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5-HT_1 AND 5- HT_{1A} BINDING SITES IN DEPRESSED SUICIDES

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Altered serotonergic (5-HT) function has long been implicated in suicide and depression. In this study 5-HT_1 and 5-HT_{1A} binding sites were quantitated in post-mortem brain tissue from suicide victims (15M, 6F; mean age ± s.e.m., 40 ± 3 years) and a control group (15M, 6F; 41 ± 2 years) dying suddenly of causes not involving the CNS. The suicide group included only those subjects in whom a firm diagnosis of depression in the absence of symptoms of other psychiatric and neurological disorders could be made. Both drug-free and antidepressant-treated subjects were studied.

[^{3}H]5-HT binding (8 concs, 0.5-32nM) was performed on well-washed membranes using coded samples. Binding to the total population of 5-HT_1 sites was defined by 10μM unlabelled 5-HT, and to 5-HT_{1A} sites by 300nM 8-OH-DPAT. Equilibrium dissociation constants (K_D) and the maximum number of binding sites (B_{max}) were determined by non-linear regression analysis.

Table 1 5-HT_1 binding sites in drug-free depressed suicides and controls.

	Frontal cortex (n=12)		Hippocampus (n=13)	
	K_D	B_{max}	K_D	B_{max}
CONTROLS	3.4 ± 0.3	579 ± 46	4.5 ± 0.3	1444 ± 71
DEPRESSED SUICIDES	3.4 ± 0.3	601 ± 48	3.8 ± 0.3	1149 ± 118*

Values are means ± s.e.m. K_D, nM; B_{max}, fmol/mg protein * $P > 0.05$.

No significant differences in number or affinity of 5-HT_1 or 5-HT_{1A} binding sites were found in the frontal or temporal cortex between drug-free or antidepressant-treated depressed suicides and controls (Table 1). There was however a significantly lower number of 5-HT_1 binding sites (by 20%), affinity unaltered, in hippocampus of drug-free suicides (Table 1), whereas the number of sites in amygdala did not differ. No differences in 5-HT_1 sites were found between antidepressant-treated suicides and matched controls in hippocampus or amygdala. However the number of 5-HT_1 binding sites was significantly higher (by 40%) and the binding affinity lower (by 31%) in the hippocampus of the antidepressant-treated suicides when compared to the drug-free suicides.

Previous studies have reported no significant differences in cortical 5-HT_1 binding between suicides (Crow et al., 1984; Owen et al., 1986) or elderly depressed patients (McKeith et al., 1987) and controls. In agreement we found no differences in cortical 5-HT_1 or 5-HT_{1A} binding in depressed suicides compared to controls. Lowered hippocampal 5-HT_1 binding was however apparent in the drug-free depressed suicides. We have recently reported a significant reduction in hippocampal 5-HT_2 sites in this same group of drug-free suicides (Cheetham et al., 1988).

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2P

8-OH-DPAT FAILS TO DECREASE 5-HT RELEASE IN RATS TREATED CHRONICALLY WITH A 5-HT_2 ANTAGONIST, SETOPERONE

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Recently, selective 5-HT_2 receptor antagonists such as ritanserin and setoperone have been shown to have antidepressant, anxiolytic and antischizophrenic properties (Ceulemans et al., 1985; Reyntjens et al.,1986). In such disease states these drugs will be given chronically and, as yet, little information is available on their long term effects. In rats treated chronically with setoperone or ritanserin, ex vivo binding studies have shown a decrease in 5-HT_2 receptor number with no change in affinity (Leysen et al., 1986). Based on such studies we have decided to observe the effects of chronic treatment with setoperone, a mixed 5-HT_2 and DA_2 receptor antagonist, on the release of 5-HT and 5-HIAA using brain microdialysis.

Male Wistar rats (200-270g) were treated orally with water or setoperone, 5 mg/kg, twice a day for 2 weeks. 5 days after the last injection the rats were anaesthetised with 2% halothane and dialysis loops were implanted stereotaxically in the frontal cortex. The loop was perfused with artificial CSF (pH 7.4) at a flow rate of 1 µl/min and samples were collected every 20 min for analysis of 5-HT and 5-HIAA by HPLC-EC (Brazell et al., 1985).

Sample collection was started after a two hour equilibration period and basal release was measured. Setoperone treated rats (n = 9) had slightly lower basal levels of 5-HT (0.107 $\pm$ 0.025 pmoles/20 µl) and 5-HIAA (0.41 $\pm$ 0.072 pmoles) than control rats (5-HT, 0.151 $\pm$ 0.021 pmoles and 5-HIAA, 0.47 $\pm$ 0.084 pmoles, n = 9). 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT, 0.32 mg/kg, s.c.), a 5-HT_{1A} agonist which has been shown to decrease 5-HT release in vivo (Sharp et al., 1988), was given when basal release levels had stabilised. 8-OHDPAT significantly inhibited the release of 5-HT in control rats, reaching a maximum (-25.8 $\pm$ 10.5%) 60 min post-drug with a smaller decrease in 5-HIAA. In setoperone treated rats 8-OHDPAT did not significantly alter either 5-HT release (+21.09 $\pm$ 14.74 %, t = 80 min) or the level of 5-HIAA, though in all animals a small increase rather than a decrease was observed . These increases in amine levels were significantly different from the decreases seen in the control rats 80 min post-8-OHDPAT.

These data suggest that chronic treatment with the 5-HT_2 antagonist setoperone alters the basal levels of released 5-HT and attenuates the effects of 8-OHDPAT at 5-HT_{1A} receptors. This effect on 5-HT_{1A} receptor function is not connected with a change in either 5-HT_{1A} receptor number or affinity (Leysen et al., 1986), and the mechanism remains to be determined including the possible involvement of dopamine receptors.

We acknowledge the support of Janssen Pharmaceutica and their gift of setoperone. E.J. Kidd has a CASE-SERC grant.

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NEUROENDOCRINE AND TEMPERATURE EFFECTS OF GEPIRONE IN HUMANS

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In rats, the 5-HT_{1A} receptor agonist, 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) dose dependently elevates plasma prolactin (PRL) levels, an effect attenuated by the non-selective 5-HT antagonist, metergoline but not by the selective 5-HT_2 antagonist, ritanserin (Aulakh et al, 1988). In contrast, 8-OH-DPAT decreases growth hormone (GH) levels in rats (Aulakh et al, 1988). In rodents, 8-OH-DPAT causes hypothermia, probably by a pre-synaptic action, as in mice this response is antagonised by pretreatment with the selective 5-HT neurotoxin, 5,7-dihydroxytryptamine and the 5-HT synthesis inhibitor, parachlorophenylalanine (Goodwin et al, 1985). Gepirone is a selective 5-HT_{1A} agonist (Traber and Glaser 1987) which has recently become available for use in humans. The present study was designed to investigate the effects of oral gepirone on PRL and GH secretion and temperature (Temp) in human volunteers.

Twelve normal male volunteers, tested on three occasions separated by at least one week, received placebo, 10mg gepirone and 20mg gepirone in a balanced order, single blind design. Testing occurred on a research unit where an intravenous cannula was inserted at 08.30 following an overnight fast. After baseline blood sampling, gepirone or placebo was administered orally at 09.30 and blood samples taken at +15, +30 and then at 30 minute intervals until +180 mins. Oral Temp was taken by glass-mercury thermometer (10 minutes incubation) at 30 minute intervals. PRL and GH were measured by radioimmunoassay. The hormone results are expressed as area under the response curve minus baseline (trapezoid method) and Temp as maximum change from baseline.

Table 1: The effects of gepirone on PRL, GH and Temp in 12 human volunteers

Measure	Placebo	Gepirone 10mg	Gepirone 20mg
PRL (mIUmin/l)	-5473 ± 1899	-10635 ± 2407*	-3396 ± 3798
GH (mUmin/l) †	67.5 ± 62.8(N=10)	560.0 ± 236.5(N=9)	999.0 ± 212.0(N=10)**
Temp change (°C)	0.01 ± 0.07	-0.04 ± 0.07	-0.30 ± 0.06***

Results are Mean ± SEM. † Subjects with baseline GH 10mU/l were excluded
* $p<0.05$ vs placebo and gepirone 20mg, ** $p<0.01$ vs placebo
*** $p<0.01$ vs placebo and gepirone 10 mg (two tailed paired t-test).

Gepirone caused an increase in plasma GH and a decrease in Temp which was significant at the higher dose and appeared to be dose related. PRL secretion was however significantly decreased by the lower dose of gepirone, an effect that disappeared at the higher dose (Table 1). The GH result may be due to gepirone acting on post-synaptic receptors while the PRL and Temp effects may reflect a pre-synaptic action as in animals gepirone has agonist action at both pre- and post-synaptic 5-HT_{1A} receptors (Traber and Glaser 1987).

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4P

IS THE EATING RESPONSE INDUCED BY 8-OH-DPAT MEDIATED BY 5-HT1A RECEPTORS IN THE DORSAL RAPHE?

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Peripheral injection of the 5-HT1A ligand 8-OH-DPAT increases food intake in free-feeding rats. Hutson et al (1988) reported that this effect is attenuated by compounds which show moderate to high affinity for the 5-HT1A binding site, such as spiperone, metergoline and (-) pindolol but not by ketanserin or haloperidol. The effect of 8-OH-DPAT is prevented also by central 5-HT depletion, and can be induced following direct application of 8-OH-DPAT into the dorsal raphe nucleus (DRN) (Bendotti and Samanin, 1986; Hutson et al, 1986). These results have led to the proposal that 8-OH-DPAT induces feeding by acting on 5-HT1A receptors located on 5-HT cell bodies in the DRN The present experiments were designed to test this hypothesis.

Male Sprague-Dawley rats were anaesthetized with sodium pentobarbital.and, stainless steel guide cannulae were implanted,aimed at the DRN. Animals were allowed 1 week to recover. Food and water were freely available. During experiments food intakes were determined 1h after injection of 8-OH-DPAT (3.125 nmol) or sterile 0.9% saline (0.5 µl over 1 min) into the DRN. Antagonists (or vehicle solutions) were injected peripherally or into the DRN 30 minutes prior to 8-OH-DPAT or saline. A repeated measures design was used in each experiment with 3 drug free days between test days.

In 7 groups of rats (n = 8-12) saline treatment induced very little eating (range 0.2±0.1g to 0.5±0.1g). 8-OH-DPAT significantly increased food intake in all groups (range 1.2±0.1 to 1.8±0.3g). Peripherally injected spiperone (0.13 µmol/kg), haloperidol (0.27 µmol/kg) and (±)propranolol (16.9 µmol/kg) significantly attenuated the effect of 8-OH-DPAT. None of these antagonists reduced the eating induced by injection of noradrenaline into the paraventricular nucleus of the hypothalamus. Metergoline (2.5 and 12.5 µmol/kg) did not reduce the effect of 8-OH-DPAT, although the higher dose induced an eating response of 1.1±0.2g in its own right. When injected directly into the DRN neither spiperone (10 and 50 nmol) nor metergoline (10 nmol) reduced the effect of 8-OH-DPAT. Propranolol (20 nmol) slightly attenuated the response.to 8-OH-DPAT. Higher doses of metergoline (50 nmol) and propranolol (100 nmol) elicited eating responses of 2.0±0.2 g and 2.5±0.5 g respectively.

Spiperone, metergoline and propranolol all show affinity for the 5-HT1A binding site,in addition to their other pharmacological properties. None of these compounds significantly attenuated the effect of 8-OH-DPAT when injected into the DRN, and spiperone failed to mimic the effect of 8-OH-DPAT. Although metergoline and propranolol induced eating following DRN injection, these effects do not appear to correlate with their relative affinities for the 5-HT1A binding site (Hoyer, 1988). Thus, these results are inconsistent with the hypothesis that 5-HT1A receptors in the DRN mediate the eating response induced by 8-OH-DPAT. The dopamine antagonists spiperone and haloperidol injected peripherally prevented the effect of 8-OH-DPAT at doses which spare behavioural performance. These findings implicate the involvement of dopaminergic systems "downstream" from the DRN in the eating response induced by 8-OH-DPAT.

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DRUG-INDUCED MODULATION OF THE RELEASE OF TOTAL TRITIUM AND [^{3}H]5-HT FROM RAT HYPOTHALAMIC SLICES

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Inhibitors of 5-HT uptake and monoamine oxidase both increase synaptic levels of 5-HT (by reducing reuptake and metabolism, respectively). Whereas uptake inhibitors do not modify, by themselves, the electrically induced release of tritium from rat hypothalamic slices prelabelled with [3H]5-HT (Galzin et al., 1985), pargyline decreases it (Moret and Briley, 1988). In addition, uptake blockers attenuate the effect of 5-HT autoreceptor agonists on tritium release (Langer and Moret, 1982 ; Galzin et al., 1985 ; Moret and Briley, 1988), whereas pargyline does not (Galzin et al., 1985). To further understand the action of these drugs, the release of total tritium ([3H]total) and [3H]5-HT was studied.

Rat hypothalamic slices were prelabelled with [3H]5-HT and superfused with Krebs' solution. Two periods (S_1 and S_2) of electrical stimulation were applied and drugs were added before S_2. Each aliquot of superfusate was divided into two and assayed for [3H]total and [3H]5-HT after separation on disposable strong cation exchange columns. Results are expressed as percent fractional release (± SEM, * $p<0.05$), during S_2, of [3H]total or [3H]5-HT in each aliquot.

		n	[3H]total	[3H]5-HT
Control		16	2.03 ± 0.18	0.63 ± 0.07
LSD	0.1 µM	7	1.09 ± 0.27*	0.44 ± 0.09
Methiothepin	1 µM	4	5.59 ± 0.82*	1.86 ± 0.41*
Pargyline	3 µM	7	0.88 ± 0.25*	0.67 ± 0.12
Citalopram	1 µM	5	1.77 ± 0.27	1.32 ± 0.25*
Control		16	2.17 ± 0.14	1.30 ± 0.08
LSD	0.1 µM + citalopram (1 µM) (S_1-S_2)	7	1.45 ± 0.12*	0.99 ± 0.07*
Methiothepin	1 µM + citalopram (1 µM) (S_1-S_2)	8	6.03 ± 0.57*	4.34 ± 0.26*
Pargyline	3 µM + citalopram (1 µM) (S_1-S_2)	5	1.84 ± 0.53	1.62 ± 0.25

Under control conditions and in the presence of 0.1 µM LSD or 1 µM methiothepin, 30-40 % of released [3H]total was in the form of [3H]5-HT. Exposure to LSD decreased the release of [3H]total and [3H]5-HT, whereas methiothepin increased them both. In the presence of 1 µM citalopram, the release of [3H]total was unchanged but the proportion of [3H]5-HT was increased to 60 to 70 %. Citalopram attenuated the LSD-induced inhibition of the release of [3H]total and [3H]5-HT. The methiothepin-induced increase of [3H]5-HT release was also enhanced. Pargyline (3 µM) decreased the overflow of [3H]total without modifying that of [3H]5-HT. In the presence of citalopram, the pargyline-induced inhibition of [3H]total release was attenuated whereas [3H]5-HT release was enhanced.

Thus electrically evoked [3H]total overflow reflects [3H]5-HT release provided that uptake inhibitors or monoamine oxidase inhibitors are not present. The results in the presence of such compounds are compatible with a functional interaction between the 5-HT uptake mechanism and the 5-HT autoreceptor.

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6P

EFFECT OF THE 5-HT RECEPTOR ANTAGONIST BMY 7378 ON CENTRAL 5-HTP SYNTHESIS

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Neurochemical studies have shown that 5-HT_{1A} receptor agonists including 8-OHDPAT, buspirone and LY 165163 reduce serotonergic turnover in vivo (Hjorth & Carlsson, 1983; Fuller et al, 1986) via an action on somatodendritic 5-HT_{1A} receptors on dorsal Raphe cells. There is as yet no information on the antagonism of these effects in this model. In the present study we have investigated the neurochemical effects of the novel selective 5-HT_{1A} receptor antagonist, BMY 7378, (Yocca et al, 1987) in vivo.

Male Sprague-Dawley rats were treated subcutaneously with vehicle, BMY 7378, 8-OHDPAT, buspirone or LY 165163. Thirty minutes later all animals were given the aromatic amino acid decarboxylase inhibitor NSD 1015 (100 mg/kg i.p.). After a further 30 minutes the animals were killed, the brain removed and the cortex, hippocampus and striatum dissected out. The tissue samples were analysed for 5-HT, DA their precursors and metabolites by HPLC using electrochemical detection.

All of the 5-HT_{1A} receptor agonists examined produced a significant reduction in 5-HTP levels compared to controls. For example, in the cortex 5-HTP levels were reduced from 173.4 ± 9.6 ng/g in vehicle treated rats to 63.3 ± 8.9 ng/g by 8-OHDPAT (0.3 mg/kg), 77.1 ± 4.6 ng/kg by buspirone (1 mg/kg) and 81.8 ± 13.7 ng/g by LY 165163 (2 mg/kg). Similar results were found for the hippocampus and striatum.

CORTEX

BMY (mg/kg)	% Control 5-HTP		% Conrol DOPA	
	Vehicle	+8-OHDPAT (0.05 mg/kg)	Vehicle	+8-OHDPAT (0.05 mg/kg)
0	100.0 ± 3.9	41.5 ± 3.0*	100.0 ± 8.2	97.4 ± 7.9
0.01	81.4 ± 5.3	42.9 ± 2.4	105.9 ± 12.0	89.2 ± 6.2
0.10	91.2 ± 6.0	42.0 ± 2.6	148.2 ± 11.6*	126.5 ± 6.4#
1.00	62.6 ± 2.9*	49.6 ± 1.6*#	219.0 ± 19.3*	186.9 ± 6.2#

Students t-test * $p < 0.05$ compared to veh/veh
# $p < 0.05$ compared to veh/8-OHDPAT

BMY 7378 also had an agonist-like effect in this in vivo model in that it lowered 5-HTP levels when administered alone (see table). However at a dose of 1.0 mg/kg s.c. BMY 7378 significantly reversed the effects of 8-OHDPAT on 5-HT synthesis. These results are consistent with BMY 7378 having a partial agonist action on 5-HT_{1A} receptors in the dorsal Raphe. However, the reversal of the effect of 8-OHDPAT at higher doses may be due to other interactions e.g. dopaminergic, as BMY 7378 0.1 and 1.0 mg/kg significantly raised DOPA levels. This is currently under investigation.

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5-HT_3 RECOGNITION SITES IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM: AUTORADIOGRAPHIC AND RADIOLIGAND BINDING STUDIES

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The pharmacology and distribution of 5-HT3 recognition sites were investigated in the central and peripheral nervous system of various species by means of radioligand binding and quantitative autoradiographic studies using the selective and potent 5-HT3 receptor antagonist 3H-ICS 205-930.

Radioligand binding studies were performed with 3H-ICS 205-930 in cat vagus nerve (NV) and superior cervical ganglion (SCG) as described previously (Hoyer and Neijt, 1987, 1988). The rank order of affinity for various drugs was similar in both tissue preparations. The following pK_D values (-log mol/l, $n \geq 3$) were obtained in SCG and VN respectively: ICS 205-930 (8.4, 8.9), BRL 43694 (8.6, 8.5), quipazine (7.9, 7.9), MDL 72222 (7.9, 7.5), 2-methyl-5-HT (6.2, 7.0), 5-HT (6.4, 6.7). Affinity values obtained in the equivalent rabbit tissues were similar and correlated significantly with those found in NG 108-15 and N1E-115 cells (Hoyer and Neijt, 1987, 1988; Neijt et al, 1988).

Autoradiographic studies performed with 3H-ICS 205-930 in human and mouse brain as described previously (Waeber et al, 1988), revealed very high concentrations of 5-HT3 recognition sites in the nuclei of the tractus solitarius, vagus nerve, trigeminal nerve and the area postrema (300-1400 fmol/mg protein). In contrast forebrain areas showed much lower concentrations of 5-HT3 sites (70-150 fmol/mg): hippocampus, amygdala, enthorinal cortex and nucleus accumbens. Other brain areas showed very low to undetectable specific binding (striatum, thalamus, hypothalamus, and cerebellum). The spinal cord was another area of very high concentrations of 5-HT3 sites, especially the dorsal horn (monkey and man).

The data show that the sites labelled with 3H-ICS 205-930 in the central and peripheral nervous systems are similar to the sites labelled in rat brain with 3H-GR 65630 (Kilpatrick et al, 1987), 3H-quipazine (Peroutka and Hamik, 1988), 3H-Q ICS 205-930 (Watling et al, 1988) and 3H-zacopride (Barnes et al, 1988). The sites labelled by these radioligands appear to be homogeneous, although functional studies suggest the existence of subtypes of 5-HT3 receptors.

The distribution of 5-HT3 sites in the central and peripheral nervous system supports a role of 5-HT3 receptors in sensory processes and is also consistent with behavioral effects observed with 5-HT3 receptor selective antagonists (see Kilpatrick et al, 1987).

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8P

[^{3}H]-GR67330, A VERY HIGH AFFINITY LIGAND FOR CENTRAL 5-HT$_3$ RECEPTORS

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5-HT$_3$ receptor binding sites were originally identified in the CNS using the radioligand ^{3}H-GR65630 (Kilpatrick et al., 1987). Since then, central 5-HT$_3$ have also been labelled using the ligands ^{3}H-quipazine (Peroutka & Hamik, 1988), ^{3}H-zacopride (Barnes et al., 1988) and ^{3}H-quaternised ICS 205-930 (Watling et al., 1988). We now describe the characteristics of a new 5-HT$_3$ receptor ligand, with an affinity 10 times greater than any reported to date, ^{3}H-GR67330 (1,2,3,9-tetrahydro-3-[(5-methyl-1H,-imidazol-4-yl)methyl]-9-C^{3}H$_3$-4H-carbazol-4-one).

Pooled rat brain tissue (entorhinal cortex was used routinely) was prepared as described previously (Kilpatrick et al., 1987). The ^{3}H-GR67330 binding assay was performed as described previously for ^{3}H-GR65630, except that a final assay volume of 1ml was employed (0.35-0.5mg protein). ^{3}H-GR67330 was used at 0.1nM except in saturation assays, where it was varied between 0.01 and 2nM. Using homogenates of entorhinal cortex, total ^{3}H-GR67330 binding was 2500- 2700dpm. This was reduced to 800-900dpm in the presence of metoclopramide (30μM; used to define non-specific binding). Specific binding associated and dissociated rapidly (K_{+1} 1.47±0.36 x10^8 M^{-1} S^{-1}; K_{-1} 7.85±0.41 x10^{-3} S^{-1}; mean ± S.E., n=3). Specific ^{3}H-GR67330 binding was saturable (B_{max} 22.6±0.2fmol/mg protein) and to a single site of very high affinity (K_d 0.038±0.003nM).

Agonists and antagonists of the 5-HT$_3$ receptor inhibited ^{3}H-GR67330 binding potently (Table 1). Using the entorhinal cortex tissue (ECX) these compounds inhibited up to 70% of total binding, in hippocampal(HP) and nucleus accumbens/olfactory tubercle (NA/OT) homogenates some 40% of total binding was inhibited. An exception to this was GR38032F, which inhibited up to 90% of total binding in each tissue. This additional inhibition of non-specific binding was due to a second lower affinity site unrelated to the 5-HT$_3$ receptor. Hill coefficients were close to unity (0.89-1.14) except for 5-HT and quipazine, which were significantly greater than 1 (1.45-1.91). Drug IC$_{50}$s were similar in each tissue.

Table 1 The IC$_{50}$s of 5-HT$_3$ antagonists and 5-HT to inhibit specific ^{3}H-GR67330 binding

	IC$_{50}$ (nM)			
	ECX	HP	NA/OT	
Quipazine	0.8	1.2	1.4	
Zacopride	1.0	1.6	1.2	
ICS 205-930	3.2	4.3	5.6	
BRL 43694	4.2	4.9	5.0	
GR38032F	1.6*	1.3*	1.5*	
5-HT	130	200	270	* = Ki

In conclusion, ^{3}H-GR67330 is a high affinity ligand for 5-HT$_3$ receptors. The drug competition profile was similar in ECX and HP and NA/OT, suggesting that 5-HT$_3$ receptors, labelled by ^{3}H-GR67330, are the same in each brain area.

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ARE TFMPP, mCPP AND CGS 12066 SELECTIVE FOR 5-HYDROXYTRYPTAMINE 5-HT_{1B} RECEPTORS?

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Based on radioligand binding studies, 1-(m-trifluoromethylphenyl)piperazine (TFMPP), 1-(m-chlorophenyl)piperazine (mCPP) and 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo-[1,2-a]-quinoxaline-1 (CGS 12066), have been described as 5-HT1B receptor selective ligands (Sills et al, 1984; Neale et al., 1987). We have investigated this claim by analysing the effects of 5-HT, TFMPP, mCPP and CGS 12066 on functional 5-HT1A, 5-HT1B, 5-HT1C and 5-HT1D receptors. Following models were used: forskolin (10 uM)-stimulated adenylate cyclase activity, measured in crude homogenates of calf hippocampus (5-HT1A, Schoeffter and Hoyer, 1988), rat and calf substantia nigra (respectively 5-HT1B: Bouhelal et al, 1988 and 5-HT1D: Schoeffter et al, 1988) and stimulation of inositol phosphate production in pig choroid plexus (Hoyer, 1988a, 1988b). The results are presented in table 1 and are expresssed as pEC_{50} values (-log mol/l) and % maximal inhibition ± SEM ($n \geq 3$) (5-HT = 100 %).

Table 1: Effects of 5-HT, TFMPP, mCPP and CGS 12066 on 5-HT1 receptor subtypes

Drug	5-HT1A	5-HT1B	5-HT1C	5-HT1D
5-HT	7.6 ± 0.2	7.8 ± 0.2	6.3 ± 0.1	7.5 ± 0.1
	100%	100%	100%	100%
TFMPP	6.7 ± 0.2	6.9 ± 0.4	6.8 ± 0.1	5.8 ± 0.2
	67 ± 5%	74 ± 10%	59 ± 13%	54 ± 6%
mCPP	5.9 ± 0.1	6.5 ± 0.3	6.9 ± 0.1	*5.1 ± 0.3
	40 ± 5%	60 ± 8%	65 ± 7%	<30%
CGS 12066	6.4 ± 0.2	7.6 ± 0.2	4.1 ± 0.1	7.1 ± 0.2
	91 ± 11%	102 ± 10%	35 ± 3%	83 ± 5%

All compounds acted as agonists at the different 5-HT1 receptor subtypes. Due to the weak partial agonist activity of mCPP at 5-HT1D receptors, this compound was tested as an antagonist (* pK_B value). TFMPP was almost equipotent at 5-HT1B, 5-HT1C and 5-HT1A receptors. mCPP was slightly more potent at 5-HT1C than at 5-HT1B receptors, whereas CGS 12066 was only slightly more potent at 5-HT1B than at 5-HT1D receptors (2.8 fold).

The present data illustrate the substantial agonist activity of TFMPP and mCPP at 5-HT1C receptors, as suggested from behavioral studies by Kennett and Curzon (1988). The lack of selectivity of TFMPP, CGS 12066 and mCPP for 5-HT1B receptors predicted from radioligand binding studies (Hoyer, 1988b) is illustrated in the present study.

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10P

ACTIVATION OF 5-HT_2 RECEPTORS EXCITES RAT NUCLEUS ACCUMBENS NEURONES BY REDUCING INWARD RECTIFIER POTASSIUM CONDUCTANCE

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The nucleus accumbens receives a moderately dense innervation from the raphe nuclei of the midbrain, and direct application of 5-hydroxytryptamine (5-HT) into the nucleus affects motor performance (Costall et al, 1978). The purpose of the present experiments was to examine the actions of 5-HT on single neurones of the nucleus accumbens, and to determine the ion conductances and receptor subtypes involved. Intracellular recordings were made from rat nucleus accumbens neurones in slices (300 μm) superfused *in vitro* with a physiological saline solution (potassium concentration 2.5 mM). 5-HT (1-100 μM) depolarized 170 of 203 neurones; this was associated with an increase in the input resistance. 5-HT applied to neurones under voltage-clamp caused an inward current at the resting potential (typically about -80 mV); this reversed to outward at a potential (E_{rev}) that was related to the extracellular potassium concentration ($[K_o]$, range 2.5 - 10.5 mM) by $E_{rev} = 59\log[K_o]/160$. The conductance reduced by 5-HT was 3-10 times larger when the current was outward (potentials negative to E_{rev}) than when it was inward. Barium (1 - 300 μM) reduced a conductance with similar properties, and the action of 5-HT was occluded by barium. The depolarization caused by 5-HT persisted in tetrodotoxin (1 μM) and was not abolished by a solution that contained lower levels of calcium (0.24 instead of 2.4 mM), higher levels of magnesium (5 instead of 1.2 mM), and cobalt (2 mM). The effect of 5-HT was competitively antagonized by ketanserin and mianserin with apparent dissociation equilibrium constants of about 3 nM and 45 nM, respectively. The depolarization was not mimicked or blocked by a number of agonists and antagonists selective for 5-HT_1 and 5-HT_3 receptors, nor was it blocked by sulpiride (1 μM). The results indicate that 5-HT acts at 5-HT_2 receptors to depolarize nucleus accumbens neurones by closing a set of inwardly rectifying potassium channels. Conn & Sanders-Bush (1986) have shown that 5-HT_2 receptors on these cells are also involved in the stimulation of phosphatidyl inositol hydrolysis; a product of this hydrolysis therefore may be involved in the reduction of the potassium conductance.

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EVIDENCE FOR THE INVOLVEMENT OF 5-HT_{1A} RECEPTOR MECHANISMS IN THE MEDIATION OF DEFEAT ANALGESIA IN MALE MICE

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In male mice, the ecologically-relevant experience of social defeat is associated with a non-opioid form of pain inhibition (Rodgers & Randall 1986a). The involvement of anxiety in the initiation of this response has been supported by the observations that (1) an identical reaction occurs in animals merely exposed to the scent of an aggressive conspecific and (2) both defeat- and scent-induced analgesia can be blocked by diazepam and flumazenil (Rodgers & Randall 1986b, 1987; Kavaliers & Innes 1988). Since recent evidence has indicated a major role for 5-HT_{1A} receptors in anxiety and its modulation (e.g. Traber & Glaser 1987), we have examined the effects of four 5-HT_{1A} receptor ligands on basal nociception and the non-opioid analgesic consequences of defeat experience. Compounds chosen for study were 8-OH-DPAT, buspirone, ipsapirone and gepirone.
Subjects were 10-18 week old male DBA/2 mice (Bantin & Kingman, Hull), housed in groups of 10 in a temperature-controlled room (24±1°C) in which a reversed 12hr light cycle was operative. All testing was conducted under dim red light during the dark phase of the cycle. Nociceptive latencies were assessed by traditional tail-flick assay using a cut-off of 8 seconds. For defeat experience, intruder DBA/2 mice were individually placed into the home cage of an aggressive resident conspecific and removed immediately upon display of the species-typical upright submissive (defeat) posture. Tail-flick latencies were established both before and after defeat. All drugs were dissolved in physiological saline and administered intraperitoneally (i.p.) in a volume of 10ml/kg 15 minutes before testing. In a separate series of experiments, 8-OH-DPAT was also administered subcutaneously (s.c.) 20 minutes prior to test. Doses cited refer to the salts.
Statistical analysis (ANOVA) indicated that, with the exception of 20mg/kg buspirone which produced weak but significant analgesia ($p<0.05$), none of the compounds exerted significant intrinsic activity on the mouse tail-flick assay (dose ranges: 8-PH-DPAT=0.1-2.0mg/kg; buspirone HCl=0.5-10mg/kg; ipsapirone HCl=0.05-20mg/kg; gepirone HCl=0.1-10mg/kg). In defeat studies, all control groups showed pronounced analgesia ($p<0.001$) a reaction that was totally blocked by all four compounds; rank-order potency (minimum effective doses):- ipsapirone (0.05mg/kg) > gepirone (0.1mg/kg)=8-OH-DPAT (s.c., 0.1mg/kg) > buspirone (0.5mg/kg) > 8-OH-DPAT (i.p., 1.0mg/kg). It is important to note that display of defeat behaviour was not impaired at these effective doses. Significantly, efficiacy in inhibiting defeat analgesia was lost at 20mg/kg buspirone, 10mg/kg ipsapirone and 1mg/kg gepirone. While further studies are required, present data suggest that serotonergic substrates, and the 5-HT_{1A} receptor in particular, may be critically involved in the mediation of this biologically-meaningful form of pain inhibition.

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12P

EFFECTS OF THE 5-HT_3 RECEPTOR ANTAGONIST, ICS 205-930, ON BEHAVIOUR OF MICE DURING SOCIAL ENCOUNTERS

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There is accumulating evidence that 5-HT_3 receptor antagonists are of potential importance as a new class of drugs for the treatment of several conditions (Costall et al., 1986; Jones et al., 1987; Williams et al., 1985). Their psychopharmacological actions include putative anxiolytic activity and they appear also to be of value in the treatment of drug dependence (Costall et al., 1988). Since 5-HT_3 receptors in the peripheral nervous system subserve a variety of functions, their effects in the central nervous system also may be wide-ranging. The present study, using ethological procedures, thus examines effects of the selective antagonist, ICS 205-930, on the whole range of unrestrained behaviour in mice during social encounters.

The drug was given by i.p. injection (10 ng/kg, 1 μg/kg and 0.1 mg/kg) to group-housed adult male mice while controls received an equivalent volume of physiological saline. Behaviour was examined under red light in a neutral cage for a 7 min period at 45 min after injection during encounters with untreated isolated males. Effects on the frequency and duration of behavioural categories and elements were analysed as described by Dixon (1982). Body temperatures of injected mice were recorded before and after each behavioural observation.

In encounters where aggression by the partner animal was low (4.3±2.0% of the encounter period, mean±s.e.m.) the administration of all doses of ICS 205-930 increased the time spent in social investigation ($P<0.05$, Mann-Whitney U test and Kruskall Wallis ANOVA). Agonistic behaviour also was increased. Aggression was raised in mice treated with 1 μg/kg of ICS 205-930 ($P<0.05$, Mann-Whitney U test) whereas occurrence of the flight element "retreat" was increased ($P<0.05$, Mann-Whitney U test) in mice receiving 0.1 mg/kg of the drug. When encounters were with more aggressive partners (aggression occupying 20.3±5.0% of the encounter period, mean±s.e.m.) behavioural change in drug-treated mice could no longer be detected. In all animals, body temperature prior to the behavioural encounters was not affected by ICS 205-930, but the drug at 10 ng/kg and 1 μg/kg significantly increased temperature above control levels by the end of the encounter period (Controls 36.6±0.3°C; Treated; 10 ng/kg, 37.2±0.1°C, $P<0.05$; 1 μg/kg, 37.3±0.1°C, $P<0.01$; mean±s.e.m.).

It is suggested that the induced hyperthermia and increased responsiveness of drug-treated mice to their social situation may have a common cause, and that one explanation for the observed findings is enhancement of social arousal by ICS 205-930.

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THE EFFECT OF STRESS ON MONOAMINE OXIDASE ACTIVITY IN THE HIPPOCAMPUS AND AMYGDALA OF THE RAT

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Recently it has been shown that rats subjected to a cold stress have a decreased monoamine oxidase (MAO) activity and benzodiazepine receptor (BDR) binding in the heart and kidney (Armando et al, 1988). Although social isolation has been regarded as stressful, isolates (ISOL) show no changes in central BDR binding compared to socially reared (SOC) rats (Rich et al, 1988). However, it is possible that on exposure to an additional stress, significant neurochemical differences between SOC and ISOL rats may be detectable. In the present study, MAO activity in the brains of SOC and ISOL rats exposed to a novel environment, and in animals left undisturbed in their home cages, was compared.

Weanling male Wistar rats were housed individually (ISOL) or in groups of four (SOC) for 21 days. At the end of this period, some of the SOC and ISOL rats were given a 5 minute trial in the light/dark shuttlebox (Stanford et al, 1988). MAO activity was measured fluorimetrically in the hippocampus and amygdala of these rats immediately after removal from the test apparatus (SOC-STS and ISOL-STS), and in controls taken direct from their home cage (SOC-CON and ISOL-CON).

ISOL took significantly longer to emerge from the dark compartment (ISOL:131$\pm$34s, SOC:62$\pm$23s; mean $\pm$ s.e.m. for n=12; $P<0.025$) and made fewer transitions between the two compartments (ISOL:5.9 $\pm$ 1.2, SOC:9.1$\pm$ 1.4;$P<0.05$) than SOC rats. However, neither isolation nor stress, altered the activity of MAO in the two brain regions (Table 1).

Table 1. MAO Activity in the Hippocampus and Amygdala.

	HIPPOCAMPUS	AMYGDALA
SOC-CON	31.9 $\pm$ 2.3	30.8 $\pm$ 1.4
SOC-STS	28.2 $\pm$ 2.2	27.2 $\pm$ 2.8
ISOL-CON	32.3 $\pm$ 2.0	32.8 $\pm$ 4.5
ISOL-STS	33.5 $\pm$ 1.6	31.2 $\pm$ 1.7

Each value is the mean enzyme activity (nmol 4-hydroxyquinoline/mg protein/h) $\pm$ s.e.m. for n=8.

The results from this study show that while behavioural differences between SOC and ISOL were evident, there were no changes in MAO activity in the hippocampus and amygdala. It is possible that in ISOL rats exposed to a novel environment, the degree of stress is not great enough to increase the production of the endogenous MAO/BDR binding inhibitor, tribulin. However, even where a more severe stressor was used, such changes were detectable in only one quarter of the tissues examined (Armando et al, 1988). It remains to be seen whether MAO activity is altered in other brain regions, or peripheral tissues of ISOL rats subjected to a mild stress.

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14P

EFFECT OF IDAZOXAN ON PASSIVE AVOIDANCE BEHAVIOUR IN ADULT AND IN AGED RATS

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Brain noradrenaline (NA) has long been associated with mechanisms of attention, learning and memory (Roberts 1981, Robbins 1984). Treatments which enhance NA-function in rodents, such as the alpha-2 adrenoreceptor antagonists idazoxan and yohimbine, improve acquisition of food rewarded lever pressing (Huang et al 1987) and reduce forgetting of maze strategies (Sara 1986). We have studied the effects of idazoxan, co-dergocrine mesylate (co-dm, Hydergine) and scopolamine on passive avoidance when given before or after training or before retesting.

Adult (240g-330g) and aged (340g-760g, 22 months) male Sprague-Dawley rats were placed into the illuminated side of a two compartment step-through avoidance chamber and allowed to explore and cross to the darkened side. In one trial, rats were trained to avoid the darkened side by presenting a single electrical foot-stimulus (0.5s., 0.5mA) 3s. after entry. Retention was tested 24h later. Rats whose latencies exceeded the 95% confidence interval of the respective training day latency distribution were said to have learned successfully. The effects of drugs given to adults before training are shown in the Table.

		Training Trial	24hr Retention Trial		
Pre-treatment	N	Latency(s) Med(range)	Latency(s) Med(range)	% exceeding criterion	Probability Fisher
vehicle, 1ml/kg, 60min	10	22(5-31)	104(18-600)	40	-
co-dm, 1 mg/kg	10	20(0-260)	434(7-600)	80	0.085
co-dm, 3 sc	10	14(4-160)	318(25-600)	60	ns
vehicle, 1ml/kg, 30min	16	22(0-105)	71(1-600)	44	-
idazoxan 0.3 mg/kg	15	26(1-217)	208(11-600)	60	ns
idazoxan 1 sc	16	50(4-393)*	377(10-600)	82	<0.05
vehicle 1 ml/kg, 10min	10	23(3-40)	142(1-445)	40	-
scopolamine 0.3 mg/kg	10	20(3-134)	63(7-520)	10	ns
scopolamine 1 ip	10	18(3-84)	71(12-121)	0	<0.05

* P<0.01 Kruskall-Wallis plus multiple comparison test vs vehicle (2 tailed).

Similar to adult rats, aged rats given idazoxan (1 mg/kg) showed significantly increased latency to enter the dark side during training (saline 25(12-50), idazoxan 126(16-600), n=7,9, P<0.05) and increased latency during retesting (saline 10(3-44), idazoxan 36.5(15-256), n=7,5, P<0.05, Mann Whitney U-test, 2 tailed). None of the drugs tested significantly altered passive avoidance responding when given 5 min. after training. Furthermore, idazoxan did not affect retention performance when given before retesting or before both training and retesting. Scopolamine (1mg/kg) given before both training and retesting significantly impaired performance, an effect which was not blocked by idazoxan (1 mg/kg). The results indicate that idazoxan improves passive avoidance responding in both adult and aged rats but only when given before training. This suggests an effect on perception and/or attention rather than on memory or retrieval mechanisms.

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EFFECTS OF THE CCK ANTAGONIST MK-329 ON ANALGESIA INDUCED BY SOCIAL CONFLICT IN MICE

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Recent reports have indicated that cholecystokinin (CCK) inhibits, and CCK antagonists enhance, morphine- and environmentally-induced analgesia (e.g. Watkins & Mayer 1986). In view of these findings, a number of studies have been conducted to assess the effects of a novel and highly potent CCK antagonist MK-329 (1-methyl-3-(2-indolyl) amino-5-phenyl-3h-1,4-benzodiazepin-2-one) on social conflict-induced analgesia in male mice. This compound has recently been shown to enhance morphine analgesia and restraint analgesia in rodents (Dourish et al. 1988a, 1988b).

In the present studies, male DBA/2 mice (Bantin & Kingman, Hull) were tested for baseline Tail-Flick latencies (TFL) and randomly assigned to one of eight groups (n=8/9):- vehicle (5% methyl cellulose), 0.00001, 0.00005, 0.0001, 0.0005, 0.001, 0.005 or 0.01 mg/kg MK-329. Injections were given intraperitoneally (i.p.) in a volume of 10ml/kg twenty minutes before testing. Injected mice were individually introduced into the home-cage of an aggressive male conspecific and, following previously reported methods (Rodgers & Randall 1985), were subjected to opioid-activating attack. Animals were then immediately retested for TFL, a procedure repeated at 20, 40 and 60 minutes post-encounter. Data were initially analyzed using Analysis of Variance, with between- and within-groups comparisons subsequently performed using appropriate error terms from the ANOVA. Data are summarised below.

		Time Post-encounter (mins.)			
mg/kg MK-329	pre	0	20	40	60
0	2.1±0.2	4.4±0.8*	3.2±0.5*	2.9±0.4*	2.8±0.2
0.00001	1.8±0.2	4.3±0.6*	2.8±0.2*	2.8±0.4*	2.0±0.2
0.00005	2.0±0.2	4.1±0.7*	3.5±0.5*	3.7±0.5*	2.6±0.3
0.0001	2.4±0.1	4.1±0.6*	2.6±0.5	3.1±0.6	2.7±0.3
0.0005	1.8±0.3	4.4±0.5*	2.2±0.2	2.3±0.2	2.2±0.2
0.001	2.5±0.2	3.3±0.4*	2.3±0.2	2.2±0.3	2.4±0.3
0.005	2.1±0.2	3.7±0.5*	2.5±0.3	2.5±0.4	2.3±0.2
0.01	2.3±0.2	3.1±0.3*	2.4±0.2	2.4±0.3	2.2±0.3

* = $p<0.05$ from pre-encounter baseline

From this analysis, it is apparent that MK-329 shortens the duration of the analgesic reaction seen in controls, leaving a residual acute analgesia that is present only immediately after encounters. Further studies have shown that this residual component is resistant to the opiate antagonist, naloxone. Together, present data indicate that (i) opioid and non-opioid forms of analgesia are present immediately post-encounter, (ii) MK-329 is without effect on the non-opioid form and (iii) MK-329 prevents the normal expression of opioid analgesia seen in mice subjected to conspecific attack. As these findings are clearly contrary to previously reported data, further studies are required to clarify the nature of the interaction between CCK and endogenous opioid substrates.

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BUSPIRONE WITH CLENBUTEROL AND/OR CHLORDIAZEPOXIDE DIFFERENTIALLY AFFECTS MOUSE ACTIVITY AND BLOCKS BACKWARD WALKING

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Buspirone is a non-benzodiazepine anxiolytic which also has antidepressant-like actions (Kennett et al., 1987). We have previously shown that co-administration of single moderate doses of clenbuterol or conventional antidepressants with chlordiazepoxide (CDZP) depresses mouse locomotor activity and induces progressive bouts of immobility, which culminate in conspicuous and prolonged backward locomotion (Davies et al., 1988). Clenbuterol 5.0 mg/kg with CDZP 5.0 mg/kg is the 'optimal' dose combination, inducing backward walking in about 80% of mice. We have therefore extended earlier experiments, in which only half this ingredient dose of clenbuterol was used, to examine whether buspirone could substitute for either drug, when given in combination with the other.

Groups of adult 129SV mice were tested with buspirone (3.0 and 6.0 mg/kg) alone, or with buspirone (1.5, 3.0, 6.0 mg/kg) combined with either 5.0 mg/kg clenbuterol or 5.0 mg/kg CDZP; the effects were compared with those of a clenbuterol 5.0 mg/kg-CDZP 5.0 mg/kg combination. Buspirone 3.0 mg/kg was, in addition, co-administered with a combination of clenbuterol 2.5 mg/kg and CDZP 5.0 mg/kg. All drugs were given i.p. in 10 ml/kg saline; 30 minutes later, 5-minute Y-maze tests were carried out (Davies et al., 1986).

