wistar rats (260-300 g, n=8) were decapitated and the thoraco-lumbar spinal cord separated into dorsal and ventral portions and stored at -80°C prior to the assay of CGRP. Spinal cords from New Zealand White rabbits (2.3-2.8 kg, n=6) were frozen on an aluminium tray on a bed of solid carbon dioxide in order to remove 2nd-4th cervical, 4th-6th thoracic and 2nd-4th lumbar segments. Each segment was subdivided into lateral white, and dorsal and ventral white and grey regions, as described previously (Fone et al., 1987). The CGRP antiserum, raised in sheep against tyr^0CGRP_{28-37} (Peninsula) conjugated to Keyhole Limpet haemocyanin, was used in a working dilution of 1:40000 and showed no cross-reactivity with substance P, neurokinins A and B, thyrotrophin-releasing hormone, proctolin or calcitonin but measured rat $CGRP_{1-37}$ (<10 pg/tube) using $[^{125}I]tyr^0CGRP_{28-37}$ as label. CGRP was extracted from tissue by sonication for 10 s in ethanol:0.1M acetic acid containing 1.05M sodium metabisulphite (50:50 v/v), followed by centrifugation (2300 g for 10 min at 4°C). Supernatant aliquots were dried (60°C -25mmHg, vortex evapourator) and assayed in triplicate using a 0.06M phosphate buffer pH 7.4 containing 0.1M EDTA and 0.5% albumin.

CGRP-LI levels in the dorsal and ventral (274.8 $\pm$ 60.4 and 32.4 $\pm$ 5.3 pmol g^{-1} wet weight, mean $\pm$ s.e. mean, respectively) sections of the rat thoraco-lumbar spinal cord agree with values reported previously using a rabbit antiserum (Gibson et al., 1984), with the recovery of CGRP from spinal cord being 71 $\pm$ 8%. In the rabbit, CGRP-LI levels were much higher in the cervical dorsal white (195.9 $\pm$ 22.6 pmol g^{-1} wet weight) and dorsal grey (152.1 $\pm$ 10.1) matter than in cervical lateral (6.7 $\pm$ 3.3) or ventral white (2.1 $\pm$ 0.6) or grey (3.5 $\pm$ 0.5) regions. Lumbar and thoracic segments contained similar CGRP levels to the cervical cord except that the amount of CGRP in the thoracic dorsal white (61.2 $\pm$ 11.6) was significantly lower ($P<0.05$, Student's t-test) than that in the other regions.

A sensitive radioimmunoassay has been developed to measure CGRP-LI and preliminary results using the antiserum show that the distribution of this peptide in the rabbit spinal cord is analogous to that in other species.

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THE EFFECT OF AN ANABOLIC STEROID AND CHRONIC EXERCISE ON NOCICEPTION AND PLASMA CORTICOSTERONE LEVELS IN RATS

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Athletes take anabolic steroids to improve their physical performance during exercise. Exercise, which is a form of stress, elevates plasma levels of the endogenous opioids β-endorphin and met-enkephalin (Howlett et al., 1984) and this mobilisation is thought to be responsible for acute exercise induced antinociception (AEIA). We have investigated whether chronic administration of nandrolone to either naive or chronically exercised rats affects AEIA and the corticosterone (CS) response to exercise.

All rats were placed on a treadmill for the same time each weekday for 4 weeks; one group received exercise whilst for the control group the treadmill was not run. The exercise schedule was as follows: on days 1 and 2 rats were run for 5 mins at a speed of 8m/min; the duration of run (at constant speed) was then increased every other day to a maximum of 30 mins at day 16; on day 17 the speed was increased to 13.3m/min with a run duration of 10 mins; this duration was then increased by 5 mins every other day to a maximum of 25 minutes at day 26.

Two days after the end of chronic exercise the rats, both exercised and non-exercised, were subjected to an acute run of 5 mins at 13.3m/min and the sensitivity of each rat to a noxious stimulus was measured by the paw pressure test before (p1) and immediately after (p2) the run. Blood was collected 10 mins after the run for plasma corticosterone assay by the fluorimetric method.