By itself, buspirone somewhat depressed mouse locomotor activity (n.s.). In combination with clenbuterol, all doses of buspirone reduced arm entries to about a quarter of the mean saline score of 21.1 entries/5 min, (df 4,56, $F=15.6$, $P<0.001$); rearing was virtually abolished (df 4,56, $F=9.71$, $P<0.01$). When combined with CDZP, only the two highest doses of buspirone depressed activity, with a roughly log-linear dose-response relationship (entries, df 2,15, $F=3.90$, $P<0.05$; rears, $F=28.17$, $P<0.001$). None of the three buspirone-clenbuterol or buspirone-CDZP combinations induced backward walking; indeed, some of the mice quickly dashed forward. The standard clenbuterol-CDZP combination, as expected, also reduced locomotor activity, especially arm entries, which were less than half the saline score (df 1,23, $F=13.8$, $P<0.01$), and backward walking occurred in 73% of the mice ($P<0.001$, binomial test).

Buspirone co-administered with the clenbuterol 2.5 mg/kg-CDZP 5.0 mg/kg mixture also depressed locomotor activity (entries, df 2,51, $F=13.82$, $P<0.001$; rears, $F=7.21$, $P<0.01$). Backward walking, though slightly more frequent than with saline, was not significant. In mice given this clenbuterol-CDZP mixture only, the incidence of backward walking was about 62% ($P<0.001$, binomial test).

It appears that buspirone can differentially affect mouse locomotion if combined with clenbuterol or CDZP. Buspirone cannot, however, substitute for either drug in inducing backward locomotion, but, on the contrary, blocks backward walking brought about by their combination.

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A CYTOPROTECTIVE EFFECT OF HEPARIN ON THE ENDOTHELIUM IN RABBIT

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Heparin (H) is known to prevent experimental venous thrombosis induced by local stasis and systemic hypercoagulability in rabbit (1-2) but no study has been done to investigate whether this antithrombotic effect of H is accompanied by a protection of the vasal endothelial cells (EC).

The aim of the present work was to evaluate at the Scanning (SEM) and Trasmission Electron Microscopy (TEM) the protective effect of H on venous endothelial cells in stasis conditions.

Male, New Zeland rabbits, 2.5 kg. b.w. anesthetized with urethane (1.5 g/kg/i.p.) were used. The right and left jugular veins were isolated. Two series of experiments were carried out.

I. The jugular veins were proximally ligated 5' after i.a. administration of H (0.2-1 mg/kg) or saline. After a stasis of 120' the jugular veins were excised and examined. Incidence and weights of the thrombi were recorded.

II. The lower effective dose of H (0.2 mg/kg) was administered 5' or 40' before the ligature. Blood samples were taken for anti Xa and APTT evaluation. Groups of six rabbits were sacrificed respectively 15' and 120' after the ligature and samples of the veins were taken for SEM and TEM.

I. In the saline group the incidence of thrombosis was 70% and the mean weight of the thrombi was 6.3 $\pm$ 1.7 mg.At the highest dose tested, H completely prevent thrombosis; at the lower dose the presence and the weight of thrombi was significantly reduced as compared with controls (10%, 0.1 $\pm$ 0.5).

II. As observed at SEM and TEM, after a 15' stasis, the endothelial cells in the control group were significantly damaged (intercellular widening, organules changes, membrane vesiculation), while after a 120' stasis there was a complete destruction of the cellular component with exposure of the subendothelial matrix.

A marked protection of the endothelial cells (nuclei less prominent, the sunc - tions between the cells normal) was evident after a 15' stasis in the animals pretreated 5' or 40' with the lower dose of H, but after a 120' stasis a signi - ficant protection of the endothelial cells were achieved only when H was administe red 5' before the ligature.

Anticoagulant activity of 0.2 mg/kg of H in the rabbit serum was low and completely disappeared after 30'.
These findings suggest that H protects the endothelial cells from stasis induced damage independently of its anti-coagulant effect.

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L-662,583: A TOPICALLY EFFECTIVE OCULAR HYPOTENSIVE CARBONIC ANHYDRASE INHIBITOR IN EXPERIMENTAL ANIMALS

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Systemically administered carbonic anhydrase (CA) inhibitors have been used to treat glaucoma for over thirty years. The agents are very effective but patient compliance is often poor. This is due to unwanted side effects stemming from the extraocular inhibition of the enzyme. Attempts to obtain a topically effective CA inhibitor in the past have been unsuccessful. However, the preclinical pharmacology of MK-927 (d,l-5,6-dihydro-4-(2-methylpropylamino)-4H-thieno[2,3b]thiopyran-2-sulfonamide-7,7-dioxide HCl) is such (Sugrue et al., 1988) that the compound was evaluated topically in glaucoma patients. The instillation of one drop of a 2% solution of MK-927 elicited a significant reduction in intraocular pressure (IOP) (Hennekes et al., 1988) and it is the first reported CA inhibitor to possess this profile.

L-662,583, 5-(3-dimethylaminomethyl-4-hydroxyphenylsulfonyl)thiophene-2-sulfonamide HCl, is a water-soluble CA inhibitor that is structurally different from MK-927. The IC_{50} value for L-662,583 against human CA II in vitro was 0.7 nM. Corresponding values for MK-927, acetazolamide and methazolamide were 13.0 nM, 10.8 nM and 21.2 nM. L-662,583 was a good penetrator of the albino rabbit eye; in an ex vivo model, a 1 hr pretreatment with 0.5% L-662,583 (one drop, 50 µl) blocked CA activity in an iris + ciliary body homogenate by 96%. The maximum concentration of L-662,583 in the iris + ciliary body of albino rabbits was determined by HPLC to be 9.61 µg/g (25.5 µmoles/kg) after 2% dosing. Corresponding peak values for cornea and aqueous humor were 47.4 µg/g (126 µmoles/kg) and 4.51 µg/ml (12.0 µM).

Ocular hypertension was induced in cynomolgus monkeys under ketamine-induced sedation by laser-induced photocoagulation of their trabecular meshwork (Lee et al., 1985). The topical administration of one 50 µl drop of 0.5%, 1% and 2% solutions of L-662,583 dose-dependently decreased the elevated IOP. The maximum decline after 2% dosing was 8.3 mm Hg at 3 hr and this was a 22.5% reduction from the baseline value of 36.8 mm Hg. A 2% solution of L-662,583 also decreased the IOP of ocular normotensive cynomolgus monkeys. The elevated IOP of alpha-chymotrypsinized rabbits (CT) (Sugrue et al., 1985) was very sensitive to L-662,583. The instillation of 0.01%, 0.1% and 0.5% solutions maximally decreased IOP by 4.5, 6.2 and 9.8 mm Hg, respectively. L-662,583 was found to have a local site of action because the unilateral instillation of 0.5% L-662,583 into the contralateral eye failed to lower the elevated IOP of the right eye. A 2% solution of L-662,583 maximally lowered the IOP of normal, albino rabbits by 2.3 m Hg. Two percent (2%) L-662,583 was devoid of undesirable effects in the rabbit, such as changing regional ocular blood flow, pupil diameter and the ability of the cornea to respond to tactile stimuli.

These preclinical observations indicate that L-662,583 is a water-soluble CA inhibitor which, on topical administration, lowers IOP by virtue of an action confined to within the eye.

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NIMODIPINE IN COMBINATION WITH ATROPINE AND DIAZEPAM AS AN ANTIDOTE TO LETHAL ORGANOPHOSPHORUS INTOXICATION

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Nimodipine, a calcium channel antagonist with central effects, given in association with atropine displays a significant protective action against a lethal dose of acetylcholine administered to the rat. Flunarazine, another calcium modulator with central effects, in combination with atropine is an effective antidote to nicotine lethal toxicity in the rat. In the present studies lethal intoxication by paraoxon, a cholinesterase inhibitor, was treated with the following drugs: atropine, diazepam, and nimodipine given separately or in combination (presently, intoxication with organophosphorus compounds is treated with a combination of atropine and diazepam, a treatment which may not prevent fatal outcome).

Fifty six fasting Sprague Dawley rats weighing 300±20 g are fitted, under ether anesthesia, with a caudal artery catheter connected to a recorder for on-line recording of arterial blood pressure which is analyzed by a microcomputer. Measurement of heart rate and pulse pressure are displayed every 30 seconds. When the animal has awakened it is placed in a restraining grid. All animals are administered I.P. 1.5 mg/kg of paraoxon, a lethal dose. Survival time of the 7 controls is 14'55"±3'54". Either atropine, diazepam, or nimodipine given alone did not alter survival time. Atropine in combination with either diazepam or nimodipine increased survival time. A combination of all three compounds prevented lethality in 6 out of 7 animals (Table). Such treatment rapidly normalizes life-threatening respiratory, cardiac, and central nervous system disturbances due to the overstimulation of sympathetic and cholinergic systems induced by paraoxon.

No. of rats	Atropine[a]	Diazepam[b]	Nimodipine[c]	Survival Time
7	0	0	0	14'55"±3'54"
7	900 µg/kg	0	0	12'32"±8'25"
7	0	2.25 mg/kg	0	10'46"±9'32"
7	0	0	30µg/kg	13'32"±6'48"
7	900 µg/kg	2.25 mg/kg	0	38'20"±15'21"[d]
7	900 µg/kg	0	52µg/kg	29'35"±4'42"[d]
7	0	2.25 mg/kg	30µg/kg	17'48"±5'21"
7	900 µg/kg	2.25 mg/kg	71µg/kg	6:>24h, 1:36'

(a) administered 3 min after intoxication, half I.P., half S.C.
(b) administered 4 min after intoxication, 0.75 mg/kg intraarterial, 1.5 mg/kg I.P.
(c) administered 5 min after intoxication, intraarterial bolus of 30 µg/kg initially and of 15 µg/kg up to 72 µg/mg total.
(d) $p < 0.05$

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20P

THE DISPOSITION OF ETHIOFOS IN THE ISOLATED PERFUSED RAT LIVER (IPRL)

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Ethiofos [S-2(3aminopropylamino) ethylphosphorothioic acid] has progressed to clinical trials as a radioprotectant and chemoprotectant (Turrisi et al 1986, Glover et al 1986). It is considered to be a pro-drug, which is dephosphorylated to the active thiol WR 1065. Although highly effective intravenously, ethiofos is without activity when given orally (Davidson et al 1980). We have investigated the disposition of [^{14}C] labelled ethiofos (20mg base, 4μCi) in the IPRL preparation in order to determine the hepatic contribution to the poor bioavailability of the drug.

Ethiofos was administered directly to the reservoir of the IPRL as a solution in 0.9% saline. Samples (0.3ml) were taken pre-dose; then at intervals for up to four hours after administration. Ethiofos and WR 1065, were quantified by HPLC (Swynnerton et al 1986a, Swynnerton et al 1986b). Clearance of ethiofos by the IPRL was very slow, (10.6 $\pm$ 3.3 ml hr^{-1}) and only amounted to 1.2 $\pm$ 0.03% of perfusate flow. The elimination half life was calculated at 7.1 $\pm$ 1.9hr. The $AUC_{0\rightarrow4}$ hr for ethiofos (2858 $\pm$ 314nM.hr ml^{-1}) was not significantly different from that of [^{14}C] (3038 $\pm$ 692 nM.hr ml^{-1}), indicating a low level of metabolism. The apparent volume of distribution for ethiofos (102.2 $\pm$ 6.8 ml) corresponded approximately to the volume of the IPRL circuit (100ml) underlining the minimal hepatic drug uptake. The $AUC_{0\rightarrow4}$ hr for the thiol metabolite, WR 1065 (37.5 $\pm$ 23.3 nM.hr ml^{-1}), was less than 2% of the AUC of ethiofos, indicating almost negligible conversion of parent drug to the thiol. The combined biliary elimination of ethiofos, WR 1065 and [^{14}C] amounted to less than 1% of the total dose. At 4hr, 7.9 $\pm$ 1.9% of the dose of radioactivity remained in the liver. Less than 1.5% could be identified as either ethiofos (0.12 $\pm$ 0.09%), or WR 1065 (1.1 $\pm$ 0.05%). The remainder of the liver radioactivity was unidentified.

Overall, it is likely that contribution of the liver to the presystemic biotransformation and poor bioavailability of ethiofos in the rat is relatively minor.

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INHIBITORS OF GLYCOGEN PHOSPHORYLASE

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Glycogen phosphorylase (GP) is a key regulatory enzyme in the metabolism of glycogen, its action being to catalyse the first step in glycogen catabolism. Hepatic GP plays a crucial role in the maintenance of blood glucose levels and may be a suitable target for the design of hypoglycaemic agents (Kasvinsky et al., 1978).

In this study, a number of compounds have been tested for GP inhibition as a first step (lead generation) in the design of GP inhibitors as potential hypoglycaemics. These compounds were chosen on the basis of structural diversity and established hypoglycaemic activity, where the mechanism of this activity is unknown or not resolved. The method of Hu and Gold (1975) was used to measure inhibition of rabbit muscle GP because the crystal structure of that enzyme has been solved to high resolution in this laboratory (Johnson et al., 1988). The overall homology between liver and muscle GP is 80% and the primary structure is completely conserved at important binding sites (Newgard et al., 1986). Measurements were repeated at least four times for each of two concentrations of the test compound and results are presented in Table I. Caffeine, a standard GP inhibitor, has a K_i of 0.1mM (Merida et al, 1984). None of the compounds show activity as strong as that of caffeine but a significant result of this study is the identification of GP inhibition in a variety of different chemical structures.

The high resolution structure of rabbit muscle GP is being used as the model for the design of novel inhibitors by the method of receptor fit using program GRID (Goodford, 1985). The aim of this work is to discover ligands which possess potent GP inhibitory activity and to investigate the relationship between GP inhibition and hypoglycaemia.

Table 1 Inhibition of Rabbit Muscle Glycogen Phosphorylase

Compound	Concentration	Inhibition (mean±s.e.m.)
Caffeine	2mM	69%±5%
	20mM	94%±1%
Phenylbutazone	2mM	19%±5%
	5mM	66%±3%
Salicylate	2mM	2%±1%
	20mM	13%±2%
Propranolol	2mM	6%±3%
	20mM	67%±8%
Serotonin	2mM	4%±2%
	20mM	95%±0%
L-5-hydroxy-tryptophan	2mM	-1%±6%
	20mM	35%+3%

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22P

INHIBITION OF MOUSE MAMMARY TUMOURIGENESIS BY THE ANTIOESTROGEN TAMOXIFEN

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On April 7, 1936 Lacassagne presented a review lecture before the annual meeting of the American Association for Cancer Research on the essential role of oestrogens in the tumourigenesis of breast cancer. He predicted that antagonistic drugs would be developed to prevent the development of the disease. Fifty years later tamoxifen, a non-steroidal antioestrogen, is considered to be the primary endocrine therapy for the treatment of breast cancer and the drug is proving to be of value in treating earlier stages of the disease (Jordan 1988).

Spontaneous mammary cancer occurs with high incidence in inbred strains of mice. Tumours occur most frequently after repeated pregnancies although more than 50% of virgin animals can have tumours at 12 months of age. Ovariectomy prevents the appearance of these tumours. Clearly an antioestrogen would be predicted to be effective as an inhibitor of tumourigenesis. However tamoxifen paradoxically causes oestrogen-like increases in ovariectomized mouse uterine weight and vaginal cornification (Jordan 1984). Tamoxifen could therefore conceivably increase tumour incidence in susceptable mice.

Two types of experiment were therefore conducted: an evaluation of the oestrogenic activity of tamoxifen in the mouse uterus and the effect of tamoxifen on mouse mammary tumourigenesis. Female mice of the C3H/OuJ strain were obtained from the Jackson Laboratories (Bar Harbor, Maine, USA). Groups (6 mice/group) of intact female mice (6 weeks old) were implanted with either a 2 cm silastic capsule (medical grade silastic tubing inside diameter 0.078" outside diameter 0.125" plugged both ends with medical grade elastomer; Dow Corning, Midland, MI) containing approximately 28 mg of tamoxifen to release drug (150 µg/day) for about 6 months or left empty as control. Uterine weight in tamoxifen treated animals (60.8 ± 3.4mg) was below control (93 ± 6.4mg) after 8 weeks and remained so throughout the experiment. Ovariectomized mice had uterine weights of 25.8 ± 2.6 mg at 8 weeks.

To evaluate the antioestrogenic effects of tamoxifen groups (6 mice/group) of ovariectomized mice (6 weeks old) were implanted with control or tamoxifen silastic capsules. At 2, 4, and 6 months groups of control or tamoxifen treated animals were implanted with oestradiol pellets (1.7 mg oestradiol in cholesterol; 8 week sustained release), or placebo pellets, and uterine weights compared 6 weeks later. Tamoxifen did not increase uterine wet weight above control but inhibited oestradiol-stimulated rises in uterine wet weight at each time point.

Following a single pregnancy-weaning cycle at 2 months of age, spontaneous mammary tumours started to appear at six months of age and all animals had tumours by 12 months of age.

Implantation of a silastic capsule containing tamoxifen following the pregnancy-weaning cycle prevented the appearance of mammary tumours. At 12 months all control mice (n=13) had tumours whereas 80% of tamoxifen treated mice (n=31) were tumour free.

Tamoxifen exhibits antioestrogen properties in the mouse uterus after long-term administration and prevents the appearance of spontaneous mammary tumours in the C3O/OuJ strain.

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EXCRETION OF WARFARIN IN THE MILK OF LACTATING RATS

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It has been reported that warfarin cannot be detected in human milk following its oral administration to lactating women (Orme et al, 1977). As part of an investigation of the transfer of drugs into milk, and into the use of an animal model to predict drug excretion in humans, warfarin was administered i.v. to lactating rats. In these studies warfarin was found in milk at levels between 17-36% of the corresponding plasma level at the same time, the mean concentration ranging from 0.22 and 0.56µg/g

Warfarin was administered at a dose of 0.5mg/kg i.v. to 10 lactating Wistar rats (299±44g) on day 14 post-partum. It was given as a solution of warfarin sodium in phosphate buffer (0.15M, pH7.4). The pups were removed from the dams at the time of dosing. Milk samples were collected under light ether anaesthesia 2, 4, 6, 8 and 24 hours after dosing by a modification of the method of McBurney et al (1964). Oxytocin (1 iu/kg i.p.) was administered 15 minutes before each milk collection and venous blood samples were taken immediately after. Total warfarin levels in the milk and plasma were assayed by high performance liquid chromatography following the "normal phase" method of Shearer (1986).

The warfarin levels in the milk and plasma samples at various times after dosing are shown in Table 1. The mean levels in milk rose from 0.37µg/g at 2 hours after dosing to 0.56µg/g at 8 hours after dosing. By 24 hours the level had fallen to 0.22µg/g. Mean plasma levels of total warfarin declined steadily throughout this period from 2.12 to 0.64µg/g, with a half-life of 13.1 hours.

Table 1. Mean total warfarin levels (µg/g + s.e.mean) in milk and plasma during 24 hours after intravenous administration

Time (h)	2	4	6	8	24
Milk	0.37 ± 0.08	0.45 ± 0.08	0.45 ± 0.07	0.56 ± 0.05	0.22 ± 0.04
Plasma	2.12 ± 0.08	1.75 ± 0.08	1.67 ± 0.05	1.57 ± 0.05	0.64 ± 0.06
Milk/Plasma	0.17	0.26	0.27	0.36	0.34

It may be concluded that warfarin is excreted into rat milk, although why such high initial levels should be achieved is unclear. Warfarin in plasma is highly protein bound with free levels of less than 2% of the total. It is postulated that during the period immediately after injection, when plasma levels are very high (about 17µg/g) warfarin moves into milk, and that although some transport back into plasma may occur, this is relatively slow and that once warfarin is present in milk, it becomes trapped and therefore accumulates. The evidence favours a diffusion mechanism.

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24P

EFFECTS OF CHRONIC ETHANOL CONSUMPTION ON INSULIN SENSITIVITY IN NORMAL AND DIABETIC MICE

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We have previously reported that chronic ethanol drinking in mice produces hypoglycaemia and an increase in tissue glucose uptake (Connelly et al., 1987), and that the same regime in hyperinsulinaemic obese diabetic male CBA mice (Connelly & Taberner, 1985) produces a fall in plasma insulin (Connelly & Taberner, 1986). In this study, the hypoglycaemic response to an acute dose of insulin has been compared in normal and diabetic mice under control or ethanol-drinking regimes.

Adult CBA/Ca mice of either sex and inbred male diabetic CBA/Ca-derived mice were used. They were housed in groups of 8 at 22°-24° and given food and water ad libitum. Experimental groups were provided with alcohol (20 % w/v) in tap water as sole fluid according to the method described previously (Unwin & Taberner, 1981). Insulin (human mono-component, Actrapid, Novo) was injected subcutaneously (6.67 iu/kg in a volume of 0.05 ml/10 g body weight). Blood samples (50 µl) were taken from the tail at 0, 1, 2, 3, 4 and 6h after the insulin, and the glucose level (BGL) measured using a Glucochek II monitor (Medistron). The integrated insulin response was calculated for each animal from the BGL-time curve.

Table 1 Values are means ± s.e.means of 7-8 observations.

Group	Blood glucose level (mM)		Integrated insulin response ($mmol.l^{-1}.h$)	
	Control	Ethanol	Control	Ethanol
Male normal	8.53 ± 0.30	7.43 ± 0.51	10.60 ± 1.02	10.61 ± 1.06
Male obese	11.40 ± 0.40	9.80 ± 0.47	22.39 ± 2.53	13.63 ± 1.73
	* $p < 0.001$	$ $p < 0.05$	* $p < 0.05$	$ $p < 0.02$
Female	8.31 ± 0.27	7.50 ± 0.27	12.70 ± 1.60	9.90 ± 0.96

* obese vs normal, $ ethanol vs control, uncorrelated 2-tailed t-test

The results confirm that the ethanol drinking reduces the BGL in all 3 groups, the greatest effect being observed in the obese diabetic mice. The integrated insulin response was similar in normal animals of either sex, and there was no significant difference after ethanol drinking. The obese mice showed a greatly enhanced insulin response, and this was attenuated by the ethanol treatment. The results suggest that the diabetic mice are not insulin insensitive, and that ethanol is a more effective hypoglycaemic agent in diabetic mice.

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ANTIBODIES RAISED AGAINST A NON-TOXIC HOMOLOGUE WILL NEUTRALISE A MYOTOXIC FRACTION OF NOTECHIS SCUTATUS VENOM

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Bites by the Australian tiger snake, Notechis scutatus, cause a severe necrotising myopathy (Hood & Johnson, 1975). Two fractions in the venom are responsible for this activity. They are homologous polypeptides of 119 amino acids, structurally similar to mammalian pancreatic phospholipase A_2 and are extremely toxic (see Harris, 1984 for details).

The toxicity of notexin and notechis II-5 makes it difficult to use them as the antigen in immunological studies. The techniques usually adopted are to inoculate animals with as much as 1 x LD_{50} of the toxin, with an adjuvant, and accept the resulting mortality or to modify the toxin chemically and risk distorting the tertiary structure of the molecule.

Notechis II-1, a consituent of the venom, is a non-toxic homologue of notexin and notechis II-5. We used notechis II-1 as an antigen to raise antibodies in rabbits. The immune sera were tested for the ability to neutralise the myotoxic effects of notechis II-5.

Five New Zealand white rabbits were immunised three times at monthly intervals with 0.1 mg notechis II-1. Test bleeds were taken after the second and third injections.

Antibody activity was assessed by incubating dilutions of the sera on microtitre plates to which notechis II-1 and notechis II-5 (500 ng/well) had been applied. The plates were then incubated with radioactive second antibody before separating the individual wells and counting them in a gamma counter.

Toxins (total volume always 0.2 ml) were injected subcutaneously into one hind limb (Harris & Johnson, 1978). At 24h injected and contralateral muscles were removed, and paraffin-embedded sections cut. Muscle fibre loss was assessed in all injected muscles.

Notechis II-5 (2.0 μg s.c.) caused an estimated loss of 26 ± 4% of muscle fibres in rat soleus muscles (n = 14). Undiluted serum from the five inoculated rabbits was incubated (37°C 1h) at an excess of 100:1 by volume with 2.0 μg toxin. The incubate was inoculated into a total of eight rats. Of the five rabbits used (see above) two produced a neutralising serum after two inoculations of antigen and four after three inoculations.

We attempted to neutralise the myotoxic effects of the crude venom using the immune rabbit sera. 1 μg of venom was used as the standard dose. This dose destroyed approximately 50% of the muscle fibres in the rat soleus. Sera from three rabbits were tested. All neutralised the effects of the crude venom.

One problem arose during these experiments for which we have no immediate explanation. Pre-immune rabbit serum potentiated the myotoxicity of notechis II-5 even though the sera alone were completely innocuous.

The use of a non-toxic, natural homologue to raise neutralising antibodies against toxic fractions of snake venom was clearly successful. We believe it could lead to novel ways of identifying the 'active regions' of toxic polypeptides.

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26P

LYMPHOCYTE CHEMOATTRACTANT ACTIVITY IN NORMAL HUMAN SKIN AND PLASMA

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During studies of the role of chemoattractants in the induction of the lymphocytic infiltrates seen in inflammmatory skin diseases, we have detected lymphocyte locomotor activity in samples from normal human skin. In view of the evidence for the presence of lymphocyte infiltrates in normal human skin (Smolle et al., 1985; Bos et al., 1987), we have partly characterised this activity and have compared it with that found in normal plasma.

The preparation of human mixed peripheral blood lymphocytes and measurement of locomotion, by using a 48-well microchemotaxis assay was carried out as previously described (Bacon et al., 1988). Results have been expressed as a mean maximal migration index [MMI; area of the lower surface of the filter occupied by cells in response to chemoattractant (mm^2)/ area of filter occupied by unstimulated cells (mm^2)]. Stratum corneum (s.c.) samples were obtained by abrading the heels of normal volunteers. Supernatants from aqueous s.c. homogenates (15mg in 0.8ml buffered Eagle's minimal essential medium [MEM]) were successively ultrafiltered through Amicon YM30 then YM10 filters. MEM (1ml) from skin chambers applied to abraded normal skin for 30 min (Brain et al., 1984) was ultrafiltered as described above. Heparinised fresh normal human plasma (1ml) was successively filtered through YM100 then YM10 membranes. Dilution-related lymphocyte locomotor activity was consistently present in the <10kD fractions of the s.c. and plasma samples, and to a lesser extent in chamber fluid (MMI $\pm$ s.e.mean being, 2.08$\pm$0.19, 1/16 dilution, n=6; 2.35$\pm$0.17, 1/8 dilution, n=7; and 1.37$\pm$0.09, 1/4 dilution, n=5, respectively). Lipid extracts of the <10Kd samples from both s.c. (n=5) and plasma (n=3) failed to reveal any lymphocyte locomotor activity and the presence of the protease inhibitors aprotinin ($5\mu g ml^{-1}$) and phenylmethylsulphonylfluoride (0.5mM) did not affect the activity present in samples from either source (n=3). Further analysis of <10kD plasma (0.5ml) and s.c. (50mg) samples was carried out using reversed phase h.p.l.c. (Brownlee Aquapore RP-300 C8 column eluted with a linear 0.1% trifluoroacetic acid/acetonitrile gradient, 100:0 - 0:100, over 30 minutes at 1 ml min^{-1}). Analysis of collected fractions, evaporated and resuspended in 120μl MEM, consistently showed a peak of lymphocyte locomotor activity eluting at approximately 9 min, after h.p.l.c. of both plasma and s.c. samples (MMI $\pm$ s.e. mean being 2.54 $\pm$ 0.15, 10-20 fold dilution, n = 6 and 3.00 $\pm$ 0.34, 10-20 fold dilution, n = 4, respectively). The activity in plasma fractions was associated with a single, clearly resolved u.v. absorbing peak (210 nm).

The identification of active components eluting at similar times on reversed phase h.p.l.c. of both plasma and s.c. samples, suggests that a plasma-derived factor may be responsible, at least in part, for eliciting the lymphocytic traffic which plays an important role in the physiological immunosurveillance mechanism operating in normal human skin. The structural identity and in vivo properties of this material, and its potential as a therapeutic target, await further study.

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A COMPARISON OF THE EFFECTS OF CROMAKALIM, NORADRENALINE AND ISOPRENALINE IN GUINEA-PIG ISOLATED TAENIA CAECI

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Cromakalim (CK) and α-adrenoceptor agonists produce relaxation in intestinal smooth muscle associated with the opening of at least two different types of K-channel (Weir & Weston, 1986). The activation of ß-adrenoceptors by isoprenaline (ISO) also causes relaxation of intestinal smooth muscle. The objective of the present experiments was to obtain further evidence about the mechanism of the inhibitory action of CK, NA and ISO in guinea-pig taenia caeci.

In microelectrode studies CK and NA maximally relaxed the tissue and this effect was associated with cessation of spontaneous spike activity and a hyperpolarisation of the muscle membrane (Table 1). In the presence of phentolamine (1μM), isoprenaline (0.32-1.28μM) maximally relaxed the tissues within 1-2 min. This was associated with abolition of spike activity and hyperpolarisation (Table 1). In some tissues, however, spike activity returned in the continuing presence of ISO although maximum relaxation was maintained.

Table 1: Hyperpolarisation produced by CK, NA and ISO in taenia caeci

	CK (μM)		NA (μM)		ISO (μM)	
	1.6	6.4	1.6	3.2	.32	1.28
Maximum Hyperpolarisation (mV)	11±2 (10)	15.1±1.7 (9)	6.9±1.2 (9)	8.8±1.8 (7)	7.5±1 (5)	12.5±2 (4)

Data represent mean ± s.e. mean (number of observations)

ISO, CK, and NA had no effect on cGMP levels ($p > 0.05$, $n = 5$) whereas sodium nitroprusside (10μM) used as a standard significantly increased cGMP levels 4-fold. In contrast, NA (3.2μM) significantly increased cAMP levels 2.5-fold ($p < 0.01$, $n = 5$); CK (1.6 & 3.2μM) increased cAMP levels approximately 2-2.5-fold and ISO (0.32 & 1.28μM) increased levels by 3-fold ($p < 0.01$, $n = 5$).

In a ^{42}K efflux study, segments of taenia caeci were impaled on fine needles and loaded with ^{42}K ($1.6\mu Ci.ml^{-1}$ for 180 min). The rate of ^{42}K efflux was determined at 2 min intervals. CK (6.4μM) and NA (3.2μM) significantly increased the mean basal ^{42}K efflux rate measured between the 20th and 28th minutes of the efflux by approximately 25% and 30% respectively ($p < 0.05$, $n = 6$). ISO, however, was without effect.

This study has confirmed previous findings that CK and NA can open K-channels in guinea-pig taenia caeci. In addition, the actions of CK, NA and ISO are associated with an elevation of intracellular cAMP levels. Whether an increase in cAMP levels by CK and NA is necessary for the inhibitory action of these agents in this tissue is currently being investigated.

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ENDOGENOUS OPIOID INHIBITION OF OXYTOCIN RELEASE FROM THE RAT NEUROHYPOPHYSIS MAY INVOLVE ACTIVATION OF POTASSIUM CHANNELS

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It has been shown that opioids may cause reduction of calcium currents as well as increase of potassium conductions in different neurons (see North, 1986). As nerve terminals are not directly accessible to electrophysiological measurements, the mechanisms involved in the presynaptic inhibitory effects of opioids are as yet unknown. The release of oxytocin from the isolated neurohypophysis is under inhibitory control of endogenous opioids (Bicknell & Leng, 1982; Racké et al., 1987). Due to the fact that the neurohypophysis contains only axons and nerve endings, it follows that this organ is superbly suited to serve in illuminating the mechanisms involved in opioid-effects at the level of nerve endings.

Isolated neural lobes with their stalks fixed to a platinum wire were superfused with Krebs solution. Oxytocin and vasopressin released into the medium were determined by RIAs. In some experiments the glands were first incubated for 1 h in medium containing 5-8 µCi 86-Rb prior to determining the 86-Rb efflux during superfusion. Hormone release was evoked after 1 h of superfusion by raising the potassium concentration to 30 mM for 30 min. In all experiments tetrodotoxin (1 µM) was present from the onset of superfusion onwards. Test drugs were also added to the superfusion medium from the onset of superfusion.

Table 1.: Effects of naloxone (Nal, 10 µM) and of the potassium channel blockers tetraethylammonium (TEA, 10 mM), quinidine (Quin) and barium (Ba, 0.5 mM) on the high potassium (30 mM) evoked release of vasopressin and oxytocin (mU/30 min)

Test substances	Oxytocin	Vasopressin
Controls	2.01 ± 0.26 (6)	0.45 ± 0.09 (6)
Nal	2.46 ± 0.36 (6)	0.57 ± 0.22 (5)
TEA	3.07 ± 0.48 (7)+	2.13 ± 0.66 (8)++
TEA + Nal	7.13 ± 0.92 (7)*	1.33 ± 0.52 (5)
TEA + Quin (100 µM)	5.36 ± 0.38 (4)	1.36 ± 0.20 (4)
TEA + Quin (100 µM) + Nal	9.83 ± 0.46 (4)*	2.06 ± 0.68 (4)
TEA + Quin (300 µM)	5.00 ± 1.22 (4)	0.79 ± 0.24 (4)
TEA + Quin (300 µM) + Nal	4.93 ± 0.33 (4)	0.79 ± 0.20 (4)
TEA + Ba	4.63 ± 0.75 (6)	2.69 ± 0.81 (6)
TEA + Ba + Nal	6.14 ± 0.64 (6)	2.11 ± 0.36 (6)

Means ± s.e.m of (n) experiments. Significance of differences from the corresponding controls, + $P < 0.05$; ++ $P < 0.01$; from the corresponding value in the absence of Nal: * $P < 0.01$.

Raising the potassium concentration to 30 mM caused an increase of 86-Rb efflux from 3 %/min to 8 %/min. TEA (10 mM) inhibited the high potassium induced increase of 86-Rb efflux by 45 %. In the presence of TEA (10 mM) naloxone (10 µM) reduced the high potassium induced increase of 86-Rb efflux by 40 %. This was not the case in the absence of TEA.

The experiments shown in Table 1 suggest that opioid receptors inhibiting oxytocin release in the neurohypophysis may be linked to potassium channels.

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EFFECTS OF PINACIDIL AND CROMAKALIM ON THE UNSTABLE RAT BLADDER

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Preliminary data (Foster and Brading, 1987) have shown that the potassium channel opener chromakalim may abolish spontaneous bladder contractions in pigs with bladder instability. In the present study the effects of cromakalim and pinacidil, another potassium channel opener, on the bladder function in normal rats and rats with bladder instability secondary to outflow obstruction were investigated.

Under methohexital sodium anaesthesia (50 mg x kg^{-1}, i.p.) the urethra in female Sprague-Dawley rats was partially ligated as described previously (Lindnér, Mattiasson, Persson and Uvelius, 1988). Six weeks after the establishment of obstruction, the urethral ligature was removed under methohexital sodium anaesthesia (50 mg x kg^{-1}). A catheter was placed in the urinary bladder and tunneled subcutaneously in these rats and in age-matched controls. Cystometries were performed two days thereafter in the conscious animals before and one hour after administration of solutions of cromakalim or pinacidil 1 mg x kg^{-1} orally. Data are given as mean ± s.e.m. Statistical evaluation was made using Student's t-test for paired data.

Partial outflow obstruction for six weeks induced a significant bladder hypertrophy. During cystometry rats with bladder hypertrophy exhibited spontaneous detrusor contractions exceeding 15 cm H_2O prior to micturition. In these animals the most prominent effect of both cromakalim and pinacidil was a decrease of the spontaneous bladder contractions to 22 ± 7 % ($p<0.001$, $n=5$) and 26 ± 12 % ($p<0.01$, $n=6$), respectively of that seen prior to drug administration. Further, in both control rats and rats with bladder hypertrophy cromakalim and pinacidil decreased micturition pressure. This effect was most pronounced in rats with bladder hypertrophy. After both cromakalim and pinacidil rats with bladder hypertrophy tended to develop residual urine. Pinacidil and cromakalim had no effects on bladder capacity, basal bladder pressure, threshold pressure, bladder compliance or on bladder wall tension neither in control rats nor in rats with bladder hypertrophy.

The findings of an almost complete disappearance of spontaneous detrusor contractions but a remaining voiding ability after pinacidil or cromakalim indicate that these substances might be a possible therapy in the treatment of bladder instability secondary to outflow obstruction. The mechanism leading to depression of spontaneous bladder contractions cannot be explained from the present data. Further studies need to be performed in order to clarify this and to confirm the present results in man.

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30P

ROLE OF RENAL PERFUSION PRESSURE ON THE RENAL ACTIONS OF ANGIOTENSIN II

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Angiotensin II (AII) has a biphasic action at the kidney, being antinatriuretic and antidiuretic at low and natriuretic and diuretic at high doses. These latter doses also cause an increase in renal perfusion pressure (RPP) which itself could influence the reabsorptive processes. This possibility was studied by comparing the renal responses to AII when RPP was allowed to rise and when it was regulated at control levels.

In pentobarbitone anaesthetised Sprague-Dawley rats (350-400g) cannulae were inserted into the right carotid artery for monitoring blood pressure and sampling, the left jugular vein for saline and drug infusions and the ureter of the left kidney exposed abdominally. Cilazapril was given as a bolus ($0.35 mgkg^{-1}$ plus $0.035 mgkg^{-1}h^{-1}$ iv) to suppress endogenous AII production. Two 15 min urine samples were collected, AII (at 5, 10, 20, 45 and $150ngkg^{-1}min^{-1}$ iv) was infused for 35 min, and urine collected during the last 15 min, 30 min after AII was stopped two further 15 min urine samples were taken. In a second group, the left femoral artery was cannulated to monitor RPP which was regulated by constricting the aorta just above the renal artery.

Table 1 Percentage changes in response to angiotensin II infusion

RPP	MAB	RBF	GFR	UF	FE_{Na}	AII $ngkg^{-1}min^{-1}$
Uncont	7±2**	-16±3***	-10±2*	-22±2***	-12±4**	5
Cont	5±1***	-19±2***	-9±4*	-36±6***	-29±7**	
Uncont	13±2***	-23±3***	-9±3*	-21±4**	-14±8	10
Cont	10±1***	-27±2***	-7±3*	-37±5***	-29±5***	
Uncont	20±2***	-31±3**	-10±6	35±18	34±11*	20
Cont	17±2***	-34±3***	-12±3*	-45±6**	-43±6**	
Uncont	33±6***	-38±4**	-10±7	100±12***	122±13**	45
Cont	19±2***	-44±3***	-13±4*	-51±7***	-46±8***	
Uncont	38±3***	-58±5***	1±10	175±39**	154±36**	150
Cont	23±2***	-59±3***	-27±10*	-72±6***	-68±8***	

- Uncont = uncontrolled (n=7), Cont = controlled (n=8), RPP = renal perfusion pressure, MAB = mean arterial pressure (113±1mmHg), RBF = renal blood flow ($20±1ml min^{-1}kg^{-1}$), GFR = glomerular filtration rate ($3.2±0.1ml min^{-1}kg^{-1}$), UF = urine flow ($56±3\mu l min^{-1}kg^{-1}$), FE_{Na} = Fractional sodium excretion (22.7±0.1%). * = P<0.05, ** = P<0.01, *** = P<0.001.

When RPP was permitted to increase, the low dose AII induced an antinatriuresis and antidiuresis but above $20ngkg^{-1}min^{-1}$ became increasingly natriuretic which paralleled the rise in blood pressure. By contrast, when RPP was controlled the antinatriuresis was enhanced at low doses and became greater at higher doses of the peptide. These findings support those of Olsen et al (1985) in the dog and indicate that RPP is responsible for the natriuresis at high doses of AII.

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EFFECT OF 8-CYCLOPENTYL-1,3-DIPROPYLXANTHINE ON GLYCEROL-INDUCED ACUTE RENAL FAILURE IN THE RAT

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We have previously shown that 8-phenyltheophylline (8-PT), a non-selective adenosine antagonist, reduces the severity of glycerol-induced acute renal failure (ARF) in the rat (Bowmer et al., 1986). Haleen et al., (1987) have reported that 8-cyclopentyl-1,3-dipropylxanthine (CPX) is a highly selective A_1 adenosine antagonist in vitro. In this study, we have evaluated the potential of this compound to ameliorate ARF.

In preliminary studies, CPX (0.1mg kg^{-1} i.v.) produced a 30-fold increase in the dose of adenosine required to produce a 1.0ml min^{-1} fall in kidney blood flow. This dose was selected to treat rats with ARF. Rats were injected with glycerol (10ml kg^{-1} i.m.) immediately following i.v. treatment with either CPX (0.1mg kg^{-1}) or vehicle (1.0ml kg^{-1}; 1.0% DMSO, 0.75% 1M NaOH in saline). CPX or vehicle treatment was repeated 12, 24 and 36h later. One group was injected with glycerol and received no treatment. Blood samples for plasma urea and creatinine determinations were taken prior to and at 24 and 48h after glycerol administration. The clearances of [^{3}H]-inulin (C-IN) and [^{14}C]-p-aminohippurate (C-PAH) were estimated at 48h after glycerol injection.

TABLE 1.

		Group 1 no treatment (n=15)	Group 2 vehicle treated (n=15)	Group 3 CPX treated (n=15)
Plasma urea (mg dl^{-1})	0h	42 ± 1	45 ± 3	40 ± 1
	48h	262 ± 19	273 ± 22	94 ± 8*+
Plasma creatinine (mg dl^{-1})	0h	0.49 ± 0.01	0.52 ± 0.03	0.47 ± 0.02
	48h	3.23 ± 0.41	4.15 ± 0.46	0.85 ± 0.08*+
C-IN (ml min^{-1} $100g^{-1}$ b.w.)		0.18 ± 0.02	0.14 ± 0.02	0.54 ± 0.06*+
C-PAH (ml min^{-1} $100g^{-1}$ b.w.)		0.76 ± 0.07	0.59 ± 0.08	2.39 ± 0.29*+

Mean ± s.e. mean and number of rats in parenthesis.
* $P<0.001$ relative to group 1; + $P<0.001$ relative to group 2 (ANOVA).

Rats treated with CPX had significantly lower plasma urea and creatinine levels compared to untreated and vehicle treated rats. Furthermore, the C-IN and C-PAH of CPX treated rats were significantly higher than in untreated and vehicle treated rats (Table 1). These results show that the adenosine antagonist CPX can ameliorate glycerol-induced acute renal failure in the rat. The beneficial effect of CPX is comparable to that previously noted with 8-PT treatment. However, with CPX this was achieved with a dose 100 times less than with 8-PT.

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GENTAMICIN TOXICITY IN PROXIMAL TUBULAR CELLS ISOLATED FROM RAT RENAL CORTEX

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Gentamicin administered i.p. 50mg/kg/day to Sprague Dawley rats (250-300 g) results in renal proximal tubular damage, manifest by an increase in the excretion of N-acetyl glucosaminidase (NAG) and a decrease in glomerular filtration rate (GFR). Urinary NAG is significantly elevated by day 4 (183 ± 53 compared with 72 ± 9 nmol 4MU/h/kg, $p<0.05$), whereas GFR is only impaired by day 10 (189 ± 84 compared with 385 ± 124 ml/h/kg, $p<0.05$). The aim of this study was to determine if the toxicity elicited *in vivo* could be reproduced *in vitro*.

Proximal tubular (PT) cells were isolated as previously described (Gordon *et al.*, 1987) from rats after 4 and 10 day gentamicin administration and compared with cells isolated from control animals. Transmission electron microscopy revealed an increase in the number and size of lysosomes and the appearance of myeloid bodies in the PT cells isolated from rats treated for 10 days, although cell yield and viability did not differ between the three groups as shown below (Table 1).

Table 1 Characteristics of PT cells isolated from rats treated for 4 or 10 days with gentamicin (50mg/kg/day i.p.)

	Control	4d	10d
Viability (%)	92.4 ± 0.4	92.4 ± 0.7	91.2 ± 0.7
γ-glutamyl transpeptidase (γ-GT) (μmol/min/10^6 cells)	5.10 ± 0.43	3.59 ± 0.33*	3.01 ± 0.44*
PAH uptake (nmol/20min/10^6 cells)	6.7 ± 1.1	15.9 ± 4.2	40.0 ± 12.9*
Cellular NAG (nmol 4MU/h/10^6 cells)	3459 ± 363	5531 ± 631*	3514 ± 584
Lipid peroxidation (pmol MDA/min/10^6 cells)	1.11 ± 0.12	1.92 ± 0.41	1.64 ± 0.36

Results are expressed as mean ± SEM ($n=10$), *$p<0.05$, when compared with control using one way ANOVA with significance assigned using Dunnett's test.

The increase in PAH uptake is similar to that described using renal cortical slices (Lapkin *et al.*, 1977; Gordon *et al.*, 1987). The effects on γ-GT and cellular NAG indicate brush border and lysosomal involvement respectively, which may be secondary to gentamicin induced changes in membrane phospholipids. The sequence of early events in gentamicin toxicity was examined *in vitro* by the addition of gentamicin to PT cells in suspension (100-500μg/ml) for 2h or cultured for 24h (100μg/ml) in 50:50 Dulbecco's MEM : Ham's F12 medium, containing insulin, transferrin, hydrocortisone and sodium selenite. No significant effects on any of the parameters described above were observed, confirming the data of Marche *et al.*, (1987) where phosphoinositide metabolism was more sensitive to the *in vivo* rather than the *in vitro* administration of gentamicin.

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POTENT PRO-INFLAMMATORY PROPERTIES OF AN INTERLEUKIN 1-LIKE MATERIAL PURIFIED FROM THE STRATUM CORNEUM OF NORMAL HUMAN SKIN

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Large amounts of interleukin 1 (IL 1)-like material may be recovered from normal human stratum corneum (s.c.) (Gahring et al, 1985; Fincham et al, 1988). Recombinant (r) IL 1α , however, possesses potent pro-inflammatory properties on intradermal injection in human skin (Dowd et al, 1988). We have therefore determined the inflammatory properties of the s.c. derived IL 1-like material.

Samples of s.c. (150 mg each) obtained from the heels of three normal volunteers by using a file, were homogenised in 0.1% trifluoroacetic acid (TFA) and supernatants ultrafiltered through YM 10 membranes (Amicon). Retentates were subjected to reversed phase h.p.l.c. on a Brownlee RP 300 C8 column eluted with 0.1% TFA in water/acetonitrile (0-100% acetonitrile over 30 min at 1 ml min^{-1}). An aliquot (20 ul) of each 1 min fraction was evaporated and tested in an IL 1 bioassay incorporating the EL-4 NOB-1 cell line (Gearing et al, 1987). A further 100 ul of each fraction was evaporated, reconstituted in 60 ul sterile phosphate buffered saline (PBS) and injected intradermally in each corresponding volunteer. Inflammatory activity was recorded as area of erythema (mm^2) at intervals up to 24 h. Selected biologically active fractions were pooled and individually repurified on a TSK DEAE 5 PW anion exchange column eluted with a 0.1M ammonium acetate gradient (pH 8 to 4 over 30 min, at 1 ml min^{-1}). Portions of each 1 ml fraction (50 ul and 200 ul) were evaporated and respectively tested for IL 1 activity and pro-inflammatory activity in each corresponding volunteer, as described.

After reversed phase h.p.l.c. of each sample, a discrete peak of IL 1 activity was seen in fractions 15-17, and closely co-eluted with a peak of pro-inflammatory activity which occupied the same fractions. There was no consistent biological activity in other fractions. After repurification of pooled fractions (15-17) by anion exchange h.p.l.c., the major IL 1 activity was consistently found in fraction 21 (effluent pH approximately 5), with minor and inconsistent activity eluting at neutral pH. Comparison of IL 1 activity in fraction 21 against standard rIL 1 dose responses curves showed approximately 320, 1000 and 1800 pg IL 1 equivalents ml^{-1} for the three donors respectively. Fraction 21 contained the major pro-inflammatory activity in each case, the reactions being characterised by slight tenderness, swelling and erythema which first became detectable at 1-2 h, reached a maximum at 9-12 h (mean maximum area of erythema = 403 mm^2) and faded by 48 h.

These results indicate that pI 5 IL 1 is the major IL 1 species in normal human heel s.c., as determined by the described purification and bioassay methods. It induces pronounced, persistent inflammatory reactions of a similar magnitude and duration to rIL 1α (Dowd et al, 1988), and appears to be the major >10kD pro-inflammatory compound in normal s.c. Its mechanism of control and release in normal skin, and its role as a mediator of inflammatory skin disease, await further study.

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POTENTIATION OF ENDOTHELIN-INDUCED GASTRIC MUCOSAL DAMAGE IN THE RAT BY INDOMETHACIN

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Endothelin is a novel 21-residue vasoconstrictor peptide synthesized by vascular endothelial cells (Yanagisawa et al., 1988). We have recently demonstrated that endothelin can potently induce gastric mucosal damage in the rat (Whittle & Esplugues, 1988). Since the mucosal formation of vasodilator and protective mediators such as prostacyclin may attenuate such ulceration, we have now investigated whether inhibition of mucosal cyclo-oxygenase would augment the gastric damage induced by local intra-arterial infusion of endothelin.

Male rats (230-250g) were anaesthetised with pentobarbitone, the stomach exposed and the left gastric artery cannulated with a 23g Teflon cannula (Esplugues & Whittle, 1988). After ligating the oesophagus and the pyloric sphincter, 2ml of acid-saline (100mM HCl) were instilled into the gastric lumen via a 25g needle inserted through the forestomach wall. Twenty min following termination of the infusion, the stomach was removed and gastric damage was assessed macroscopically by computerized planimetry. Segments of mucosa were fixed in formalin for subsequent histological evaluation.

Close-arterial administration of the vehicle, saline ($10\mu l\ min^{-1}$) for 10 min induced minimal mucosal damage (0.1 ± 0.1% total mucosal area, n = 5). However, close-arterial infusion of endothelin (4-100 pmole $kg^{-1}\ min^{-1}$) for 10 min induced a dose-dependent increase in the macroscopically assessed damage, that involved 61 ± 13% (n = 4) of the total area of the mucosa with the highest dose. This damage was apparent as areas of vasocongestion, haemorrhage and necrosis, which was confirmed histologically. Pretreatment (15 min) with indomethacin ($5mgkg^{-1}$ s.c.), in a dose reducing the ex vivo generation of prostacyclin by mucosal tissue by over 90% (Whittle, 1983) did not induce any macroscopically detectable damage over the 30 min experimental period. However, pretreatment with indomethacin significantly augmented the effects of a threshold local infusion of endothelin (4 pmole $kg^{-1}\ min^{-1}$), increasing the area of damage from 16 ± 7% (n = 7) to 51 ± 10% of the total mucosal area (n = 4; $P<0.01$). This damage appeared as diffuse areas of haemorrhagic necrosis across the mucosa.

Thus, local intra-arterial infusion of low doses of endothelin induced extensive gastric mucosal damage, which may involve constrictor actions on the gastric microcirculation. Inhibition of gastric cyclo-oxygenase by indomethacin, by preventing the endogenous formation of vasodilator prostanoids such as prostacyclin, could enhance any vasoconstrictor effects of endothelin on the microvasculature. This action could account for the substantial potentiation by indomethacin of the mucosal damage induced by threshold doses of endothelin. These findings suggest that an imbalance between these endogenous endothelial cell-derived vasoactive mediators could thus contribute to gastro-intestinal ulceration, such as that associated with the administration of non-steroid anti-inflammatory agents.

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LOCAL OPIATE-SENSITIVE NEURONAL ACTIVITY MODULATES PAF-INDUCED RAT GASTRIC DAMAGE

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Local intra-arterial infusion of Paf-acether (Paf) induces mucosal damage and motility changes in the rat stomach (Esplugues & Whittle, 1988a,b). Following termination of the local Paf infusion, a prolonged phase of gastric motility has been observed, brought about by activation of local non-cholinergic, non-adrenergic neurones (Esplugues & Whittle, 1988b). We have now investigated whether local neuronal mechanisms can also influence the degree of Paf-induced gastric mucosal damage.

Male rats (230-250g) were anaesthetised with pentobarbitone, the stomach exposed and the left gastric artery cannulated with a 23g Telfon cannula. After ligating the oesophagus and the pyloric sphincter, 3ml of saline were instilled into the gastric lumen via a 25g needle inserted through the forestomach wall.

Close arterial infusion of Paf (50 ng kg^{-1} min^{-1} for 10 min, 12.5µl min^{-1}), with the stomach being removed 20 min later, induced macroscopically apparent damage affecting 30 ± 4% (n = 13) of the total area of the mucosa, as determined by computerised planimetry. Neither vagotomy nor pretreatment with atropine (1.5 mg kg^{-1}, i.v.) nor hexamethonium (5mg kg^{-1}), phentolamine (10 mg kg^{-1} i.v.) and propanolol (1 mg kg^{-1} i.v.) significantly modified the extent of Paf-induced damage. However, local intra-arterial infusion of tetrodotoxin (TTX; 0.05 and 0.1 µg kg^{-1} min^{-1} with 3ml of 5 and 10 µM TTX respectively instilled into the gastric lumen), significantly potentiated the degree of Paf-induced gastric damage (to 72 ± 7%, n = 4 and 97 ± 3%, n = 3 of total area, respectively, $P<0.01$). Administration of TTX alone, in these doses, however, did not induce any macroscopically apparent mucosal damage.

Administration of morphine (0.75 and 3 mg kg^{-1} i.v.) also significantly increased the extent of mucosal damage induced by Paf (to 52 ± 6% and 73 ± 12%, n = 8 of total area, respectively $P<0.01$). N-methyl morphine (15mg kg^{-1} i.v.), a peripherally acting analogue (Smith et al. 1982), likewise augmented damage (to 65 ± 5%, n = 5, $P>0.001$), while both morphine or N-methyl morphine, alone in these doses, did not damage the mucosa. The potentiation of Paf-induced damage by morphine (3 mg kg^{-1} i.v.) was significantly inhibited by pretreatment with the opiate antagonist, naloxone (1 mgkg $^{-1}$ i.v.) to 32 ± 8% of total area (n = 5; $P<0.05$), a level comparable to that induced by Paf alone. Pretreatment with N-methyl nalorphine (3 mg kg^{-1} i.v.) a peripherally-acting quaternary opioid antagonist (Smith et al., 1982), likewise reduced this morphine-potentiated damage to 30 ± 4% (n = 4; $P<0.05$).

This study suggests that a local non-cholinergic, non-adrenergic neuronal mechanism can attenuate the extent of Paf-induced gastric mucosal damage, since TTX but not cholinergic or adrenergic blockade could potentiate such damage. The findings that morphine and N-methyl morphine could likewise augment Paf-induced damage and that such enhancement could be prevented by both naloxone and N-methyl nalorphine suggests that a peripheral opiate-sensitive neuronal pathway may modulate gastric mucosal integrity.

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SELECTIVE INHIBITION OF 5-LIPOXYGENASE BY ICI 207968: IN VITRO AND EX VIVO STUDIES

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Progress in elucidating the biological roles of leukotrienes has been hampered by the lack of 5-lipoxygenase inhibitors with adequate selectivity and oral activity. We report here a novel series of 5-lipoxygenase inhibitors, 2-substituted indazolinones, which combine high selectivity and oral activity and describe the in vitro and ex vivo profile of a member of this series, ICI207968. Biological studies with this compound are described elsewhere (Aked & Foster, this meeting; Foster, Aked, McCormick & Potts, this meeting).

Activity against 5-lipoxygenase was assesssed in a continuous spectrophotometric assay using enzyme prepared from RBL-1 cells (Masters et al, 1988). In vitro and ex vivo 5-lipoxygenase potency and selectivity with respect to cyclo-oxygenase were investigated by measuring LTB_4, TXB_2 and PGE_2 release from whole blood stimulated with calcium ionophore A23187 (McMillan et al, 1986). In vitro effects of inhibitors in the absence of plasma were studied on eicosanoid release from mouse peritoneal macrophages stimulated with zymosan.

Inhibition of isolated 5-lipoxygenase by 2-substituted indazolinones was characterised by extended lag phases, reduced reaction rates and decreased substrate conversion. Cell-free potency correlated strongly with lipophilicity but not with redox potential. Cellular potency and ex vivo activity did not correlate with lipophilicity but the nature of the N2 substituent was critical. The N2 unsubstituted indazolinone was orally inactive, the N2 methyl analogue was orally active but inhibited both 5-lipoxygenase and cyclo-oxygenase whilst the N2 benzyl derivative was selective and possessed weak oral activity in the rat. Further structural modification identified 2-(3-pyridylmethyl)-indazolinone (ICI207968) which combined oral potency and selectivity. ICI207968 inhibited RBL 5-lipoxygenase with an IC_{50} value of 3μM. ICI207968 inhibited LTB_4 generation in murine macrophages and in human and rat blood with similar potency (IC_{50} values = 3μM, 6μM and 1.5μM respectively) and the compound was 200 times less potent as an inhibitor of cyclo-oygenase. In rat blood an additional weak inhibitory action on thromboxane synthase was also observed (IC_{50} = 100μM). In ex vivo studies ICI207968 inhibited LTB_4 release by rat blood (ED_{50} = 3mg/Kg, p.o.) and did not inhibit PGE_2 release at doses up to 300mg/Kg.

We conclude that ICI207968 is an orally active and selective inhibitor of 5-lipoxygenase and will be a valuable agent for clarifying the biological roles of leukotrienes and the therapeutic potential of 5-lipoxygenase inhibitors.

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ANTI-INFLAMMATORY EFFICACY OF ICI207968, A SELECTIVE 5-LIPOXYGENASE INHIBITOR

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One of the key issues surrounding selective 5-lipoxygenase inhibitors is whether such agents will have anti-inflammatory properties. Until recently, however, no suitable model has been available which would allow evaluation of the potential anti-inflammatory efficacy of selective 5-lipoxygenase inhibitors. Intradermal injection of arachidonic acid (AA) in the rabbit causes a PMNL-dependent inflammatory reaction (Aked et al, 1986) which depends, in part, on the local generation of LTB4 (Aked & Foster, 1987). This appeared, therefore, to be an appropriate model of inflammation in which to evaluate the anti-inflammatory efficacy of ICI207968 (2-(3-pyridylmethyl) -indazolinone; an orally active selective 5-lipoxygenase inhibitor (McMillan et al, this meeting).

The local effect of ICI207968 on AA-induced inflammation was investigated. ICI207968 (0-100ug/site) was co-injected intradermally with AA (100ug/100ul pyrogen-free saline) and its effect on plasma extravasation (PE) and neutrophil accumulation was measured, over 60 min, using 125I-albumin and 111In-labelled PMNL respectively, by the methods previously described (Aked et al, 1986; Rampart & Williams, 1988). The bioavailability of ICI207968 in rabbit blood was measured following an oral dose of 30 and 100mg/kg suspended in HPMC (0.5% hydroxypropylmethylcellulose in 0.1% polysorbate 80). Blood was taken at various times, challenged with the calcium ionophore A23187, and LTB4 and PGE2 levels were measured by radioimmunoassay. To assess the effect of ICI207968 on AA-induced inflammation, groups of 10 rabbits were dosed orally with 100mg/kg of compound or vehicle alone. Blood was taken 50 min later and A23187-stimulated eicosanoids were measured. One hr after dosing, AA (5-100ug/site) was injected intradermally and plasma extravasation (PE) was determined 60 min later.

Local administration of ICI207968 caused a dose-dependent inhibition of AA-induced PE (approx.IC30 1.5ug/site) and PMNL infiltration (approx.IC30 9.0ug/site). ICI207968 (100mg/kg p.o.) caused 100% inhibition of LTB4 generation by ex vivo A23187-stimulated blood, for at least 2 hr post-dose, whilst 30mg/kg caused 50% inhibition at 2 hr. No inhibition of PGE2 generation was detected thus supporting the selective action of ICI207968 for 5-lipoxygenase in the rabbit. Orally dosed ICI207968 (100mg/kg) caused approximately 40% inhibition ($P<0.05$) of AA-induced PE for each concentration of AA injected.

These studies support a role for LTB4 in AA-induced inflammation in rabbit skin, and indicate that this is a useful model for evaluating the inflammatory efficacy of 5-lipoxygenase inhibitors. The demonstration of the anti-inflammatory efficacy of ICI207968 supports the view that selective 5-lipoxygenase inhibitors will have therapeutic benefits in the treatment of inflammatory disease.

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CYTOPROTECTIVE PROPERTIES OF ICI207968, A SELECTIVE 5-LIPOXYGENASE INHIBITOR, ON THE RAT GASTROINTESTINAL MUCOSA

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ICI207968 (2-(3-pyridylmethyl)-indazolinone) is an orally active selective 5-lipoxygenase inhibitor (McMillan et al, this meeting) with anti-inflammatory properties (Aked et al, this meeting). Because of the gastrointestinal (G.I.) toxicity associated with inhibitors of prostanoid biosynthesis, (eg.NSAIAs), it is important to establish that any new agent, with therapeutic potential, which interferes with eicosanoid metabolism does not damage the G.I. mucosa. In addition, since leukotrienes have been implicated as mediators of gastric ulceration (Peskar et al, 1986, Rainsford,1987, Broughton-Smith & Whittle, 1988) inhibition of their formation may prevent mucosal damage. Therefore, the effects of ICI207968 on the rat gastric mucosa have been investigated.

Rats were dosed p.o., twice daily for 7 days and once on day 8, with ICI207968 (100mg/kg) suspended in 0.5% hydroxypropylmethylcellulose in 0.1% polysorbate 80 (HPMC) or vehicle alone. One hr after the last dose the rats were killed and the gastrointestinal tracts were examined macroscopically and microscopically (histology) for signs of mucosal damage. In addition, fasted rats were pretreated p.o. or i.p. with ICI207968 or vehicle, 0.5hr prior to the p.o. administration of 50% aqueous ethanol (1ml) and killed 1hr later. In further studies, fasted rats were pretreated p.o. with ICI207968 or vehicle, 0.5 hr prior to a s.c.injection of 30mg/kg of indomethacin in HPMC and killed 5 hr later. Ethanol-induced gastric damage was assessed by measuring the total length of the haemhorrhagic streaks in the stomach and the indomethacin-induced ulceration was assessed by counting the number and measuring the total length of ulcers in the stomach.

Macroscopic and microscopic examination of the gastrointestinal tract, from stomach to terminal ileum, did not reveal any significant difference in the appearance of the mucosal surface of the guts taken from vehicle- or ICI207968-treated rats. In rats treated with ethanol, ICI207968 (100mg/kg) dosed p.o. or i.p. caused 73.5% ($P<0.05$) and 85.3% ($P<0.05$) inhibition of the ethanol-induced gastric damage. In rats treated s.c. with indomethacin gastric ulceration was evident 5 hr after its administration. The number and total length of stomach ulcers were inhibited 96% ($P<0.001$) and 98% ($P<0.001$) respectively by 100mg/kg ICI207968 and 39% ($P<0.02$) and 67% ($P<0.003$) by 10mg/kg.

The lack of damage to the G.I., tract following chronic dosing of ICI207968, suggests that selective 5-lipoxygenase inhibitors are unlikely to have the G.I. toxicity problems associated with NSAIAs. The results described here also clearly demonstrate that ICI207968 protects the rat gastric mucosa against ethanol- and indomethacin-induced damage and support the view that leukotrienes may mediate damage to the gastric mucosa. Such agents therefore may have therapeutic potential for protecting against NSAIA-induced gastric ulceration and may also have utility for the treatment of peptic and inflammatory bowel disease.

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ZYMOSAN INDUCES PMN DEPENDENT AND INDEPENDENT INFLAMMATORY RESPONSES IN THE RAT

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Increased vascular permeability at sites of inflammation can be induced by mediators which act directly on the vascular endothelium e.g histamine, bradykinin or which act via polymorphonuclear (PMN) cells e.g. C5a, LTB_4 (Wedmore and Williams 1981). In a previous communication to the society we have described the inflammatory response to zymosan injected into the peritoneal cavity of rats (Wood et al 1988). We have investigated the mechanisms involved in causing increased vascular permeability in this model.

Charles River CD Rats (250g) were given an i.p. injection of zymosan (10 mg) to induce an inflammatory response. Thirty minutes prior to killing the animals they were given an i.v. injection of pontamine sky blue (50 mg/kg) to label plasma proteins. After killing the animals the peritoneal cavity was lavaged with 5 ml phosphate buffered saline. Aliquots of each sample were taken for total and differential cell counts and spectrophotometric determination of dye labelled plasma protein (Abs 610 n.m.).

Injection of zymosan caused a rapid increase in vascular permeability which then decreased slightly prior to a second larger increase which was maximal at 2.5-3 hours (Table). This second phase of permeability coincided with the onset of PMN influx into the peritoneal cavity (1.1 ± 0.5 x 10.6 PMN/rat t=0, 23.5 ± 3.9 x 10.6 PMN/rat t=3 hours, 43.0 ± 4.8 x 10.6 PMN/rat t=6 hours, mean ± s.e., n=7)

Time after injection zymosan (hours)	Dye extravasation in 30 minutes (Abs 610)
0-0.5	0.37 ± 0.06
0.5-1	0.23 ± 0.03
1.5-2	0.29 ± 0.02
2.5-3	0.66 ± 0.05
5.5-6	0.59 ± 0.06

Results show mean ± s.e., n=8

We studied the effects of phenidone (50 mg/kg p.o.), dexamethasone (10 mg/kg s.c.) and colchicine (2 mg/kg s.c.) on the vascular permeability changes between 0-0.5 hours and 2.5-3 hours. Phenidone, a selective 5-lipoxygenase inhibitor in this model inhibited the early response (67 ± 2% inhibition (I) n=13) but had no effect on the secondary response (-9 ± 10% I n=14) or PMN influx (-3 ± 17% I). In contrast dexamethasone and colchicine had no effect on the early response (-12 ± 13% I, n=7 and -7 ± 11I%, n=14 respectively) but significantly ($p<0.05$) inhibited the secondary response (74 ± 3% I n=7 and 61 ± 5% I n=12) and PMN influx (84 ± 5% I and 99 ± 1% I).

These results suggest that zymosan causes an early PMN independent increase in vascular permeability followed by a later PMN dependent response.

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40P

EFFECT OF PERTUSSIS TOXIN ON NEUTROPHIL ACCUMULATION IN VIVO AND NEUTROPHIL SURFACE CD18 EXPRESSION IN VITRO

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The role of chemoattractant receptors in neutrophil stimulation in vitro is well established, however, the precise mechanisms underlying local leukocyte accumulation at inflammatory sites in vivo have not been defined. In vitro studies have shown that a GTP-binding protein sensitive to pertussis toxin (PT) plays a central role in mediating neutrophil activation induced by chemotactic factors (Becker et al., 1986). In this study, we have investigated the effect of in vitro pretreatment of radiolabelled rabbit neutrophils with PT on their accumulation in vivo in response to zymosan, zymosan-activated plasma (ZAP, source of C5a des arg), FMLP, LTB4 and IL-1. Further, we have studied the effect of such pretreatment with PT on the surface expression of neutrophil CD18 antigen which is thought to be involved in neutrophil adherence (Anderson & Springer, 1987).

Rabbit neutrophils were separated from whole blood and labelled with ^{111}In (Rampart & Williams, 1988). Prior to the final wash, ^{111}In-neutrophils from each donor rabbit were divided into two aliquots one of which was treated with PT (1.0 μg/ml of cell suspension containing 2-5x10^7 neutrophils) for 60 min at 27°C. The second cell aliquot was incubated with the appropriate vehicle. Cells were then washed and resuspended in autologous plasma before i.v. injection into two recipient rabbits. Neutrophil infiltration in rabbit skin was measured over 4h as the local accumulation of ^{111}In-neutrophils. Stimuli were injected intradermally in 0.1ml volumes. Immunofluorescence flow cytometry was used to measure the neutrophil surface expression of CD18 antigen as assayed by binding of monoclonal antibody (MoAb) 60.3 (Wallis et al., 1986). Pretreatment of neutrophils with PT resulted in a significant inhibition of C5a-stimulated granular enzyme release in vitro, e.g. C5a (10^{-8} M) induced release of β-N-acetylhexoseaminidase (from cytochalasin B treated cells) was inhibited by 81±9% (n=4). However, degranulation induced by phorbol 12-myristate 13-acetate was unaffected, indicating that in this study PT was inhibiting responses mediated via chemotactic receptors (Becker et al., 1986). Such pretreatment of ^{111}In-neutrophils with PT resulted in significant ($p<0.05$) inhibition of cell accumulation in vivo in response to all stimuli (numbers shown are ^{111}In-neutrophils accumulating per site for control vs. PT treated cells); ZAP 2816±686 vs. 699±165; FMLP (5x10^{-11}moles) 1948±552 vs. 806±29; LTB4 (5x10^{-10}moles) 1752±525 vs. 617+189 ; zymosan (300 μg) 4026±1024 vs. 1321±428; IL-1 (10 U) 1468±176 vs. 613±180; saline control 402±71 vs. 476±104 (ns) (mean±s.e.mean, n=5 rabbit pairs). The pretreatment of neutrophils with PT had no effect on the basal binding of MoAb 60.3. However, PT inhibited the FMLP-induced increase in expression of CD18. The surface expression of CD18 on toxin treated cells after stimulation with FMLP was in fact below the level of unstimulated cells.

Our results suggest that a PT-sensitive GTP-binding protein mediates chemoattractant-induced neutrophil activation in vivo. Interestingly , pretreatment of neutrophils with PT inhibited IL-1 induced cell accumulation , in spite of the reports that IL-1 lacks neutrophil chemoattractant activity in vitro.

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CONTROL OF THE HUMAN ALVEOLAR MACROPHAGE: EFFECT OF ENPROFYLLINE AND 5'-(N-ETHYL) CARBOXAMIDO ADENOSINE (NECA)

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The alveolar macrophage is an important cell in airway host defence. Once activated, the release of inflammatory mediators gives it a pro-inflammatory role in airway disease. Previously (Baker & Fuller, 1988) we have shown that the release of membrane derived inflammatory mediators thromboxane A_2 and superoxide (O_2^-), could be inhibited by stimulation of adenylate cyclase, however, the release of the phagocytosis marker (N-acetyl-glucosaminidase (NAG) was not inhibited. We also demonstrated that these cells were without functional ß-receptors and could not be inhibited by the phosphodiesterase inhibitor Ro-201724. Xanthines have a proposed anti-inflammatory action which may be related to phosphodiesterase inhibition, adenosine receptor antagonism or through an undetermined mechanism (Persson, 1986). In order to study the effect of xanthines on macrophage activation and the mechanism of their inhibition, we have studied the effect of the xanthine enprofylline, which differs from theophylline in its potency as a phosphodiesterase inhibitor and adenosine antagonist. We have also examined the effect of the adenosine receptor agonist NECA and the combination of the two agents.

Alveolar macrophages isolated by culture in patients undergoing diagnostic bronchoscopy were studied. Cells from 8 patients were incubated in the presence or absence of zymosan, Ro-201724 and enprofylline (0.1-100 µg), from 6 patients with NECA (10^{-7} to 10^{-4} M) and in a further 4 patients with enprofyllin and NECA 10^{-6}M. After 30 minutes the supernatant was harvested for analysis of thromboxane B_2 (TXB_2) by radioimmunoassay and NAG by specific enzymic assay.

In all experiments there was an effect of zymosan which caused a 200 ± 49% increase in TXB_2 release 42 ± 8% NAG release. The table shows the mean ± SE % change in TXB_2 or NAG from the zymosan stimulated control.

	NECA				Enprofylline				NECA 10^{-6} & Enprofylline			
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	0.1	1	10	100	0	1	10	100
	(M)				(µg)				(µg)			
TXB_2	100 ±7	96 ±5	90 ±12	84 ±11	106 ±5	94 ±10	108 ±6	72 ±9	90 ±7	75 ±9	80 ±4	78 ±2
NAG	103 ±10	108 ±5	99 ±5	104 ±7	140 ±58	150 ±41	110 ±34	184 ±71	126 ±28	102 ±26	120 ±22	85 ±21

Therefore thromboxane release was inhibited by the highest dose of either NECA or enprofylline but by all doses of enprofylline in combination with NECA. NAG release was not altered by either treatment.

The release of inflammatory mediators from human alveolar macrophages can therefore be inhibited by the xanthine enprofylline and the adenosine antagonist NECA. The inhibitory effect of enprofylline cannot therefore be due either to phosphodiesterase inhibition (as Ro-201724 was presented in all experiments) or adenosine inhibition as it increased rather than decreased the effect of NECA. The mechanism of the inhibitory effect of enprofylline requires further study.

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BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH PSORIASIS EXHIBIT AN ENHANCED ADHERENCE TO CULTURED VASCULAR ENDOTHELIUM

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Psoriasis is a common skin disease characterized by inflammatory changes including infiltration of blood mononuclear cells into the dermis. Aqueous extracts of psoriatic lesions contain interleukin-1 (IL-1)-like activity (Camp et al, 1986) and treatment of cultured endothelium with IL-1 and other cytokines (Cavender et al, 1986; 1987) is reported to enhance the adhesion of blood mononuclear cells. The aim of this study was to determine if there was an increased attachment of blood mononuclear cells from patients with psoriasis to cultures of untreated and cytokine-treated endothelial cells when compared with normal subjects and, if so, whether this was related to the extent of disease activity.

Blood mononuclear cells were isolated from 20 normal healthy subjects and 20 patients with chronic stable plaque psoriasis. Their adherence was quantitated by measuring the binding of ^{51}Cr labelled mononuclear cells (1 x 10^5 cells) to confluent monolayers of human umbilical vein endothelium grown in microwells. Endothelial cells were pretreated with recombinant IFN-γ for 24 hours and with recombinant IL-1β and tumour necrosis factor (TNF) for 4 hours. Each test was performed in quadruplicate. After incubation at 37°C for 1 hour the non-adherent mononuclear cells were aspirated and the endothelial monolayers washed 5 times to remove non-adherent cells. The endothelium was osmotically disrupted and the radio-lysate counted. Disease activity was assessed and sera screened for immune complexes, acute phase proteins (α1 anti-trypsin, α2-macroglobulin, α1 AGP and C-reactive protein) and complement levels (CH100, C3 and C4).

Mononuclear cells from psoriatic patients showed a greater adherence (mean 55% increase $p<0.01$) to untreated endothelium than cells prepared from normal controls. Treatment of endothelium with TNF (0.02 to 2 units/well) produced a mean 46% increase ($p<0.01$) in adhesion of mononuclear cells for both groups of subjects. There was no significant enhancement of the attachment of normal and psoriatic cells to IL-1-treated endothelium. Incubation of endothelium with rIFN-γ (10-50 units/well) produced an 11% and 23% increase ($p<0.01$) in the adhesion of mononuclear cells from control and patients respectively. The augmented adhesion of psoriatic mononuclear cells to untreated endothelium was unrelated to the disease activity of the patients. In a few patients serum levels of immune complexes, acute phase proteins and complement components were abnormal.

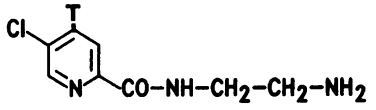
In conclusion, blood mononuclear cells from patients with psoriasis exhibit an increased adherence to cultures of untreated vascular endothelium when compared with cells from normal healthy subjects. These findings imply that abnormalities of mononuclear cell-endothelial interaction may be a factor in the pathogenesis of psoriasis.

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QUANTITATIVE ENZYME RADIOAUTOGRAPHY WITH [^{3}H]Ro 19-6327: LOCALISATION OF MAO-B IN RAT CNS, PERIPHERAL ORGANS AND HUMAN BRAIN

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Monoamine oxidase (EC 1.4.3.4., MAO), a flavin-containing enzyme located in the outer membranes of neuronal and glial mitochondria, exists in two forms, MAO-A and MAO-B, which are identified by their inhibitor sensitivity and by their substrate selectivity [Fowler and Ross, 1984]. Since both forms are important for neurotransmitter regulation, fluctuation in functional MAO activity may be associated with CNS disorders such as depression, Parkinson's disease and Alzheimer's disease. Previous attempts to map the regional and cellular distribution of MAO in the CNS have used enzyme- and immunohistochemical techniques [Arai et al., 1986; Westlund et al., 1988]. The recent development of reversible, selective MAO inhibitors, e.g. Ro 16-6491 and Ro 19-6327 (MAO-B inhibitors) [Da Prada et al., 1986, 1987], as specific radioligands [Cesura et al., 1987], has allowed the identification of their cellular sites of interaction in rodent CNS, peripheral organs and human brain by quantitative enzyme radioautography and image analysis.



([^{3}H]Ro 19-6327

Preclinical profile: short acting, complete reversibility, high potency and specificity, no tyramine potentiation, prevention of MPTP-neurotoxicity.

Cryostat sections of fresh frozen rat brain, spinal cord, pituitary, liver, kidney, skeletal muscle and human brain were incubated in 20 nM [^{3}H]Ro 19-6327 (s.a. 18.9 Ci/mmol) in 50 mM Tris/HCl buffer for 90 min at 20°C, rinsed, rapidly dried and exposed (with tritium brain-paste standards) to LKB Ultrofilm^R for up to 3 weeks at 4°C. Labelling specificity was determined in competition binding with 0.5 µM selegiline or clorgyline.

A high density of in vitro binding sites was observed in discrete regions of rat brain, spinal cord, pituitary, kidney and throughout the liver. No binding was detected in skeletal muscle. The binding was particularly intense in the olfactory nerve layer, ependyma (including all circumventricular organs), hippocampus (lacunosum moleculare), median habenula, paraventricular thalamus, raphe nuclei, interpeduncular nucleus, cerebellum (Bergmann glia cells), nucleus of the solitary tract, inferior olives, spinal cord (substantia gelatinosa), neurohypophysis, renal cortex and renal artery. Intense discrete labelling was also observed in the hippocampus and substantia nigra of human postmortem brain. Binding to all tissues was completely inhibited by selegiline but not by clorgyline.

In conclusion, Ro 19-6327 is a highly selective ligand for MAO-B in brain and peripheral organs; in the former it is probably localized in neurones as well as in glia. It is a potentially useful tool to study, by quantitative enzyme radioautography, MAO-B levels in discrete areas of human brain e.g. in Parkinson's disease and Alzheimer's disease. Since human liver MAO-B has recently been cloned [Bach et al., 1988], the expression of the enzyme can now be studied by in situ hybridization histochemistry.

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CHARACTERISATION OF BINDING SITES FOR [^{3}H]-L644,711, A NOVEL ANTI-OEDEMA AGENT IN MAMMALIAN BRAIN

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L-644,711, (+)-[(5,6-Dichloro-2,3,9,9a-tetrahydro-3-oxo-9a-propyl-1H-fluoren-7-yl)oxy]acetic acid is a non-diuretic fluorenyl derivative of oxyacetic acid loop diuretics, which reduces mortality and neurological morbidity after traumatic head injury in mammals. In particular it prevents the associated astrocytic swelling (Baron et al, 1988) by inhibiting the stilbene derivative (DIDS or SITS) sensitive anion carrier thereby inhibiting the Cl^-/HCO_3 exchange system while having negligible effect on the Na^+, K^+ $2Cl^-$ cotransport system (Garay et al, 1986). The aim of this study was to determine whether binding sites for this substance are present in mammalian brain, and thence their distribution.

Binding of [9^{3}H]-L644,711 (5nM, 21.86Ci/mmole) to rat brain crude synaptic membranes (Bowery et al, 1983) was obtained by incubation in Tris-HCl buffer (50mM, pH7.4) at 20°C for 20 minutes, followed by centrifugation at 10,000g and a 5 min incubation of the pellet in fresh buffer at 4°C. This was centrifuged and radioactivity associated with the pellet measured. Non-specific binding was obtained with unlabelled L644,711 (500µM). Specific binding was 65-80% of total binding in Tris-HCl buffer, which was approximately double that obtained in Krebs-Henseleit buffer. Binding was also estimated to freeze-thawed rat brain sections (10µm), some of which were further processed for autoradiography by the procedure described by Wilkin et al (1981). Sections were labelled by incubation of [9-^{3}H] L644,711 (5-100nM) in Tris-HCl (50mM) for 20 min, followed by a 20 min rinse at 0°C.

The binding of [9-^{3}H] L644,711 to rat brain membranes and slices was saturable and preliminary Scatchard analysis indicated the presence of at least 2 sites with affinities (Kd) in the region of 0.5µM and 5µM. Binding was enhanced in the presence of Ca^{2+} up to 2.5mM. Zn^{2+} (0.1-1mM) could substitute for Ca^{2+} whereas Mg^{2+} did not. Numerous compounds were tested for their ability to alter the binding of [9-^{3}H]L644,711 to brain sections. The anion channel inhibitors SITS, DIDS and 2,4-dichlorophenoxyacetic acid significantly reduced the binding by 33%, 58% and 30% respectively at concentrations between 100-500µM. Interestingly, the antidepressants imipramine and desipramine also depressed the binding (by 44% and 35% respectively at 100µM) whereas amitriptyline and mianserin significantly enhanced binding in a concentration dependent manner (% enhancement at 100µM was 55% and 72% respectively). Frusemide, ouabain, verapamil and diltiazem were without effect at 100µM, and similarly no effect was obtained with ligands for acetylcholine, monoamine and amino acid receptor complexes. L644,711 binding sites were uniformly distributed throughout the brain, although binding was higher in brain stem regions such as the pontine nucleus, and in the cerebellar nucleus, thalamic nuclei and inferior colliculus.

In conclusion, these data suggest that binding sites for L644,711 exist on neuronal membranes in rat brain but do not exclude the possibility of an additional glial cell location. An association with anion channels is also indicated.

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RECEPTOR AFFINITY OF TRH ANALOGUES IN CENTRAL AND PERIPHERAL TISSUES OF DIFFERENT SPECIES: POSSIBLE THERAPEUTIC IMPLICATIONS

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The tripeptide, thyrotropin releasing hormone (TRH), evokes hormone secretion from the pituitary (PIT) gland and has a variety of actions in the mammalian central nervous system (CNS). Although TRH has a short biological half-life it has produced beneficial effects in spinal injury, motoneuron disease (MND), circulatory shock, Alzeheimer's disease (AD) and seizure disorders (Metcalf, 1982). Numerous TRH analogs have been synthesised in order to overcome its lability and to differentiate the CNS and endocrine actions of this peptide. In the present study we have determined the relative affinity of these compounds for TRH receptors in the central and peripheral tissues of different species.

[^{3}H]MeTRH bound to TRH receptors (Sharif and Burt,1983) in rodent, rabbit and dog brain and spinal cord (SC), and in sheep PIT with high affinity (K_ds = 4-7 nM; n = 3) but to different densities of TRH receptors (rabbit SC > sheep PIT >> dog brain > rat SC; range = 27-145 fmol/mg protein). TRH analogs competitively inhibited [^{3}H]MeTRH binding in these tissues (n = 3-6) with K_is of (nM): MeTRH (4-7), TRH (14-65), CG3703 (29-142), RX77368 (112-406), MK-771 (143-245), TRH-Gly-NH_2 (175-808); CG3509 (10-32 μM), $NVal^2$-TRH (12-56 μM), TRH free acid (35-92 μM) and cyclo-His-Pro (> 1 mM). There was an excellent correlation between the rank order of potency of TRH analogs at inhibiting TRH binding in the brain, SCs and PIT of these species, indicating a pharmacological similarity of CNS and PIT TRH receptors. However, RX77368 appeared to exhibit a lower affinity for the sheep PIT (K_i= 406 nM) compared to dog brain, and rat, G.Pig and rabbit SC, suggesting that this analog may be CNS-selective. Interestingly, $NVal^2$-TRH, which has been claimed to be CNS-selective (Szirtes et al.1984), was neither very active nor CNS-selective in any of the above species. Similarly, although CG3509 has potent <u>in vivo</u> activity (Metcalf,1982), it appeared to be much less active than CG3703, RX77368 and MK-771 in all our receptor binding assays. In contrast, CG3703 was more efficacious than TRH and the latter analogs in analeptic, hyperthermic and locomotor stimulant tests (Metcalf, 1982) and does have a high TRH receptor affinity. Interestingly, CG3703 was a more potent competitor of TRH binding in the rabbit brain than in the dog and rat brain, and more potent in the rabbit SC than in sheep PIT ($P < 0.05 - 0.01$). In the dog brain RX77368, TRH-Gly-NH_2 and $NVal^2$-TRH were more potent binding displacers than in sheep PIT, rabbit SC, and rabbit and rat brain, suggesting some species differences.

In conclusion, we have shown that CNS and peripheral (PIT) TRH receptors have a similar pharmacological specificity, but that some TRH analogs appear to be more active at inhibiting TRH receptor binding in some tissues and species than in others. Detailed <u>in vivo</u> studies are now necessary in order to attach biological significance to these <u>in vitro</u> observations. However, in view of the greater metabolic stability of CG3703, CG3509, RX77368 and MK-771 than TRH, these analogs should prove useful as therapeutic agents in combating MND, AD and other neurological disorders mentioned above.

We thank Grunenthal GmbH (CG3703,CG3509), Reckitt & Colman (RX77368) and Merck, Sharp & Dohme (MK-771) for generously providing samples of their analogs.

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MUSCARINIC CHOLINERGIC RECEPTOR BINDING IN THE BRAINS OF PATIENTS WITH IDIOPATHIC PARKINSON'S DISEASE

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Alterations of central cholinergic function in Alzheimer-type dementia are well established. Choline acetyltransferase (CAT) activity and the number of M-2 muscarine receptors are reduced in the cerebral cortex, with no change in the number of M-1 receptors (Mash *et al.*, 1985). In Parkinson's disease (PD) cognitive impairment and dementia also occur (Brown & Marsden, 1984), and CAT activity is decreased in the cortex and hippocampus (Ruberg *et al.*, 1982). We have examined whether altered cholinergic function is associated with changes in muscarinic receptor binding in the frontal cortex (Brodman area 9), caudate nucleus and hippocampus of patients with PD.

Brains were obtained from 7 patients with PD (average age at death 74.7 ± 3.0 years, mean ± s.e.mean) and from 7 control subjects (average age at death 79.1 ± 2.8 years) without neurological or psychiatric diseases. Among Parkinsonian cases, dementia had been present in 3. Two Parkinsonian subjects had received anticholinergic medication prior to death. Saturation analyses were performed on washed membranes with [^{3}H]quinuclidinylbenzilate (QNB; total number of muscarinic receptors; concentrations 9.4 pM - 0.3 nM), [^{3}H]pirenzepine (M-1 receptors; concentrations 0.5 - 64 nM) and [^{3}H]oxotremorine-M (M-2 receptors; concentrations 0.5 - 64 nM). Non-specific binding was defined as the amount of tritiated ligand bound in the presence of 1μM atropine.

Table 1. B_{max} values for muscarinic receptor binding (fmol/mg protein; means ± s.e.mean)

		[^{3}H]QNB binding	[^{3}H]Pirenzepine binding
Frontal cortex:	Control	674 ± 32	438 ± 51
	PD	895 ± 48 *	561 ± 42 *
Hippocampus:	Control	336 ± 24	175 ± 19
	PD	360 ± 33	164 ± 25
Caudate nucleus:	Control	1095 ± 49	746 ± 68
	PD	1130 ± 40	690 ± 53

*) Significantly different from controls ($P < 0.05$, Mann-Whitney U test)

In the caudate nucleus and the hippocampus of patients with PD, no changes in densities of total muscarinic receptors, M-1 receptors or M-2 receptors were observed. In the frontal cortex of Parkinsonian subjects, the concentrations of total muscarinic receptors and postsynaptic M-1 receptors were increased whereas the number of M-2 receptors was slightly reduced.

The increase of muscarinic receptors in the cortex may be induced by treatment with anti-cholinergic drugs prior to death, but this was also seen in patients who had not received these drugs and may reflect denervation supersensitivity due to decreased pre-synaptic cholinergic activity. Receptor supersensitivity is not invariably associated with a cortical cholinergic deficit, since it is not observed in patients with Alzheimer's disease (Mash *et al.*, 1985).

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CONTINUOUS I.C.V. INFUSION OF HEMICHOLINIUM-3 DISRUPTS PERFORMANCE OF RATS IN A T-MAZE REINFORCED ALTERNATION TASK

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Cholinergic dysfunction has long been associated with age related memory deficits indicated by biochemical, electrophysiological and pharmacological evidence (see review by Bartus et al 1984). Hemicholinium-3 [2,2-(4,4-biphenylene)-bis-(2-hydroxy-4-dimethylmorpholinium)-bromide] (HC-3) acts to inhibit acetylcholine synthesis 'in vitro' by blocking the uptake of choline into the cholinergic neurone (Gardiner 1961). This inhibition of choline uptake also provides explanation of its effects 'in vivo' (Freeman et al 1979). The following experiments were devised in an attempt to provide a link between persistent disruption of central cholinergic function and disruption in performance in which a rodent is required to retain information of a spatial task. HC-3 (0.01-10.0μg/day) was infused persistently into the lateral ventricles via chronically indwelling cannulae stereotaxically located (Ant. 5.8, Vert. 2.5, Lat. ±2.0) and coupled to subcutaneously implanted Alzet osmotic minipumps.

Impairments in performance of animals receiving HC-3 were compared with those induced by scopolamine (0.25mg/kg i.p. b.d.) and aged animals (18 months old), and those induced by lesions of the nucleus basalis (ibotenic acid, 5.0μg/0.5μl in phosphate buffer). The male Lister Hooded rats (250-300g OLAC) subject to these treatments were trained on a food reinforced alternation task using an elevated T-maze (modification of the protocol of Salamone et al 1982). Food was withdrawn 2 days prior to the test, and throughout testing animals were deprived of food for 23h per day, but with water available ad libitum (85% normal body weight maintained). Rats were allowed 10 min habituation to the maze on day 1 (both arms baited, banana flavoured reward pellets) and were subject to a pretraining period of reinforced alternation on days 2-5 of the test, with training on days 6-9. All training consisted of paired trials (each pair constituting a "run"), the first being "forced" in that one arm was blocked whilst the other arm was baited. The second was a choice trial in which reward pellets were placed in the arm opposite to that reinforced in the first trial of the pair. A correct choice was when the rat entered the arm containing the food on the choice trial. In addition to correct/incorrect choice, latency to reward was recorded for both forced and choice trials.