Unexercised rats treated with ethyl oleate (1ml/kg twice a week for 4 weeks, i.m. n=4) showed AEIA (p2-p1=5.25, $p < 0.05$ using Mann Whitney U-test) but in similar animals treated with nandrolone (20mg/kg twice a week for 4 weeks, n=4) AEIA was absent (p2-p1=3) confirming an observation previously made in mice (Cowan et al. 1987). AEIA was also absent in oleate treated rats that had been exercised (p2-p1=1.75) but was present in nandrolone treated rats that had been exercised (p2-p1=5.5, $p<0.05$). The acute run produced an elevation of plasma CS in non-exercised oleate treated rats of 15.3±3ug/100ml ($p<0.05$) whereas no rise occurred in chronically exercised rats. The rise in plasma CS occurred in both the non-exercised (17.7±6ug/100ml, $p<0.05$) and exercised (22.4±5ug/100ml, $p<0.05$) animals receiving nandrolone.

These results show that the chronic administration of an anabolic steroid affects the response to exercise stress differently in chronically exercised and non-exercised rats.

SCF is a Sports Council Science Scholarship Student.

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PROSTACYCLIN ANALOGUES CONTRACT GUINEA-PIG ILEUM LONGITUDINAL SMOOTH MUSCLE BY MORE THAN ONE MECHANISM

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The contractile action of PGI_2 on guinea-pig ileum longitudinal muscle is abolished by tetrodotoxin (TTX) and appears to be due to activation of enteric neurones (Gaion & Trento, 1983). PGE_2 in contrast has both direct and indirect actions (Sanner, 1971). As part of our prostanoid receptor studies we have examined the effects of three stable prostacyclin analogues on guinea-pig ileum (Tyrode's soln., 95%O_2 + 5%CO_2, 37°C, isometric recording).

Cicaprost (ZK96480) (Stürzebecher et al, 1986) was a highly active contractile agent - EC_{50} ~ 2nM. Submaximal responses were abolished by 100nM TTX (IC_{50} = 13nM) and more conveniently by 200nM morphine (IC_{50} = 12nM). However matching responses to iloprost (ZK36374) and 6a-carba PGI_2 were only partially inhibited by even higher concentrations of TTX and morphine (Table 1). The PGE receptor antagonist AH6809 (Coleman et al, 1987) had little effect on cicaprost action but markedly suppressed responses to PGE_2, iloprost and 6a-carba PGI_2. SC25191 (Sanner et al, 1973) was less specific.

Table 1 Inhibition of submaximal contractile action on guinea-pig ileum

% Inhibition (± s.e.m., n = 4 or 5)

Agonist	Morphine (0.64μM)	TTX (1μM)	AH6809 (1μM)	SC25191 (2μM)	SC25191 (10μM)
Cicaprost	100 ± 0	100 ± 0	13 ± 5	26 ± 8	36 ± 10
Iloprost	60 ± 4	63 ± 7	55 ± 6	63 ± 5	73 ± 9
6a-Carba PGI_2	33 ± 5	39 ± 5	46 ± 8	49 ± 6	65 ± 9
PGE_2	38 ± 6	37 ± 4	67 ± 6	63 ± 7	77 ± 5

Concentration-response curves obtained in the presence of 1μM morphine showed cicaprost to have no contractile activity up to a concentration of 1.5μM. Iloprost and 6a-carba PGI_2 had slightly shallower curves than PGE_2; 30% maximal responses were given by about 2nM PGE_2, 3nM iloprost and 5nM 6a-carba PGI_2. This ranking of agonist activity is similar to that reported previously by us (Dong et al, 1986) for PGE-sensitive contractile systems and confirms the high specificity of cicaprost for PGI receptors.