The most effective dose of HC-3 to produce a disruption in performance, with minimal peripheral effects, was 1.0μg I.C.V./day. The % correct response of control animals increased from 21.9±5.7 to 78.6±3.1% over the 9 day test period. The response of rats receiving 1.0μg/day HC-3 remained at 51.9±3.3% on day 9 ($P<0.025$ Dunnett's t-test).

The impaired performance of the rats receiving continuous infusions of HC-3 was indistinguishable from that of the aged animals (% correct was 51.9±4.8% on day 9, $P<0.025$), that of scopolamine treatment throughout testing (% correct was 52.4±2.4% on day 9, $P<0.025$) or where central cholinergic function was disrupted by lesions of the nucleus basalis (% correct was 49.9±5.3% on day 9, $P<0.025$).

The present data indicates that, in addition to the impairments seen after scopolamine treatment, after nucleus basalis lesions or in aging, rats subject to continuous ICV infusion of HC-3 also show marked cognitive impairments which may be valuable in analyses of cognitive dysfunction in the rodent.

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REGIONAL DIFFERENCES IN NEUROTRANSMITTER RESPONSES TO A SINGLE ELECTROCONVULSIVE SHOCK (ECS) IN RATS

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Although a number of receptor and behavioural responses change after ECS, the direct effect of the seizure on neurotransmitter release has not been studied. We investigated this by using microdialysis to measure neurotransmitter concentrations in three brain areas before and after a single ECS.

Male Sprague-Dawley rats were stereotaxically implanted with microdialysis probes under chloral hydrate anaesthesia, which continued throughout the experiment. The probes were inserted into either the frontal cortex (FCx), striatum (Str) or nucleus accumbens (NAcc). Artificial CSF was perfused at 1.3 ul/min, and samples were collected every 15 mins, 30 mins after probe insertion, and were immediately analysed by HPLC with ESA coulometric detection. After 4 baseline samples a single tonic-clonic seizure was produced by ECS (80mA, 10 sec, ear-clip electrodes). Control animals only had ear-clips attached.

Baselines were stable in all regions prior to ECS or clipping. Neurotransmitter responses to a single ECS varied markedly between regions, as determined by 2-way analysis of variance with repeated measures compared with controls.

Table 1. Maximum regional DA and NA levels after ECS.

	DA (fmole ± sem)		NA (fmole ± sem)	
Region	Baseline	Maximum Level	Baseline	Maximum Level
FCx	6 ± 1	12 ± 2*	65 ± 10	195 ± 22*
NAcc	16 ± 3	9 ± 2*	28 ± 2	35 ± 2
Str	87 ± 11	100 ± 11	not detected	

(* $p<0.05$)

Concentrations of the dopamine metabolites DOPAC and HVA, and serotonin metabolite 5-HIAAA, increased by 20-50% over baseline in all 3 areas studied, and these did not show any relationship with the neurotransmitter changes seen. No changes were seen in any neurotransmitter or metabolite concentrations in the control animals.

This demonstrates that a single ECS has specific and differential regional effects on extracellular neurotransmitter levels. These may provide insight into the mechanism of the therapeutic effect of this treatment. It is also of interest that these changes are not accompanied by similar changes in metabolite concentrations, perhaps suggesting that these are less useful indicators of neurotransmitter activity than study of the parent compound.

BRAIN ASCORBATE AND MOTOR ACTIVITY IN RESPONSE TO TAIL-PINCH IN RATS AFTER ANXIOLYTIC DOSES OF DIAZEPAM

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When a striatal carbon paste electrode is held at a potential slightly above the peak of the ascorbic acid oxidation current measured by linear sweep voltammetry, a current is recorded which is abolished by the microinjection of ascorbic oxidase; this implies that the current is due solely to the oxidation of ascorbate. The current shows the same diurnal variation as the ascorbate peak in a linear sweep voltammogram and can be recorded continuously. A tail-pinch applied through a paper clip causes a rapid rise in the ascorbate current, whose onset and offset coincides with the application and removal of the paper clip.

We have monitored changes in motor activity and changes in ascorbate, using this novel constant potential voltammetric technique, in response to anxiolytic doses of the benzodiazepine diazepam (DZP). Male Sprague-Dawley rats (350-300g) were implanted with carbon paste electrodes in the striatum and nucleus accumbens under chloral hydrate anaesthesia and experiments were carried out after a 24h recovery period.

In rats given a s.c. injection of 3mg/kg DZP there was an increase in spontaneous motor activity during a 60 min post-injection period compared to vehicle injected controls.

A 5 min tail-pinch was administered 90 min after drug injection which produced a vigorous chewing response followed by a general increase in motor activity after the removal of the tail clip. The duration of this motor activity was defined by the occurrence of the first 2 min silent period after the end of the tail-pinch. In the DZP-injected rats there was a reduction in the duration of the post-stimulus motor activity compared to the vehicle-injected rats.

The DZP injection produced no change in the basal ascorbate current in either striatum or nucleus accumbens. In the vehicle-injected rats the tail-pinch caused a rapid increase in ascorbate current of 1.4+0.34 nA in the striatum and 1.05+0.85 in nucleus accumbens. The height of the ascorbate current response was reduced by the DZP injection to 0.85+0.27 nA in the striatum and 0.62+0.16 nA in nucleus accumbens ($P<0.001$).

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CHOLINERGIC INNERVATION OF THE FELINE ESOPHAGUS

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Our aim was to determine the relative importance of the cholinergic innervation of the feline esophagus and lower esophageal sphincter (LES). Circular strips were subjected to electrical stimulation [1 msec, 2 Hz and submaximal (80 %) voltage, 10 sec/8 min]. Esophageal strips responded with a contraction of 12.9 $\pm$ 1.4 grams. The responses were abolished by tetrodotoxin or atropine. Cisapride enhanced the contractions to 126 $\pm$ 9 % at 5.4 x 10^{-6} M. LES strips responded to electrical stimulation with a relaxation (on-response) of 3.3 $\pm$ 0.7 grams, followed by a small contraction (off-response). The evoked LES-responses could be blocked by tetrodotoxin but were not affected by atropine or cisapride, indicating a neurogenic non-cholinergic nature. When continuous stimulation was applied [1 msec, 0.1 Hz, submaximal (80 %) voltage], the esophageal strips responded with contractions (8.3 $\pm$ 0.6 grams) that were atropine- and tetrodotoxin-sensitive, whereas the LES strips showed only weak contractions (1.4 $\pm$ 0.2 grams). Cisapride doubled the esophageal contractions (190 $\pm$ 34 % at 1.4 x 10^{-6} M). In this respect, it was about 4 times as potent as metoclopramide. Cisapride did not significantly enhance the LES contractions.

In conclusion: Whereas the esophageal body is mainly cholinergically innervated, the lower esophageal sphincter is mainly innervated via non-cholinergic nerves. This explains the distinct motility enhancing effects of cisapride on the esophagus of the cat.

3-AMINOPROPYL PHOSPHINIC ACID INHIBITS THE CHOLINERGIC TWITCH IN THE GUINEA-PIG ILEUM THROUGH $GABA_B$ RECEPTOR AGONIST ACTIVITY

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The guinea-pig ileum has previously been used to evaluate the $GABA_B$ agonist activity of compounds such as GABA and baclofen. $GABA_B$ receptors located presynaptically on cholinergic neurones,mediate a reduction in ACh output in the transmurally stimulated guinea-pig ileum preparation (Kleinrok & Kilbinger 1983).Several compounds have been reported to show $GABA_B$ receptor activity (Bowery,1982), but to date, none have shown an increase in potency over baclofen.

3-Aminopropyl phosphinic acid (3-APPA), a compound, which has been reported to bind to $GABA_B$ receptors in rat brain membranes with a greater affinity than (±) baclofen (Dingwall et al.,1987), has now been tested on the guinea-pig ileum.

Male guinea-pigs (300-450g) were killed, the abdomen opened and the ileum removed into Kreb's solution. Two cm segments were suspended in organ baths containing Kreb's solution under 1g isometric tension. Preparations were electrically stimulated (0.5ms pulse duration, 0.2Hz,25-35V) via platinum electrodes placed transmurally. 3-APPA, like GABA and (±) baclofen, caused a concentration dependent inhibition of the cholinergic twitch contraction in the guinea-pig ileum. Unlike GABA, but like (±) baclofen, 3-APPA did not produce an initial contraction. The EC_{50} for inhibition of the cholinergic twitch for 3-APPA was 1.84±0.23μM (n=12). This compares with EC_{50} values for GABA and (±) baclofen of 13.0+1.63μM (n=8) and 12.6+1.8μM (n=8) respectively. The effects of 3-APPA were not significantly antagonised by bicuculline (10μM), phentolamine plus propranolol (both 1μM), yohimbine (1μm), naloxone (1μM), impromidine (1μM) or 8-phenyltheophylline (10μM). When preparations were made tachyphylactic by 2-3 administrations of (±) baclofen (100μM), there was no further response to 3-APPA (10μM) (n=4).

The results indicate that 3-APPA is a potent, selective $GABA_B$ agonist in the guinea-pig ileum. 3-APPA was found to be seven times more potent than GABA or (±) baclofen on $GABA_B$ receptors in the guinea-pig ileum.

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PENILE ERECTILE RESPONSE IN PITHED RAT; INHIBITION BY 5-HYDROXY-TRYPTAMINE (5-HT)

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The neuropharmacology of penile erection has received considerable attention in recent years, following the introduction of pharmacological treatment for impotence. Recently, an inhibitory role for 5-HT on erection has been demonstrated in conscious rats with spinal transection (Mas et al, 1985). We have used a modification of the pithed rat preparation of Gillespie et al. (1970) in order to stimulate the parasympathetic nerve supply to the penis, and obtain a reproducible erectile response as shown by increase in corpus cavernosum pressure. The spinal electrode (exposed length = 1mm) was positioned at the region of the upper sacral vertebrae. Corporal pressure was measured with a saline-filled 26g hypodermic needle connected to a pressure transducer, and bladder pressure measured after ligation of the urethra. Stimulation of the spinal electrode using square-wave pulses (1ms duration) at 40V, 1 to 30 Hz, produced an increase in both bladder and corporal pressure with no change in arterial pressure. The corporal pressure response was blocked by chlorisondamine (2mg/kg i.v.), whereas atropine (1mg/kg) reduced bladder response but had no effect on corporal response.

Intravenous injection of 5-HT caused a brief pressor response (doses above 8 µg/kg) which was accompanied by inhibition of the corporal pressure response to spinal stimulation. Complete inhibition of the corporal response occurred at doses of 5-HT above 64 ug/kg, with an IC50 value of 14.5±3.3 (SEM, n=6) µg/kg. Bladder contraction in response to spinal stimulation was inhibited by a maximum of 35±4.3%(SEM n=6) by 5-HT. Neither 8-OH-DPAT nor LSD produced a similar inhibition of the corporal response. Both the inhibitory effect of 5-HT on corporal pressure, as well as the pressor effect of 5-HT, were blocked by methysergide (1mg/kg), whereas ketanserin (20 µg/kg) blocked the pressor response to 5-HT without altering the corporal response. These observations indicate the presence of a peripheral 5-HT receptor, inhibitory to the erectile process, which belongs to the 5-HT1 subtype, but cannot at present be related to 5-HT1 A, B or C subtypes.

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ACTIVITIES OF SOME 5-HT ANALOGUES IN 5-HT_3 RECEPTOR MEDIATED RELEASE OF [^{3}H]-ACh IN GUINEA-PIG ILEUM

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It now seems likely that the direct and indirect components of the contractile action of 5-HT in the guinea-pig ileum are mediated by the 5-HT_2 and 5-HT_3 receptor subtypes respectively of the Bradley *et al* (1986) scheme, though there are a number of uncertainties. These include the lack of a selective 5-HT_2 agonist, the involvement of substance P as well as ACh in the excitatory neuronal component (Buchheit *et al* 1985), an inhibitory component also apparently mediated by 5-HT_3 receptors (Gunning & Humphrey, 1987), 5-HT_1-like inhibitory neuronal receptors (Fozard & Kilbinger, 1985) and non-5-HT_3 excitatory neuronal receptors (Sanger, 1987). We therefore examined in greater detail the contractile response to 5-HT through the use of α-Me-5-HT and 2-Me-5-HT claimed to show some selectivity for 5-HT_2 and 5-HT_3 receptors respectively in other systems. Their activities were compared to 5-HT with respect to contraction and to [^{3}H]-ACh release, with ketanserin and GR38032F (Butler *et al* 1988) being used to aid definition of 5-HT_2 and 5-HT_3 receptors respectively. In addition, this provided a direct estimate of the affinity of GR38032F at the prejunctional site.

Concentration-response curves were determined in longitudinal muscle-myenteric plexus strips from the distal ileum of male Dunkin-Hartley guinea-pigs (400-550g) using isometric recording in Krebs' medium containing mepyramine (1μM), ibuprofen (1μM) and hexamethonium (10μM). Under these conditions the three agonists gave curves of similar shape and maxima with activity ratios 5-HT : (±)α-Me-5-HT : 2-Me-5-HT of c. 1.0:0.1:0.2 (EC_{50}, 5-HT 2μM). GR38032F (1μM) gave c. 10-fold displacement to the right of all three curves, but ketanserin (1μM) had no significant effect.

Release of [^{3}H] label after incubation with [^{3}H]-choline was used to estimate ACh release from superfused preparations using methodology similar to Fosbraey *et al* (1984) with evoked output expressed as mean increase in rate coefficient over basal during the 3 min exposure period. Relative activities of the agonists were estimated using a 3x3 Latin Square design, using the NK-3 receptor agonist senktide (10nM) as an internal control. Using this protocol the estimated activity ratios 5-HT : (±)α-Me-5-HT : 2-Me-5-HT were c. 1.0:0.1:0.3 (EC_{50}, 5-HT 2μM). Experiments of similar format showed evoked release of [^{3}H]-ACh by all three agonists was greatly attenuated by the 5-HT_3 antagonist GR38032F (1μM) whereas ketanserin (1μM) was essentially without effect.

It was concluded that under the particular conditions described, the 5-HT_2 receptor contribution to contractile responses of the three agonists was rather small, even though quite large direct responses could be shown in the presence of GR38032F, atropine or TTX. A major part of these agonist responses seem due to ACh release following activation of a 5-HT_3-like receptor whose properties could be defined in terms of the affinity of GR38032F. Comparison of the activities of the agonist ligands studied under these conditions suggests all three are potent at 5-HT_3 receptors, and that therefore (±)-α-Me-5-HT has limited usefulness in this system as a selective 5-HT_2 agonist.

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MECHANICAL, ELECTRICAL AND BIOCHEMICAL EFFECTS OF AH 21-132 IN GASTROINTESTINAL SMOOTH MUSCLE

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AH 21-132 (Bewley & Chapman, 1988; Boyle et al, 1988) has non-specific relaxant activity in guinea-pig isolated trachealis. The tracheal relaxant action of AH 21-132 in some ways resembles that of theophylline but AH 21-132 may be discriminated from theophylline in that (a) its relaxant action is potentiated by epithelial removal (b) it does not release Ca^{2+} from intracellular stores and (c) it exhibits selectivity as an inhibitor of cAMP-PDE as opposed to cGMP-PDE (Boyle et al, 1988).

In transmurally-stimulated (supramaximal voltage; 0.5ms; 0.2Hz) guinea-pig ileum neither theophylline (1mM) nor AH 21-132 (1 and 10μM) antagonised twitch suppression induced by noradrenaline. Theophylline (1mM) antagonised twitch suppression induced by adenosine whereas AH 21-132 (1 and 10μM) had no such effect. Higher concentrations (100μM and 1mM) of AH 21-132 themselves caused twitch suppression. Log concentration/spasm curves for acetylcholine (ACh), histamine and KCl were constructed in guinea-pig isolated ileum. In concentrations greater than 10μM, AH 21-132 depressed the concentration/response curves of each of the spasmogens. Simultaneous extracellular electrophysiological and mechanical recording from guinea-pig isolated taenia caeci showed that AH 21-132 (100μM-1mM) inhibited spontaneous tension waves and their associated bursts of electrical spike activity. Equivalent recording using intracellular microelectrodes showed that the mechano-inhibitory effect of 1mM AH 21-132 was accompanied by suppression of spontaneous spike activity but little, if any, hyperpolarisation.

Table 1 Longitudinal smooth muscle of guinea-pig ileum
Phosphodiesterase (PDE) inhibition; -log molar IC_{50} (mean ± s.e.m.)

	cAMP-PDE	cGMP-PDE
Theophylline	3.41±0.09	3.21±0.11
AH 21-132	5.08±0.17	4.19±0.30

Both AH 21-132 and theophylline inhibited cAMP-dependent and cGMP-dependent PDE derived from longitudinal smooth muscle of guinea-pig ileum. AH 21-132 was the more potent inhibitor in each case (Table 1) and, in contrast to theophylline, exhibited selectivity for cAMP-PDE.

We conclude that, unlike theophylline, AH 21-132 does not act as an antagonist at the A_1 receptors (Paton, 1981) for adenosine which modulate ACh release from ileal cholinergic nerves. AH 21-132 exerts non-specific smooth muscle relaxant effects in gastrointestinal smooth muscle but such effects are observed at greater concentrations than those required to suppress the spontaneous tone of guinea-pig trachea. The lower relaxant potency of AH 21-132 in the ileum cannot be accounted for by lower potency as an inhibitor of ileal cAMP-PDE or cGMP-PDE.

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cGMP AND cAMP CONTENT OF THE RAT ANOCOCCYGEUS DURING RELAXATIONS INDUCED BY DRUGS OR BY NANC STIMULATION

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Relaxations of the mouse anococcygeus muscle to non-adrenergic, non-cholinergic (NANC) field stimulation and to nitrovasodilator drugs are selectively inhibited by N-methyl-hydroxylamine (Gibson, 1988) and selectively enhanced by M&B 22948 (Gibson et al., 1988). These observations suggest that the NANC transmitter of the anococcygeus may resemble, both in structure and mode of action, the nitrovasodilators. This being so, NANC stimulation and the nitrovasodilators should activate the same cellular second messenger systems. Nitrovasodilators are believed to act via generation of cGMP (Waldman & Murad, 1987); for this reason, we have measured cGMP levels, and those of cAMP, in the rat anococcygeus muscle during relaxations induced by drugs and NANC stimulation.

Anococcygeus muscles from male rats (Wistar ; 200 - 400 g) were set up for the recording of isometric tension responses. Relaxations of carbachol (50 μM)-induced tone to drugs or field stimulation were obtained ; at the peak relaxation the organ bath was dropped away from the tissue, which was rapidly frozen in isopentane, cooled to the temperature of liquid N_2. cGMP and cAMP levels in acid extracts of the frozen tissues were measured using standard assay kits (Amersham International). All results are expressed as pmoles nucleotide / g tissue.

Control levels of cGMP (27 ± 5) and cAMP (305 ± 91) were unchanged after exposure to carbachol (26 ± 6 and 231 ± 38 respectively). Sodium nitroprusside (SNP ; 0.1 - 1000 μM) produced concentration-related relaxations of carbachol-tone. Lower concentrations of SNP, which reduced tone by 20% (0.2 μM) and 42% (1 μM), produced no change in cGMP; higher concentrations, which reduced tone by 78% (10 μM), 68% (100 μM), and 78% (1000 μM) did produce significant ($P < 0.05$) increases in cGMP, to 67 ± 12, 88 ± 21, and 106 ± 15 respectively. cAMP levels were unchanged by any of the concentrations of SNP used.

Forskolin also caused concentration-related relaxations; 27% at 0.5 μM, 56% at 2 μM, 99% at 25 μM, and 90% at 250 μM. These concentrations of forskolin increased cAMP levels to 879 ± 101, 1655 ± 360, 3674 ± 304, and 3663 ± 476 respectively, but were without effect on cGMP levels.

IBMX also relaxed carbachol-tone, by 30% at 10 μM, and by 90% at 100 μM.Neither concentration significantly affected cGMP content, but both increased cAMP, to 341 ± 28 and 445 ± 69 respectively.

NANC field stimulation (10 Hz ; 20 sec train) produced a maximum relaxation of 52% but had no effect on either cGMP or cAMP. A combination of such NANC stimulation and 1 μM SNP caused a greater relaxation (84%) than either acting alone (52% for NANC ; 44% for 1 μM SNP); although neither procedure on its own affected cyclic nucleotide levels, the combination of the two resulted in significant elevation of cGMP content (to 78 ± 10) while leaving cAMP unaffected.

Thus, SNP caused a selective elevation of cGMP, although only at concentrations producing relaxations of > 60%; NANC stimulation also selectively increased cGMP, but only when its relaxations were augmented to > 60% by SNP. The results are consistent with the hypothesis that the NANC transmitter and nitrovasodilators are similar, but the lag in detecting elevation of cGMP content requires explanation.

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ATP-INDUCED NON-ADRENERGIC NON-CHOLINERGIC RELAXATIONS OF NEURAL ORIGIN IN THE CANINE ILEUM AND ILEOCOLONIC JUNCTION

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In a previous study, we have provided evidence for the existence of an inhibitory non-adrenergic non-cholinergic (NANC) innervation in the canine ileum and ileocolonic junction (ICJ) (Pelckmans et al, 1987). In the present study, we have attempted to evaluate the role of ATP in this system by means of pharmacological manipulations.

After removal of the mucosa, circular muscle strips of the ileum and ICJ were prepared and mounted in organ baths filled with aerated Krebs-Ringer solution for isometric tension-recording. Results are shown as the mean ± standard error of the mean (s.e.mean) for the number of experiments indicated between brackets.

Under basal conditions, ATP (10^{-6}-10^{-3}M) induced concentration-dependent relaxations and contractions in both ileum and ICJ. Relaxations in the ICJ (10^{-3}M ATP, 0.58 ± 0.10 g, n = 11) were significantly higher than in the ileum (10^{-3}M ATP, 0.17 ± 0.05 g, n = 9), whereas the contractions in the ileum (10^{-3}M ATP, 1.75 ± 0.34 g, n = 9) were greater than in the ICJ (10^{-3}M ATP, 0.57 ± 0.12 g, n = 11). Tetrodotoxin (TTX, $6x10^{-7}$M, n = 5) blocked the relaxations, but not the contractions. Contractions were blocked by both meclophenamate ($3x10^{-5}$M, n = 6) and indomethacin ($3x10^{-5}$M, n = 6). Guanethidine ($3x10^{-6}$M, n = 5) and atropine ($3x10^{-7}$M, n = 5) did not affect the responses to ATP.

During a noradrenaline ($3x10^{-5}$M)-induced contraction, ATP (10^{-4}M) induced transient relaxations, similar to the NANC relaxations obtained by electrical stimulation (ES) and acetylcholine (ACh, $3x10^{-5}$M). TTX, lidocaine and domperidone inhibited the relaxations to ATP (Table 1). Atropine ($3x10^{-7}$M), guanethidine ($3x10^{-6}$M), timolol (10^{-5}M), hexamethonium ($5x10^{-4}$M), bicuculline (10^{-5}M) and desensitisation to γ-aminobutyric acid failed to block the relaxations to ATP. Repeated administration of ATP ($3x10^{-5}$M) resulted in specific desensitisation to ATP, whereas the relaxations to ES and ACh were not affected.

Table 1 Effects of TTX, lidocaine and domperidone on ATP- induced relaxations.

	ICJ		Ileum	
	control	experiment	control	experiment
TTX ($6x10^{-7}$M)	58.06 ± 7.41	0	48.36 ± 7.42	0
Lidocaine (10^{-4}M)	54.35 ± 3.35	12.83 ± 5.54	49.47 ± 8.33	19.05 ± 6.81
Domperidone (10^{-5}M)	58.40 ± 6.62	23.87 ± 7.08	50.61 ± 8.09	27.65 ± 9.81

Relaxations are expressed as percentage of the noradrenaline-induced contraction and shown as the mean ± s.e.mean for n = 6.

In summary, our results indicate that the NANC relaxations to ATP are of neural origin, possibly involving dopamine receptors. Since ATP desensitisation had no effect on the relaxations to ES and ACh, it is unlikely that ATP is the inhibitory NANC neurotransmitter in this model. The contractions to ATP under basal conditions are probably mediated by prostaglandins.

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BOVINE ENDOTHELIUM AND SMOOTH MUSCLE CELLS IN ISOLATION AND CO-CULTURE: RESPONSES TO CALCITONIN GENE-RELATED PEPTIDE

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Human calcitonin gene-related peptide (hCGRP) is a potent vasodilator, but doubt remains over the requirement of endothelium for this response (Brain et al, 1985; Greenberg et al, 1987) and, if so, whether this is a result of the release of endothelium-derived factor (EDRF). EDRF causes relaxation by stimulation of guanylate cyclase in vascular smooth muscle (Rapoport & Murad, 1983).

We have developed a co-culture system, in which bovine aortic endothelial cells (BAE) and bovine aortic smooth muscle cells (BASM) are grown either separately or together. This has enabled us to determine the cyclic nucleotide responses of the cells to vasoactive agents and thus to assess the ability of hCGRP to induce the release of EDRF.

BAE were obtained by brief collagenase (0.5mg/ml) treatment of aortae. BASM were grown from medial explants. Co-culture preparations were obtained either by seeding pre-confluent BAE with BASM or by allowing BAE to become overgrown by contaminating BASM from the original isolation. BAE were used between passages 1-4 and BASM between passages 3-7.

cGMP levels were measured by radioimmunoassay of 0.1M HCl extracts of cells grown in 36mm wells. Bradykinin (BK) induced small and variable rises in cGMP in BAE alone with a maximal response to 1μM BK of 0.208±0.15 pmol/mg protein (mean±S.D. n=4) compared with unstimulated levels of 0.079±0.07 pmol/mg protein. BK had no effect on cGMP in BASM alone; 1μM BK 0.04±0.03 pmol/mg protein versus control 0.096±0.09 pmol/mg protein. However, BK caused large dose-dependent rises in cGMP in co-culture; maximal response was observed with 0.1μM BK, 4.65±0.80 pmol/mg protein compared with control levels of 0.25±0.21 pmol/mg protein (n=3). This effect of BK was inhibited by methylene blue (20μM). These changes were all measured at one minute after addition of BK when cGMP responses were at their greatest. cGMP levels fell to control values by four minutes. In contrast, hCGRP (1nM-1μM) applied to co-cultures caused no change in cGMP in experiments where BK was active; basal 0.275±0.14; 1μM hCGRP 0.34±0.25; 1μM BK 2.495±1.76 pmol/mg protein (n=3).

Adenylate cyclase activation was measured in membrane preparations of BAE and BASM by the method of Salomon et al, 1974. hCGRP caused a dose-dependent rise in cAMP accumulation in both BAE and BASM from basal levels of 4.66±1.14 (n=2) and 5.33±1.77 (n=3) pmol/min/mg protein respectively. K_{act} for BAE was ∿180nM and for BASM ∿350nM hCGRP but V_{max} was 6 pmol/min/mg protein in BASM considerably lower than in BAE 15.5 pmol/min/mg protein

These studies demonstrate that hCGRP directly interacts with BAE and BASM to cause activation of adenylate cyclase but in co-culture there is no rise in cGMP in response to this agent. It, therefore, seems unlikely that vasodilator responses to hCGRP involve EDRF release. The role of the cAMP changes induced by this agent in BAE remains to be determined.

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RISE OF INTRACELLULAR CALCIUM IONS IN EOSINOPHILS INDUCED BY PLATELET ACTIVATING FACTOR

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Eosinophils exhibit chemotactic locomotion (Wardlaw et al., 1986), endothelial adhesion (Kimani et al., 1988), and leukotriene C_4 generation (Bruynzeel et al., 1987) when exposed to platelet activating factor (PAF). In addition, we have demonstrated that human and guinea pig eosinophils are stimulated by PAF to release enzymes and basic proteins stored in their specific granules (Kroegel et al., 1988). PAF-induced degranulation was almost completely abolished in the absence of extracellular Ca^{2+}. This results prompted us to investigate the role of Ca^{2+} in the PAF-induced activation of eosinophils. Eosinophils where obtained from polymyxin B treated guinea pigs by peritoneal lavage. The cells were purified using a discontinuous gradient of isomolar Percoll solutions. Eosinophils (purity >97%; viability >99%) then were loaded with fura-2 and fluorescence of $3x10^6$ cells was monitored in a spectrophotofluorimeter at 37°C. Addition of PAF caused a dose-dependent elevation of the intracellular calcium concentrations ($[Ca^{2+}]_i$) above basal values of 120 $^+$ 36.5 nM (n=10) up to approximately 1000 nM. The effect of PAF was rapid, transient and returned to basal levels within 3 min. The response to PAF was dose-related with a mean EC_{50} of 17.6 nM (n=7) and maximal at 1000 nM. The PAF receptor antagonist WEB 2086 specifically inhibited the elevation of $[Ca^{2+}]_i$ induced by PAF with an mean IC_{50} of 59.5 nM (n=5).

Leukotriene B_4 (LTB_4) and fMet-Leu-Phe (fMLP) also increased the $[Ca^{2+}]_i$ in eosinophils. Whereas PAF and LTB_4 showed a similar time course, fMLP was much slower in onset but with a longer duration of action. After initial exposure to PAF the same cells were fully responsive to subsequent application of LTB_4 or fMLP. In contrast, application of PAF rendered the eosinophils nonresponsive to a second exposure to PAF, indicating homologous desensitization. Incubation of eosinophils with PAF (100 pM - 1 uM) in Ca^{2+}-free buffer in the presence of 5 mM EGTA or 1 mM Ni^{2+} led to a significant inhibition of the rise in $[Ca^{2+}]_i$ of $85.6^+5.4$% ($p<0.05$; n=12) and $95.8^+2.1$%($p<0.05$; n=12), respectively. In contrast, the dihydropyridine antagonist nimodipine up to concentrations of 10 uM did not effect the response seen with PAF. These results indicate that the PAF-induced rise in $[Ca^{2+}]_i$ arises mainly from an influx of extracellular Ca^{2+} and that voltage-dependent Ca^{2+} channels are not involved.

In conclusion, our results suggest that an increase in $[Ca^{2+}]_i$ is involved in PAF-mediated eosinophil activation and is due to entry through receptor-operated Ca^{2+} channels.

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RENAL EFFECTS OF CYTOCHROME P450-DEPENDENT METABOLITE OF ARACHIDONIC ACID: 12(R) HYDROXYEICOSATETRAENOIC ACID

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12(R) hydroxyeicosatetraenoic acid (HETE) is the principal cytochrome P450-dependent arachidonic acid metabolite of the corneal epithelium (Schwartzman et al., 1987). In contrast to the 12(S) enantiomer formed by lipoxygenase activity, 12(R) HETE is a potent inhibitor of Na^+-K^+-ATPase (Schwartzman et al., 1987) and attenuates potassium-induced vascular relaxation (Masferrer and Mullane, 1987). Like the cornea, the thick ascending limb of the loop of Henle metabolizes arachidonic acid via cytochrome P450 forming another Na^+-K^+-ATPase inhibitor, 11,12 hydroxyeicosatrienoic acid, the hydrolysis product of 11,12 epoxyeicosatrienoic acid (Schwartzman et al., 1985). Theoretically, 12(R) HETE could arise from this same precursor. Moreover, both tissues exhibit furosemide inhibitable sodium chloride co-transport. We, therefore, studied the renal effects of 12(R) HETE using the isolated, perfused rat kidney.

Male Sprague Dawley rats (325-400 g) were anesthetized with phenobarbital (60 mg/kg, i.p.). Catheters were placed in the ureter, lower abdominal vena cava and into the right renal artery via the mesenteric artery. The kidney was perfused, in a recirculating system, with modified Krebs' buffer which included 20 amino acids, urea (6 mM), lactate (5 mM) and pyruvate (5 mM) and contained 10 g/L bovine serum albumin and Ficoll 70, 35 g/L as oncotic agents. An intrarenal perfusion pressure of 90 mmHg was maintained by adjusting flow. After 15 min equilibration, urine was collected for a 15 min control clearance period following which the effect of 12(R) HETE (10-40 nM) or vehicle was examined over three subsequent periods.

Urine volume increased immediately in response to 40 nM 12(R) HETE (n=4) to 115 ± 39 μL/min (mean $\pm$ SEM) compared to 40 ± 7 μL/min in the vehicle group. At 20 nM (n=5) urine volume increased gradually to 92 ± 18 μL/min by period 3. 10 nM (n=5) was without effect. Sodium excretion also increased in a dose- and time-dependent manner, reaching a maximum of 7.41 ± 4.27 μEq/min in period 2 with 40 nM 12(R) HETE vs. 2.28 ± 0.86 μEq/min in the control. Potassium excretion was elevated within the first clearance period after 20 and 40 nM 12(R) HETE being 3.78 ± 10.44 and 4.33 ± 1.06 μEq/min, respectively, compared to a control of 2.24 ± 0.44 μEq/min. We conclude that 12(R) HETE can influence transport function in the whole organ, presumably through its ability to affect Na^+-K^+-ATPase.

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60P

TACHYPHYLAXIS TO THE VASOCONSTRICTOR EFFECTS OF ARACHIDONIC ACID IN THE RAT ISOLATED, PERFUSED KIDNEY

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We have previously shown that arachidonic acid (AA) elicits vasoconstrictor responses in the isolated perfused kidney of the rat and that these responses are dependent upon conversion of AA by cyclooxygenase to endoperoxides (1). However, repeated administration of AA leads to progressively diminished vasoconstrictor responses. The present study was undertaken to characterize the development of tachyphylaxis.

Ten male Wistar rats, 8-10 weeks of age, were used in these experiments. Rats were anaesthetized with pentobarbitone (60 mg/kg i.p.) and the right kidney exposed by midline laparotomy. The vena cava below the right renal vein was cannulated for the collection of perfusate. The right renal artery was cannulated via the mesenteric artery and the ureter transected. The kidney was then perfused with oxygenated Krebs'-Henseleit solution at 37°C. During a 10 minute equilibration period, flow was adjusted to obtain a stable perfusion pressure (PP) of approx. 80 mmHg. The renal venous effluent was collected for 1 min before any drug administration. Drugs were given close arterially as 10 μl bolus injections. AA (3 μg) was given every 10 min for a total of 4 administrations and PP changes recorded. Perfusate collections were made for 1 minute periods (control) 2 minutes before the administration of AA, and for 2 mins after the administration of AA. In 5 preparations, PP responses to 100 ng U46619 were obtained 10 mins before the first administration and 10 mins after the last administration of AA to detect any changes in sensitivity to endoperoxides or TxA_2. In four of the preparations, angiotensin II (AII), 10 ng, was given before and after AA to determine prostaglandin release and vasoconstrictor responses. Prostanoids in the renal venous effluent were measured directly using specific radioimmunoassays.

The basal perfusion pressure and flow rate for all preparations were 80±2 mmHg and 9.3±0.5 mls/min, respectively. Repeated administration of 3 μg AA led to a progressive decline in the vasoconstrictor response obtained. Thus, the four consecutive doses of AA produced PP changes of 124±12, 86±12, 41±14 and 17±9 mmHg, respectively ($p<0.05$). The vasoconstrictor responses to AII and U46619 were unaffected by the development of tachyphylaxis to AA. U46619 and AII increased PP by 72±18 mmHg and 91±14 mmHg, respectively, before AA administration compared to 82±19 mmHg and 98±13 mmHg, respectively, after AA.

The development of tachyphylaxis to the vasoconstrictor effects of AA was accompanied by progressive decrements in prostanoid release. PGE_2 efflux fell from 8.2±1.4 ng/min to 2.1±0.2 ng/min ($p<0.01$) after the first and fourth doses of AA, respectively, while 6-keto-$PGF_{1\alpha}$ release declined from 9.6±0.9 ng/min to 1.5±0.2 ng/min ($p<0.01$). Similar reductions were observed for $PGF_{2\alpha}$ and TxB_2. In contrast, prostanoid release to AII, given before and after the development of tachyphylaxis, was enhanced. Thus, before AA, AII increased the release of PGE_2 and 6-keto-$PGF_{1\alpha}$ by 3.8±1.7 ng/min and 1.1±0.6 ng/min, respectively, whereas after AA, the values were 17.0±4.4 ng/min ($p<0.05$) and 2.8±1.3 ng/min, respectively.

This study demonstrates that the development of tachyphylaxis to the renal vasoconstrictor effects of AA is associated with a decline in conversion of AA to prostanoids suggesting inhibition of cyclooxygenase. However, as AII-stimulated prostanoid release was not decreased the possibility exists that uptake of AA may be affected.

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EFFECTS OF BN 52021 IN DIFFERENT FORMS OF ACTIVE ANAPHYLAXIS; IMPORTANCE OF THE BOOSTER INJECTION

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The role of Platelet-activating factor (PAF) in acute anaphylaxis is now better recognized, particularly after antagonists, such as BN 52021 or WEB 2086, which block bronchoconstriction (BC) of passive anaphylactic shock in the guinea-pig (Lagente et al., 1987; Pretolani et al., 1987) were described. When guinea-pigs were sensitized with high amounts of antigen in a single injection (no booster), BN 52021 also inhibited BC of active anaphylaxis (Berti et al., 1986). In contrast, we found that BN 52021 fails to inhibit BC of active anaphylaxis when guinea-pigs are sensitized with low amounts of ovalbumin [10 μg in Al $(OH)_3$] injected i.p. twice at two weeks interval, being challenged seven days later by intravenous antigen. BN 52021 also failed to inhibit anaphylactic BC and release of thromboxane B_2 and histamine from perfused lungs provided by actively sensitized guinea-pigs. In this model, Pretolani et al. (1988) demonstrated that active sensitization of guinea-pigs leads to *in vitro* bronchopulmonary hyperresponsiveness to PAF. Nevertheless, the model of systemic anaphylactic shock is complex, because of the multiplicity of targets and mediators involved. This led us to deliver the antigen directly into the trachea of actively sensitized guinea-pigs, which induced immediate BC, with thrombocytopenia (30-40 %) and leucopenia (20-30 %) occuring during the 30 minutes following the challenge. The time-course of the anaphylactic response, the amounts of circulating IgG and the ability of serum of actively sensitized animals to passively sensitize lung strips of normal guinea-pigs were studied. No anaphylactic BC, nor IgG or transferable sensitizing activities were detected up to 14 days after the sensitizing injection, but if an antigen booster was administered at day 14, the amounts of IgG and the capacity of serum to sensitize passively was raised up to day 21, decreasing very slowly thereafter. Under those conditions, BN 52021 administered by the intra-trachal route (1 mg, 30 min before ovalbumin, also given intra-tracheally at 100 μg) failed to inhibit BC, leucopenia and thrombocytopenia of guinea-pigs which were boosted at day 14 and challenged at days 21, 42, 70 or 98.

The serum of animals which were sensitized without being boosted and were bled at days 21 or 70 after the ovalbumin injection contained very low amounts of IgG and failed to passively sensitize lung strips from normal guinea-pigs. Nevertheless, these sensitized but non-boosted guinea-pigs challenged at days 21 or 70 did develop an anaphylactic BC to ovalbumin administered intra-tracheally at 1 mg. This BC was blocked by 1 mg of BN 52021 (day 21, 60.9% and day 70, 74.8 % inhibition) but leucopenia or thrombocytopenia were not affected by BN 52021.

Another group of guinea-pigs was sensitized by a first injection of ovalbumin on day 1, was boosted on day 98 and challenged 7 days later. The amount of circulating IgG detected were equivalent to those of guinea-pigs which were challenged 7 days after the booster injection and here also BN 52021 failed to inhibit BC (non-significant reduction of 32.2 %) and trombocytopenia or leucopenia.

In conclusion, our results demonstrate that the booster injection determines whether the anaphylactic BC in actively sensitized animals will or will not be suppressed by the PAF antagonist BN 52021. This may not be a special case, because in other circumstances PAF antagonists may also fail to inhibit not only antigen-induced BC but also PAF-induced effects *in vitro*, whenever the tissues are provided by boosted guinea-pigs (Pretolani et al., 1988). The booster appears to operate as an inflammatory challenge, modifying tissue reactivity, possibly via a shift to the lipoxygenase pathway (Pretolani et al., submitted).

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EFFECTS OF PROSTAGLANDIN E_2 AND CYCLOPENTYLADENOSINE ON NEUTROTRANSMISSION IN GUINEA-PIG ILEUM: INFLUENCE OF PERTUSSIS TOXIN

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Prostanoids enhance and adenosine inhibits electrically-induced contractions of the guinea-pig ileum longitudinal muscle-myenteric plexus preparation (GPI_L-MP; Ehrenpreis et al 1973; Gintzler and Musacchio 1975). These agents may produce their effects through stimulation and inhibition respectively of adenylate cyclase (Shimizu et al 1982; Tucker and Von Uexkull 1985). In order to determine whether the guanine nucleotide binding protein (Gi), which couples receptor activation to adenylate cyclase inhibition, contributes to the responses of GPI_L-MP to prostaglandin E_2 (PGE_2) and the adenosine A_1-receptor agonist, cyclopentyladenosine (CPA; Williams et al, 1986), we have investigated the effects of pre-treatment of guinea-pigs with pertussis toxin (PTX). PTX is a bacterial exotoxin which permanently inactivates G_i and 'G_i-like proteins', but has no effect on Gs which couples receptor activation to adenylate cyclase stimulation (Murayama and Ui, 1983; Northup, 1985).

Segments of proximal ileum were removed from male guinea-pigs (400-500g), 72hrs following intraperitoneal injection of either 50µg/kg purified PTX (Porton Products Ltd) or sterile saline. Longitudinal muscle strips with the myenteric plexus intact were prepared and stimulated electrically as described previously (Poll et al, 1988). Statistical significance was determined using an unpaired Student t-test.

PGE_2 (0.3-300nM) produced a concentration-related potentiation of field-stimulated GPI_L-MP contraction in tissues taken from either control or PTX pre-treated animals, with EC_{50} values of 4.0 ± 0.2nM (n=3) and 3.0±1.0nM (n=3) respectively. These values were not significantly different ($p>0.05$). CPA (0.3-300nM), in contrast to PGE_2, caused a concentration-related inhibition of field-stimulated GPI_L-MP contraction, with IC_{50} values of 2.2±0.5nM (n=5) and 41 ± 0.5nM (n=6) being obtained in tissues taken from control and PTX- pre-treated animals respectively. These values were significantly different ($p<0.001$). In addition, in three separate experiments, prior administration of CPA (300nM) abolished the potentiation obtained with PGE_2 (0.3-300nM) in control tissues giving a concentration ratio of >100. However, in tissues taken from PTX pre-treated animals, this inhibitory effect of CPA was significantly reduced, with a mean concentration ratio (95% C.L.) of 5.5 (3-10, n=3).

This study demonstrates that PGE_2-induced augmentation of field-stimulated GPI_L-MP contraction does not involve a PTX-sensitive G-protein. In contrast, the inhibitory effect of CPA both on responses to electrical stimulation and on the enhancement of those responses by PGE_2, appears be sensitive to PTX and may involve a Gi-protein.

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AH-6809 DISTINGUISHES TWO TYPES OF DIRECT CONTRACTILE ACTION FOR PROSTANOIDS ON GUINEA-PIG ILEUM LONGITUDINAL MUSCLE

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In the presence of tetrodotoxin, morphine or atropine, PGE_2 directly contracts guinea-pig ileum longitudinal muscle. This action is blocked by SC 19220 and AH 6809 and has been attributed to the activation of EP_1-receptors (Kennedy et al, 1982; Coleman et al, 1987a). We have found however that AH 6809 blocks the contractile actions of PGE analogues on the ileum to different extents.

Isometric contractions were recorded from lengths of terminal ileum suspended in Tyrode's solution gassed with 95% O_2/5% CO_2 at 37°C. In the presence of 1μM morphine, ICI 80205, PGE_2 and 17-phenyl PGE_2 gave maximum responses equal to about 85% of the acetylcholine maximum : EC_{25} values are given in Table 1. AH 6809 (2μM) produced roughly parallel rightward shifts of the agonist log concentration-response curves, consistent with a pA_2 of 6.8 - 7.1. Carbacyclin, one of several PGI_2 analogues with potent PGE-like contractile activity (see Dong et al, 1986), was similarly blocked by AH 6809. Sulprostone however was shifted to a small extent by AH 6809 at the 10-30% response level, but to a larger extent at the 50-70% level (dose ratio ≈ 16). Finally MB 28767 (Banerjee et al, 1985) gave a shallow log concentration-response curve reaching 55% of the acetylcholine maximum at 1μM: AH 6809 had little effect on the contractile activity of MB 28767.

Table 1 Potencies of prostanoids on guinea-pig isolated preparations

Prostanoid	Ileum (1μM Morphine) EC_{25} (nM)			Trachea EC_{25} (nM)	Vas deferens IC_{50} (nM)
	control	AH 6809 2μM			
ICI 80205	0.77	13	(17)*	0.06	1.2
PGE_2	5.3	150	(28)	5.5	1.5
17-Phenyl PGE_2	4.4	60	(14)	1.0	11
Carbacyclin	28	840	(30)	14	87
Sulprostone	17	50	(2.9)	3.7	0.21
MB 28767	15	25	(1.7)	900	1.1

n = 4. * Bracketted values are dose ratios for AH 6809 antagonism.

Our experiments on guinea-pig tracheal rings (1 μM EP 092 was used to block TP-receptors) show that MB 28767 has little EP_1-receptor agonist activity (Table 1). This would account for its lower maximum on the ileum. Both MB 28767 (Table 1) and sulprostone (Coleman et al, 1987b) are highly potent inhibitors of the twitch response of the guinea-pig vas deferens to electrical field stimulation. The latter action has been proposed to derive from activation of EP_3-receptors (Coleman et al, 1987b). It is possible therefore that direct contraction of guinea-pig ileum longitudinal muscle can be mediated via activation of both EP_1 and EP_3-receptors. Our experiments point to the difficulty of determining the mechanism and potency of EP_1-receptor antagonists on guinea-pig ileum.

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POSSIBLE REQUIREMENTS OF A LIPOXYGENASE METABOLITE FOR INTERLEUKIN-1 INDUCED PROSTAGLANDIN BIOSYNTHESIS

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The relationship between interleukin-1 and arachidonic acid metabolism, particularly the induction of prostaglandin biosynthesis from various cell types, has been extensively studied. The mechanism for this induction is not clear although recent studies have shown interleukin-1 to stimulate PGE_2 production by increasing cellular synthesis of cyclo-oxygenase (O'Neill et al., 1987). Furthermore, enhanced levels of lipoxygenase products (Smith et al., 1987) and phospholipase A_2 activity (Chang et al., 1986) have been observed following interleukin-1 stimulation. Augmented prostanoid synthesis and phospholipase A_2 activity have also been demonstrated using leukotriene D_4 (Clark et al., 1986). In this paper we have investigated the modulation of human recombinant interleukin-1 beta (hrIL-1β)-induced prostaglandin release using selective inhibitors of arachidonic acid metabolism and protein synthesis.

Rat stomach strips, prepared as previously described (Vane, 1957), were suspended under 2.5 g load in 20 ml organ chambers and bathed with Krebs solution. Tissue contractions following the addition of calcium chloride ($CaCl_2$) to the bathing solution were detected by isotonic transducers. Concentration-effect curves were repeated in the presence and absence of hr IL-1β as well as combinations of the interleukin with indomethacin, nordihydroguaiaretic acid (NDGA), actinomycin-D or cyclohexamide.

$CaCl_2$ (0.5-5 mM) caused concentration dependent contraction of tissues. Indomethacin (5.6 μM) inhibited these responses (94.4±2.5%; n=5) but NDGA (20μM) was without effect (n=3). Indomethacin had no effect on contractions induced by acetylcholine (200 μM). hr IL-1β (30 pM) added 20 min prior to the $CaCl_2$, augmented $CaCl_2$ maximum response (62.4±5.1%; n=20). Indomethacin decreased $CaCl_2$ contraction in the presence of hrIL-1β such that approximately 20% of control response was evident, this being reduced further to less than 5% by NDGA (n=5). In contrast, NDGA inhibited only the augmented response to hrIL-1β (n=5). Actinomycin-D (10 μM) and cyclohexamide (356 μM), added 15 min prior to hrIL-1β, reduced the enhancement of $CaCl_2$ contraction such that only a small percentage of the augmentation was evident (22.4±5.1 and 24.0±3.0 respectively; n=4). In both cases NDGA further reduced this contraction to that obtained by control maximum (n=4).

These results indicate that $CaCl_2$ induced contraction is mediated by the production and release of prostaglandins. The increase in prostaglandin formation by hrIL-1β appears to be mediated by a lipoxygenase metabolite which might be requisite for the induction of de novo protein synthesis.

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PROSTANOID-INDUCED CONTRACTION OF MYOFIBROBLASTS: EFFECT OF THE THROMBOXANE (TP) RECEPTOR ANTAGONIST, GR32191

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Dupuytren's contracture is a condition for which surgical intervention is of limited success (Schneider, 1986), and there is no successful therapeutic treatment. The myofibroblast is considered to be responsible for initiation of the contractures and we now report a possible approach to the selective inhibition of this response.

Palmar nodules, either contained within cords or macroscopically distinct, were obtained from patients undergoing surgery for Dupuytren's contracture (n =18). Each tissue was used within 60 minutes of excision, cleared of extraneous material and cut into strips along the longitudinal axis. Four strips were examined per sample. Macro- and microscopically uninvolved tissues, obtained from the same cords, were used as controls. The preparations were superfused as previously described (Cross et al, 1988), and cumulative dose-response curves were constructed to PGD_2 (7×10^{-6}M-10^{-4}M), PGE_1 (7×10^{-6}M-10^{-4}M), PGE_2 (7×10^{-7}M-10^{-5}M), $PGF_{2\alpha}$ (7×10^{-7}M-10^{-5}M) and the stable thromboxane mimetic, U46619 (7×10^{-7}M-10^{-5}M), on tissue strips from seven patients. In tissues taken from another group of patients (n = 11), responses to U46619 were determined in the absence and presence (10^{-9}-10^{-7}M) of the thromboxane (TP) receptor antagonist, GR32191 (Lumley et al, 1987). After both the agonist and antagonist studies, the tissue response to the non-prostanoid contractile agonist, mepyramine (0.5mM) was determined.

Control tissues failed to respond to any of the agents used. In contrast, PGD_2 contracted 2/7, PGE_1 and PGE_2 relaxed 3/7, and $PGF_{2\alpha}$ contracted 1/7 preparations from Dupuytren's nodules. In the same experiments, U46619 contracted 3/7, 4/7, 4/7 and 6/7 tissues. In the presence of GR32191, a concentration-related inhibition of responses to U46619 was observed (pA_2 value between 8 and 9). In the same concentration range the antagonist had no effect on responses to mepyramine.

In all of the tissues that responded to prostaglandins, U46619 produced concentration-dependent and repeatable contractions. These responses showed a slow reversibility on washing (>40 min for complete recovery). The rank order of potency was U46619 >> $PGF_{2\alpha}$ > PGD_2 > PGE_1 and PGE_2 and this, together with the finding that GR32191 antagonised responses to U46619, suggests that myofibroblasts from Dupuytren's nodules contain TP-receptors (Kennedy et al., 1984). As yet, the involvement of TP-receptors in Dupuytren's contracture is unknown, but these findings demonstrate for the first time that a TP-antagonist such as GR32191 is capable of selectively inhibiting the contractile activity of the myofibroblast.

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EFFECT OF GOSSYPOL ON cGMP RELEASE FROM THE ISOLATED PERFUSED RAT KIDNEY

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Vasodilatation mediated by endothelium-derived relaxing factor (EDRF) is a well established phenomenon in arterial tissue which has been proposed to be mediated by stimulation of cGMP (Griffith et al, 1985). Little is known however about the importance of EDRF to the renal vascular bed, although in the rat kidney (Burton et al., 1988) as in the rabbit coeliac artery (Alheid et al., 1987) vasodilatation induced by acetylcholine (Ach) can be selectively inhibited by the EDRF antagonist gossypol. In the present experiments, performed in the isolated perfused rat kidney, the effect of gossypol on the renal release of cGMP has been measured following dilatation with either ACh or sodium nitroprusside (SNP).

Kidneys obtained from male Wistar rats (250-300g) were perfused at constant flow with Krebs-Henseleit containing theophylline (1mM) at 37°C. In control kidneys following preconstriction with methoxamine, vasodilatation was induced by 0.3μM ACh or 1μM SNP, each kidney receiving only one dilator dose. In experimental kidneys, gossypol (15μM) was administered for a 10 minute period followed by 30 minutes washout prior to administration of ACh or SNP. Renal perfusate effluent was collected and boiled for 2 minutes following which 4 ml samples underwent cationic exchange chromatography. The freeze-dried eluate was acetylated and immunoassayed for cGMP.

Gossypol markedly inhibited the vasodilator response to ACh without any reduction in the dilatation induced by SNP.

Table 1 Effect of gossypol on the renal release of cGMP (fmol/min)

	CONTROL	GOSSYPOL	P
BASAL	275 ± 34 (n=18)	103 ± 14 (n=16)	<0.001
ACh	908 ± 234 (n=6)	100 ± 20 (n=6)	<0.01
SNP	1380 ± 470 (n=5)	1090 ± 240 (n=5)	N.S

The selective inhibition by gossypol of both the increase in cGMP release and the dilator activity of ACh is consistent with the presence of EDRF mediated vasodilatation in the isolated perfused rat kidney. Moreover the reduction by gossypol of basal cGMP release is also consistent with the basal release of EDRF by this preparation.

G.A. Burton is an SERC student.

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A STUDY OF EP 157, EP 092 AND ILOPROST ON THE INHIBITION OF COLLAGEN-INDUCED FALL IN PLATELET COUNT IN RATS AND GUINEA-PIGS

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Rats (300-450gm) were anaesthetised with 60mg kg^{-1} pentobarbitone IP and guinea-pigs (350-600gm) were anaesthetised with urethane (280mg kg^{-1}) and allobarbituric acid (70mg kg^{-1}) IP. The trachea was cannulated to maintain free airway and in guinea-pigs the cannula was connected to a respiratory pump. The carotid artery was cannulated with a specially designed cannula which removed blood and immediately anticoagulated the blood with 1% EDTA. The jugular vein in rats and guinea-pigs were cannulated to inject collagen which activated platelets causing a fall in the circulating platelet count.

EP 092 (rac 5-endo(6'-carboxyhex-2'Z-enyl)-6-exo{1"-[N-(phenylthiocarbamoyl) hydrazono]ethyl}-bicyclo[2,2,1]heptane) was administered in rats and guinea-pigs by bolus IV injection. EP 157 (5 endo(6/-hydroxy hex-2Z-enyl)6 exo (diphenyl 1 mino methyl) bicyclo [2'2'2'] oct/2 Z ene) was injected by IV bolus and by infusion, iloprost was infused. Collagen (40ug kg^{-1}) was injected at 15 min intervals in rats and 30 min in guinea-pigs. In rats EP 157 was injected 1 min after the injection of collagen. In rats and guinea-pigs, the first dose of collagen is 5 min after the injection of the compounds or vehicle. EP 157 and iloprost were infused for 15, 30 or 45 min.

In rats EP 092(2.5, 5 and 10mg kg^{-1}) inhibited collagen induced fall in circulating platelet count dose dependently with a maximum inhibition of 29%. EP 157 2,5 and 10 mg kg^{-1} induced 14%, 40%, 52% inhibition respectively. When collagen was injected 1 min after the injection of EP 157 (5mg kg^{-1}) there was a 50% inhibition. When EP 157 was infused (200, 50, 50 and 10ug kg^{-1} min^{-1}) for 45, 30 15 and 15 min respectively in rats there was a dose-dependent inhibition of collagen-induced fall in the circulating platelet count. Iloprost (0.25, 0.5 and 1ug kg^{-1} min^{-1}) was infused for 30 min and produced an inhibition of the fall in platelet count (47%, 78%, 69% respectively). When the infusion was terminated with 1 and 0.25ug kg^{-1} min^{-1} there was an increase in the level of platelet count. When EP 092 (10mg^{-1} kg^{-1}) was injected at the start of a 30 min infusion with iloprost 0.25ug kg^{-1} min^{-1} there was a significant increase in inhibition of the fall in platelet count compared with single infusion of iloprost. When the infusion was terminated there was no increase in the level platelet count.

In guinea-pigs, EP 092 (0.1, 0.25 and 1mg^{-1} kg^{-1}) was shown to be much more potent than in rats; there was a greater inhibition than was observed in rats 44.6, 52 and 64% respectively. EP 157 (50ug kg^{-1} min^{-1}) was infused for 30 min and 44.3, 40.7 and 38.9% inhibition was observed after 5, 35 and 65 min respectively. In conclusion guinea-pigs are more sensitive to thromboxane A_2-receptor antagonists than rats. EP 157 has shown that it has a dual activity blocking thromboxane A_2-receptor and has a prostacyclin-like activity. Unfortunately it has a very short half-life in the blood system which will have to be investigated.

INHIBITION OF THE PLATELET ANTI-AGGREGATING ACTIVITY OF RAT NEUTROPHILS BY N^G-MONOMETHYL-L-ARGININE

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Nitric oxide (NO), which relaxes vascular smooth muscle and inhibits platelet aggregation, accounts for the biological activity of endothelium-derived relaxing factor (EDRF; Palmer et al, 1987; Radomski et al, 1987). Endothelial NO is derived from L-arginine, and its synthesis can be inhibited by the substrate analogue N^G-monomethyl-L-arginine (L-NMMA) (Palmer et al, 1988a,b). Rat neutrophils also release a factor that can relax vascular smooth muscle (Rimele et al, 1988), while incubation of rabbit neutrophils with superoxide dismutase (SOD) can inhibit FMLP-induced neutrophil aggregation, as does NO (McCall et al, 1988). Furthermore, incubation of human neutrophils with washed platelets inhibits subsequent platelet aggregation (Cynk et al, 1988). We have now investigated whether the NO synthesis inhibitor, L-NMMA, can inhibit the platelet anti-aggregatory activity of rat neutrophils.

Following i.p. injection of 0.2% oyster glycogen (10ml), leukocytes were harvested 16h later from the rat peritoneum. A 95%-pure preparation of neutrophils was obtained following density gradient centrifugation on Ficoll-Hypaque and resuspended in Tyrodes solution containing indomethacin (5μM). Human washed platelets were prepared by the method of Radomski et al (1987). Following incubation for 50 min at 37°C, aliquots of the neutrophil suspension were incubated for 4 min with the washed platelet suspension (0.5 ml containing indomethacin, 5μM) and platelet aggregation was induced by a submaximal concentration of thrombin (20 - 40 mU/ml).

Incubation of rat neutrophils (2×10^5 - 2×10^6 cells) with human platelets induced a cell concentration-related inhibition of platelet aggregation, with 2×10^6 cells inducing a 96 ± 3% inhibition (n = 4; $P<0.05$). The anti-aggregatory action was enhanced by incubation with SOD (60 U/ml) at a concentration not directly affecting platelet aggregation. Thus, the platelet inhibition with 2×10^5 cells (15 ± 8%, n = 3) was significantly potentiated to 58 ± 9% (n = 3, $P<0.05$) by SOD. The anti-aggregating action of 6×10^5 neutrophils (63 ± 10% inhibition, n = 3) was reduced by haemoglobin (200nM) to 18 ± 7% inhibition (n = 3, $P<0.05$). This action of rat neutrophils was not simply a particle effect since fixation with glutaraldehyde (0.1%) reduced their maximal activity to 10 ± 4% inhibition (n = 3, $P<0.05$). Preincubation of rat neutrophils (50 min at 37°C) with L-NMMA (30-300μM), in doses not directly affecting platelet aggregation, attenuated the platelet anti-aggregatory activity, with L-NMMA (100μM) inducing a 56 ± 10% reduction (n = 4, $P<0.001$) of this action. This effect of L-NMMA was abolished (n = 4) by concurrent incubation with L-arginine (100μM), but not with D-arginine (100μM).

These findings indicate that rat peritoneal neutrophils can release NO, derived from L-arginine. Thus, L-NMMA is a useful pharmacological tool to evaluate the biological relevance of NO release in different cells and tissues.

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THE THROMBOXANE RECEPTOR ANTAGONIST, GR32191 INHIBITS PLATELET DEPOSITION ON HUMAN ARTERIES *IN VITRO*

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The thromboxane A_2 receptor antagonist, GR32191 is a powerful inhibitor of platelet aggregation (Lumley et al, 1987) and secretion. We have now compared GR32191, prostacyclin and aspirin as inhibitors of platelet deposition on human and rabbit arterial segments *in vitro* in a Baumgartner (1973) chamber. Citrated blood containing functional platelets labelled with ^{111}Indium (Hawker et al, 1980) was circulated over everted, de-endothelialised segments of human umbilical artery (HUA), human cerebral artery (HCA) or rabbit aorta (RA) at 160mls/min for 10 min at 37°C. The segments were washed free of blood and counted for radioactivity. The experiment was then repeated using fresh pieces of artery and the same blood in the presence $1x10^{-15}$-$1x10^{-4}$M of GR32191, prostacyclin or aspirin. Platelet deposition was calculated from measurement of the radioactivity of the blood and the tissues and the surface area of the tissues exposed to blood. Inhibition was calculated as a percentage of the initial deposition. Recirculation of untreated blood over fresh tissues did not significantly reduce deposition (<10%).

On HUA, the maximum inhibition of deposition which could be obtained with GR32191 ($1x10^{-10}$M) or aspirin ($1x10^{-4}$M) was approximately 55%. Half of the maximum achievable effect (IC_{50}) of GR32191 was observed at concentrations of $1.2\text{-}7.2x10^{-13}$M (n=4). Similar potency was observed using HCA as a surface for deposition (IC_{50} $5\text{-}12x10^{-13}$M; n=5). Prostacyclin reduced inhibition on HUA by 80% ($1x10^{-8}$M). Whilst the extent of inhibition achievable with prostacyclin was greater than with GR32191 or aspirin comparison of their half maximal effects showed that GR32191 was approximately 1000-times more potent than prostacyclin (IC_{50} $0.5\text{-}7x10^{-10}$M; n=4) and $1x10^7$-times more potent than aspirin (IC_{50} $0.4\text{-}4x10^{-6}$M; n=5). On RA, each compound caused maximum inhibition of deposition of 75-80%. Concentrations of GR32191 which caused half of the achievable effect ranged from $1.2\text{-}16x10^{-10}$M; n=5). The potency of GR32191 as an inhibitor of deposition on human arteries is much greater than can be explained solely by an effect on platelet aggregation since GR32191 has an IC_{50} of 7.0 ± $0.1x10^{-9}$M (mean ± S.E.) against U46619 ($1x10^{-6}$M) induced aggregation of human platelet rich plasma. Analogues of GR32191 also showed some selectivity for human arterial tissue, but this was not seen with structurally-unrelated antagonists. The unique potency of GR32191 as an inhibitor of platelet deposition on human tissues may have profound implications for its use as an anti-thrombotic agent, with potential in the treatment of vascular proliferative disease.

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MODULATION OF RABBIT PLATELET THROMBOXANE B_2 GENERATION BY PROTEIN KINASE C

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We and others have shown that activators of protein kinase C (PKC) inhibit fluoride or agonist-stimulated thromboxane B_2 generation by human platelets (Poll et al., 1986,1987; Krishnamurthi et al., 1986). The aim of this study was to compare the effect of a PKC activator, TPA with a PKC inhibitor, staurosporine (Tamaoki et al.,1986) on rabbit platelet TxB_2 generation induced by PAF, ionomycin or arachidonic acid (AA). Cytosolic free calcium concentration ($[Ca^{++}]_i$), dense granule release and TxB_2 generation were measured as described previously (Poll et al., 1986) using prostacyclin washed rabbit platelets loaded with Fura 2. Results are the mean ± se mean of 3-6 experiments.

Incubation of platelets with 1, 3, 10 and 30 nM 12-0-tetradecanoyl phorbol 13-acetate (TPA) for two mins reduced submaximal PAF (3 nM) elevated $[Ca^{++}]_i$ from 455 ± 34.5 to 400.5 ± 4.2, 303 ± 26, 157 ± 11.5 and 21 ± 6 nM respectively and the corresponding TxB_2 generation (30 nM PAF) from 18.4 ± 1.8 to 19.77 ± 1.4, 6.7 ± 1.0, 2.4 ± 0.5 and 0.64 ± 0.1 pmol/10^8 platelets respectively. However TPA produced only a weak non-significant inhibition of PAF induced 5HT release. In contrast, TPA at 1, 3, 10, and 30 nM increased ionomycin (5µM)-induced TxB_2 generation from 161.2 ± 9.7 to 137.6 ± 31.7, 200.6 ± 9.3, 254 ± 29 and 259.6 ± 12 p mol/10^8 platelets respectively and 5HT release was potentiated from 59.6 ± 2.24 to 75.4 ± 5.9, 84.8 ± 17.1, 95.85 ± 4.1 and 99.6 ± 0.4% respectively. The AA (10 µM)-induced elevation of $[Ca^{++}]_i$ was reduced from 192 ± 17 to 143 ± 19, 29 ± 13.5 with no detectable $[Ca^{++}]_i$ elevation when TPA values reached 10 nM and above. With all concentrations of TPA examined AA-induced TxB_2 generation was not modified ($p>0.05$).

Incubation of platelets with 30, 100, 300 and 1000 nM staurosporine for two minutes did not modify PAF (3nM) elevated $[Ca^{++}]_i$ (N=4) nor was there any significant potentiation of TxB_2 generation with 30 nM PAF except with 1 µM staurosporine. However, 5HT release with 3 nM PAF was inhibited from 58.4 ± 2.5 to 52.7 ± 6, 46.1 ± 6.6, 43 ± 3.7 and 20.1 ± 8.1 % respectively (N=4). As with TPA, staurosporine did not modify $[Ca^{++}]_i$ elevated by 5 µM ionomycin (N=3) however in direct contrast to the results obtained with TPA, TxB_2 generation with 5 µM ionomycin was inhibited from 151.4 ± 13.4 to 142.3 ± 30, 79.5 ± 9.7, 66.8 ± 7.4 and 34.8 ± 9.8 pmol/10^8 platelets respectively, and 5HT release was reduced from 54.4 ± 3.5 to 47.2 ± 4.7, 42.2 ± 0.4, 8.8 ± 6.5 and 6.15 ± 1.6 %. Staurosporine (30-1000 nM) as with TPA did not modify the amount of TxB_2 generated in response to submaximal AA (30 µM) (N=3).

Therefore these results suggest that receptor stimulated elevation of $[Ca^{++}]_i$ and TxB_2 generation are not controlled by endogenous PKC activators, as demonstrated with staurosporine. Although if the $[Ca^{++}]_i$ is not limiting, as with ionomycin, TxB_2 generation can be markedly enhanced or inhibited with PKC activators or inhibitors respectively. The site of modulation by PKC is probably not the cyclo-oxygenase or thromboxane synthetase enzymes as AA-induced TxB_2 generation is not modified.

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PROSTANOID RECEPTORS MEDIATING CONTRACTION AND RELAXATION OF GUINEA-PIG UTERINE ARTERY

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Prostanoids can both contract and relax isolated vascular smooth muscle, these responses being mediated by TP- and EP_3-receptors and by IP-, DP-, and EP_2-receptors respectively (Ahluwalia et al., 1988; Coleman, 1987; Coleman et al., 1980). In the present study, we have attempted to characterise the prostanoid receptors in guinea-pig uterine artery (GPUA).

Uterine arteries were obtained from diethylstilboestrol-treated guinea-pigs (600-800g). Arterial rings were mounted in organ baths under 0.4g resting tension, in Krebs solution at 37°C, containing indomethacin (2.8μM) and bubbled with O_2/CO_2 (95%/5%).

Prostanoids caused concentration-related contractions of GPUA. The stable thromboxane-A_2 mimetic, U-46619 was a potent contractile agonist (EC_{50} (95% C.L.) = 31 (20-50)nM, n=9). PGE_2 was also potent, but displayed biphasic activity, causing contraction at low concentrations (1-100nM) with an equipotent concentration ratio (EPC, U-46619=1) of 3.4 (0.8-14, n=7) and relaxation at higher concentrations (300-3000 nM). $PGF_{2\alpha}$, PGD_2 and PGI_2 were weak contractile agonists (EPC = 76 (29-198, n=8), 489 (198-1211, n=9) and >1000 (n=5), respectively). The TP-receptor blocking drug, GR32191 caused concentration-related rightward shifts of responses to U-46619 (pA_2 = 10.2 (9.4-11.0), slope = 0.88 (0.5-1.2); n=5). A high concentration of GR32191 (1μM) abolished responses to U-46619 and PGI_2, caused a reduction in the maximum response to PGD_2, and had little or no effect on responses to PGE_2, $PGF_{2\alpha}$ or phenylephrine. The potency of PGE_2 in contracting this preparation is consistent with an EP-receptor involvement and therefore the EP-receptor selective agonists, sulprostone (EP_1/EP_3) and rioprostil (EP_2/EP_3) (Coleman et al., 1988) were tested. Both were potent in inducing contraction (EC_{50} = 3 (1-9)nM, n=9 and 18 (9-36)nM, n=5, respectively). Other prostanoid receptor agonists, fluprostenol (FP) and BW245C (DP) were inactive. The responses to sulprostone, rioprostil and PGE_2 were not affected by the EP_1-receptor blocking drug, AH6809 (3μM).

In preparations where tone was induced by phenylephrine (0.3μM), prostanoids caused a concentration-related relaxation of GPUA. The standard agonist, PGI_2 had an EC_{50} of 65 (26-163)nM, (n=6), whereas the stable PGI_2 analogue, iloprost was more potent (EPC = 0.1 (0.07-0.14), n=6). PGE_2 was of moderate potency (EPC = 16 (3-75), n=4) and BW245C was weak (EPC = 969 (range = 35-2000, n=3). $PGF_{2\alpha}$, PGD_2 and rioprostil did not cause relaxation, even at concentrations as high as 30μM.

These data are consistent with the presence in GPUA of both TP- and EP-receptors mediating contraction, and of IP-receptors mediating relaxation. Furthermore, these results suggest that the EP-receptors which mediate contraction are of the EP_3-receptor subtype.

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METABOLISM AND GENERATION OF CYSTEINYL CONTAINING LEUKOTRIENES BY HUMAN AIRWAYS IN VITRO

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The cysteinyl-containing leukotrienes (LTs), LTC_4, LTD_4 and LTE_4, are potent constrictors of human airways and can be released by lung tissue following both immunological and non-immunological stimulation (Kumlin et al, 1987). In this study we have investigated the metabolism of cysteinyl-containing LTs by both human lung parenchyma (HP) and bronchus (HBr) as well as the contribution by LTs to the contraction of the HBr following anti-IgE challenge.

Samples of macroscopically normal HP and HBr were chopped, suspended in Tyrode's solution (1g/5ml) and agitated at 37°C. LTC_4, LTD_4 or LTE_4 was added (final concentration 1nM) with tracer amounts of the respective ^3H-LT. Aliquots were removed at time intervals 0-120 min and partially purified using C_{18} Sep Paks. The material was further purified by RP-HPLC (Techsphere 5ODS column, solvent MeOH:H_2O:HAc 75:25:0.01, pH 5.4, flow rate 1ml/min). Spirally-cut strips of HBr were superfused with Tyrode's solution containing a mixture of antagonists to 5-HT, ACh, histamine and α-and β-adrenoceptors. Indomethacin (2.8μM) and/or FPL 55712 (1.9μM) were infused where appropriate (Samhoun & Piper, 1984). Anti-human IgE (Sigma) was given as bolus injections (0.03/1600-3/1600 dilution). In all cases n=3-5.

LTC_4 incubated with HP was metabolised to LTD_4 and LTE_4, as determined by separation of labelled products using HPLC. At the concentration used, the t½ for LTC_4 was approximately 4 min and maximal formation of LTD_4 was observed at 5 min. Subsequently a decrease in level of LTD_4 occurred concomitantly with an increase in that of LTE_4. At 60 min 4±1% and 63±5% of the radioactivity was associated with LTD_4 and LTE_4 fractions respectively. When LTD_4 was incubated with HP it was converted into LTE_4 with a t½ of approximately 11 min. In all experiments further metabolism of LTE_4 could not be detected. When incubated with HBr, LTC_4 was metabolised to LTD_4 and LTE_4 with a t½ of approximately 7 min. Peak formation of LTD_4 occurred at 20 min. At 60 min 25±5% and 46±5% of the radioactivity eluted with the LTD_4 and LTE_4 fractions respectively.
Stimulation of HBr with anti-IgE, in the presence of indomethacin, caused long-lasting contractions which closely resembled those induced by LTs. In addition similar protracted contractions were induced by anti-IgE on HBr from two asthmatics. All responses were reduced in the presence of FPL 55712 suggesting the involvement of cysteinyl-containing LTs.

Metabolism of LTC_4 proceeds via formation of LTD_4 and then LTE_4 in both HP and HBr. Whereas conversion of LTC_4 to LTD_4 occurs at a similar rate in both preparations, that of LTD_4 to LTE_4 appears to be slower in HBr. LTE_4, although less active than LTC_4 or LTD_4, still retains considerable activity and induces sustained contractions of HBr (Samhoun and Piper, 1984). Formation and stability of LTE_4 as well as its pharmacological profile on HBr, which is very similar to that due to anti-IgE, suggest that this LT may be important in airway diseases.

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EFFECT OF NEDOCROMIL SODIUM ON LUNG HYPERRESPONSIVENESS AND EOSINOPHIL ACCUMULATION IN THE GUINEA-PIG

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We recently demonstrated (Pretolani et al., 1988) that active sensitization induces a guinea-pig lung hyperresponsiveness to various mediators including platelet-activating factor (PAF-acether). This phenomenon, which is triggered by the booster injection of antigen, is accompanied by an increase in the number of eosinophils in bronchoalveolar lavage (BAL). These results led us to investigate the interference of nedocromil sodium (Tilade ® †), an anti-asthma drug with a potential protective effect upon migration of inflammatory cells, with PAF-acether- and antigen-induced bronchoconstriction (BC) and mediator release in lungs from actively sensitized guinea-pigs and with cell composition of BAL. Guinea-pigs were actively sensitized by two s.c. injections of 10 μg ovalbumin in 1 mg Al $(OH)_3$ at two weeks interval. One week after the second injection (booster injection), lungs were removed and perfused with Krebs solution containing 0.25 % bovine serum albumin (Krebs-BSA). PAF-acether was injected at 10 min intervals into the pulmonary artery at doses of 1 and 100 ng in 0.1 ml of 0.1% BSA. Ovalbumin was administered at 1 μg at the end of each experiment and the % increase of BC, the release of leukotriene-like material (detected by a bioassay using a guinea-pig tracheal strip located under the lung) and histamine secretion (measured by a spectrofluorometric method in 3 min fractions of the effluent) were measured. In another series of experiments, actively sensitized animals were anaesthetized and their bronchoalveolar cells were collected in 20 successive lavages using 5 ml aliquots of NaCl 0.9 % through a polyethylene tracheal cannula. Cells were counted and differentiated by May-Grünwald Giemsa staining test and results expressed as % eosinophils of the total cell populations.

When the direct effect of nedocromil sodium was studied, the drug was dissolved at 10 μM in the Krebs-BSA solution for lung perfusion or it was administered for one week, from the day of the booster injection to the day of the experiment, at a dose of 30 mg/kg/day s.c. No inhibition of PAF-acether- and antigen-induced BC or mediator release from sensitized lungs was observed when nedocromil sodium was added directly to the Krebs-BSA solution. By contrast, when guinea-pigs were treated with nedocromil sodium for one week after the booster injection of ovalbumin, BC to 1 ng PAF-acether was reduced by approximately 50% ($P < 0.05$) and release of leukotriene-like material was significantly inhibited by 62% ($P < 0.05$). The action of the LT-like material was antagonized by the LT antagonist FPL 55712. No inhibition by nedocromil sodium was observed for 100 ng PAF-acether and 1 μg ovalbumin. Furthermore, nedocromil sodium markedly impaired histamine secretion induced by both PAF-acether and antigen administration. Nedocromil sodium did not affect the concentration of circulating ovalbumin-specific IgG as detected by an enzyme-linked immunosorbent assay since the titers were of 3375 ± 1276 and 3062 ± 1169, as measured in the serum of sensitized vehicle-injected and nedocromil sodium- injected guinea-pigs, respectively. The week's treatment with nedocromil sodium also reduced markedly the number of eosinophils in the BAL of sensitized guinea-pigs. Indeed, 25.0 ± 5.5 and 12.2 ± 4.0 % eosinophils ($P < 0.05$) were found in BAL obtained from vehicle- and nedocromil sodium-treated guinea-pigs, respectively. Our results indicate that nedocromil sodium does not interfere directly with the enhanced effect of PAF-acether on lungs obtained from actively sensitized guinea-pigs, nor with the effects of injected antigen. On the other hand, it prevents hyperresponsiveness (increased mediator release and BC) and eosinophil migration into the airways, when administered after the booster injection of antigen. The latter acts as an inflammatory stimulus, sensitive to nedocromil sodium and which accounts for the development of hyperresponsiveness.

† registered trade mark of Fisons plc.

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PLATELET ACTIVATION AND PAF-INDUCED AIRWAY HYPERREACTIVITY IN THE ANAESTHETISED GUINEA-PIG

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It has previously been reported that intravenous infusion of PAF fails to induce airway hyperreactivity in guinea-pigs depleted of platelets (Mazzoni et al., 1985). To ascertain whether release of platelet secretory products might account for airway hyperreactivity, ADP and thrombin have been compared with PAF. In addition, iloprost, a stable PGI2 mimetic, has been used to inhibit platelet activation by PAF. Airway resistance (Rl, cm H2O/l/sec) was measured in anaesthetised (phenobarbitone 100 mg/kg i.p. and pentobarbitone 30 mg/kg i.p.) ventilated (1Hz, 8 ml/kg) guinea-pigs with a Buxco respiratory analyser (Model 6). Animals received an i.v. injection of ^{111}Indium-labelled platelets and platelet accumulation in the thorax was measured using a crystal scintillation detector system (Page et al., 1982). Successive injections of PAF (32 ng/kg) at 10 minute intervals yielded decreased intrathoracic platelet accumulation but progressively larger Rl, attributable to airway hyperreactivity as shown by responses to histamine (1.0 & 1.8 ug/kg i.v.) prior to, and after, PAF injection (Table). Single injections of thrombin (100 U/ml) or ADP (100 ug/kg) caused platelet accumulation and increased Rl comparable with PAF (32 ng/kg), but on successive administration neither substance produced increased reactivity to histamine (1.8 ug/kg) (thrombin, pre Rl 52±18, post Rl 96±36; ADP, pre Rl 108±58, post Rl 136±68). Platelet depletion, by use of anti-platelet serum (APS) or infusion of iloprost (10 ng/kg/min) inhibited increased Rl due to PAF (32 ng/kg) and the increased airway response to histamine (1.8 ug/kg), even though at this dose, iloprost did not diminish PAF-induced intrathoracic platelet accumulation.

Interaction between PAF and platelets to yield a material which causes increased airway hyperreactivity could account for the differing pattern of responses to platelet stimuli. Such material may be related to PAF, since injections of lyso-PAF (10 ug/kg) also causes significant airway hyperreactivity to histamine when injected into the guinea-pig (pre Rl 83±30, post Rl 217±61).

Test substance	n	Increase in R_1 (cm H2O/l/sec) HIST (pre)	PAF (32 ng/kg i.v.)	PAF	PAF	HIST (post)	P
PAF 32 ng/kg	24	127±25	337±68	464±86	505±85	486±57	0.0005
PAF + APS	5	140±80	8±4	5±2	4±2	158±60	NS
PAF + ILOPR	5	117±35	20±8	43±22	29±8	187±37	NS

HIST= histamine 1.8 ug/kg; Student's t-test for paired observations was used for statistical evaluation of responses to histamine

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INCUBATION OF PLATELETS WITH PAF PRODUCES A FACTOR WHICH CAUSES AIRWAY HYPERREACTIVITY IN GUINEA-PIGS

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Development of airway hyperreactivity in response to intravenous injections of PAF depends upon the presence of intact circulating platelets. The capacity of platelets to generate an intermediate material capable of inducing airway hyperreactivity has been investigated. Airway resistance (Rl, cm H2O/l/sec) was measured in anaesthetised (phenobarbitone, 100 mg/kg and pentobarbitone, 30 mg/kg, i.p.) ventilated (1 Hz, .8 ml/kg) guinea-pigs with a Buxco respiratory analyser (Model 6). Guinea-pigs were made thrombocytopaenic by intravenous injection of a specific rabbit anti-guinea-pig platelet serum (APS), 24 hours prior to test. Airway hyperreactivity has been expressed as incremental increase of Rl ($\underline{d}$Rl) in response to PGF2alpha (32-56 ug/kg) before and after injection of test material. Intravenous injection of PAF (32 ng/kg) in APS-treated guinea-pigs neither provoked an increase in Rl nor enhanced airway responsiveness to PGF2alpha. However, in thrombocytopaenic guinea-pigs, the supernatant from guinea-pig PRP (1 ml, at 37°C) incubated with PAF (32 ng/kg in 10 ul) for 1 minute induced airway hyperreactivity. After three successive injections of supernatants, at 10 minute intervals, $\underline{d}$Rl was significantly greater for both doses of PGF2alpha than in animals receiving supernatants from BSA treated PRP (Table).

TREATMENT	n	$\underline{d}$Rl (cm H2O/l/sec) to PGF2alpha 32 ug/kg	56 ug/kg	P
BSA+PRP x 3	5	44±15	40±33	
PAF+PRP x 3	28	170±33	330±34	0.001
PAF+PRP+ILO	5	22±15	39±25	NS

Student's t-test for groups of unequal size was used to assess the statistical significance of differences between BSA-treated group and other treatments using PGF2alpha 56 ug/kg responses.

The generation of this platelet derived factor was inhibited by incubation of PRP with iloprost (10 ng/kg) prior to activation with PAF. When platelet supernatants were stored at 37°C in the absence of platelets for 10 minutes, increased $\underline{d}$Rl was not observed (PGF2alpha 56 ug/kg, $\underline{d}$Rl = 29±35, n=5, NS), whereas following storage in the presence of platelets, no diminution was observed for up to 60 minutes (PGF2alpha 56 ug/kg, $\underline{d}$Rl = 279±46, n=5, P<0.003). If, however, PRP was stored at 4°C, there was a progressive loss of capacity to induce $\underline{d}$Rl. Neither physical disruption (freeze/thaw) of platelets nor activation by ADP (PGF2alpha 56 ug/kg, $\underline{d}$Rl = 43±25, n=5, NS) led to the generation of a factor capable of inducing airway hyperreactivity. These observations imply that exposure of platelets to PAF only initiates secretion or formation of a mediator of airway hyperreactivity. The chemical nature of this material remains unidentified, but since this biological activity can be produced by washed human platelets, there is a prospect of using this test system to evaluate the action of anti-asthma drugs on human platelets.

ACTIONS OF KETOTIFEN ON PAF-INDUCED AIRWAY HYPERREACTIVITY IN THE ANAESTHETISED GUINEA-PIG

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The development of airway hyperreactivity that follows intravenous infusion of platelet activating factor (PAF) can be inhibited by ketotifen (Kristersson et al, 1988). Hence, we have tested whether ketotifen inhibits formation or action of the platelet derived factor which induces airway hyperreactivity (Sanjar et al, 1988). Airway resistance (Rl, cm H2O/l/sec) was measured in anaesthetised (phenobarbitone 100 mg/kg i.p. and pentobarbitone 30 mg/kg i.p.) ventilated (1 Hz, 8 ml/kg) guinea-pigs with a Buxco respiratory analyser (model 6). Airway hyperreactivity, expressed as the incremental increase ($\underline{d}$Rl) of Rl to PGF2alpha (56 ug/kg) before and after treatment, was observed in thrombocytopaenic guinea-pigs following 3 successive injections (10 minute intervals) of supernatant from platelet rich plasma (PRP) incubated for 1 minute with PAF (32 ng/kg). Intravenous infusion of ketotifen (0.1 or 1.0 ug/kg/min for 30 min.) into recipient guinea-pigs inhibited the development of airway hyperreactivity (Table). Infusion of prednisolone at 1.0 ug/kg/min also inhibited the hyperreactivity, whereas the same dose of DSCG or aminophylline had no effect (Table). When ketotifen (10 ug/kg) or prednisolone (10 ug/kg) was incubated with PRP for 10 minutes prior to addition of PAF, injection of supernatants (x 3) into thrombocytopaenic recipients gave a reduced $\underline{d}$Rl (Ketotifen 101±39, $P<0.018$; prednisolone 161±45, $P<0.047$). Incubation of PRP with cromoglycate (10 ug/kg) did not effect significant inhibition of airway hyperreactivity ($\underline{d}$Rl 245±55, NS).

TREATMENT	n	AIRWAY RESISTANCE (cm H2O/l/sec) PGF2alpha BEFORE	PGF2alpha AFTER	INCREMENTAL INCREASE	P
PAF+PRP x 3	20	107±14	405±46	296±37	
" + KETO 0.01	5	63±15	359±27	296±23	NS
" + KETO 0.1	5	102±41	220±43	118±62	0.036
" + KETO 1.0	5	59±14	163±17	104±10	0.001
" + DSCG 1.0	5	78±18	393±108	315±98	NS
" + PRED 1.0	5	73±13	185±49	112±52	0.028
" + AMIN 1.0	5	128±22	380±107	252±92	NS

PGF2alpha=56 ug/kg i.v.; Students t-test for groups of unequal size was used to compare incremental increases of drug treated groups with the control group.

Kristersson, A. et al. (1988) Br. J. Pharmac. 93, 53P
Sanjar, S. et al. This meeting

THE GENERATION AND METABOLISM OF LEUKOTRIENES IN IONOPHORE-STIMULATED BLOOD OF NORMAL AND ASTHMATIC SUBJECTS

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The potent actions of leukotrienes (LTs), suggest a mediator role in asthma. Isolated granulocytes from asthmatics have been shown to release more LTs than those of normal subjects with A23187 stimulation (Wang et al,1986). In whole blood, complex cellular interactions may modulate LT generation and metabolism (Gresele et al,1986). We therefore investigated the A23187-stimulated release and metabolism of LTs in blood of normal (N) and atopic asthmatic (AA) subjects.

Five AA subjects were recruited with a positive skin prick test and a forced expiratory volume in 1 second of <70% of their predicted value. Heparinised AA and N venous blood was incubated at 37°C with A23187, and the reactions terminated by immersion in ice at times up to 2 hours. [^{3}H]-LTs were added as internal standards. After extraction, LTs B_4,C_4,D_4 and E_4, were separated by RP-HPLC and assayed by RIA. Ionophore-stimulated blood was also incubated with [^{3}H]-LTB_4 or [^{3}H]-LTC_4 (0.1 µCi) and labelled metabolites were detected by RP-HPLC.
LTB_4 and LTC_4 were generated in response to ionophore in a dose-dependent manner (EC50 25µM). A23187 (25µM) elicited a rapid rise in LTB_4 levels to a plateau after 30-60 mins. LTB_4 levels were significantly higher ($p>0.05$) in AA blood at all time points, with peak levels (mean ± s.e.mean) at 30 mins of AA:436±73ng/ml and N:246±26ng/ml ($n=5$,$p<0.025$). Exogenous [^{3}H]-LTB_4 was slowly metabolised in ionophore-stimulated N and AA blood to two polar products that co-eluted with authentic 20-carboxy-LTB_4 and 20-hydroxy-LTB_4. LTC_4 generation was greater ($p<0.01$) in AA blood, with peaks after 10 mins of AA:106±24.8 ng/ml ($n=5$), and N:39.7±12.7ng/ml ($n=8$), decreasing to <6ng/ml at 2 hours. The LTD_4 response peaked at 20 mins (AA:39.8±10.0 ng/ml and N:15.2±1.0 ng/ml;$n=5$,$p<0.01$). LTE_4 concentrations rose rapidly to levels consistently higher in the AA blood (at 30 mins, AA:103±14.0ng/ml and N:59.4±9.9ng/ml, $n=5$, $p<0.05$). Exogenous [^{3}H]-LTC_4 was metabolised rapidly in A23187-stimulated N and AA blood to two products that co-eluted with LTD_4 and LTE_4.

These results show that in AA and N blood, ionophore-generated LTB_4 is metabolised by w-oxidation, and LTC_4 by the glutathione detoxification pathway. Importantly, we have demonstrated a significantly enhanced capacity for LTB_4 and LTC_4 generation in the blood of atopic asthmatics, suggesting the existence of an inherent cellular difference in the leukocytes of these subjects.