One is tempted to assume that the TTX-sensitive component of iloprost or 6a-carba PGI_2 action is due entirely to activation of PGI receptors. However, by analogy with the direct action (1μM morphine) data, a contribution from PGE receptor activation is possible.

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POTENTIATION BY AH6809 OF ILOPROST-INDUCED RISES IN PLATELET CYCLIC AMP LEVELS

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AH6809 specifically blocks EP_1 receptors on smooth muscle (Kennedy et al, 1983). In addition, it inhibits the anti-aggregatory action of PGD_2 on human platelets, but slightly potentiates the action of PGI_2 (Keery and Lumley, 1985). We have investigated the effects of AH6809 alone and in combination with iloprost and PGD_2 on cAMP levels in washed platelet preparations from man, rat and rabbit. Preincubation for 2 minutes with 30μM AH6809 inhibits platelet aggregation induced by ADP* or PAF† in the three species. This is accompanied by small increases in platelet cyclic AMP levels (Table 1).

Table 1 Effect of AH6809 (30μM, 2 min) on washed platelet preparations

	HUMAN†	RAT*	RABBIT†
Cyclic AMP (x basal)	1.47; 1.23; 1.00	1.08; 1.14; 1.93	1.16; 1.0; 1.07
% Inhibn. of aggregation	83; 78; 74	49; 35; 83	37; 31; 30

AH6809 also produces a marked potentiation of iloprost-induced rises in platelet cyclic AMP in all three species. Table 2 gives the mean values of three experiments in each case. In contrast, AH6809 (30μM) inhibited rises in cyclic AMP induced by 2.8μM PGD_2 in human platelets by 41, 89 and 87% and in rabbit platelets by 12, 34 and 31%, consistent with an antagonist action at PGD_2 receptors.

Table 2 Effect of AH6809 (30μM, 2 min) on increases in basal cyclic AMP induced by iloprost

	HUMAN		RAT		RABBIT	
Iloprost (nM)	C	AH	C	AH	C	AH
0.28	1.32	1.65	-	-	-	-
2.8	7.3	10.5	1.98	2.86	1.75	1.86
28	16.7	30.5	15.1	31.3	3.12	3.09
280	21.0	48	45.8	127.8	14.1	29.4
2800	-	-	37	121.4	20.5	41.6

The cAMP changes seen with AH6809 resemble those produced by low concentrations (10-30nM) of forskolin (Siegl et al, 1982). However SQ22536 (100μM, 2 min) an inhibitor of adenylate cyclase (Harris et al, 1979) enhances the inhibitory action of AH6809, whereas it reduces the anti-aggregatory of iloprost (1.4nM) and isobutylmethyl xanthine (30μM) as expected. Further studies on the interaction of AH6809 with the platelet adenylate cyclase system are in progress.

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BRADYKININ-STIMULATED RELEASE OF PEPTIDOLEUKOTRIENES FROM RAT LUNG AND ITS INHIBITION BY VASOACTIVE INTESTINAL PEPTIDE

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We have previously shown that the biosynthesis of the peptidoleukotrienes (pLT) LTC_4, LTD_4, and LTE_4 when stimulated by platelet activating factor and ovalbumen challenge can be inhibited by vasoactive intestinal peptide, whereas when calcium ionophore A23187 is used the inhibition occurs to a much smaller extent (Di Marzo et al, 1986, 1987). We have shown that cyclic AMP (cAMP) is the second messenger responsible for this inhibition and suggested that cAMP acts by an effect on the phosphoinositol (PI) cycle (Di Marzo et al, 1987). This study examines the effect of bradykinin (Bk), which also acts through the PI cycle (Yano et al, 1984), on pLT biosynthesis in rat lung and its inhibition by VIP. Aliquots (400mg) of rat chopped lung tissue were incubated as previously described (Di Marzo et al, 1986) with four concentrations of Bk, 0.01, 0.1, 1 and 10μM, and the effect of a 6μM VIP pre-incubation was examined on three of these concentrations. Supernatants were extracted on C18 sep paks, purified by reverse phase HPLC and the pLTs quantitated by RIA using an antiserum directed against LTD_4 (Beaubien et al, 1984). Statistically significant inhibition was determined by paired Student's t test. The effect of BK on pLT biosynthesis was a dose-related stimulation for LTD_4, LTE_4 and total pLTs and VIP inhibition was highest on LTC_4 levels, in agreement with previous studies with PAF. VIP inhibition of total pLT biosynthesis was maximal when 1μM BK was used.