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THE ARACHIDONATE CASCADE AND ALVEOLAR MACROPHAGES, BUT NOT NEUTROPHILS, MEDIATE BRONCHOCONSTRICTION BY FMLP IN GUINEA-PIGS

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The granulocyte secretagogue N-formyl-methionyl-leucyl-phenylalanine (FMLP) injected i.v. to the anaesthesized guinea-pig at 0.3, 1, 3 a nd 10 μg/kg induces dose-dependent bronchoconstriction (BC) and leucopenia, with no significant thrombocytopenia (Boukili et al., 1986). Since these effects of FMLP were accompanied by ultra-structural evidences of intrapulmonary entrapment and activation of circulating neutrophils, we used vinblastine (O.75 mg per kg, injected i.v. 72 hours before experiment) as a tool to obtain neutrophil-depleted animals, which were challenged with i.v. FMLP. BC was measured by the Konzett-Rössler method. In no instance was BC reduced, when neutrophils were depleted by 82 ± 3% (controls: 4200 ± 523 per μl vs. 725 ± 14 per μl in vinblastine-treated animals; means ± S.E.M., $p < 0.001$). After thus demonstrating that leucopenia does not cause BC, we limited the potential targets of FMLP, by delivering it by aerosol (10, 100 or 1000 μg/ml in the aerosolator's cuvette for 2 minutes) and confirmed that BC is independent from direct activation of circulating cells. Indeed, aerosolized-FMLP was accompanied by dose-dependent BC, activation of alveolar macrophages and their accumulation in the pulmonary alveolar lumens. Non-activated eosinophils were observed in the lungs and a few platelet micro-aggregates in the pulmonary microvasculature. Concomitantly, no significant accumulation of ^{131}I-labelled albumin, ^{111}In-labelled neutrophils or ^{111}In-labelled platelets was detected in the lungs after the aerosolization of FMLP. Repeated aerosolizations of FMLP were accompanied by desensitization to BC by FMLP itself, showing that prior exposure of the guinea-pig pulmonary alveolar macrophages to FMLP alters their subsequent function. In contrast, the intravenous administration of FMLP was accompanied by neutrophil sequestration and increase of extravascular albumin in the lungs. Aspirin, administered i.v. or by aerosol reduced significantly BC by aerosolized FMLP. Indomethacin was only effective when given by aerosol and in this case it inhibited BC by aerosolized or i.v. FMLP. The dual cyclooxygenase/lipoxygenase inhibitor compound BW 755C suppressed BC by the highest dose of aerosolized FMLP used, whereas the histamine H_1-antagonist mepyramine was inactive.

In conclusion, our studies show that BC by FMLP administered by the i.v. and aerosol routes to the guinea-pig is probably mediated by TxA_2 and/or by another cyclooxygenase metabolites associated to lipoxygenase derivatives, as indicated by the effectiveness of BW 755C and the surmontability of inhibition by aspirin and indomethacin. Finally, macrophages are important target cells for FMLP given into the airways, whereas neutrophils appear as less important, whatever the route of administration.

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COMPARISON OF CONSTRICTION IN PERFUSED BRONCHIAL SEGMENTS AND ISOMETRIC FORCE IN STRIPS OF AIRWAY WALL TO CARBACHOL AND HISTAMINE

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The effect of bronchoconstrictor drugs on the airways *in vivo* (eg. on FEV_1 in man) and on isometric force in the airways *in vitro* does not appear to be correlated (Douglas *et al.*, 1977, Vincenc *et al.*, 1983). This suggests that the commonly measured force parameter in strips of airway *in vitro* may not fully reflect smooth muscle shortening and airway narrowing produced by bronchoconstrictors in the intact airway in the lung.

We have used a segment of cylindrical airway, continuously perfused through the lumen with a Krebs solution at 37°C, to compare pharmacological responses to histamine and carbachol. The airways examined were all segmental bronchi obtained from 50-60 kg pigs. Two perfusion systems were examined, one in which the flow rate was held constant (ie. isovolumic) and one where the perfusion pressure was held constant (ie. isobaric). The resting transmural pressure was approximately 5-6 cmH_2O in the two systems. From the changes in pressure or in flow, resistance could be calculated. Concentration-response characteristics to histamine and carbachol were also compared with those obtained from isometric force from 8 x 2 mm strips of airway wall which had been dissected from the same bronchi.

Histamine and carbachol both contracted the bronchial strips. At high concentrations the responses plateaued with carbachol producing a 2-fold greater maximum response than histamine (0.85±0.06 and 0.42±0.04 mN/mg wet weight respectively, n=4, $P<0.001$). Carbachol was also 22 times more potent than histamine (negative log EC_{50} [pD_2] 6.22±0.08 vs. 4.94±0.11 respectively, $P<0.001$). When bronchial segments were perfused in the isovolumic system the concentration-response (resistance) characteristics were similar to those in the strips except that 10-30 times higher concentrations were needed for maximal and half maximal responses (n=4, $P<0.001$).

In contrast, when segments were perfused isobarically (n=4) the sigmoidal response curves (ie. with a plateau at high concentrations) described above were not seen, instead resistance rose in an exponential manner. Furthermore, high concentrations of both drugs produced the same maximal response and reduced flow rate by more than 95%. Ratios of EC_{50}s between carbachol and histamine were less in perfused segments compared with the strips but the differences were not significant.

We suggest that *in vitro* preparations in which smooth muscle shortening and airway wall forces are represented may reveal airway responses which differ strikingly from those recorded isometrically in opened strips of airway wall. This appears to be most evident in the perfused preparation in which the transmural pressure remains constant, where the response characteristics are reminiscent of those seen in provocation studies in hyperresponsive human subjects.

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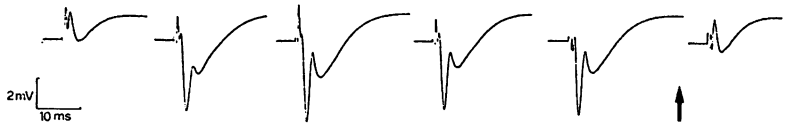
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EFFECT OF THE COGNITION ENHANCING AGENT OXIRACETAM ON ELECTRICAL ACTIVITY OF HIPPOCAMPAL SLICES

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Oxiracetam belongs to a series of 2-pirrolidinone derivatives which facilitate learning and memory and prevent the impairment of cognitive function induced by drugs and brain insults (Schindler et al.,1984). The mechanisms of these actions are still unknown. However, oxiracetam and its analogue aniracetam act on brain cholinergic system (Spignoli and Pepeu, 1986), and aniracetam enhances long term potentiation (LTP) in guinea pig hippocampal slices (Satoh et al., 1986).
In this work the effect of oxiracetam on evoked electrical responses in rat hippocampal slices was investigated.
Transverse slices, 400 μm thick, prepared from hippocampi removed from adult male Wistar rats were superfused with oxigenated artificial cerebrospinal fluid (Corradetti et al., 1983). Test pulses (80 μs - 0.1 Hz) were delivered through bipolar electrodes positioned in the stratum radiatum. Evoked orthodromic potentials were extracellularly recorded from the pyramidal cells of the CA1 region.
As shown in Fig.1, oxiracetam added for 10 min to the superfusion medium at concentrations ranging from 0.1 to 100 M brought about an increase in the amplitude of the evoked potentials and the appearance of a population spike, promptly reversible with washing. The maximum effect occurred with 1 μM.



Oxiracetam - 0.1 1.0 10 100 μM

Figure 1 Effect of oxiracetam on evoked potentials recorded from pyramidal cells of the stratum radiatum; ↑ washing

LTP produced by 2 trains (100 Hz,0.4 s each,5 min interval) was also enhanced by oxiracetam. A 70% increase in the initial slope of the field e.p.s.p. was observed after 10 min perfusion with 0.1 μM. Washing restored LTP to pre-drug level.
Since much evidence connect hippocampus with memory mechanisms, the increase in hippocampal synaptic excitability brought about by oxiracetam may be relevant to its cognition enhancing properties.

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CHARACTERISATION OF OPIOID RECEPTORS IN THE NEONATAL RAT SPINAL CORD

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Nociceptive reflexes occur in the neonatal rat spinal cord and can be depressed by opioids (Perkins & Dray, 1986). We have now characterized the opioid receptors involved using both ligand binding and functional tests for antinociception.

In equilibrium binding studies ^{3}[H]-DAGO (mu), ^{3}[H]-DPLPE (delta) and ^{3}[H]-EKC (kappa in the presence of DAGO) were used as selective opioid ligands. Crude membranes were prepared from the neonatal spinal cord and incubated with an appropriate radioligand at room temperature for 60 min, filtered, washed and counted. Non saturable binding was measured in the presence of 10μM naloxone. In the functional studies the spinal cord and attached tail was removed from 1 day old rats. The cord and tail were separately superfused with oxygenated physiological salt solution. Activation of peripheral fibres by noxious (capsaicin, heat at 48°C) and innocuous stimuli was assessed by measuring the depolarization evoked in a spinal ventral root (L_3-L_5). Opioids were administered in the spinal cord superfusate 15 min before and during peripheral stimulation. The potency of naloxone against DAGO, DPLPE and the kappa ligands U50488, U69593 (Lahti et al, 1985) and Cambridge-20 (PD117302) (Clarke et al, 1988) was measured using a paradigm similar to that for a pA_2 determination. This is expressed as the naloxone A_2 value.

Saturable binding was observed with ^{3}[H]-DAGO (K_d = 0.93 ± 0.09 nM; B_{max} 220 ± 40 fmol/mg protein) and ^{3}[H]-EKC (K_d = 0.66 ± 0.08 nM; B_{max} 280 ± 50 fmol/mg protein) but not with ^{3}[H]-DPLPE. The binding of EKC (K_d = 0.54 ± 0.24; B_{max} 89 ± 10 fmol/mg protein) in the presence of 100nM cold DAGO suggested that about 70% of EKC was bound to mu receptors. The remaining EKC sites probably represent kappa receptor binding. In the spinal cord, opioids produced a dose dependent reduction of capsaicin and noxious-heat evoked responses.

Agonist	ED_{50}(nM)	Naloxone A_2(nM)
DAGO	2.6	2.0
DPLPE	180	7.0
U50488	200	>500
U69593	5	55
Cambridge-20	8	52

Relative potency and naloxone sensitivity of opioids on the spinal cord.

DAGO, U69593 and Cambridge-20 were potent agonists whereas U50488 and DPLPE were relatively weak. Agonists could be divided into 3 groups based on naloxone sensitivity, with DAGO and DPLPE being very sensitive, U69593 and Camb-20 less so and U50488 relatively insensitive.

The neonatal rat spinal cord contains mu and possibly kappa opioid binding sites. Mu, delta and kappa ligands produce antinociception but, based on naloxone sensitivity, DPLPE appears to act via a mu receptor. In keeping with activation of kappa receptors, U69593 and Cambridge-20 act via a mechanism 10-20 times less sensitive to naloxone than a mu ligand. U50488 was relatively insensitive to naloxone and is likely to act through a different site from other kappa ligands.

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SYNAPTIC EVENTS PERSIST IN THE PRESENCE OF EXCITATORY AMINO ACID RECEPTOR BLOCKADE IN GUINEA-PIG NEOCORTEX IN VITRO

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Previous experiments in neocortical slices have shown that selective antagonists of the N-methyl-D-aspartate (NMDA) sub-type of excitatory amino acid receptor block different types of epileptiform activity and components of normal synaptic transmission (Aram et al., 1988, Fletcher et al., 1988). The contribution from activation of non-NMDA receptors to these synaptic events has been studied with 6-nitro-7-cyano-quinoxaline-2,3-dione (CNQX, Honore et al., 1988), a new selective competitive antagonist of quisqualate and kainate responses. During these experiments we have observed conditions in which excitatory and inhibitory synaptic events persist following blockage of fast synaptic transmission by CNQX.

Coronal slices (500uM thick) of guinea pig neocortex were maintained in an interface chamber at 32°C. Extra and intracellular recordings were made from layers II-V using electrodes filled with 3M NaCl or K acetate respectively. Antagonists were bath applied for 30-60min at doses that were selective against NMDA receptors (D-2-amino-5-phosphonovalerate (AP5, 10-20uM), or against non-NMDA receptors (CNQX, 0.5-2uM). Tungsten bipolar electrodes were used to electrically stimulate the underlying white matter.

Normal synaptic transmission was studied using medium containing 1.6mM magnesium. Stimulus amplitudes close to threshold elicited e.p.s.p.s of up to 50msec duration which exhibited classical current-voltage relations and were reduced or abolished by CNQX. At higher stimulus strengths the amplitude and duration of synaptic events increased, often due to the appearance of i.p.s.p.s that were depolarising at resting membrane potential. CNQX alone reduced, or in combination with AP5, abolished these p.s.p.s. At still higher stimulus strengths (4-10 times threshold) a late i.p.s.p lasting 200-500msec could also be elicited which remained in the presence of CNQX. This may reflect direct activation of inhibitory neurones (see Collingridge et al., 1988).

Three types of epileptiform activity were investigated and found to have different sensitivities to excitatory amino acid receptor antagonists. Firstly, perfusion of slices with magnesium-free medium led to spontaneous and evoked epileptiform bursts of 1-5sec duration that were abolished by AP5. CNQX, however, had little effect whether added prior to, or following, removal of magnesium (Fletcher et al., 1988). Secondly, 50-100uM picrotoxin (n=4) or 4-aminopyridine (n=6) added to normal medium induced interictal (1 sec) or ictal (1-2min) epileptiform bursts respectively that were progressively blocked by separate application of either AP5 or CNQX. Thirdly, in the combined presence of AP5 (20uM) and CNQX (up to 10uM) 4-aminopyridine was able to elicit regular synchronised spontaneous and evoked depolarising potentials of 500-2000msec duration. These synaptic potentials were blocked by 0.5mM calcium/10mM magnesium and were coupled to field potentials recorded simultaneously.

In conclusion, although both NMDA and non-NMDA receptors mediate normal excitatory synaptic transmission and interictal and ictal epileptogenesis in the neocortex, 4-aminopyridine elicited epileptiform potentials which persisted following competitive block of receptors activated by NMDA, quisqualate and kainate.

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THE EFFECT OF 1,9-DIDEOXY FORSKOLIN ON SPONTANEOUS EPILEPTIFORM ACTIVITY IN AN IN VITRO RAT CORTICAL SLICE MODEL

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We have previously shown that forskolin, a potent activator of adenylate cyclase, has a dual effect on epileptiform activity in vitro (Boulton & O'Shaughnessy, 1988). Its epileptogenic actions at low concentrations may be attributable to cyclic AMP formation. Forskolin has recently been shown to also close K^+ channels (Hoshi et al, 1988) and this action may account for its convulsant activities. In order to clarify the mechanisms of action of forskolin further, we have tested the effects of 1,9-dideoxy forskolin, a drug which does not stimulate adenylate cyclase, but is equipotent with forskolin in closing voltage-dependent K^+ channels (Hoshi et al, 1988)

Cortical wedges comprising cortex and corpus callosum were mounted in tissue baths using grease-seal techniques to separate grey from white matter (Harrison & Simmonds, 1985). Magnesium-free artificial C.S.F. was superfused over the cortex at a rate of 2ml/min and all drugs were added on this side of the barrier. Slices were allowed to equilibrate for around two hours or until stable responses were obtained. Spontaneous epileptiform activity, recorded using silver/silver chloride electrodes, consisted of large negative-going potentials with a number of afterpotentials superimposed on the decay phase.

Superfusion of 1,9-dideoxy forskolin over the slices at 0.01μM had no effect upon spontaneous epileptiform activity. 0.1μM drug however caused a significant reduction in the number of bursts per minute to 87.2% ± 2.6% ($n = 4$, $P<0.05$ paired t-test). 10μM 1,9-dideoxy forskolin reduced the number of bursts per minute, afterpotentials per minute and afterpotentials per burst significantly after 30 min of superfusion ($n = 4$, $P<0.05$). All effects were completely reversed on washout of the drug. None of the concentrations tested increased epileptiform activity.

These results suggest that the convulsant effects of forskolin are not due to K^+ channel blockade; its epileptogenic actions are more likely due to cyclic AMP formation.

*C.L.B. is an SERC scholar.

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MANIPULATION OF AMINO ACID NEUROTRANSMISSION IN THE CONTROL OF EPILEPTOGENIC EEG ACTIVITY IN THE RAT

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Epileptogenic activity may be controlled to varying degrees by drugs that augment the function of GABA or block excitatory amino acid receptors, particularly NMDA. We have studied the effects of a range of such compounds on EEG spiking induced in the urethane-anaesthetised rat by 5 min. superfusion of the $GABA_A$ receptor antagonist bicuculline (200μM), across the surface of the exposed cortex using a cortical cup (4mm i.d.) incorporating EEG recording electrodes (Kent and Webster, 1986). Total spike voltage, total number of spikes produced as well as the average size of the compound spikes were monitored. Drug effects were measured as the percentage changes induced in these parameters by varying concentrations of compounds superfused continously in artificial CSF for 30 min. before bicuculline exposure and until cessation of abnormal epileptogenic activity. One hour was left between successive bicuculline applications and no tachyphylaxis to repeated bicuculline exposures was observed. The results (mean ± s.e.mean) for selected drug doses are given in the Table:

Compound	No. of expts.	Percentage changes in; total integrated voltage	total number of spikes	average size of each spike
AP 7 (100μM)	6	-49 ± 9**	+08 ± 9	-52 ± 8**
AP 5 (100μM)	5	-34 ± 12*	-01 ± 16	-35 ± 3**
MK801 (10μM)	6	-29 ± 9*	+22 ± 17	-39 ± 5**
Mg^{++} (10mM)	5	-33 ± 4**	+42 ± 11*	-52 ± 3**
GAMS (1mM)	6	-38 ± 5**	-31 ± 7*	-08 ± 5
CNQX (20μM)	6	-46 ± 12*	-39 ± 9*	-14 ± 6
Clonazepam (100μM)	5	-45 ± 8*	-10 ± 18	-35 ± 7*
Muscimol (30μM)	4	-48 ± 9*	-19 ± 11	-37 ± 5**
Baclofen (30μM)	4	-32 ± 2**	-34 ± 5*	+04 ± 8

*P<0.05, **P<0.001, compared to control, paired t-test.

All compounds reduced total integrated voltage. At concentrations having similar effect on this measure, those reducing NMDA activated events (APH,APV and MK801), as well as increased Mg^{++} reduced the average size of the spikes whereas the more specific non-NMDA antagonists GAMS (Davies and Watkins, 1985) and CNQX (Honoré et al,1988) reduced the total number of spikes. Similarly activation of $GABA_A$ receptors directly by muscimol or indirectly by clonazepam appears to mainly reduce the average size of the spikes whereas the $GABA_B$ receptor agonist, baclofen, leads to a reduction in spike number without affecting the spike size. These results suggest that the initiation of epileptogenic activity may be controlled by mechanisms involving amino acid receptors that differ from those affecting its severity.

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A PUTATIVE GLYCINERGIC CELL IN RAT CEREBELLUM WHICH MAY MODULATE NMDA RECEPTOR FUNCTION IN GRANULE CELLS

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The inhibitory neurotransmitter role of glycine has already been well characterized in brain stem and spinal cord regions, where glycine is known to act upon a strychnine-sensitive glycine binding site (Zarbin et al 1981). ^{3}H-strychnine binding sites are present in a few of the more rostral regions of the CNS, however little evidence exists to support the role of glycine as an inhibitory neurotransmitter in these regions. In higher centres of the brain the possibility exists that glycine may have a completely different role and Johnson and Ascher (1987) have shown that it can modulate N-methyl D-aspartate (NMDA) receptor activation via strychnine-insensitive sites.

Previous electron microscopic autoradiographical studies on ^{3}H-glycine and ^{3}H-GABA uptake (Wilkin et al 1981) and immunocytochemical studies by Ottersen et al (1988) point to populations of Golgi cells which are either glycinergic or GABAergic, as well as to a further population positive for both amino acids. Our current studies have indicated a population of Golgi inhibitory interneurones in the cerebellum cortex which both transport ^{3}H-glycine with a high affinity and release it in a Ca^{2+} dependent manner. Furthermore, ^{3}H-glycine binding sites and subsequent autoradiographical analysis confirm the presence of strychnine insensitive binding site in the cerebellum which could be the site of action of the released glycine.

400um vibratome cut slices of male SD rat cerebellum were preincubated for 60min in Krebs-Henseleit solution at 37°C, followed by incubation for 45min in Krebs containing 100nM ^{3}H-glycine. Slices were perfused (0.1ml/min) for 40min prior to stimulation with concentrations of K^{+} up to 55mM, in the presence or absence of Ca^{2+}, for 3 or 5 mins. Autoradiography and ^{3}H-glycine binding studies were performed on 10um cryostat sections, as described by Bristow et al (1986).

Release at 3min was increased by 136±16.5% over basal release (equivalent to an increase of 0.4%±0.2% of total radioactivity) and by 144±21.7% at 5 min (0.3%±0.15% n=8 in both cases). In the absence of Ca^{2+} no increase over basal levels was observed at either 3 or 5min. Scatchard analysis of the binding of ^{3}H-glycine to cerebellar sections indicated that it was saturable with a single component (Kd: 288±122nM, Bmax 1540±31.4 fmol/mg tissue (mean±SEM n=6)). Image analysis of autoradiograms indicated that in excess of 90% of these strychnine-insensitive binding sites were localised in the granule cell layer. The distribution of the glycine sites within the cerebellum concurs with that of NMDA receptors (Monaghan and Cotman 1985). Moreover glycine can enhance NMDA-mediated conductance in mechanically dissociated granule cells (S. Cull-Candy, personal communication). Taken together, these data might suggest a neurally-mediated modulation of NMDA function within the cerebellum.

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THE DISCRIMINATIVE STIMULUS PROPERTIES OF ANTAGONISTS OF N-METHYL-D-ASPARTATE

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Recent research has shown that phencyclidine has N-methyl-D-aspartate (NMDA) antagonist properties which may be responsible for at least some of its behavioural effects. For example, in studies of the discriminative stimulus properties of phencyclidine it has been found that stimulus control established by this drug will generalise to other NMDA antagonists including systemically administered MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate) and N-allylnormetazocine, and 2-amino-7phosphonoheptanoic acid injected into the cerebral ventricles (Tricklebank et al, 1987 ; Willetts et al, 1986). However, it was recently reported that systemic administration of two other NMDA antagonists, CPP (3-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid) and ifenprodil, did not produce phencyclidine-like discriminative stimulus properties (Jackson and Sanger, 1988).

In order to further investigate the discriminative stimulus properties of NMDA antagonists rats were trained to discriminate a low dose of MK-801 from saline. Thus the animals were required to respond on one lever in a standard operant test chamber after administration of MK-801 (0.05 mg/kg ip) and on the other lever after saline. The rats learned this discrimination very rapidly, reaching criterion performance in a mean of 32 $\pm$ 3 sessions. The MK-801 discrimination was dose-related after both intraperitoneal and oral administration and response rates were consistently increased after the training dose of MK-801. When substitution tests were carried out with other NMDA antagonists, it was found that both phencyclidine (1 - 4 mg/kg) and N-allylnormetazocine (2.5 - 20 mg/kg) produced dose-related responding on the MK-801 lever. Ifenprodil (5-15 mg/kg ip) produced only a low level of substitution for MK-801 whereas the ifenprodil derivative SL 82.0715 (Carter et al, 1988) did not substitute (2.5 - 10 mg/kg). These results show that the discriminative stimulus properties of MK-801 are similar to those of phencyclidine in that they generalise to some, but not all, compounds shown to be antagonists of NMDA.

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INDIRECT EFFECTS OF GLUTAMATE ON CYCLIC AMP FORMATION IN GUINEA-PIG BRAIN SLICES

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The stimulation of cyclic AMP formation by neurotransmitters via receptors directly linked to adenylate cyclase is frequently modulated by other endogenous agents. In this study we have examined the indirect effects of the excitatory amino acid, glutamate, on cyclic AMP production in guinea-pig brain slices.

Cyclic AMP formation was measured using the [^{3}H]-adenine prelabelling method as described previously (Donaldson et al., 1987) using slices of cerebral cortex from male and female Hartley guniea-pigs. Enzyme inhibitors and receptor antagonists were added 20 min prior to agonist administration. Where appropriate 2-chloroadenosine and glutamate or histamine were added simultaneously. In studies of the histamine H_1-receptor effect on cyclic AMP accumulation, cimetidine (0.3mM) was included in the incubation medium to remove the direct effects of H_2-receptor stimulation.

At a concentration of 3 mM (at which, saturation had not been reached) glutamate stimulated cyclic AMP formation by 5.9 $\pm$ 1.1 fold (n=4). This effect appeared to depend upon endogenous adenosine since it was reduced to 1.5 $\pm$ 0.1 fold (n=7) in the presence of adenosine deaminase (ADA, 1.2 U/ml) and was abolished by the adenosine antagonist cyclopropyltheophylline (0.1mM). Glutamate (3mM) also enhanced the direct effect of the adenosine analogue 2-chloroadenosine (2-CA, 30 μM) in the presence of ADA from 12.7 $\pm$ 1.2 fold to 38.9 $\pm$ 3.4 fold over basal levels (n=6). Glutamate was probably acting via a non-NMDA receptor since the cyclic AMP response to a combination of glutamate plus 2-CA was unaffected by the non-competitive NMDA antagonist MK801 in concentrations up to 0.1mM. Some excitatory amino acid effects are inhibited by low concentrations of forskolin (Baba et al., 1988). In our hands the enhanced response to glutamate was reduced by 50.2 $\pm$ 5.1 % (n=5) in the presence of 1 μM forskolin. Dideoxyforskolin (0.1 - 10 μM) had no such effect. In addition the histamine H_1-receptor-mediated augmentation of the 2-CA cyclic AMP response was not inhibited by forskolin.

These data support the idea that glutamate both releases endogenous adenosine and augments the final effect of the released adenosine on cyclic AMP accumulation. The mechanism underlying the augmentation of adenosine-stimulated cyclic AMP accumulation produced by glutamate, which might involve a forskolin-sensitive G protein, however, appears to differ from that due to histamine H_1-receptor activation.

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NMDA-STIMULATED ENDOGENOUS DOPAMINE RELEASE FROM RAT STRIATAL SLICES CAN BE REGULATED BY D-2 AUTORECEPTORS

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Glutamate receptors of the N-methyl-D-aspartate (NMDA) type are thought to be present on striatal dopaminergic nerve terminals, and activation of NMDA receptors has been shown to stimulate the release of dopamine (DA) both *in vivo* and *in vitro* (Carter *et al* 1988; Jhamandas & Marien 1987). NMDA receptors are thought to open receptor-operated channels (ROC) gating calcium entry into cells (Lodge 1987), thus raising intracellular calcium levels by a mechanism different to that of conventional depolarising stimuli which open voltage-sensitive calcium channels (VSCC). Endogenous DA release stimulated by such depolarising stimuli is known to be regulated by D-2 autoreceptors (Herdon *et al* 1987), so in the present studies the regulation of NMDA-stimulated DA release by these receptors has been investigated.

Endogenous DA release from superfused striatal slices was measured using HPLC with electrochemical detection as described previously (Herdon *et al* 1987) except that magnesium-free Krebs buffer was used for superfusions. DA release was stimulated by applying two 4 min pulses of NMDA 50 min apart (S1 & S2); other drugs were added 20 or 30 min before S2 and remained present for the rest of the experiment. Results are presented as means ± SEM (n=4-8) of S2/S1 ratios normalised to percentages of the relevant control values. NMDA stimulated DA release in a concentration-dependent manner; 0.1mM NMDA produced an 8-10 fold increase in release above spontaneous levels with an S2/S1 ratio of 0.71 ± 0.02. This release was calcium-dependent, abolished by $MgCl_2$ (1.2mM) and reduced to 32 ± 4% of control values by tetrodotoxin (1μM), confirming other studies (Jhamandas & Marien 1987). However, it was unaffected by atropine (1μM), in contrast to a report on DA release *in vivo* (Carter *et al* 1988). Sulpiride (1μM) increased NMDA-stimulated release to 151 ± 7% of control values, whilst pergolide (1μM) reduced release to 89 ± 3% of control and also prevented the sulpiride-induced increase in release. The neuronal VSCC-blocking peptide ω-conotoxin GVIA (10nM) reduced NMDA-stimulated release to 79 ± 2% of control values, but in the presence of ω-conotoxin the same sulpiride-induced increase in release was still observed (149 ± 7% of control).

These results show that NMDA-stimulated endogenous DA release can be regulated by D-2 autoreceptors in a manner quantitatively similar to that observed for release evoked by conventional depolarising stimuli such as veratrine (Herdon *et al* 1987). The mechanism by which NMDA stimulates DA release remains unclear, though its partial inhibition by tetrodotoxin suggests that it might involve an indirect effect requiring activation of voltage-sensitive sodium channels as well as a direct effect on calcium influx into dopaminergic terminals. Although NMDA receptor activation might result in terminal depolarisation and the resultant opening of VSCC, such a process may not play a major role in the stimulation of DA release, since this was not greatly reduced by ω-conotoxin which blocks neuronal VSCC and produces large reductions in depolarisation-evoked DA release (Herdon & Nahorski, in press). It has been proposed that presynaptic D-2 receptors act via inhibition of calcium influx through VSCC, but the finding that the sulpiride-induced increase in NMDA-stimulated DA release was unaffected by ω-conotoxin suggests that D-2 autoreceptors can also regulate calcium influx through ROC or, more probably, that they modulate the release process at an intracellular level.

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EFFECTS OF PARTIAL DOPAMINE RECEPTOR AGONISTS ON D-AMPHETAMINE DISCRIMINATIVE CUE: COMPARISON WITH DOPAMINE ANTAGONISTS

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The dopamine (DA) analogue 3-(3-hydroxyphenyl)-N-n-propylpiperidine, (-)-3-PPP, and transdihydrolisuride (TDHL) are DA agonists with low relative efficacy (Clark et al., 1985). In normal animals, these compounds act as partial agonists at DA autoreceptors, but as antagonists at postsynaptic DA receptors. Although (-)-3-PPP exhibits a behavioural profile which is similar in many regards to classical DA antagonists, it does not produce serious motor impairments such as catalepsy and muscular rigidity. To further compare the behavioural profile of (-)-3-PPP, TDHL and classical DA antagonists, we have examined their effects on the discriminative cue produced by d-amphetamine.

Male, Sprague Dawley rats were taught to discriminate d-amphetamine (0.5 mg/kg, i.p.) from saline in operant boxes containing two retractable levers. Similar methods to those of Cunningham and Appel (1986) were used. During 25 minute training sessions, animals had to respond on the correct lever (determined by the prior injection) to obtain food reinforcement on a FR 20 schedule. Once the discrimination had been learnt (n=42 rats), the effects of various compounds on the d-amphetamine cue were examined in groups of eight rats. The percentage of responses on the drug-appropriate lever prior to the first reinforcement, and time taken to complete this were recorded.

The classical antipsychotic haloperidol, the selective D2 antagonist raclopride and D1 antagonist SCH 23390 all completely blocked the d-amphetamine discriminative cue, inducing saline-appropriate responding (ED_{50} by log. probit analysis: 0.05, 0.4 and 0.025 mg/kg, respectively). On the other hand, (-)-3-PPP (ED_{50}:1.0 mg/kg) only partially inhibited (38-52%) the d-amphetamine cue across a wide range of doses (1-16 mg/kg). The dose-dependent effects of TDHL (ED_{50}: 0.4 mg/kg) were more similar to those observed with classical DA antagonists; statistically, complete blockade of the cue was observed. However, TDHL and (-)-3-PPP were similar in regards to the marked variations in responsiveness of individual rats to the inhibitory effect of these drugs (revealed by ANOVA "subject" factor). In some animals, the cue was blocked by low doses of these drugs, while other rats were remarkably more resistant to these inhibitory effects. This variation does not appear related to differences in drug metabolism, since "insensitive" rats showed a dose-dependent decline in response rate during these test sessions. Dopamine antagonist-induced inhibition of the cue was consistent across animals.

The present study illustrates a further difference between (-)-3-PPP, and TDHL to some extent, and classical DA antagonists. The partial inhibitory effect of (-)-3-PPP on the d-amphetamine cue contrasts with its complete antagonism of the locomotor hyperactivity produced by this stimulant drug. This may reflect a dissociation between the two behavioural models. Alternatively, the repeated d-amphetamine administration used in the present study may induce DA receptor changes which would be expected to influence the functional effects of partial agonists and may vary considerably between animals.

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THE ANTIPARKINSON POTENTIAL OF SK&F 101468-A REVEALED IN RODENT AND PRIMATE TESTS

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SK&F 101468-A (4-[2-9(dipropylamino)ethyl]-1,3-dihydro-2H-2-one HCl) is a potent selective agonist at dopamine D_2 receptors. Peripherally this action results in neuronal inhibition of noradrenaline release with consequent hypotension (Gallagher et al. 1985; Eden et al. 1988). Animal studies have shown that tolerance develops rapidly to these peripheral effects. The present studies show that SK&F 101468-A additionally has activity in the CNS with a profile that provides a basis for development of this agent for the treatment of Parkinson's disease.

SK&F 101468-A was initially assessed in male BKW mice (25-30g) which had received unilateral 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra (8μg 6-OHDA over 60s). Asymmetric posturing was scored on a 4 point scale 7 to 21 days after surgery. Maximum effect was when animals exhibited marked asymmetry with body bent in one direction only, unable to turn in the opposite direction, pivoting nose to tail on initiation of locomotor activity. In these animals apomorphine (0.5mg/kg s.c.) caused marked contralateral asymmetries whilst amphetamine (1.25mg/kg i.p.) caused marked ipsilateral responses. SK&F 101468-A treatment, like apomorphine, caused contralateral asymmetries (threshold dose 0.01mg/kg i.p., maximum asymmetry at 0.1mg/kg i.p.). Subsequent studies concentrated on analyses of effects on motor impairments induced in the common marmoset (Callithrix jacchus) by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), 20μg/24h, 7-13 days, infused unilaterally into the substantia nigra. Mature male and female marmosets (250-450g) were used. Locomotor activity, speed of locomotion, head movements and jumps were measured in addition to behavioural assesssments including response to novel stimuli and ability to alter facial expression. All of these parameters were influenced adversely by MPTP, animals exhibited hypokinesia, bradykinesia, and loss of facial expression and response to novel stimuli. These effects were all antagonised by treatment with SK&F 101468A (0.05-1.0mg/kg s.c. or 0.1mg/kg p.o.). For example in one experiment, % time spent in locomotor activity prior to MPTP treatment was 51.2%, after MPTP this was reduced to 10.1%. Subsequent oral treatment with SK&F 101468A, 0.1mg/kg, restored locomotion to 46.2%.

The MPTP-induced deficits could also be antagonised by L-dopa (12.5mg/kg i.p. or 50mg/kg p.o. given 30 min after benserazide 12.5mg/kg i.p.). However, whilst the L-dopa antagonism of the MPTP response was associated with emesis and behaviour characteristic of nausea, antagonism of MPTP deficits by SK&F 101468-A could be effected at doses devoid of these side-effects. In both the rodent and primate studies the compound was found to have an onset of action within 10-20 min of administration, with a duration in excess of 2h.

These studies therefore show SK&F 101468-A to exert activity consistent with that of a directly acting dopamine agonist in the extra-pyramidal system, with no evidence of tolerance. This would encourage human studies to assess the potential of the compound for the treatment of Parkinson's disease.

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REGIONAL DOPAMINE DOSE-RESPONSE DIFFERENCES TO COCAINE STUDIED BY MICRODIALYSIS

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Cocaine acts at dopamine (DA) neurons by blocking presynaptic reuptake, and at high doses, may also cause DA release. Although there are regional differences in basal DA levels within the brain, there have been no studies looking at regional differences in DA responses to cocaine. This was studied by infusing various doses of cocaine through a microdialysis probe and looking at subsequent DA responses in three brain regions, frontal cortex, striatum and nucleus accumbens.

Male Sprague-Dawley rats were kept anaesthetised with chloral hydrate, and microdialysis probes were inserted stereotaxically in either frontal cortex, striatum or nucleus accumbens. Artificial CSF was perfused through these at 1.33 ul/min. 30 mins after probe insertion, samples of dialysate were collected every 15 mins and injected immediately into an HPLC using an ESA coulometric detector. After three baseline samples, cocaine in artificial CSF was substituted for the artificial CSF for one 15 min collection period, then artificial CSF was restarted for 45 mins. Four concentrations of cocaine (0.01, 0.1, 1.0, and 10.0mM) were used.

Basal DA levels were: striatum 94 fmole $\pm$ 20 s.e.m., nucleus accumbens 42 fmole $\pm$ 7 s.e.m., frontal cortex 18 $\pm$ 4 s.e.m. Significant DA release was seen in all areas at every dose of cocaine.

Table: Peak % DA dose responses (+/- s.e.m.) to cocaine.

Cocaine conc.	Frontal Cortex	Nucleus Accumbens	Striatum
0.01mM	213 $\pm$ 33	571 $\pm$ 36	854 $\pm$ 264
0.1mM	221 $\pm$ 25	1640 $\pm$ 368	1486 $\pm$ 426
1.0mM	271 $\pm$ 31	2551 $\pm$ 655	2165 $\pm$ 488
10.0mM	267 $\pm$ 34	4563 $\pm$ 398	6723 $\pm$ 1222

(all values mean $\pm$ s.e.m.; n=4).

Clear dose response relationships were seen in striatum and nucleus accumbens, with similar relative (percent) increases in DA at all doses of cocaine used. However, significantly greater absolute DA responses (as area under the curve) were seen in striatum compared with nucleus accumbens at all doses, perhaps due to the greater DA reserves in striatum. In frontal cortex, DA responses were not dose related. Although this may be a reflection of the low tissue levels, it may imply that other processes than just reuptake blockade are occurring in striatum and nucleus accumbens at higher cocaine concentrations.

DOPAMINE D-2 AGONISTS DECREASE STIMULATED DOPAMINE RELEASE IN RAT NUCLEUS ACCUMBENS BUT NOT IN CAUDATE : IN VIVO VOLTAMMETRIC DATA

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Whereas most studies have shown that dopamine (DA) agonists decrease DA release from nerve terminals, 2 recent in vivo reports have shown a transient elevation of striatal DA release following quinpirole and apomorphine (Sharp et al, 1987; Stamford et al, 1987). The present study sought to extend this work by examining the effects of a series of agonists upon stimulated DA release in the rat caudate (CPu) and nucleus accumbens (Acb).

Male Sprague Dawley rats (250-350g) were anaesthetised with chloral hydrate (400 mg/kg i.p.). Carbon fibre microelectrodes were implanted into the CPu and Acb while reference (Ag/AgCl) and auxiliary electrodes were placed on the skull. Electrical stimulations (50 Hz, 100 ± 10μA r.m.s., 2s train) of the median forebrain bundle (MFB) were applied at intervals of 5 minutes throughout the experiment. Evoked DA release was monitored using fast cyclic voltammetry (Stamford et al, 1987). Drugs or vehicle were administered after the sixth stimulus train and DA release on the subsequent 12 stimulations was averaged and expressed as a percentage of release in the pre-drug period (S1-6). The results are shown in Table 1.

Drug	Dose (mg/kg i.p.)	Mean DA release on S7-18 (% of pre-drug values) CPu	Acb
Controls		87.6 ± 9.5	92.5 ± 3.6
Apomorphine	1	103.6 ± 18.5	50.5 ± 4.8***
Quinpirole	1	97.6 ± 11.0	59.5 ± 4.9***
Bromocriptine	10	86.9 ± 9.6	91.9 ± 10.7
Pergolide	1	101.2 ± 8.2	70.6 ± 6.6*
(+) 3PPP	20	73.0 ± 4.3	58.4 ± 4.6**
(-) 3PPP	20	200.1 ± 35.9**	113.7 ± 13.1

All values are means ± s.e.m. (n = 5-8)
*P < 0.05, ** P < 0.01, *** P < 0.001 vs vehicle controls (Mann Whitney U test).

Bromocriptine had no effect upon DA release in either nucleus, consistent with its reported postsynaptic selectivity (Seeman, 1981). The (-) isomer of 3PPP behaved as an antagonist in the CPu and slightly, though not significantly, elevated DA release in Acb. The remaining agonists all reduced DA release in Acb without any effect in the CPu. The heterogeneity of the striatal (CPu) response was evident from the generally larger error bars. Whereas some animals showed decreased DA release, others showed sharp elevations. The response in CPu was not determined by the level of DA release on previous stimulations.

In conclusion, the data indicate that the DA receptors controlling release in CPu and Acb differ in their response to DA agonists. Differences in agonist binding sites (Leonard et al, 1987) or in the linkage to adenylate cyclase (Stoof and Verheijden, 1986) as already reported for D2 receptors in striatal and limbic regions may underly these differences.

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AGONIST AND ANTAGONIST INTERACTIONS WITH THE D-1 DOPAMINE RECEPTOR

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Pseudo non-competitive interactions between agonists and their receptors have been reported to occur at D-2 dopamine (Sibley & Creese, 1980) and enkephalin (Rothman & Westfall, 1982) binding sites. At the D-1 dopamine receptor the partial agonist CY 208-243 inhibits the binding of ^{3}H-SCH 23390 in a mixed (competitive/non-competitive) fashion (O'Boyle & Waddington, 1988b). The present study investigates the nature of the interactions with D-1 receptors of a weak partial agonist, a full agonist and an antagonist.

Rat striatal D-1 receptors were labelled with ^{3}H-SCH 23390 as previously described (O'Boyle & Waddington, 1987). The compounds under investigation were SK&F 75670 (weak partial agonist), dopamine (full agonist) and R-SK&F 83566 (antagonist, O'Boyle & Waddington, 1988a). ^{3}H-SCH 23390 saturation curves were constructed using 0.1-6.0 nM ligand and were measured in the absence and presence of approximately 1/10 Ki, Ki and 5Ki concentrations of each test agent. Scatchard analysis was used to determine changes in K_d and B_{max}. Results are summarised in the Table.

		K_d nM		B_{max} pm/g	n
SK&F 75670	control	0.29 ± 0.02	*	73.2 ± 4.4	6
	10 nM	0.42 ± 0.01		69.5 ± 5.6	3
	200 nM	2.81 ± 0.39		70.4 ± 6.6	3
	500 nM	9.24 ± 1.21		79.5 ± 12.6	4
Dopamine	control	0.33 ± 0.02	**	70.6 ± 5.5	6
	500 nM	0.49 ± 0.02		61.6 ± 6.4	6
	5000 nM	0.88 ± 0.06		57.4 ± 4.5	5
	20000 nM	1.26 ± 0.04		42.2 ± 4.3#	6
SK&F 83566	control	0.49 ± 0.05	**	68.1 ± 4.2	3
	0.1 nM	0.59 ± 0.14		62.0 ± 2.3	3
	1.0 nM	2.01 ± 0.29		70.2 ± 1.8	3
	5.0 nM	4.68 ± 0.87		65.0 ± 4.4	3

means ± s.e.mean. *$p<0.05$ **$p<0.005$ for change in K_d (Kruskall Wallis). #$p<0.005$ compared with control (1-way ANOVA, t-test)

All three agents caused a significant, dose-related reduction in the affinity of ^{3}H-SCH 23390 for its binding site. The partial agonist SK&F 75670, which has a low intrinsic activity relative to dopamine, and the antagonist SK&F 83566 did not alter the density of D-1 binding sites. However, in the presence of dopamine the number of D-1 receptors was reduced by 40%. These data expand our previous finding that a partial agonist with moderate intrinsic activity (CY 208-243) decreases D-1 receptor density by 27% (O'Boyle & Waddington, 1988a). Thus, D-1 agonists and antagonists may be distinguished in binding assays by their effects on the binding parameters of the ligand. It is interesting to speculate that the extent to which a D-1 agonist appeared to reduce receptor number may be related to its intrinsic activity.

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MODULATION OF POTASSIUM-STIMULATED [^{3}H]DOPAMINE RELEASE FROM THE RAT NUCLEUS ACCUMBENS BY (-)N-n-PROPYLNORAPOMORPHINE

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Behavioural studies have previously demonstrated an inhibition of locomotor activity following the injection of dopamine agonists into the nucleus accumbens of the rat and mouse (Costall et al. 1980; Bradbury et al. 1984). It was hypothesised that the behavioural inhibition may reflect a presynaptic action on dopamine receptors to reduce dopamine release. In the present study we investigate this hypothesis by analysing the influence of a potent dopamine receptor agonist, (-)N-n-propylnorapomorphine [(-)NPA], on the release of [^{3}H]dopamine from the nucleus accumbens.

Studies used female Hooded-Lister rats (Bradford bred) weighing 275-350 g. The nucleus accumbens was removed and cross-chopped at 90^o to produce slices of tissue 0.35 mm by 0.35 mm. The tissue was suspended in normal Krebs solution (mM: NaCl 118.0, KCl 4.75, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.0), continuously gassed with 95% O_2, 5% CO_2, and was initially depolarised in a buffer containing 39.7 mM KCl prior to incubation in Krebs buffer containing 0.11 μM [^{3}H]dopamine (specific activity 49.9 CimM^{-1}) for 45 min. The tissue was washed in Krebs buffer and loaded into Swinnex chambers and superfused with Krebs buffer at a rate of 0.5 ml min^{-1}. After a 30 min washout period, 4 min samples of perfusate were collected. At 12 min (S1) and 48 min (S2) the slices were depolarised for 4 min in a 20.0 mM KCl buffer.

In the absence of any drug the S2/S1 ratio was 0.92±0.02 (n = 10). The addition of (-)NPA (10^{-12}-10^{-9}M) caused a concentration-dependent inhibition of potassium-stimulated [^{3}H]dopamine release. The higher concentrations of (-)NPA inhibited approximately 60% of the [^{3}H]dopamine release induced by potassium. The inclusion of the dopamine D_2 antagonist (-)sulpiride (10^{-7}-10^{-5}M) in the media antagonised the inhibitory effects of (-)NPA in a concentration-dependent manner with values returning to control levels. However, (-)sulpiride superfused alone (10^{-6}M) did not alter the release of [^{3}H]dopamine from the nucleus accumbens tissue. The selective dopamine D_1 antagonist SCH 23390 (10^{-10}-10^{-6}M) failed to alter the inhibition of [^{3}H]dopamine release caused by (-)NPA and did not affect the potassium-stimulated [^{3}H]dopamine release when superfused alone (10^{-7}M). Idazoxan, prazosin, GR38032F, zacopride, propranolol, atropine, hexamethonium, mepyramine, naloxone, fluphenazine, ranitidine, ritanserin and methysergide (1.0 μM) superfused alone failed to modify the potassium-induced release of [^{3}H]dopamine. With the exception of fluphenazine (10^{-9}-10^{-6}M), all the above agents failed to antagonise the (-)NPA induced inhibition of potassium-stimulated [^{3}H]dopamine release from accumbens tissue.

The present studies show that (-)NPA is extremely potent and effective to reduce [^{3}H]dopamine release from tissue slices of the rat nucleus accumbens. The biochemical effects of (-)NPA may be mediated via presynaptic dopamine receptors and are indicated to be of dopamine D_2 receptor type. This action may provide a biochemical correlate for the action of (-)NPA to reduce locomotor activity on injection into this limbic nucleus.

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HAEMODYNAMIC AND PHARMACOLOGICAL PROFILE OF ENDOTHELIN IN PITHED RATS

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Endothelin, a 21 aminoacid peptide produced by vascular endothelium, is one of the most potent natural vasoconstrictor known to date (Yanagisawa *et al.*, 1988). The aim of this study was to investigate its cardiovascular mechanism of action in pithed rats.

Male normotensive Sprague Dawley rats (250 g) were anaesthetised with ether, placed under artificial ventilation and pithed *via* the right orbit with a stainless steel rod. Blood pressure was measured from the cannulated left carotid artery and injections were given *via* the femoral veins. Doppler blood flow velocity probes were placed on the aorta at its exit from the diaphragm (cardiac output estimation), renal and mesenteric arteries and the terminal aorta (hindquarter blood flow). The effects of various i.v. bolus injections (0.125, 0.25, 0.5, 1.0 nmol/kg) were studied in independent groups of rats. Furthermore, the pressor effects of 0.25 nmol/kg dose were investigated in animals pretreated 10 min earlier with i.v. saline (0.25 ml/kg), phentolamine (5 mg/kg), enalapril (0.3 mg/kg), methysergide (0.1 mg/kg), desipramine (0.1 mg/kg), SK&F 100273 (V_2 vasopressin antagonist : 15 µg/kg), BW 755c (cyclo- and lipo-oxygenase inhibitor : 5 mg/kg), diltiazem (30 µg/kg/min), verapamil (12.5 µg/kg/min), nitrendipine (5 µg/kg/min) and syrosingopine (5 mg/kg given i.p. 18 h before experiment). The effects of endothelin were also studied in pithed rats in which the low level of blood pressure was elevated by an infusion of vasopressin (7.5 mIU/kg/min).

I.v. bolus injection of endothelin (0.125, 0.25, 0.5, 1.0 nmol/kg) induced dose-related increases in blood pressure which attained a maximum (15.6 ± 1.5, n = 5; 28.7 ± 2.7, n = 7; 45 ± 0.6, n = 6; 59.1 ± 3.1, n = 6) after 2 min and waned after approximately 30 min. Endothelin (0.25 nmol/kg) increased renal blood flow (30 %), decreased mesenteric blood flow (-39 %) but changed neither cardiac output nor hindquarter blood flow. Thus, mesenteric vascular resistance (200 %) increased more than systemic (65 %), hindquarter (55 %) and renal (22 %) resistances. The highest dose of endothelin substantially decreased (45%) cardiac output. The vasopressor effect induced by endothelin (0.25 nmol/kg) was not modified by phentolamine, enalapril, methysergide, desipramine, SK&F 100273 or BW 755c but was enhanced (40 %) by pretreatment with syrosingopine. In contrast, diltiazem, nitrendipine and verapamil inhibited (50 to 60%) the endothelin pressor response. In vasopressin-supported, pithed rats, endothelin produced an initial, short lasting (2 min) fall (-48 ± 2 from 116 ± 5 mmHg, n=6) in blood pressure followed by a moderate increase (19.1 ± 2.6 mmHg).

These results confirm that endothelin is a potent vasoconstrictor with a long duration of action. Haemodynamically, its effects can be accounted for by an elevation in systemic vascular resistance and, for the highest doses studied, a fall in cardiac output. Several blockers of classical receptors mediating vasoconstriction did not modify the pressor effects of endothelin which, in contrast, were inhibited by calcium entry blockers. Thus, endothelin appears to activate specific receptors which may use calcium as second messenger. Finally, endothelin possesses also transient vasodepressor activity the mechanism of which remains unrevealed.

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ENDOTHELIN HAS POTENT ULCEROGENIC AND VASOCONSTRICTOR ACTIONS IN THE STOMACH

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Endothelin is a 21-amino acid peptide derived from vascular endothelial cells which has been suggested to play a pathophysiological role in conditions characterized by vascular spasm (Yanagisawa et al., 1988). Since the gastric mucosa is a highly vascularized tissue in which the regulation of vascular tone appears to be of paramount importance in determining whether or not the mucosa will withstand exposure to its potentially damaging luminal contents, we propose that endothelin may be an endogenous mediator of gastric ulceration.

Male, Wistar rats (200-225 g; n=4 to 6 per group) were anaesthetized with sodium pentobarbital (60 mg/kg i.p.) and an ex vivo gastric chamber was prepared as described by Wallace et al. (1982). In some experiments the rats were pretreated with indomethacin (5 mg/kg s.c.) 60 minutes before the experiment. The mucosa was bathed with 0.05 M HCl for 20 minutes, followed by a 10-minute exposure to 20% ethanol (v/v). During the final 30 minutes of the experiment the mucosa was once again bathed with 0.05 M HCl. At minute 15 of the experiment, an intravenous or intra-arterial infusion of synthetic human/porcine endothelin (Peninsula Laboratories; 10^{-10} to 10^{-6} M) was started. The infusion was continued for 10 minutes at a flow rate of 0.1 ml/min. At the end of the experiment the extent of haemorrhagic damage was quantified planimetrically (Wallace et al., 1982). Systemic arterial pressure was measured continuously throughout the experiments. The effects of endothelin on gastric vascular tone were examined using an in vitro vascularly perfused stomach preparation (Salvati & Whittle, 1981). The effects of 5-minute infusions of endothelin (10^{-11} to 10^{-8} M) on perfusion pressure were assessed.

Topical challenge of the gastric mucosa with 20% ethanol did not produce haemorrhagic damage when the vehicle for endothelin (phosphate-buffered saline; pH 7.4) was infused systemically. Infusion of endothelin resulted in concentration-dependent increases in the extent of haemorrhagic damage. At a concentration of 10^{-6} M (i.v.), the haemorrhagic damage covered 57 $\pm$ 9% of the total glandular mucosa. Intra-arterial infusions of endothelin produced marginally greater damage than intravenous infusions of the same concentrations, and at the highest concentration tested intra-arterially (10^{-6} M), none of the rats survived the infusion. Significant increases in gastric damage were observed with concentrations of endothelin (10^{-9} to 10^{-8} M) which were without significant systemic hypertensive actions. Indomethacin significantly augmented the damage-promoting and hypertensive actions of endothelin. Endothelin produced significant increases in gastric tone at concentrations in the 10^{-9} to 10^{-8} M range. At the higher concentration, perfusion pressure increased by 68 $\pm$ 10 mm Hg during the infusion period, and remained elevated above resting levels for 10-15 minutes after completion of the infusion.

These results demonstrate that in nanomolar concentrations, endothelin can render the gastric mucosa susceptible to damage by a topical irritant and significantly increase gastric vascular tone, presumably through vasoconstriction. The observation that intra-arterially administered endothelin had greater effects than intravenously administered endothelin is consistent with the observation of Antunes et al. (1988) that endothelin is inactivated in the lung. The factors regulating the synthesis of endothelin and the balance between production of endothelial-derived constricting and relaxing factors may be of critical importance in the pathogenesis of gastric ulceration.

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ENDOTHELIN CONTRACTS VASCULAR SMOOTH MUSCLE VIA RECEPTORS THAT DIFFER FROM DIHYDRO-PYRIDINE SENSITIVE CALCIUM CHANNELS

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Endothelin (ET) is a 21-amino acid vasoconstrictor peptide from porcine endothelial cells reported to activate voltage dependent calcium channels (Yanagisawa et al., 1988). Here we have compared the activity of ET and of a voltage dependent calcium channel agonist, Bay K 8644 (Schramm et al., 1983), on different smooth muscle strips and assessed the effect of a dihydropyridine-sensitive calcium channel blocker on these two agonists.

Spirally cut strips of rabbit jugular vein (RbJV), rabbit mesenteric vein (RbMesV) and rabbit mesenteric artery (RbMesA) denuded of endothelium were superfused at 5 ml min^{-1} in a cascade (Vane, 1964) with warmed (37°C) and gassed (95%O_2/5%CO_2) Krebs' solution containing indomethacin (5.6 μM).

Endothelin (2.5-5 pmol) induced substantial contractions of the RbMesV and RbJV which were rapidly reversible (n=10). Higher concentrations of ET (10-25 pmol, n=8) were needed to contract the RbMesA (D'Orleans-Juste et al., 1988). Bay K 8644 promoted sharp contractions of the RbMesV and the RbMesA (2.7 and 0.27 nmol respectively, n=9). Injections of Bay K 8644 up to 27 nmol were ineffective on the RbJV. Nicardipine (200 nM), a dihydropyridine calcium channel antagonist, did not affect the contractions induced by ET on the three vascular preparations whereas the contractions induced by Bay K 8644 on the RbMesV and RbMesA were abolished (n=6).

Our results indicate that ET acts on these three vascular preparations via specific receptors which are different from the dihydropyridine sensitive channels activated by Bay K 8644. Similar results with nicardipine were also observed in the rat aorta (Finet et al., 1988). The development of specific antagonists for ET should provide further information on the possible role(s) of ET in vivo.

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THE EFFECTS OF ENDOTHELIN IN THE ISOLATED RAT HEART

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A highly potent vasoconstrictor peptide, endothelin (ET), has been isolated from supernatants of cultured vascular endothelial cells (Yanagisawa et al., 1988). ET has long-lasting constrictory effects on a variety of vascular and non-vascular smooth muscle preparations (D'Orleans-Juste et al., 1988), and it has been suggested that ET may act as a Ca^{2+} channel agonist (Yanagisawa et al., 1988). We describe the effects of synthetic ET on the isolated perfused rat heart, and pharmacological investigations upon its possible mechanism of action.

Hearts from male Wistar rats (250-350g) were perfused by the Langendorff technique at 37°C and 10ml min^{-1} using Krebs-Henseleit buffer containing 5.9mM K^+. Bolus injections of ET (1-100 pmol, Novabiochem, Nottingham) caused a dose-dependent and maintained increase in perfusion pressure. This increase was preceded by a transient decrease in coronary tone (n=7). Over the same dose range (1-100 pmol) ET had no significant effect on heart rate but caused an initial increase in cardiac contractility. Repeated dosing with ET however resulted in an irreversible negative inotropic action.

The changes in perfusion pressure were not accompanied by the release of either 6Keto PGF1α or TxB2 as detected by radioimmuno assay. Lack of involvement of eicosanoids in the vascular action of ET was confirmed by infusion of flurbiprofen (2uM) and BW 755c (7uM), both of which were without effect. Pretreatment in vivo with dexamethasone (1mg/Kg, -3h) did not alter the responses in vitro to ET.

Since the vasodilation component could potentially be due to EDRF release, we investigate the effects of methylene blue (1uM)&superoxide dismutase (20U/ml) on ET-induced vasodilation. Neither treatment affected the response.

The effects of ET in isolated tissues is thought to be dependent on Ca^{2+} channel activation. However, verapamil (0.1uM), which inhibited responses to the Ca^{2+} agonist Bay K 8644 (1-300 pmol) did not affect the vasoconstrictor responses to ET suggesting that this effect is not mediated by Ca^{2+} channel activation.

The vasoconstrictor effects of ET in the perfused rat heart appears to be independent of eicosanoid release or Ca^{2+} channel activation. The vasodilator component also appears to be independent of eicosanoid or EDRF release. Acute pretreatment in vivo with dexamethasone does not affect the responses to ET.

D'Orleans-Juste et al., 1988 Br J Pharmac, in press.

Yanagisawa, M, et al., 1988 Nature, 332, 411-415.

THE 5,6-EPOXYEICOSATRIENOIC ACID IS AN INDIRECTLY ACTING, ENDOTHELIAL-DEPENDENT VASODILATOR

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Arachidonic acid (AA) can be converted via the cytochrome P450 monooxygenase pathway to biologically active metabolites. We have previously reported that the 5,6-epoxyeicosatrienoic acid (5,6-EET) was the sole epoxide to vasodilate the isolated, perfused rat caudal artery (Carroll et al., 1987). The present experiments were performed to determine if the responses to 5,6-EET were endothelial-dependent and, since the 5,6-EET is known to be a substrate for cyclooxygenase, we investigated whether the 5,6-EET requires further metabolism for its vasoactivity, by using inhibitors of AA metabolism.

Male Wistar rats (200-250 g) were killed by decapitation, the caudal artery excised and perfused with Krebs'-Henseleit solution at 6 ml/min. Perfusion pressure was raised to 100 mmHg by continuous infusion of phenylephrine (0.5-1.0 μM). 5,6-EET was separated from non-enzymatically formed contaminants, 5,6-diol and 5,6-δ-lactone, by HPLC immediately prior to bioassay. ACh (40 nM) and nitroprusside (NP; 50 nM) were used as known endothelial-dependent and -independent agents, respectively. These agents were given as bolus injections close arterially, while the inhibitors of AA metabolism were added to the perfusate.

The administration of 5,6-EET at doses of 6.25, 12.5 and 25.0 nM resulted in dose-dependent decreases in perfusion pressure of 9 ± 1, 15 ± 2 and 18 ± 1 mmHg, respectively. De-endothelialization, performed by passing a stream of air through the arterial lumen was verified by the loss of ACh-induced relaxation. In the absence of endothelium, the responses to the 5,6-EET were reduced by 80% to 100% ($n=4$; $P<0.05$), whilst the response to NP was unaffected.

Eicosatetraynoic acid (ETYA) (1 μM), the acetylenic analogue of AA which inhibits all AA metabolic pathways, reduced the responses to 5,6-EET by 71% to 83% ($n=4$;$P<0.05$). In the presence of indomethacin (3 μM), the responses to 5,6-EET were reduced by 77% to 95% ($n=8$;$P<0.005$). The responses to ACh were unaffected by both ETYA or indomethacin.

Inhibitors of lipoxygenase, nordihydroguairetic acid (2.5 μM), and cytochrome P450 monoxygenases; SKF525A (1.1 μM), metyrapone (150 μM) and clotrimazole (0.7 μM) had non-specific effects since these inhibitors reduced the responses to both 5,6-EET and ACh.

We conclude that the vasodilatory responses to 5,6-EET require an intact endothelium and further metabolism through cyclooxygenase for its vascular activity.

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100P

EFFECT OF ENDOTHELIN ON CANINE AND PORCINE CORONARY PREPARATIONS IN VITRO

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The effect of the peptide endothelin was examined on isolated epicardial coronary artery ring preparations of varying diameters from pigs and dogs and in the canine coronary circulation perfused *in vitro*. Porcine hearts were obtained from an abbatoir and transported to the laboratory in cardioplegic solution at 4°C. Canine hearts were obtained form anaesthetized greyhounds at the completion of *in vivo* studies. 4mm rings were prepared from the left anterior descending coronary artery (LAD) and their diameters measured. The rings were mounted between two stainless steel hooks and suspended in 5ml baths in Krebs' solution warmed to 37°C and gassed with 95%O_2/5%CO_2. Tension in the rings was adjusted to give a maximal response to 40mM KCl, which was also used as the standard to compare endothelin responses. Dose-responses to endothelin were constructed between 0.03 and 300nM.

A 1.22mm diameter catheter was inserted as distally as possible in the LAD of dog hearts in order to perfuse a small territory of intact myocardium with Krebs' solution at 37°C, gassed with 95%O_2/5%CO_2 and at a flow rate which produced a mean perfusion pressure of approximately 80mmHg. After the experiment, the LAD was perfused with methylene blue and the stained territory was cut out and weighed. Responses to endothelin were obtained with infusions of 1 to 1000pmol min^{-1}.

Endothelin contracted both porcine and canine coronary rings in a dose-related manner and had a more potent effect on the smaller diameter rings. The EC_{50}s for the porcine rings of 2, 1.5 and 1mm internal diameter were respectively 19.7, 6.9 and 1.8nM (calculated from the means of 7, 5 and 5 experiments). In canine rings the EC_{50}s were 17.3 and 9.5nM respectively for 1.5 and 1mm rings (calculated from the means of 10 and 10 experiments). There was a significant difference between responses for 2mm and 1mm diameter porcine rings at endothelin concentrations of 1, 10, and 30nM ($p<0.05$, Student's t test for unpaired data).

Endothelin also produced a dose-related increase in perfusion pressure in the constant-flow (7.7±1.5ml min^{-1}, mean±s.e.mean, n=5) perfused canine myocardium. Threshold concentration was 3pmol min^{-1} and the maximum effect of 61.5±13.7mmHg increase (mean±s.e.mean, n=4) was achieved with an infusion rate of 50pmol min^{-1}. The EC_{50} in this preparation was approximately 0.8nM.

Thus endothelin is a potent constrictor of both porcine and canine coronary arteries *in vitro* and appears to be more potent in smaller distal vessels.

CONTRACTILE EFFECTS OF ENDOTHELIN AND [Ala^{3,11}]ENDOTHELIN IN RAT ISOLATED AORTA

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Endothelin is a potent vasoconstrictor peptide produced by endothelial cells in culture. Its production is increased by stimuli such as thrombin, adrenaline and anoxia (Yanagisawa et al., 1988). Possible roles of this substance in physiological or pathological processes are unknown, but its production in the vicinity of vascular smooth muscle cells suggests that it may be involved in control of vascular tone. Therefore the contractile effects of endothelin and [Ala^{3,11}]endothelin were examined in rat isolated aortic rings.

Aortic rings from male Sprague Dawley rats were mounted for recording of tension, with or without endothelium (Schoeffter & Miller, 1986). Two hours after an initial contraction to 1 μM noradrenaline, cumulative concentration-effect curves to endothelin (0.1 to 10 nM) or its analogue (2.25 to 225 nM) were elicited. In some experiments tissues were preincubated for 1 h with 1 μM diltiazem before exposure to the peptides and in other experiments tissues were washed with a Ca^{++}-free solution for 10 min before addition of a submaximal concentration of peptide for 10 to 15 min. This was then followed by cumulative addition of Ca^{++} (10 μM to 10 mM) to the organ bath.

Endothelin and [Ala^{3,11}]endothelin both contracted rat aortic rings (Table 1). The presence of endothelium significantly affected responses to endothelin but not those to the analogue. Diltiazem (1μM) significantly increased EC_{50} values of endothelin but not those of the analogue. Contractile responses elicited by submaximal, equiactive, concentrations of endothelin (3 nM) and the analogue (75 nM) in the absence of endothelium were reduced by 84% and 48% respectively in the absence of extracellular Ca^{++}. Concentration-effect curves elicited by Ca^{++} in the presence of endothelin and [Ala^{3,11}]endothelin had significantly different EC_{50} values of 1.2 ± 1.1 mM and 55.5 ± 6.8 μM respectively ($P < 0.05$) and maximal contractions amounted to about 70% of those induced by 1 μM noradrenaline in each case. Diltiazem did not affect responses to Ca^{++} in tissues exposed to endothelin but shifted them to the right in the presence of [Ala^{3,11}]endothelin, increasing the EC_{50} value to 0.26 ± 0.13 mM ($P < 0.01$) without affecting the maximal response. The Kb value for diltiazem was about 0.2 μM.

Table 1. Effects of endothelium and diltiazem on responses to endothelin and [Ala^{3,11}]endothelin

	ENDOTHELIN			[Ala^{3,11}]ENDOTHELIN		
	EC_{50} (nM)		Emax	EC_{50} (nM)		Emax
Effect of endothelium						
With	4.0 ± 0.6*	(8)	89.7 ± 34.2	20.8 ± 9.8	(7)	146.7 ± 30.2
Without	1.3 ± 1.0	(8)	109.2 ± 22.3	16.8 ± 5.4	(7)	101.1 ± 18.6
Effect of diltiazem without endothelium						
Control	0.9 ± 0.2	(4)	104.2 ± 12.8	39.4 ± 14.5	(4)	114.7 ± 27.2
Diltiazem	3.0 ± 1.0 *	(4)	95.1 ± 13.3	28.2 ± 10.3	(4)	131.4 ± 27.0

Values are means ± s.e.mean. *$P < 0.05$ from corresponding control values. Emax is expressed as percentage of the maximal response to 1 μM noradrenaline. (n) represents number of observations.

[Ala^{3,11}]endothelin was less potent than endothelin by a factor of about 12, a ratio similar to that of their affinities for specific [^{125}I]endothelin binding sites (Jones et al., 1988). Endothelin contractions were modulated by the presence of endothelium, as are those of many contractile agonists, but responses to the analogue were not. This unexpected result may mean that the peptides interact differently with the endothelium. Contractions elicited by both compounds were relatively dependent on extracellular Ca^{++}, but diltiazem did not antagonize them in a manner typical of a calcium entry blocker. Contractions elicited by re-addition of Ca^{++} in the presence of endothelin were not antagonized by diltiazem either, although contractions elicited in the presence of the analogue were antagonized by diltiazem and the calculated Kb value was similar to those reported in vascular tissues (Godfraind et al., 1986). These results suggest considerable differences between endothelin and [Ala^{3,11}]endothelin.

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[^{125}I]ENDOTHELIN BINDING IN MAMMALIAN TISSUE: RELATION TO HUMAN ATRIAL INOTROPIC EFFECTS AND CORONARY CONTRACTION

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Endothelin (ET) is a recently sequenced 21 residue peptide isolated from porcine endothelial cells with a structure containing disulphide bonds previously unknown among bioactive peptides of mammalian origin (Yanganisawa et al, 1988). It is a potent vasoconstrictor (D'Orleans-Juste et al, 1988; Warner et al, 1988; Hughes et al, 1988). The aim of the study was to localise ET binding sites and relate their distribution to a possible physiological function.

The distribution of binding sites for ET was investigated using [^{125}I]ET (Amersham International) by in vitro autoradiography and computer-assisted image analysis (Davenport et al, 1988). Non-specific binding was defined in the presence of 1 μM unlabelled ET. In rat heart, the highest level of binding was observed in the atria, which was 1.6 times higher than that of the ventricles, 2.2 times that of the aorta and 2.5 times that of the pulmonary artery. Similar levels were obtained for human and pig atria and ventricles. Binding was detected in pig coronary arteries and nerves. ET binding was also found in rat kidneys, adrenals and brain.

The function of myocardial ET binding sites was investigated on right atrial appendages from 6 patients undergoing bypass surgery for ischaemic heart disease (IHD). Atrial strips were paced at 0.5 Hz to contract isometrically in Krebs solution at 37°C and exposed for 2 h to 6 μM phenoxybenzamine (Gille et al, 1985). ET caused positive inotropic effects with a pEC_{50} (M) of 8.92 ± 0.23 ($\bar{x}$ ± SEM, 8 strips). Maximum effects observed with 10 nM ET amounted to 34 ± 8% of the effects of 400 nM (-)-isoprenaline, administered to terminate the experiment. The positive inotropic effects of ET occurred in the presence of (±)propranolol 0.5 μM and atropine 1 μM (pEC_{50} for ET 9.10 ± 0.22, 4 strips).

Circumflex coronary arteries were dissected from the hearts of 2 IHD patients undergoing transplantation. ET administered cumulatively to 14 strips in Krebs solution at 37°C, caused tonic contractions (pEC_{50}(M) 7.78 ± 0.21, $\bar{x}$ ± SEM). Maximum ET-induced contractions (0.2 - 2 μM) were 29 ± 7% stronger than 93 mM K^+ contractions. Maximum 5HT-induced contractions were 24 ± 9% weaker than K^+ contractions.

(1) [^{125}I] binding has been detected in cardiac and vascular tissues from rat, pig and human.
(2) In humans, ET may increase atrial contractility by acting on the binding sites visualised by autoradiography.
(3) The presence of binding sites in kidney, adrenals and brain suggeststhe function of ET may not be restricted to the cardiovascular system.

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CONTRACTION OF RAT TRACHEAL SMOOTH MUSCLE BY ENDOTHELIN

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The recently described novel peptide endothelin (Yanagisawa et al, 1988) has been hypothesised as an endogenous ligand for the dihydropyridine sensitive voltage dependant calcium channel and is reported to produce sustained contractions of vascular smooth muscle. We have investigated the possibility that this peptide may also elicit spasmogenic effects on airway smooth muscle.

Male Wistar rats (150-350g) were killed by cervical dislocation and the trachea removed. Tracheal rings, consisting of 3 adjacent cartilage plates, suspended under a resting tension of 1 g were superfused with Krebs solution ($CaCl_2$ = 2.5 mM) at 2 ml/min. The superfusate was pre-gassed with 95% O_2, 5% CO_2 and dripped over the tissues at 37°C. The tissues were equilibrated for 60 min and the resting tension at the end of this period was 0.88 ± 0.03 g. Agonists were added in the superfusing buffer and were in contact with the tissue for 30 sec. Cumulative concentration-response curves to endothelin were obtained by incremental increasing concentrations at the plateau of the previous response. Changes in tension were measured using a Grass FT03C force-displacement transducer.

Binding studies, using in vitro autoradiography, with ^{125}I endothelin (200-400 pM) were carried out on 10µm cryostat sections of rat trachea (n = 6) non-specific binding being assessed on serial sections in the presence of a 1000 fold excess of unlabelled endothelin.

In Krebs solution with normal calcium concentrations endothelin (10^{-8}-10^{-5} M) produced a concentration dependent contraction of rat trachea, but was 50-100 times less potent than on rings of rat aorta under the same conditions. The endothelin induced contractions of tracheal smooth muscle were long lasting, responses to 10^{-6} M reaching a plateau (0.63 ± 0.07 g, n = 20) within 6 min and returning to resting tensions over the next 60 min. The contractions could be partially but transiently resolved by isoprenaline (10^{-6} M) or glyceryltrinitrate (10^{-5} M). Responses to endothelin (10^{-6} M) were attenuated following 1 hr incubations in calcium free solution (0.26 ± 0.08 g, n = 13) and almost completely abolished following a 1 hr incubation in calcium free solution containing 1 mM EGTA (0.05 ± 0.06, n = 7). Return to normal calcium concentrations 30 or 60 min following addition of endothelin in calcium free buffer resulted in sustained contraction of the tissues (plateaus 0.77 ± 0.14 and 0.48 ± 0.22 g over resting respectively, n = 5). Responses to endothelin, however, were unaffected by a 30 min equilibration of the tissues with 10^{-7} M nicardipine (present in the superfusing Krebs solution).

Specific binding sites for endothelin were identified in smooth muscle and nerve trunks. Binding was rapid, reaching eqilibrium within 15 min and was unaffected by coincubation with unrelated peptides (ANP, CGRP, VIP or gastrin {10-6 M}), nicardipine (10^{-7} M) or verapamil (10^{-7} M).

In conclusion, specific endothelin binding sites have been identified in rat trachea and endothelin has been shown to elicit long lasting contractions of rat tracheal smooth muscle. These contractions may be dependent on extracellular calcium but are unlikely to be due to interaction with a dihydropyridine binding site.

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EFFECTS OF ENDOTHELIN ON FIELD STIMULATED RAT VAS DEFERENS AND GUINEA-PIG ILEUM

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The recently described vasoactive peptide endothelin (Yanagisawa et al., 1988) has been shown to bind specifically to sites in the central nervous system (Jones et al. 1988). In addition, [^{125}I]endothelin binding is inhibited by two analogues of endothelin in which one or other pair of cysteinyl residues forming its disulphide bridges has been replaced by two alanyl residues, that is [Ala^{1,15}]endothelin and [Ala^{3,11}]endothelin. It is therefore of interest to ascertain whether or not it has effects on responses mediated by stimulation of peripheral nerves.

Guinea-pig ileum was mounted in an organ bath, under a tension of 0.5 g, between platinum electrodes in a physiological solution and stimulated at 150 V for 2 ms at 0.05 Hz. After 2 h equilibration it was exposed to 1 nM endothelin for 5 min and then washed over the next 45 min until twitch responses returned to control. Some tissues were incubated with nifedipine for 20 min before exposure to the second and subsequent doses of endothelin. Rat vas deferens were similarly mounted and stimulated at 150 V for 1 ms at 6 Hz. Cumulative concentrations of endothelin or of one of its analogues were added to the bath and responses allowed to reach equilibrium before increasing the concentration. In some experiments, after a first concentration-effect curve had been obtained to one peptide a second peptide was added in a cumulative fashion without washing the tissue.

Electrically stimulated guinea-pig ileum responded to 1 nM endothelin by an increase in basal tension and an inhibition of the stimulated twitch responses. The increase in basal tension amounted to 1.46 ± 0.25 g (mean ± s.e.mean, n = 7) with the first exposure to endothelin and this response decreased to 1.32 ± 0.41 g, 0.93 ± 0.23 g and 0.58 ± 0.15 g over 3 subsequent exposures. In a parallel series of experiments (n = 8) the initial increase in basal tone was 1.23 ± 0.25 g; in the presence of 10 nM nifedipine the second exposure to endothelin elicited an increase in tone of 0.60 ± 0.13 g, 30 nM nifedipine reduced the third response to 0.10 ± 0.05 g and 100 nM nifedipine abolished the fourth response. With regard to the twitch responses themselves, exposure to 1 nM endothelin reduced the first succeeding twitch to 60 ± 9% of the control twitch (1.45 ± 0.15 g; n = 7), the next twitch to 12 ± 3% and the fifth to 26 ± 5%. Recovery thereafter was gradual and the control level was reached after 45 min of washing. Second and third exposures to endothelin had similar effects to the first but a fourth exposure resulted in a lesser degree of inhibition. Nifedipine (10 to 100 nM) had no significant effect on these responses (n = 8).

Endothelin (1 to 10 nM) had small but significant effects on baseline tension in rat vas deferens and significantly increased the magnitude of the twitch response elicited by electrical stimulation such that 10 nM increased responses to 199 ± 23% of control (n = 8). [Ala^{3,11}]endothelin (0.75 to 75 nM; n = 7) also dose-dependently increased the twitch response to 211 ± 74% at 0.75 nM and to 951 ± 218% at 75 nM. In contrast [Ala^{1,15}]endothelin (1.4 to 140 nM; n = 7) had negligible effects on twitch height. Both analogues had small but significant effects on basal tone. In the presence of 10 nM endothelin, the additional effect of [Ala^{3,11}]endothelin was to increase twitch responses maximally to 334 ± 77% (n = 4) of the pre-peptide control. In contrast, combination of endothelin with [Ala^{1,15}]endothelin resulted in a twitch response that was greater than with endothelin alone.

Endothelin thus contracts ileal smooth muscle markedly but has moderate direct effects on vas deferens. The ileal contractions were antagonized by calcium channel blockade. The changes in the cholinergic twitch responses induced by the peptide were also antagonized by nifedipine. However, sympathetically-mediated twitch responses in the vas deferens were enhanced by endothelin and one of its analogues. Furthermore, in the vas deferens there appeared to be some interactions between the peptides suggesting that they might not have a common mechanism of action; although endothelin might be a partial agonist relative to [Ala^{3,11}]endothelin.

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INHIBITION OF NITRIC OXIDE SYNTHESIS INHIBITS ENDOTHELIUM-DEPENDENT VASODILATATION IN THE RABBIT ISOLATED HEART

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The formation of nitric oxide (NO) by vascular endothelial cells accounts for the biological activity of endothelium-derived relaxing factor (EDRF; Palmer et al., 1987; Radomski et al., 1987). Nitric oxide is generated enzymatically from the terminal nitrogen atom(s) of the guanidino group of L-arginine (L-Arg; Palmer et al., 1988a), a process which is stereoselectively inhibited by N^G-monomethyl-L-arginine (L-NMMA; Palmer et al., 1988b). Since acetylcholine (ACh) induces the formation of NO in the coronary circulation of the rabbit in amounts sufficient to account for its vasodilator effects (Amezcua et al., 1988), we have now studied the role of the arginine/NO pathway in the regulation of basal coronary vascular tone and in the vasodilator response to ACh in the Langendorff preparation.

The hearts of male New Zealand White rabbits were perfused at constant flow (15 to 25 ml/min) with Krebs buffer, containing indomethacin (5 μM) and gassed with 5%CO_2 in O_2 at 37°C. Heart rate (180 beats/min) was kept constant by electrical stimulation of the left ventricle. Acetylcholine and prostacyclin (PGI_2) were administered as 1 min infusions. Other treatments were given as 20 min infusions 10 min before challenging with ACh or PGI_2. Results are given as the mean ± s.e.m. of (n) experiments.

Acetylcholine (0.03 to 3 μM) caused a dose-dependent fall in the coronary perfusion pressure (CPP), which was inhibited by L-NMMA (IC_{50} = 8.6 ± 1.4 μM, n=11). The vasoconstrictor properties of ACh were unmasked by L-NMMA (30 and 100 μM) which also induced a dose-dependent, sustained increase in the CPP. The fall in CPP induced by PGI_2 (1 μM, n=3) was unaffected by L-NMMA (100 μM). L-arginine (100 μM) had no direct effect on CPP (n=3).

The release of NO into the cardiac effluent (measured by chemiluminescence; Amezcua et al., 1988) induced by ACh (0.3 μM) was significantly enhanced (to 148 ± 18% of the control, n=4, $p < 0.05$) by L-Arg (30 μM). The release of NO induced by ACh (3 μM) was inhibited by 48 ± 7% (n=4, $p<0.05$) by L-NMMA (10 μM). Furthermore, this inhibition was long lasting (1.5 h), but could be reversed by L-Arg (30 μM, n=4). The release of EDRF into the cardiac effluent induced by ACh (3 μM; measured by bioassay; Palmer et al., 1987) was enhanced in six out of seven experiments (to 175 ± 38% of the control, n=6, $p<0.05$) by L-Arg (30 μM). In contrast, L-NMMA (30 μM) caused a 61 ± 5% inhibition of ACh-induced release of EDRF (n=3, $p <0.05$). The D-isomers of Arg and NMMA (100 μM) had no effect on any of these parameters (n=3).

This study shows that the generation of NO by the endothelium from L-Arg plays a role in the regulation of vascular tone and in the mediation of the vasodilatation induced by ACh in the coronary circulation of the rabbit.

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ENDOGENOUS NITRIC OXIDE (NO) IS INVOLVED IN THE REGULATION OF BLOOD PRESSURE IN THE ANAESTHETISED RABBIT

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We have recently shown that nitric oxide (NO) is released from vascular endothelial cells and mediates endothelium-dependent relaxation (Palmer et al., 1987; Amezcua et al., 1988). Porcine aortic endothelial cells in culture synthesise NO from the terminal guanidino nitrogen atom(s) of the amino acid L-arginine (Palmer et al., 1988a). This process and the endothelium-dependent relaxation of rings of rabbit aorta are inhibited by N^G-monomethyl-L-arginine (L-NMMA; Palmer et al., 1988b; Rees et al., in press). As a result we have proposed that L-arginine is the physiological precursor for the synthesis of NO. We have now examined the actions of L-NMMA on mean arterial blood pressure and on the hypotensive effect of acetylcholine (ACh) and glyceryl trinitrate (GTN) in the rabbit in vivo.

Male New Zealand White rabbits (2-2.2 kg) were initially anaesthetised with sodium pentobarbitone (40-50 mg kg^{-1} i.v.) and the anaesthesia maintained with a continuous infusion (30 mg kg^{-1} hr^{-1}) into the ear vein. Drugs (15 sec infusion) were infused via a cannula into the femoral vein and blood pressure was monitored via a cannula in the carotid artery.

Acetylcholine (0.1-3.0 µg kg^{-1}) and GTN (1-30 µg kg^{-1}) produced a dose-dependent fall in mean arterial blood pressure (ED_{50}'s; 0.2 ± 0.04 µg kg^{-1} and 2.4 ± 0.3 µg kg^{-1} respectively; n=3). L-NMMA (3-100 mg kg^{-1}), but not D-NMMA (100 mg kg^{-1}), produced a dose-dependent increase in mean arterial blood pressure. The mean arterial blood pressure before and after L-NMMA (100 mg kg^{-1}) was 69 ± 4 mm Hg and 88 ± 5 mm Hg respectively (mean ± s.e.m., n=4). Following L-NMMA (30-100 mg kg^{-1}), but not D-NMMA (100 mg kg^{-1}), the hypotensive action of the endothelium-dependent vasodilator ACh (0.3 µg kg^{-1}) was significantly reduced; the falls in mean arterial blood pressure before and after L-NMMA (100 mg kg^{-1}) were 17 ± 2 mm Hg and 10 ± 1 mm Hg respectively (mean ± s.e.m., n=4). In contrast, the response to the endothelium-independent vasodilator GTN, at an equi-effective dose (3 µg kg^{-1}), was unaffected by L-NMMA (100 mg kg^{-1}; n=3). The actions of L-NMMA were reversed by L-arginine, but not D-arginine (both at 100 mg kg^{-1}; n=3). Indomethacin (1 mg kg^{-1}, i.v.) had no direct effect on mean arterial blood pressure and did not affect the hypotensive actions of ACh or the effects of L-NMMA (n=3).

Our results indicate that NO formation from L-arginine plays a role in the control of mean arterial blood pressure and in the hypotensive actions of ACh.

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ENDOTHELIN INFUSION IN THE ANAESTHETISED RAT: PLASMA ENDOTHELIN CONCENTRATIONS AND BLOOD PRESSURE CHANGES

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Endothelin is an endothelium-derived, 21-amino acid vasoconstrictor peptide recently isolated and purified from porcine aortic endothelium (Yanagisawa *et al*, 1988). These authors showed that bolus doses of endothelin produce a prolonged increase in arterial blood pressure in chemically denervated rats. We have recently confirmed these findings in rats treated only with anaesthetic (Spokes *et al*, 1988). The effects of endothelin infusions have now been studied.

Female Wistar rats (190-220g) were anaesthetised with fentanyl, fluanisone and midazolam (i.p.). The arterial blood pressure and heart rate were continuously monitored via the femoral artery. Pure synthetic endothelin (Peptide Institute Inc., Osaka, Japan) was infused at 20pmol/min at a rate of 2ml/h via the femoral vein for 15 or 20 min (n=8 in each case) and then 4ml blood taken, spun down and the plasma frozen until required for assay. In another group of rats the peptide was infused for 20 min and the blood taken 2 min after the end of the infusion (n=8). The endothelin was made up in saline and diluted immediately before use in saline containing 5% rat plasma, control rats received vehicle alone (n=7). In order to check that the removal of 4 ml of blood did not itself release endothelin a second 4 ml sample was taken from the control rats 5 min after the first. An infusate sample was taken at the end of each infusion. Plasma and infusate endothelin concentrations were then measured by specific radioimmunoassay.

Arterial blood pressure increased steadily during the endothelin infusions. The systolic pressure increased by 36.1 ± 4.4 mmHg after 15 min and 55.9 ± 5.9 mmHg after 20 min. The blood pressure decreased slightly, but not significantly during the two minutes after the infusion. The heart rate fell by about 50 beats/min after 15 or 20 min infusion and did not change significantly after the infusion. In the control rats the heart rate increased slightly during the vehicle infusion and the blood pressure did not change. The plasma endothelin concentration was 1500 ± 290 pmol/l after 15 min infusion and 1720 ± 240 after 20 min. This fell dramatically to 223 ± 28 pmol/l by 2 min post infusion. Endothelin was undetectable in all control samples. The infusate concentration was 575 ± 39 pmol/ml. From these results the plasma half life of endothelin was calculated to be 35 s and the apparent volume of distribution 42 ml/kg indicating that endothelin remains mainly in the vascular compartment.

The short plasma half life of endothelin found in this study is in marked contrast with its long lasting biological effect. Endothelin may thus activate a long lasting mechanism. Alternatively, endothelin may be broken down in the blood into fragments, one of which is biologically active but is not picked up by the radioimmunoassay. In summary, the results presented here confirm that endothelin is a highly potent, persistent hypertensive agent in the anaesthetised rat and is itself rapidly cleared from the circulation.

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TMB-8 INHIBITS ENDOTHELIAL PRODUCTION OF PROSTACYCLIN BUT NOT ENDOTHELIUM-DERIVED RELAXING FACTOR

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We have shown previously that pig aortic endothelial cells (PAEC) exert differential control of production of prostacyclin and endothelium-derived relaxing factor (EDRF) (White & Martin, 1987). Thus, bradykinin (Bk)-induced production of EDRF is maintained for some time, but production of prostacyclin is transient. Extracellular Ca^{2+} is required for the sustained production of EDRF, but appears not to contribute to production of prostacyclin. Release of an intracellular store of Ca^{2+} may control production of prostacyclin and contribute transiently to production of EDRF. We have explored further the possibility of differential control of production of these two vasodilators by examining the actions of TMB-8, an inhibitor of intracellular calcium release. On the basis of the above scheme, TMB-8 might be expected to inhibit production of prostacyclin but not EDRF.

Endothelial cells were isolated from pig aorta by collagenase treatment, seeded into 9.6 cm^2 multiwell plates and grown to confluence. The tissue culture medium was removed and the cells incubated in Krebs solution at 37°C. EDRF production was assessed indirectly by measuring endothelial cell cyclic GMP content (Martin et al., 1988), and production of prostacyclin was measured by radioimmunoassay of 6 keto $PGF_{1\alpha}$. In cascade bioassay experiments EDRF was generated from PAEC grown on microcarrier beads and perfused in columns. EDRF production was assayed against phenylephrine (PE)-induced tone on endothelium-denuded rings of rabbit aorta.

In control endothelial cells the resting cyclic GMP content was 20±3 $fmol.\mu g^{-1}$ DNA (n=23) and the resting production of 6 keto $PGF_{1\alpha}$, measured over 40 min, was 201±25 $pg.\mu g^{-1}$DNA (n=18). Bk (0.1 μM) induced a maximum 6.3-fold (n=24) increase in cyclic GMP content within 1 min, which declined slowly thereafter. Production of 6 keto $PGF_{1\alpha}$, measured simultaneously, increased by a maximum of 10.6-fold (n=18), and was complete within 3 min.

When TMB-8 (100 μM) was present during the 40 min incubation period the resting content of cyclic GMP rose slightly to 29±1 $fmol.\mu g^{-1}$DNA (n=24) and production of 6 keto $PGF_{1\alpha}$ fell to 88±11 $pg.\mu g^{-1}$DNA (n=18). Bk (0.1 μM) now induced a significantly greater (13.6-fold, n=23) rise in cyclic GMP content than in the absence of TMB-8, but the stimulation of 6 keto $PGF_{1\alpha}$ production was significantly smaller (2.9-fold, n=17).

In cascade bioassay experiments infusions of Bk (0.1 nM-0.1 μM) into columns of PAEC induced concentration-dependent relaxations of rabbit aortic rings: the EC_{50} for relaxation was 1.6±0.9 nM and the maximum relaxation obtained was 55±6% (n=8). When TMB-8 (100 μM) was infused continuously into columns of PAEC a 73±3% relaxation of PE-induced tone was observed in aortic rings. When the tone was raised to its original level by adding further PE, infusion of Bk (0.1 nM-0.1 μM) induced relaxations in a similar manner to that observed in the absence of TMB-8: the EC_{50} for relaxation was 1.5±0.4 nM and the maximum relaxation obtained was 53±4% (n=8).

In conclusion, TMB-8 inhibits resting and Bk-stimulated production of prostacyclin but production of EDRF is not inhibited. These results support the proposal that production of prostacyclin and EDRF is subject to differential control.

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SIN-1-INDUCED INHIBITION OF LYSOSOMAL ENZYME AND LTB_4 RELEASE IN POLYMORPHONUCLEAR LEUCOCYTES MAY BE MEDIATED BY CYCLIC GMP

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The second messenger cyclic AMP is thought to decrease the release of lysosomal enzymes and LTB_4 from stimulated polymorphonuclear leucocytes (PMN). Ignarro and George (1974) studying the effects of acetylcholine on PMN function, suggested cyclic GMP to augment PMN function. However, to date little is known about the influence on PMN function of nitrovasodilators which are the most potent activators of guanlyate cyclase in vascular tissue. Therefore, this study investigates the effect of molsidomine and its metabolites, of glyceryl trinitrate (GTN) and sodium nitroprusside (SNP) on the release of ß-glucuronidase and LTB_4 from FMLP-stimulated human PMN and assesses the intracellular levels of cyclic nucleotides under the same conditions.

PMN were prepared from fresh human blood by Ficoll-Paque gradient centrifugation as described previously (Ney and Schrör, 1988). Washed PMN were pretreated for 10 min with the agents to be studied and then stimulated with FMLP (30 nM) for another 10 min. ß-Glucuronidase release was determined as a parameter for PMN activation. Cyclic GMP levels and LTB_4 release were measured by radioimmunoassay. Cyclic AMP levels were determined by a proteinkinase binding assay.