Table 1: Release of pLT from Bk-stimulated rat lung and the effect of VIP (fmol per aliquot)

Bk	LTC_4	LTD_4	LTE_4	TOTAL
0.01	405±92	262±28	842±580	1509±683
0.1	178±63	398±147	1734±397	2309±604
	(121±49)*	(396±162)	(1589±252)	(2106±458)
1.0	788±199	442±44	2656±165	3800±351
	(513±106)*	(322±38)*	(2365±293)	(3200±430)**
10.0	647±61	952±90	3894±107	5494±223
	(447±130)*	823±16	(3652±128)*	(4922±110)*

The results are means ± s.e.m., n = 3 or 4. * $p<0.05$, ** p,0.005. Figures in brackets are pLT release in the presence of 6μM VIP.

These results demonstrate that Bk stimulates pLT biosynthesis in rat lung and that VIP is able to inhibit this effect.

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CHARACTERIZATION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASES IN CULTURED BOVINE AORTIC ENDOTHELIAL CELLS

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Endothelial cells play an important role in the regulation of vascular tone (e.g. by production of prostacyclin or endothelium-derived relaxing factor). Vasoactive agonists increase cyclic nucleotide levels in endothelial cells (Buonassisi & Venter, 1976), however little is known about cyclic nucleotide metabolism in these cells. We have thus characterized cyclic nucleotide phosphodiesterases (PDEs) of endothelial cells.

Bovine aortic endothelial cells (BAECs) were cultured as previously described (Takeda, Schini & Stoeckel, 1987). Primary and first or second passaged cell cultures were used. After removal of culture medium cells were equilibrated in a physiological solution for 1 h, then collected in a buffer containing protease inhibitors, homogenized and, after ultrasonication (10 s), centrifuged (105,000xg, 1 h). The supernatant was fractionated, stored at -80°C and analysed by HPLC (TSK-DEAE 5. PW 8 x 75mm). PDEs were eluted by a linear NaCl gradient (16-30 %) and enzyme activity assayed as previously reported (Lugnier et al., 1986). Cyclic GMP content of BAECs was determined as previously described (Miller et al., 1984).

The major peak of eluted PDE activity specifically hydrolysed cyclic AMP (cAMP-PDE). A second, much smaller PDE peak was identified which hydrolysed both cyclic AMP and cyclic GMP (cAMP/cGMP-PDE). This latter cyclic AMP hydrolytic activity was stimulated by cyclic GMP, but was detected only in 2 of 4 preparations. Somewhat surprisingly, no calmodulin-activated PDE activity was detected. The IC50s of a variety of specific PDE inhibitors were determined (substrate concentration of 0.25 µM; Table 1).

Table 1. IC50s (µM) of PDE inhibitors

	cAMP-PDE (substrate cAMP)	cAMP/cGMP-PDE (substrate cGMP)
Trequinsin	0.3	
Rolipram	0.9	>200
Papaverine	1.8	
IBMX	9.4	
AAL 05	36.0	
Zaprinast	65.0	2.7
SKF 94120	>200	

As found for cAMP-PDE of bovine aortic smooth muscle (Lugnier et al., 1986), trequinsin, rolipram and papaverine were also potent inhibitors of cAMP-PDE of BAECs. Similarly, zaprinast which selectively inhibits bovine aortic smooth muscle cGMP-PDE also inhibited cAMP/cGMP-PDE of BAECs and was relatively ineffective against cAMP-PDE. Zaprinast (30 µM) did not alter either basal or atriopeptin II (1 µM)-stimulated cyclic GMP content of BAECs.

These results suggest a major role for cAMP-PDE by comparison with that of cAMP/cGMP-PDE in BAECs. This latter minor PDE form hydrolysed both cyclic nucleotides and as in heart, brain and liver, the cyclic AMP hydrolytic activity was stimulated by cyclic GMP. In contrast to bovine aortic smooth muscle, no calmodulin-activated PDE activity was detected in BAECs.

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THE RELATIONSHIP BETWEEN ENDOGENOUS GLUCOCORTICOIDS AND EICOSANOID FORMATION IN ALTERATIONS TO GASTRIC MUCOSAL INTEGRITY

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Administration of glucocorticoids can lead to peptic ulceration (Messer et al, 1983) which may relate to inhibition of mucosal protective prostaglandins (Dandona & Jeremy, 1986). However, glucocorticoids can also prevent ulcerogenesis (Ritchie et al, 1978) which may involve inhibition of lipoxygenase products since the latter are increased in some forms of damage (Peskar et al, 1986). The following studies therefore attempted to assess the role of glucocorticoids in gastric damage utilising the cold/restraint stress model (C/RS) in which plasma corticosterone (cort.) and mucosal eicosanoid formation were measured.

Gastric damage was induced by restraining groups (n=6) of male Wistar rats (200-250g; fasted 18h) in Bollman-type cages at 4°C for periods up to 2.5h, after which the animals were killed and blood collected for estimation of cort. by radioimmunoassay. In another group (n=6) restraint only (RS) was performed and the above variables measured. Two other groups were also subjected to C/RS and mucosal 6-keto-PGF1a and LTB4 were measured by radioimmunoassay (Melarange & Rashbrook, 1986). Mucosal damage was estimated (on a 0-7 basis) by an independent observer. Results were analysed using Student's 't' test or the Mann-Whitney U test.

RS at 1h failed to raise damage above non-restrained control values (1.0 ± 0.4 vs. 0.7 ± 0.4; $p>0.05$) but significantly elevated cort. (75 ± 8.0 ng/ml vs. 750 ± 105 ng/ml; $p<0.001$). In contrast, after 1h of C/RS, damage was significantly raised (2.67 ± 0.5 vs 1.0 ± 0.4; $p<0.05$) but cort. was unchanged compared with RS ($p>0.05$). At 2.5h cort. remained elevated (813 ± 22 ng/ml) in the C/RS group but returned towards control values in the RS group (347 ± 69 ng/ml; $p<0.001$). Damage in the respective groups was 4.8 ± 0.5 and 0.67 ± 0.3; $p<0.01$). Cort. (2.5mg/kg i.m.) given 1.5h into RS treatment raised plasma cort. to 915 ± 84 ng/ml at 2.5h ($p<0.001$), yet at 2.5h damage was significantly less than the C/RS value (0.7 ± 0.2 vs 4.8 ± 0.5; $p<0.01$). There was a negative correlation between damage and 6-keto-PGF1a or LTB4 production ($r=0.68$ $p<0.001$, $n=34$ and -0.71, $p<0.01$, $n=22$ respectively).

These results suggest that cort. alone does not contribute to damage during C/RS because comparable values were found in RS where damage was absent. Moreover, administration of cort. which raised plasma levels to those found in C/RS also failed to induce damage supporting the notion that endogenous glucocorticoids are not detrimental to mucosal integrity. Decreased eicosanoid production was found in these studies possibly because circulating cort. inhibited their production (Blackwell et al, 1978). At present it is not clear whether the reduction in 6-keto-PGF1a contributes to damage but LTB4 does not appear to be involved.

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PROSTAGLANDIN E_2 AND PGD_2 HAVE OPPOSITE ACTIONS ON SHORT CIRCUIT CURRENT IN RAT ISOLATED COLONIC MUSCOSA

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Prostaglandin (PG) E_2 and PGD_2 are the most abundant prostanoids produced by rat colonic submucosa (Craven and DiReubertis, 1983). In contrast to the well established secretory effects of PGE_2 in this tissue, PGD_2 has been reported to inhibit colonic electrolyte secretion (Georg et al, 1986). In the present study we have investigated the effects of PGD_2 on short-circuit current (SCC), a measure of electrolyte secretion, under basal conditions and after stimulation by a range of secretagogues.

Male Wistar rats were killed by cervical dislocation and descending colon was removed. The mucosa was dissected from the overlying muscle layers and mounted in Ussing chambers, bathed with Krebs-Henseleit solution (containing indomethacin, 3μM) maintained at 37°C and gassed with 95% O_2/5% CO_2. The mucosa was voltage clamped at zero potential and the resultant SCC was continuously recorded.

Under resting conditions colonic mucosa produced a stable SCC of $12\pm1\mu Acm^{-2}$ (mean ± SEM; n=20). PGE_2 (0.001-10μM) increased SCC with an EC_{50} value of 0.13 (0.06-0.30)μM (95% confidence limits) and a maximum increase in SCC of $96\pm7\mu Acm^{-2}$ (n=8). In contrast PGD_2 (0.03 to 10μM) reduced SCC with an EC_{50} value of 0.59 (0.15-2.38)μM and a maximum reduction in SCC of $15\pm2\mu Acm^{-2}$ (n=8). A significant positive correlation ($P<0.01$, n=32) was observed between the magnitude of basal SCC and reduction in SCC produced by PGD_2 (3μM). Therefore the effects of PGD_2 (3μM) were studied in mucosa where SCC was increased by PGE_2, vasointestinal polypeptide (VIP) or dibutyryl cyclic AMP (dbcAMP). PGE_2 (1μM) produced a sustained increase in SCC of $40\pm4\mu Acm^{-2}$ and under these conditions PGD_2 produced a significantly greater reduction in SCC than in control tissues ($19\pm1\mu Acm^{-2}$ vs $7\pm2\mu Acm^{-2}$, n=4, $P<0.005$). VIP (0.1nM) increased SCC by $72\pm5\mu Acm^{-2}$ and the subsequent inhibition of SCC by PGD_2 was again significantly enhanced ($41\pm4\mu Acm^{-2}$ vs $6\pm3\mu Acm^{-2}$, n=4, $p<0.005$). In contrast, dbcAMP (1mM) produced large increases in SCC of $204\pm14\mu Acm^{-2}$ but this did not affect the reduction in SCC produced by PGD_2 ($7\pm1\mu Acm^{-2}$vs $6\pm2\mu Acm^{-2}$, n=4).

These studies show that PGE_2 and PGD_2 have opposing actions on SCC in rat isolated colonic mucosa. The observations that PGD_2-induced reductions in SCC were enhanced by pretreatment with the cAMP-dependent secretagogues PGE_2 and VIP, but not by dbcAMP itself suggest that PGD_2 exerts its inhibitory activity at the level of adenylate cyclase.

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INVESTIGATION OF THE ROLE OF NEUTROPHILS AND PAF IN OEDEMA FORMATION INDUCED BY ACUTE ISCHAEMIA OF THE RABBIT MYOCARDIUM

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Temporary coronary artery occlusion (CAO) followed by reperfusion results in myocardial oedema and neutrophil infiltration localised in the zone of ischaemia (Engler et al, 1986). We have evidence for the involvement of C5a in neutrophil infiltration in the rabbit heart (Williams & Rampart, 1987). Since in the skin, oedema formation induced by C5a is dependent on neutrophil accumulation (Wedmore & Williams, 1981a), we have investigated the effect of systemic neutrophil depletion on ischaemia-induced oedema in the heart.

Male New Zealand White rabbits (2.3-3.0kg) were anaesthetized with hypnorm (0.3ml/kg) i.m. followed by sagatal (30mg/kg) i.v., intubated and ventilated with room air. Blood pressure (BP) was recorded from the central ear artery together with a lead I ECG. The chest was opened at the 4th intercostal space and a ligature placed under the left circumflex coronary artery. After 30 min CAO the coronary circulation was reperfused for 3 hours. Plasma protein leakage was measured using i.v. ^{125}I-human serum albumin. Heart tissue was divided into normal zone (NZ) and ischaemic zone (IZ) by dye exclusion and levels of ^{125}I measured by gamma counting. Depletion of circulating neutrophils to 6% of control levels by nitrogen mustard (NM, 1.75mg/kg, 4 days previously) did not alter the increase in plasma protein leakage in IZ compared to NZ (+91$\pm$13% in controls, n=5; +90$\pm$16% in NM group, n=5 % increase $\pm$ s.e.mean).

Since the oedema formation was not neutrophil-dependent, the role of platelet activating factor (PAF) a direct mediator of increased vascular permeability (Wedmore & Williams, 1981b) was examined using a PAF antagonist WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno [3,2-f][1,2,4]triazolo-[4,3-a][1,4]-diazepin-2-yl]-1-(4-morphinyl)-1-propanone). Rabbit neutrophils isolated from whole blood and labelled with ^{111}In (Rampart & Williams, 1987) were also administered to allow measurement of their accumulation into the IZ. Administration of WEB 2086 (10mg/kg i.v.) 10 min prior to CAO followed by an infusion of 1.3mg/kg/h did not alter the increase in plasma protein leakage in IZ compared to NZ (+78$\pm$12%, n=6, vs. +78$\pm$13% in controls, n=6). Moreover WEB 2086 had no effect on the increase in number of ^{111}In-neutrophils in IZ compared with NZ (+243$\pm$68% vs. +281$\pm$84% in controls). However this dose of WEB 2086 prevented the fall in BP caused by 1µg PAF i.v. (-21$\pm$3 mmHg in controls vs. +2$\pm$1 mmHg with WEB 2086 n=3).

In summary, in the rabbit oedema formation induced by acute ischaemia of the myocardium is independent of circulating neutrophils. Furthermore, PAF does not appear to be involved in either neutrophil accumulation or oedema formation in this model.

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ENDOTHELIUM-DEPENDENT RELAXATION AND LOW-DENSITY LIPOPROTEINS: THE IMPORTANCE OF RECEPTOR-MEDIATED ENDOCYTOSIS & OXIDATION?

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Low-density lipoproteins (LDL) inhibit relaxation of rabbit aortic rings mediated by endothelium-derived relaxing factor (EDRF), (Andrews et al. 1987). The inhibition occurs after a lag period and is not reversed after removal of lipoproteins. These observations may be explained by (1) receptor-mediated endocytosis of LDL (Andrews et al. 1987) or (2) a time-dependent oxidative modification of LDL by exposoure to endothelial cells.

Here we investigate (1) by using Watanabe hereditary hyperlipidaemic (WHHL)rabbits which lack the high affinity LDL receptor (Tanzawa, K. et al. 1980) & (2) by the inhibition of oxidation of LDL by the ion chelator DTPA (diethylene-triamine penta-acetic acid) and its deliberate oxidation, catalysed by Cu^{2+}(Parthasarathy et al. 1985).

Normal LDL was prepared as previously described (Andrews et al. 1987) and 0.3mM DTPA was added when appropriate to prevent oxidation. The inhibition of EDRF mediated relaxations evoked by acetylcholine in intact aortic rings precontracted with serotonin was performed as before. Aortae were used from 6-month old Japanese White (control) and non-atherosclerotic segments from homozygous Watanabe hereditary hyperlipidaemic (WHHL) rabbits (plasma cholesterol: 386 ± 50 mg/dl) or in the oxidation experiments, New Zealand White rabbits. Oxidation of LDL was measured using fluorescence spectroscopy (Koller et al. 1987) and colorimetric assay of thiobarbituric acid reactive substances (TBARS) (Parthasarathy et al.1985)

ACh evoked endothelium-dependent relaxations in precontracted aortic rings from both WHHL and control rabbits. The inhibition of maximal relaxation by LDL in WHHL rabbits was 69 ± 8% (mean ± s.e. mean, n = 4) and was not significantly different from controls, 62.4 ± 11%. This result indicates that high affinity LDL receptors are not required for the inhibition.

The oxidative state of LDL was examined during the inhibition of relaxation in aortic rings from New Zealand White rabbits. No change in the oxidation state of LDL was detected. Furthermore, no correlation was observed between the kinetics of inhibition and changes in the oxidative state of LDL as altered by DTPA protection or oxidation for 24 hrs by 100μM Cu^{+}, compared to normal LDL.

We conclude that receptor-mediated endocytosis and the oxidative state of LDL are unlikely to be implicated in the LDL inhibition. Since LDL inhibition continues after the removal of the lipoproteins, the above results argue for a time-dependent transport of LDL into the subendothelial space by transytosis before it inactivates nitric oxide/EDRF (Jacobs et al). (This meeting).

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THE PHARMACOLOGY OF N-METHYL LTC_4, A POSSIBLE LTC_4 MIMETIC

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Any pharmacological investigation of leukotriene C4 (LTC4) is made difficult by its rapid bioconversion to LTD_4 by γ glutamyl transpeptidase. Consequently we have prepared and studied the pharmacology of N-methyl LTC_4, a potential LTC_4 mimetic, that is not a substrate for γ glutamyl transpeptidase.

N-methyl LTC4 produces the same maximal response as LTC4 on guinea pig ileum but is approximately ten times less active. Comparative studies with FPL55712 (a selective LTD_4 antagonist (Fleisch et al, 1986)) on ileum revealed a pKb of 7.9 (n=5) against LTD_4, whereas the pKb for N-methyl LTC_4 and LTC_4 was considerably lower at 5.9 and 5.7 respectively (n=9). N-methyl LTC_4 perfused through the airways of the isolated guinea pig lung preparation produced an increase in perfusion pressure which was approximately one tenth the potency of LTC_4. In further experiments where levels of LTs had been adjusted to produce an equal increase in perfusion pressure, FPL55712 (1μM) antagonised LTD_4 (89%) LTC_4 (84%) and N-Methyl LTC_4 (21%) induced increases in perfusion pressure. These findings suggested that in this system a significant proportion of the LTC_4 response was mediated through LTD_4. This was confirmed by HPLC studies which identified the presence of LTD_4 (16.3±6.4%) in the perfusate when LTC_4 was perfused through the lung. No conversion to LTD_4 was detected in perfusate following administration of N-methyl LTC_4.

Experiments using deeply anaesthetised artificially ventilated guinea pigs (Konzett Rössler), demonstrated that N-methyl LTC_4 administered either intravenously or by aerosol produced a weak bronchoconstrictor response, approximately 10 times weaker than LTC_4.

Studies with pharmacological antagonists revealed that the bronchospasm induced by N-methyl LTC_4 was potentiated by propranolol (1 mg/kg i.v.), but reduced by FPL55712 (1 mg/kg i.v.) and indomethacin (3 mg/kg.i.v.) N-methyl LTC_4 in this model produced a marked and prolonged increase in mean arterial blood pressure, (unlike the predominantly hypotensive response seen with natural LTs.) This hypertensive response was not altered by FPL55712 but was significantly potentiated by indomethacin. It would appear therefore that N-methyl LTC_4 is a possible mimetic of LTC_4 that shows similar <u>in vitro</u> biological activity to LTC_4, yet is stable to enzymatic breakdown by γ glutamyl transpeptidase.

An interesting feature of N-methyl LTC4 is that unlike the natural leukotrienes, it produces a hypertensive response in guinea pigs which may be mediated via the LTC_4 receptor.

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