Molsidomine, SIN-1 and SIN-1A inhibited ß-glucuronidase release from FMLP-stimulated PMN dose-dependently at 0.01 - 0.1 mM. At a maximally effective concentration (1 mM), molsidomine, SIN-1 and SIN-1A decreased enzyme release by 19 ± 6%, 37 ± 9% and 46 ± 7% of control, respectively. GTN and SNP did not affect ß-glucuronidase release from PMN. SIN-1A, SIN-1, but also SNP, at 1 mM increased cyclic GMP levels 21-fold, 9-fold and 14-fold, respectively. There was no increase in cyclic GMP in the presence of GTN and molsidomine. The two cyclic GMP analogs 8-bromo cyclic GMP and dibutyryl cyclic GMP at 1 mM decreased ß-glucuronidase release from FMLP-stimulated PMN by 12 ± 2% and 44 ± 4%, respectively. In the presence of all nitrovasodilators, intracellular cyclic AMP accumulation remained unchanged. At 0.1 mM, SIN-1A, SIN-1 and SNP decreased LTB_4 release by 70 ± 12%, 59 ± 8% and 28 ± 9% of control, respectively. (All values are given as the mean ± SEM from n = 4 - 6 experiments.)

These data show that the metabolites of molsidomine SIN-1 and SIN-1A produce a substantial inhibition of enzyme release and LTB_4 formation in FMLP-stimulated PMN and at the same time stimulate intracellular cyclic GMP. No such relation is seen with molsidomine and SNP. This suggests that in addition to cyclic GMP stimulation, other mechanisms may play a role during nitrovasodilator-induced inhibition of enzyme release. In contrast to Ignarro and George (1974), our data with cyclic GMP analogs demonstrating an inhibitory effect of cyclic GMP on PMN function, suggest a synergistic effect of cyclic AMP and cyclic GMP in PMN. This is similar to the antiaggregatory function of both cyclic nucleotides in platelets. The observed nitrovasodilator-induced decrease in LTB_4 release suggests that cyclic GMP may have an inhibitory effect on arachidonic acid release and its subsequent conversion into LTB_4. In addition to its vasodilatory and anti-platelet activity including inhibition of thromboxane and 12-HPETE-formation (Darius et al., 1984), this inhibition of neutrophils may be important for the clinical use of molsidomine in myocardial ischemia.

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ORG 30029 A NOVEL CARDIOTONIC AGENT - CARDIOVASCULAR EFFECTS IN ANAESTHETISED DOGS AND CATS

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Org 30029 (N-hydroxy-5,6-dimethoxy-benzo[b]thiophene-2-carboximidamide HCl) is a novel cardiotonic with inotropic and vasodilator activity (Shahid, et al., 1988) whose cardiovascular effects have been examined in dogs and cats.

Beagle dogs were anaesthetised with Na pentobarbitone and instrumented for i.v. drug administration and measurement of arterial blood pressure, coronary blood flow, stroke volume, left ventricular pressure and its derivatives (LV dP/dt and LV dP/dt/P). Arterial and coronary venous blood was analyzed for oxygen content and myocardial oxygen consumption (MVO_2) was determined. In a separate series of experiments, the effects of Org 30029 on blood flow in the common carotid and renal arteries and total peripheral resistance were determined. Following a stabilization period (30 min) Org 30029 (0.3 mg/kg/min) was administered by i.v. infusion over 20 min and drug effect was calculated at the end of the infusion period. The cardiovascular actions of Org 30029 were also examined in Na pentobarbitone anaesthetised cats in which cardiac function was depressed by propranolol (2 mg/kg i.v.) and Na pentobarbitone (bolus of 5 mg/kg, followed by an intravenous infusion, 15 mg/kg/h). Depression of cardiac function was stable (46±5% depression of LV dP/dt) prior to drug administration (i.v. or intraduodenal; i.d.) and control experiments showed it to remain stable throughout the experimental period. The effect of Org 30029 on heart rate, arterial blood pressure, and cardiac contractility (LV dP/dt) was monitored. All results ($n \geq 4$) are expressed as means±s.e. mean.

In the dog, Org 30029 increased cardiac contractility (LV dP/dt max: 81±7, LV dP/dt/P: 42±6), this was accompanied by a moderate increase in heart rate (18±3%) and by a fall in diastolic blood pressure (11±4%). Stroke volume (14±7) and MVO_2 increased (33±6%). Org 30029 increased coronary (41±9%), carotid (22±15%) and renal (24±8%) blood flows and reduced total peripheral resistance (29±5%). In the cardiac depressed cat, Org 30029 increased cardiac contractility and reduced blood pressure. After i.v. administration, a modest transient tachycardia was followed by a bradycardia (not shown); after i.d. administration heart rate was reduced (Table 1).

Table 1 Effects of Org 30029 in anaesthetised cardio-depressed cats

		maximal change (%)		
	mg/kg	LV dP/dt	heart rate	diastolic blood pressure
i.v.	3	51±10	14±1	-39±6
	10	80±9	10±3	-35±11
i.d.	10	36±14	-7±3	-20±14
	30	56±20	-16±1	-26±6

In conclusion, Org 30029 increases cardiac contractility in dogs after parenteral administration and in cats after both parenteral and enteral administration. The increased contractility was accompanied by vasodilation and either a transient modest tachycardia or a bradycardia. This is a potentially useful profile in a drug for the treatment of congestive heart failure and Org 30029 is presently being clinically evaluated.

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ORG 30029 A NEW CARDIOTONIC AGENT WITH POSITIVE INOTROPIC AND VASCULAR RELAXANT PROPERTIES

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We recently described the cardiovascular effects of a bradycardic cardiotonic agent, Org 10325 (Shahid, et al., 1988; Marshall, et al., 1988). In this report the activity of a structural analogue, Org 30029 (N-hydroxy-5,6-dimethoxy-benzo[b] thiophene-2-carboximidamide HCl), which is currently undergoing clinical evaluation is presented. The pharmacological effects of Org 30029 have been compared to those of milrinone on phenylephrine (10 μM) pre-contracted aortic strips, on spontaneously beating right atria and on electrically stimulated (0.4 Hz, supramaximal voltage, 1 msec duration) right ventricular papillary muscles from male NZW rabbits. Tissues were suspended in normal Krebs solution containing 2.5 mM Ca^{2+} and allowed to stabilise for 60 min before cumulative drug additions to the organ bath. Results are shown in Table 1. EC_{50} values were determined using either drug- or isoprenaline-induced maximum responses for aortic and cardiac tissues, respectively.

Table 1 The effects of Org 30029 and milrinone on isolated cardiac and vascular tissue preparations (means±s.e. mean, $n \geq 4$)

	EC_{50} (μM)		
	Aorta	Papillary Muscle	Right Atria
Org 30029	76±19	143±31	>500
Milrinone	377±75	111±32	34 6

Org 30029 produced slowly developing (20-30 min) sustained relaxant and positive inotropic responses in isolated aortic strips and papillary muscles, respectively. The maximum positive inotropic response was as great as that produced by isoprenaline and Org 30029 elicited a complete relaxation of the phenylephrine-induced contracture. The positive inotropic effects of Org 30029 were not markedly affected by atenolol (1 μM), phentolamine (10 μM) or cimetidine (10 μM). Milrinone had similar intrinsic activity and potency to Org 30029 on papillary muscle but was less potent as a vascular relaxant. In contrast to Org 30029, which had little chronotropic activity (maximum increase 15%), milrinone induced a large increase in beating frequency (from 152±9 to 284±10 beats/min), similar to that produced by isoprenaline.

At a subthreshold inotropic concentration (32 μM), Org 30029 potentiated the positive inotropic effects of isoprenaline, producing a 4 fold parallel shift to the left of the isoprenaline concentration-effect curve (the mean isoprenaline EC_{50} values before and in the presence of Org 30029 were 0.13±0.03 μM and 0.03±0.01 μM, respectively). Carbachol (0.1-30 μM) produced a concentration-dependent reversal (maximum depression 26%) of the positive inotropic response produced by Org 30029 (692 μM). The effect of carbachol was blocked by atropine (1 μM).

The results of the present study show Org 30029 to induce positive inotropism and vascular relaxation, but to have minimal chronotropic activity. Potentiation of isoprenaline and inhibition by carbachol suggests the involvement of cyclic AMP in the pharmacological actions of Org 30029.

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THE EFFECT OF VASORELAXANTS WHICH INCREASE CYCLIC NUCLEOTIDES ON PHOSPHOINOSITIDE METABOLISM

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In 1986, Rapoport claimed that vasorelaxants capable of increasing cyclic GMP also decreased the formation of inositol monophosphate (IP_1)in rat aortae treated with lithium. Since the concentrations of vasorelaxants were rather high in this study we have repeated and extended the work using concentrations producing 50% relaxation or less in rat aortic strips.

Thoracic aortae from male Sprague-Dawley rats weighing 175-200 g were cleaned and endothelium gently removed. They were split longitudinally and incubated in Krebs gassed with 95%O_2; 5%CO_2 with 8 µCi/ml of [^{3}H]-myoinositol. They were then transferred to normal Krebs for 15 minutes before addition of vasorelaxants, noradrenaline and lithium chloride. IP_1 was assayed according to Brown & Brown (1983). The pellet remaining after homogenisation was assayed for protein (Markwell et al 1987). Statistical analysis was carried out using a paired t-test.

Noradrenaline 100 nM produced a large increase in IP_1 accumulation of 923 ± 119% (n=6 $p<0.001$) when compared with unstimulated tissue. Table 1 shows the effect of various vasorelaxants on IP_1 accumulation.

TABLE 1 % Decrease in [^{3}H] IP_1 accumulation (dpm/mg protein in rat aortic smooth muscle) due to vasorelaxants in the presence of noradrenaline 100 nM

Vasorelaxants	Concentrations	% relaxation	% decrease [^{3}H] IP_1
Sodium nitroprusside	5 nM	50	28.9 ± 4.6***(n=36)
Atriopeptin II	8 nM	50	23.8* (n=6)+
Flosequinan	15 µM	10	16.4 ± 5**(n=11)
	35 µM	20	26.6 ± 4.2**(n=12)
	120 µM	50	28.8 ± 7.1**(n=18)
M & B 22948	70 µM	50	20.0 ± 6.3**(n=11)
Forskolin	25 nM	12	16.4 ± 4.7* (n=6)
	50 nM	20	25.3 ± 5.1***(n=12)
Isoprenaline	20 nM	20	22.3 ± 8.0*(n=6)
Milrinone	0.15 µM	20	26.0 ± 3.9**(n=12)
IBMX	1.2 µM	15	8.5 ± 7.3(n=17)
	10 µM	85	51.1 ± 3.7**(n=6)

*$p<0.05$; **$p<0.01$; ***$p<0.001$. + unpaired t-test.

If IBMX 1.2 µM is incubated with forskolin (50 nM) there is an additional significant decrease in IP_1 accumulation (-26.3 ± 5.6%, $p<0.01$) when compared with noradrenaline and forskolin alone.

Thus it appears that phosphoinositide metabolism resulting in accumulation of IP_1 is reduced by vasorelaxants which increase cGMP or cAMP, including some of the phosphodiesterase inhibitors. Addition of a phosphodiesterase inhibitor to forskolin which stimulates adenyl cyclase produces an augmentation of the reduction in IP_1. These effects are evident at doses producing only 10-50% relaxation of noradrenaline contractions and appear to implicate cyclic nucleotides as modulators of the production of inositol triphosphate, now known to be responsible for intracellular Ca^{++} release, in vascular smooth muscle.

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NEUROGENIC CHOLINERGIC VASODILATION OF ARTERIOLES IN GUINEA-PIG SUBMUCOSA

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Acetylcholine dilates most blood vessels; it does so by activating muscarinic receptors on vascular endothelium or on the vascular smooth muscle itself (Burnstock & Griffith, 1988). However, direct demonstrations of neurogenic cholinergic vasodilation are few because of a lack of knowledge of the source of innervating cholinergic fibres and the presence (and concomitant release) of noncholinergic vasodilator substances. We sought to test whether any of the cholinergic neurones of the guinea-pig submucous plexus provided functional innervation of submucous arterioles. We developed an optical method for tracking the outside diameter of these arterioles (*outside diameters* 20 - 80 μm). Arterioles were preconstricted with the α_1-adrenoceptor agonist, phenylephrine (2 μM), and, at the same time, a ganglion (typically containing 3 - 12 neurones) was stimulated with an extracellular electrode. Ganglionic stimulation produced a frequency dependent (2 - 20 Hz, 10 s) vasodilation which ranged from 20 - 100% of preconstricted diameter. Vasodilation was increased by eserine and blocked by tetrodotoxin and by the muscarinic receptor antagonists atropine, pirenzepine, hexahydrosilodifenidol (HSDF) and (11[[2-[(diethylamino) methyl]-1-piperidinyl] acetyl]-5,11-dihydro-6H-pryrido [2,3-b] [1,4]benzodiazepine-6-) one (AF-DX 116): IC_{50} values for HSDF, pirenzepine and AF-DX 116 were 25, 500 and 4000 nM respectively. Superfusion of the submucous plexus with muscarine produced a dose-dependent vasodilation; the EC_{50} value for muscarine-evoked vasodilation was 150 ± 25 nM (n = 10). HSDF, pirenzepine and AF-DX 116 produced a parallel, rightward shift in the muscarine concentration-response curve; K_D values for these three antagonists determined from such experiments were 30, 425 and 4300 nM respectively.

Intracellular recordings were made from submucous neurones in ganglia in which focal stimulation dilated the monitored arteriolar segment. Depolarizing current pulses (10 Hz for 10 s) evoked action potentials; in four of 15 neurones the action potentials were associated with vasodilation. This dilation was blocked by pirenzepine (2 μM). Subsequent immunohistochemistry for vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), substance P and choline acetyltransferase (ChAT) confirmed that these neurones were cholinergic; as no positive cells showed VIP but all showed ChAT.

These experiments provide a direct demonstration that neurally released ACh dilates submucous arterioles and provide the first example in which the cholinergic neurones responsible for vasodilation have been identified. They further show that the muscarinic receptor subtype activated by neurally released ACh onto these arterioles is of the M_2 glandular subtype.

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CROMAKALIM INHIBITS PHASIC CONTRACTIONS TO NORADRENALINE IN THE RAT ISOLATED MESENTERIC BED EVEN IN Ca^{2+}-FREE MEDIUM

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The novel antivasoconstrictor agent cromakalim (BRL 34915) increases the K^+ permeability of vascular smooth muscle. The resulting hyperpolarization is believed to oppose opening of voltage-operated Ca^{2+} channels (Hamilton et al; 1986). As demonstrated by investigations in large blood vessels, the antivasoconstrictor effect of cromakalim is (i) abolished in depolarizing (high K^+) solution (Hamilton et al; 1986), (ii) inhibited by glibenclamide (Quast and Cook, 1988) and (iii) restricted to the (-) enantiomer of cromakalim (Hof et al., 1988). In the present study, the inhibition by cromakalim of contractions induced by short pulses of noradrenaline has been studied in the rat isolated mesenteric bed, a preparation in which the perfusion pressure is governed by resistance vessels, and in which the endothelium is intact.

The superior mesenteric bed was isolated from male OFA rats according to McGregor (1965). The preparation was perfused with modified Tyrode's solution either with normal Ca^{2+} (1.4 mM) or in the absence of Ca^{2+} (+ 0.1 mM EGTA + 20 mM $MgCl_2$) at 37°C and at a rate of 5 ml/min. After an initial 2 h equilibration period, the mesenteric bed was challenged with 1 s pulses of noradrenaline (10^{-6}M) every 10 min and the peak increase of perfusion pressure was recorded.

In the presence of Ca^{2+}, the response to noradrenaline (increase in perfusion pressure = 61 ± 6 mm Hg, n=36) was inhibited by the dihydropyridine Ca^{2+}-antagonist, isradipine (PN 200-110, 10^{-7}M), by 21 ± 3 %. This indicated that only a small component of this contraction depended on Ca^{2+} entry through slow voltage-operated Ca^{2+} channels. It would appear that most of the Ca^{2+} for this contraction is dependent on release from internal stores and/or entry via receptor-operated channels. In contrast to isradipine, cromakalim inhibited the response to noradrenaline by 85 ± 5 % with a midpoint at $2x10^{-7}$M and a Hill slope of 1.0 ± 0.2. The effect of cromakalim satisfied the 3 criteria listed above. Perfusion with Ca^{2+}-free medium for 10 min reduced the response to noradrenaline from 100 ± 5 mm Hg to 36 ± 4 mm Hg (n=20). Cromakalim (1µM) decreased this response further to 11 ± 2 mm Hg (n=16), i.e. to 30 % of the value in absence of cromakalim. This effect of cromakalim was again stereoselective, inhibited by glibenclamide and abolished in the presence of 50 mM KCl.

These results suggest that in rat mesenteric resistance vessels cromakalim interferes with the ability of noradrenaline to release Ca^{2+} from internal stores and that this effect may depend on membrane potential. However, they are in contrast to the findings of other authors in larger vessels and to one study in the perfused mesenteric bed from spontaneously hypertensive rats (Buckingham and Parrott, 1988).

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EFFECTS OF CROMAKALIM ON PULMONARY HAEMODYNAMICS IN NORMOXIC AND HYPOXIC CONDITIONS IN ANAESTHETISED DOGS

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Cromakalim (CROM; Buckingham et al 1986) is a vascular selective antihypertensive which is thought to act by opening K^+-channels (Weir and Weston 1986). We now describe the pulmonary hemodynamic properties of CROM in anesthetized dogs under normoxia and hypoxia.
Dogs (12-18 kg) anesthetized with pentobarbitone (35 mg/kg,i.v and 5 mg/kg/h), were artificially ventilated with room air (thorax closed). Blood pressure was measured from a carotid artery and a saphenous vein was used for i.v infusion of CROM. Two Swan-Ganz catheters (size 7F) were implanted through a jugular vein into the right atrium to measure cardiac output (CO, by thermodilution) and via the right ventricle into a branch of the pulmonary artery. Effective pulmonary capillary pressure (EPCP) was determined by the methods of Holloway et al,(1983), from the biphasic pressure wave after balloon occlusion of the pulmonary artery. Blood gases and pH were measured at 15 min intervals. Diastolic carotid (DBP) and pulmonary artery pressures (DPP) and CO were used to calculate total systemic (TSVR) and pulmonary vascular resistance (TPVR). A period of normoxia (1h room air), was followed by a period of hypoxia over 1h by gassing with an air/N_2 (2:1) mixture. The effects of CROM (2ug/kg/min for 30 min) were studied under normoxia (n=4), or after hypoxia (n=6). Hypoxia (PaO_2 47.7 + 7.7 from 92.3 + 3.8 mmHg), caused an increase in DPP from 7.2 +0.5 to 16.8 +1.8 mmHg (132%), EPCP from 4.7 + 0.73 to 10.7 + 2.0 mmHg (127 %) and TPVR from 2.20 + 0.36 to 5.7 +0.80 mmHg/l/min (160 %). CO increased from 2.56 + 0.35 to 2.96 +0.31 l/min (16.0%). Neither systemic blood pressure (DBP 120 +8 from 112+11 mmHg) nor TSVR (43.8+ 3.8 from 47.3+ 6.1 mmHg/l/min) were modified. The systemic and pulmonary hemodynamic effects of CROM are shown in the Table.

		HYPOXIA			NORMOXIA		
		TSVR	TPVR	EPCP	TSVR	TPVR	EPCP
		mmHg/l/min		mmHg	mmHg/l/min		mmHg
INITIAL VALUES		43.8 +3.8	5.7 +0.8a	10.7+2.0a	55.2+7.2	3.9+0.3	6.7 +1.0
CROM	10 min	26.7 +2.7*	3.9 +0.5*	9.7+1.6	32.0+4.7*	3.1+0.4	6.5 +1.8
		(-39%)	(-32%)	(-9.5%)	(-42%)	(-20%)	(+12%)
	20 min	21.8 +2.0*	3.2 +0.4*	9.1+1.4*	26.2+5.1*	3.0+0.4	8.2 +0.7
		(-50%)	(-44%)	(-15%)	(-53%)	(-22%)	(+35%)
	30 min	18.0 +2.1*	2.4 +0.5*	9.3+1.9	23.9+4.6*	3.3+0.6	7.5 +0.5
		(-58%)	(-59%)	(-13%)	(-57%)	(-14%)	(+32%)

a : different from normoxia, * : different from initial values, p less than 0.05

CROM showed equivalent systemic vasodilator (TSVR) and CO (48-58%) effects in both models. During hypoxia, CROM significantly reduced the elevated pulmonary pressures (EPCP and DPP) and vascular resistance. PaO_2 (55.9 + 2.4) did not change. However, in normoxic conditions pulmonary vascular pressures increased after CROM as a function of the increase in CO since pulmonary resistance was little changed. In this model CROM can thus relax the preconstricted pulmonary vascular bed and maintain PaO_2 at a lower pulmonary pressure.

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THE EFFECT OF GLIBENCLAMIDE ON THE ACTION OF CROMAKALIM, DIAZOXIDE AND MINOXIDIL SULPHATE ON RAT AORTA

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The vasorelaxant drug cromakalim (BRL34915) opens K channels in smooth muscle resulting in hyperpolarisation of the cell membrane (Hamilton et al., 1986). In vitro the action of cromakalim can be inhibited by the sulphonylurea glibenclamide (Wilson et al., 1988; Quast & Cook, 1988) an agent which increases insulin secretion by blocking ATP-dependent K channels in pancreatic ß cells (Schmid-Antomarchi et al., 1987). The vasorelaxants diazoxide and minoxidil sulphate have K channel opening properties in smooth muscle (Bray et al., 1988) although diazoxide relaxed an 80mM KCl induced contraction suggesting an additional action. In the present studies the effects of glibenclamide on the vascular actions of diazoxide, minoxidil sulphate and cromakalim have been investigated. Endothelium-free strips were prepared from the thoracic aortae of male Sprague Dawley rats (300-500g) and mounted either for isometric recording in MOPS PSS at 37°C or on a manifold for ^{86}Rb efflux studies.

Glibenclamide (0.1-1µM) inhibited the ability of cromakalim, diazoxide and minoxidil sulphate to relax a 20mM KCl induced contraction. The inhibitory effects of glibenclamide against diazoxide and cromakalim were consistent with competitive antagonism. In contrast the interaction between glibenclamide and minoxidil sulphate had the characteristics of non-competitive antagonism. Glibenclamide (0.3-1µM) had no effect on the relaxant action of diazoxide against 80mM KCl. Glibenclamide (300nM, 1µM) had no effect on resting tone in rat aorta; it did not affect contractile responses to KCl (20mM) or the ability of sodium nitroprusside to relax such contractions. In segments of rat aorta loaded with ^{86}Rb, cromakalim (1-10µM) and diazoxide (1mM) increased ^{86}Rb efflux from rat aorta, but minoxidil sulphate (1-10µM) had no effect. Glibenclamide (1µM) inhibited the increase in ^{86}Rb efflux produced by cromakalim (1-10µM) and diazoxide (0.3-3mM). Glibenclamide (1µM) had no effect on ^{86}Rb efflux evoked by KCl (20mM, 80mM) in rat aorta.

It is concluded that cromakalim, diazoxide and minoxidil sulphate relax rat aorta by opening K channel(s) which can be blocked by glibenclamide. The relatively high concentrations of glibenclamide required suggest that this channel is not identical to the ATP-dependent K channel of pancreatic ß-cells (Schmid-Antomarchi et al., 1987). The inability of glibenclamide to reduce ^{86}Rb efflux stimulated by KCl (20mM, 80mM) suggests that the channel involved is not the large Ca^{2+}-dependent K channel. The K channel opening action of minoxidil sulphate is not associated with the opening of an ^{86}Rb permeable K channel.

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CENTRAL AND PERIPHERAL EFFECTS OF RILMENIDINE AND CLONIDINE, IN CONSCIOUS AND ANAESTHETISED SHRs

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In order to assess the influence of anaesthesia on the central and peripheral effects of Rilmenidine, a new alpha-2 adrenoceptor agonist antihypertensive agent, we measured blood pressure (BP) and heart rate (HR) in freely moving or pentobarbital-anaesthetized spontaneously hypertensive adult rats (SHRs), which received intracerebroventricular (i.c.v.) or intravenous (i.v.) injections of Rilmenidine. Clonidine was used as a reference drug. Aortic BP curve was continuously monitored using our computerized technique (C. Cerutti et al., 1985). I.c.v. administrations were performed through a cannula chronically implanted into the lateral cerebral ventricle. In conscious SHRs: i.v. injections of Rilmenidine (30-100-300 µg/kg) produced a sharp short-lasting and dose-dependent rise in mean BP (+12 ; + 24 and + 32 mmHg respectively), associated with a short-lasting bradycardia (-45 ; -61 and -74 bpm). This increase was followed by a prolonged depressor response (-13 ; -19 and -24 mmHg) without change in HR. Comparatively, the BP effects of Clonidine (3-10-30 µg/kg) were similar to those of Rilmenidine but, were associated with a more pronounced (-56 ; -80 and -130 bpm) and longer lasting bradycardia. The same doses of Rilmenidine or Clonidine given i.c.v. did not significantly change BP and HR. In pentobarbital-anaesthetized and artificially ventilated SHRs, i.v. injections of Rilmenidine (30-100-300 µg/kg) or Clonidine (3-10-30 µg/kg) both induced a weaker pressor response followed by a more marked hypotensive effect than in conscious SHRs. A significant bradycardia was associated with both the pressor and the depressor action of Rilmenidine and Clonidine. When given i.c.v., at the same doses as i.v., Rilmenidine and Clonidine provoked only a progressive and long-lasting depressor response associated with a bradycardia, which was more marked with Clonidine than with Rilmenidine.

The present results indicate that: 1) in conscious SHRs, the antihypertensive effect of Rilmenidine and Clonidine are essentially peripheral in origin. This is in agreement with the studies of Brody et al., 1984 and Mastrianni & Ingenito, 1987, who used Clonidine. 2) in anaesthetized SHRs, both drugs induce more marked depressor responses than in conscious animals and exhibit central effects. It is concluded that anaesthesia enhances the action of alpha-2 adrenoceptor agonists and reveals their central effects. This could be due to the increased sympathetic activity and/or the decreased baroreflex sensitivity which are associated with anaesthesia (Fluckiger et al., 1985).

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EFFECT OF THROMBOXANE SYNTHETASE INHIBITION ON BLOOD PRESSURE IN GENETICALLY HYPERTENSIVE RATS OF THE LYON STRAIN

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In order to determine if the increased renal biosynthesis of Thromboxane(Tx)A2, observed in young genetically hypertensive (LH) rats of the Lyon strain (GEOFFROY et al., 1986), could be involved in the development of their hypertension, TXA2 biosynthesis was inhibited in LH female rats (n=12) from 3 to 9 weeks of age by 2 different imidazol derivatives : Dazmegrel (75 mg/Kg/day , orally, from 3 to 5 weeks then 100 mg/Kg/day from 5 to 9 weeks,Pfizer Central Research) and OKY 046 (20mg/Kg b.i.d., sub-cutaneously, ONO Pharmaceutical Company). Simultaneously, 2 groups of 12 LH rats received vehicle (LH-Ve) and served as controls.

Systolic blood pressure (SBP) was weekly recorded by plethysmography. After 2 and 6 weeks of treatment, urinary excretion of 6-keto-prostaglandinF1alpha (U6K.V) and TxB2 (UTx.V) was measured, by specific radio-immunoassays after high pressure liquid chromatography (BENZONI et al., 1982, GEOFFROY et al., 1985). The results (x±s.e. mean) were as follows (* P<.05 ** p<.01, *** p<.001 vs LH-Ve) :

Age (weeks)		**5**	**6**	**7**	**8**	**9**	**10**
SBP (mm Hg)	LH-Ve	102±1,7	132±3,4	138±3,1	140±2,1	142±2,3	144±2,7
	LH- Daz	105±2,1	119±3,0**	130±1,6*	137±3,4	142±1,7	143±2,7
	LH-Ve	112±2,5	131±2,5	135±3,3	138±2,4	143±2,1	143±2,4
	LH-OKY	112±2,7	121±1,6**	125±1,4**	127±1,7***	133±2,4**	140±2,7
U6K.V (ng/24h)	LH-Ve	63,1±5,2				31,8±2,3	
	LH-Daz	57,8±4,0				30,9±3,7	
	LH-Ve	65,7±3,2				34,1±1,5	
	LH-OKY	69,4±3,2				33,6±1,6	
UTx.V (ng/24h)	LH-Ve	17,2±1,5				2,6±0,4	
	LH-Daz	11,2±0,9***				1,0±0,3**	
	LH-Ve	18,0±1,3				6,8±0,5	
	LH-OKY	12,7±1,2**				2,9±0,2***	

Dazmegrel and OKY 046 induced a progressive inhibition of Thromboxane synthetase without shunting of endoperoxides toward prostacyclin synthesis. The extent of the inhibition was identical with both drugs. It was associated with transient decreases in SBP which lasted much longer with OKY 046 than with Dazmegrel. These results suggest that the antihypertensive effect of OKY 046 in LH rats cannot be totally accounted for the decrease in renal production of TXA2.

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REGIONAL HAEMODYNAMIC EFFECTS OF BRADYKININ OR SUBSTANCE P IN CONSCIOUS, LONG EVANS RATS

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Both bradykinin (BK) and substance P (SP) have been shown to cause endothelium-dependent relaxation of blood vessels (see Vanhoutte et al., 1986), but their relative effects on regional haemodynamics in the same conscious rats are unknown. We have investigated the cardiovascular responses to bolus i.v. injections of equihypotensive doses of BK (0.5 nmol) or SP (0.1 nmol) in conscious, male, Long Evans rats (n=9). Animals were anaesthetized with sodium methohexitone (60 mg kg^{-1} i.p.) and had pulsed Doppler probes (Haywood et al., 1981) implanted around the left renal (R) and superior mesenteric (M) arteries and the distal abdominal aorta (H, i.e. representing flow to hindquarters). Following a period of at least 7 days, animals were briefly re-anaesthetized with sodium methohexitone (40 mg kg^{-1} i.p.) and had intra-arterial and intravenous catheters implanted; measurements were made the following day. Continuous recordings were made of mean BP, instantaneous heart rate (HR), and R, M and H Doppler shift (DS) signals; % changes in regional vascular resistances (VR) were calculated from mean BP and DS signals (Haywood et al., 1981). The table summarises changes corresponding to the nadirs in mean BP following BK or SP.

Table 1. Cardiovascular changes 1-2 min after BK or SP; values are mean (s.e.m.)

	Mean BP (mmHg)	HR (b min^{-1})	% DS R	% DS M	% DS H	% VR R	% VR M	% VR H
BK	-26(3)*	89(11)*	-2(3)	70(7)*	22(9)*	-22(3)*	-54(4)*	-36(5)*
SP	-27(3)*	102(10)*	-7(3)	-35(3)*^	70(9)*^	-18(2)*	18(5)*^	-55(3)*^

*P <0.05 vs baseline (Wilcoxon test); ^ P<0.05 BK vs SP (Wilcoxon test).

The results show that doses of BK and SP that have the same effects on mean BP have markedly different regional haemodynamic effects. BK causes mesenteric and hindquarters vasodilatation associated with increases in flow, whereas SP causes reduced mesenteric perfusion accompanied by vasoconstriction. However, SP causes a significantly greater hindquarters vasodilatation than does BK. These results raise the possibility that BK and SP interact differentially with endothelial cells in different vascular beds.

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INCREASED UPTAKE OF METHYLATED LOW DENSITY LIPOPROTEIN INDUCED BY NORADRENALINE IN CAROTID ARTERIES OF ANAESTHETISED RABBITS

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Atherosclerosis is accelerated in hyperlipidaemias but, apart from the concentration of LDL in the blood, very little is known about determinants of its flux into arterial walls. We have now shown that in anaesthetised rabbits the atherogenic uptake of LDL by arterial walls is accelerated by noradrenaline at concentrations normally present in rabbit and human blood (Bühler et al., 1978) and, even more, at the increased concentrations associated with stress (Dimsdale & Moss, 1980) as well as with cigarette smoking (Cryer et al., 1976) and hypertension (Doyle, 1978), both established risk factors of coronary artery disease (Assmann et al., 1986).

The principle of the experiments was to compare the uptake of intravenously injected radioactively labelled LDL, methylated to prevent removal by high affinity receptors, in the two carotid arteries of anaesthetised rabbits after infusing low concentrations of noradrenaline into one carotid and saline as control into the other, the volume rates of infusion being less than 1 per cent of the carotid blood flows. Human LDL, which behaves sufficiently like rabbit LDL for these purposes, was prepared, methylated and radio-iodinated by standard methods (Shafi et al., 1987). At the end of the infusions, the arteries were excised and their radioactivities determined.

Noradrenaline infused for 2 hours to produce local blood concentrations of 1 nM and higher significantly increased the LDL radioactivities of the walls of the infused carotids. Concentrations of 100 nM and higher also increased the LDL radioactivities of the walls of the control carotids; this was associated with significant increases in their blood noradrenaline concentrations.

Whatever the mechanism responsible for this noradrenaline effect, the results contribute towards an explanation for the accelerated atherosclerosis and the increased incidence of its clinical manifestations in conditions associated with elevated blood noradrenaline concentrations.

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ANALYSIS OF THE INHIBITORY COMPONENT OF CAFFEINE-INDUCED RESPONSES IN RABBIT ISOLATED AORTA

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In vascular smooth muscle caffeine produces a phasic mechanical response which results from both spasmogenic and relaxant activities (Leijten & Van Breemen, 1984). The latter component of this response may result in part from inhibition of cyclic-AMP phosphodiesterase, leading to accumulation of intracellular cyclic-AMP (Butcher & Sutherland, 1962). However, we have recently shown that an increase in both cyclic-AMP and cyclic-GMP may underlie the ability of caffeine to relax rabbit aorta (Bray et al., 1988). In the present study the role of cyclic nucleotides in caffeine-induced responses has been further examined.

Endothelium-free strips were prepared from the thoracic aorta of male half-lop rabbits (2.5-3.5Kg). For tissue bath studies, tissues were mounted for isometric tension recordings in MOPS-buffered physiological salt solution at 37°C, pH 7.4. Cyclic nucleotide levels were measured using radioimmunoassay.

Exposure to caffeine (10mM) produced a time-dependent increase in both cyclic-GMP and cyclic-AMP which correlated with mechanical relaxation (maximum increases in cyclic-GMP and cyclic-AMP at $t = 2$ min, $t = 5$ min, respectively; $n = 5$, $p < 0.01$). In addition, caffeine (1-20mM) produced a concentration-dependent increase in cyclic nucleotide levels. At the maximum concentration tested, caffeine (20mM) induced a 9.8-fold increase in cyclic-GMP and a 2.1-fold increase in cyclic-AMP ($p < 0.05$, $n = 6$). These changes were not significantly affected by addition of either potassium chloride (KCl, 40mM) or noradrenaline (NA, 300nM) prior to exposure to caffeine.

In separate experiments caffeine (0.2-10mM), sodium nitroprusside (SNP, 0.001-1μM) and cromakalim (0.3-10μM), produced complete relaxation of NA (300nM)-induced contractions (IC50 values, 2.4mM, 44nM and 3μM, respectively, $n = 6$). Preincubation with M&B22948 (100μM) potentiated the relaxant effects of SNP, (producing a 10.2-fold change in EC50, $p < 0.05$, $n = 6$), but had no significant effect on responses to caffeine or cromakalim. Methylene blue (10μM) inhibited SNP-induced relaxation and the initial caffeine (0.2mM)-induced contraction, but potentiated the relaxant actions of caffeine (0.5-10mM). Responses to cromakalim were unaffected by exposure to methylene blue. Similar results were obtained from tissues precontracted with KCl.

In conclusion, caffeine produces increases in cyclic-GMP which correlate with relaxation. However, the results with M&B 22948 and methylene blue suggest that the relaxant actions of caffeine may not be closely linked to changes in cyclic-GMP.

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A PURINERGIC COMPONENT OF NEUROGENIC VASOCONSTRICTION IN RESISTANCE VESSELS OF THE CAT INTESTINAL CIRCULATION

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The relative contribution of postsynaptic α-adrenoceptors and P_{2x}-purinoceptors (Burnstock and Kennedy 1985) in mediating neurogenic vasoconstriction was investigated in autoperfused intestinal circulation of anaesthetised cats since in this vascular bed the selective P_{2x}-purinoceptor agonist, α, ß methylene ATP, was shown to exert a powerful vasoconstrictor effect comparable with noradrenaline (Parsons and Taylor 1988).

Transmural stimulation of the sympathetic nerve plexus around the anterior mesenteric artery, in the presence of atropine, 1 mg/kg iv, and propranolol, 1 mg/kg i.v., elicited reproducible, frequency-related, increases in perfusion pressure. Prazosin, 0.5mg/kg i.a., reduced vasoconstriction to higher but not lower frequency nerve stimulation (Fig 1). Yohimbine, 1.5mg/kg i.a., reduced responses to both low and higher frequency nerve stimulation (Fig 1). In addition, yohimbine inhibited noradrenaline responses whereas prazosin was relatively ineffective. The inhibitory effects of the two antagonists were additive (Fig 1) but even after complete α-adrenoceptor blockade, nerve stimulation still elicited frequency-related increases in perfusion pressure (Fig 1). Under these conditions, repeated high doses of α, ß methylene ATP (total dose 1.5mg i.a.), to desensitise P_{2x}-purinoceptors, completely abolished the initial rapid phase of the residual vasoconstrictor response to 16Hz nerve stimulation leaving only a small, delayed onset, increase in perfusion pressure (+13.0±4.3 mmHg, n=6) which may be due to high local concentrations of noradrenaline overcoming the α-adrenoceptor blockade or to the release of other transmitters (eg peptides). The inhibitory effect of α, ß methylene ATP was maximal immediately after desensitisation and lasted 40-50min.

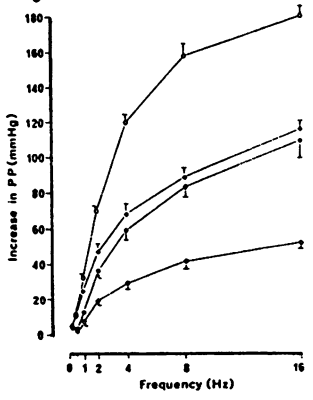


Figure 1

Effect of prazosin,0.5mg/kg i.a.(◆,n=7), yohimbine,1.5mg/kg i.a.,(●,n=6) and prazosin + yohimbine (◇,n=7) on increases in perfusion pressure(PP) elicited by sympathetic nerve stimulation (○,n=13). Mean resting value of PP=71.6±3.0mmHg.

The results suggest that neurotransmission in resistance vessels of the cat intestinal circulation is largely under adrenergic control mediated by postsynaptic α_1 - and α_2-adrenoceptors, the latter predominating at low frequency nerve stimulation and in the vasoconstrictor response to injected noradrenaline, with a possible purine involvement in the initial rapid response to nerve stimulation. As cotransmitters, noradrenaline and ATP may exert important synergistic effects.

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THE EFFECT OF IN VITRO DENERVATION ON TRANSMITTER RELEASE IN THE RABBIT BASILAR ARTERY

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Results from our earlier work (Holly & Tahir, 1988), show that transmural nerve stimulation (TNS), causes the release of 5-HT and noradrenaline (NA), in the rabbit basilar artery (RBA), but in the rabbit internal carotid artery only 5-HT was released. However, some of our unpublished studies have shown that in the rabbit internal carotid artery, noradrenaline release does occur but only at lower frequencies of stimulation.

Using superfusion techniques, in conjunction with HPLC with electrochemical detection (LCED), we have examined the effects of 6-hydroxydopamine (6-OHDA, a selective adrenergic neurotoxin; Theonen & Tranzer, 1968) and 5,7-dihydroxytryptamine (5,7-DHT, a selective serotonergic neurotoxin; Sachs & Jonsson, 1975), on the release of endogenous NA and 5-HT, in response to TNS, at frequencies between 0.5 Hz - 10 Hz.

Spiral strips of the RBA and RICA were superfused with oxygenated krebs. In one set of experiments, the vessels were superfused with krebs containing 6-OHDA, 4×10^{-4} M, (in the presence of 5-HT uptake inhibitors, monoamine oxidase inhibitor, (MAOI) and a β-blocker), in an attempt to destroy all noradrenergic neurones present. While in a second set of experiments, the krebs contained 5,7-DHT 10^{-3} M, (in the presence of NA uptake 1 and 2 inhibitors, MAOI and a β-blocker) to destroy any serotonergic neurones present. Samples were taken at regular intervals, for a total of four hours, and collected into tubes containing sodium metabisulphite. 5-HT was extracted using cation exchange chromatography, while NA was extracted using acid alumina. Both amines were assayed using LCED. The rabbit internal carotid artery was used as a non cerebral control.

In the RBA, following exposure to either 6-OHDA or 5,7-DHT, the release of both endogenous NA and 5-HT, at all frequencies tested, was totally abolished and remained thus.

In the RICA, exposure to 6-OHDA abolished the release of endogenous NA (only seen at 2 Hz and 0.5 Hz, in agreement with our earlier unpublished work). There was no change in the release of endogenous 5-HT. Following exposure to 5,7-DHT, the release of endogenous 5-HT was abolished. As 5-HT release in the RICA declined, there was a concurrent rise in the release of NA, not only at 0.5 Hz and 2 Hz, but also at 5 Hz and 10 Hz (frequencies where, under control conditions, NA release had not been previously demonstrated).

In the RBA, results suggest the co-release of NA and 5-HT, from a single neurone. In the RICA, evidence is consistent with NA and 5-HT being released from separate neurones.

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PHARMACOLOGICAL SPECIFICITY OF SYNTHETIC PEPTIDES AS ANTAGONISTS AT TACHYKININ RECEPTORS

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Definitive classifications of the receptors that mediate the actions of tachykinins have been thwarted by the lack of selective antagonists with high affinity. We report here on the profile of activities of potentially useful antagonists, obtained from a synthetic programme based on the agonist L-363,851 GlpPhePhe(R)Gly[ANC-2]LeuMet.NH_2 in pharmacological models for NK-1 (guinea-pig ileum with 5μM atropine), NK-2 (rat vas deferens), NK-3 (rat portal vein) and NK-4 (guinea-pig trachea with 5μM indomethacin) receptors, and in radio-receptor binding assays at NK-1 ([^{125}I-BH]SP in rat cortex), NK-2 ([^{125}I-BH]ELE in hamster bladder) and NK-3 ([^{125}I-BH]ELE in rat cortex) sites.

TABLE 1 Antagonist activity (pA_2) of cyclic peptides at tachykinin receptors wpa = weak, partial agonist, phosphorhamidon was present for NK-4.

	NK-1	NK-2	NK-3	NK-4
1. cyclo(GlnPhePhe(R)Gly[ANC-2]LeuMet)	5.9	4.8	<4.5	wpa
2. cyclo(GlnTrpPhe(R)Gly[ANC-2]LeuMet)	<4.5	6.7	4.9	wpa
3. cyclo(GlnTrpPheGlyLeuMet)	5.9	8.0	5.9	5.3
4. cyclo(GlntrpPhe(R)Gly[ANC-2]LeuMet)	5.0	4.9	5.3	5.5
5. cyclo(Glntrp(Me)Phe(R)Gly[ANC-2]LeuMet)	6.6	6.4	5.7	6.4
6. cyclo(Glntrp(Me)Phe(R)Gly[ANC-2]LeuMet)$_2$	7.0	5.1	5.6	4.5
7. Ac-LeuMetGlnTrpPheGly.NH_2	<4.5	6.8	<4.5	<4.5

It had been shown with L-363,851 that the constraint due to the introduction of the lactam unit ([ANC-2]) in the structure of [Glp^6]SP(6-11) induced a conformation that would be optimal in a cyclic peptide. From our findings with peptide 2 (L-659,837) and peptide 3 (L-659,877) it can be seen that the cyclisation produced maximum affinity for the NK-2 receptor, but that affinity and selectivity are maintained in the "opened" linear peptide, number 7 (L-659,874).

With these antagonists we have seen that responses in rat vas deferens to eledoisin, neurokinin A and neurokinin B are all equally sensitive to blockade, suggesting a homogeneous receptor population in this preparation, and that similar values for pA_2 are obtained using these agonists to produce contraction of rat small intestine.

Affinity and selectivity for NK-2 receptors is reduced in the peptides that contain D-Trp (Table I), but in the cyclic dimer (peptide 6) we have obtained a dodecapeptide antagonist with appreciable selectivity for the NK-1 receptor. This antagonist does not significantly effect the response in trachea to substance P or substance P-methyl ester and in combination with an NK-2 selective antagonist does not significantly effect the response to eledoisin. These observations are at odds with the view that contraction in trachea is mediated by NK-2 and/or NK-1 receptors (Ireland *et al.*, 1988).

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INHIBITION OF THE M-CURRENT BY A PUTATIVE SIGMA RECEPTOR AGONIST IN THE MOUSE HYPOGASTRIC GANGLION

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Putative sigma receptor agonists potentiate the neurogenic twitch contractions of the mouse vas deferens in vitro (Campbell et al., 1987; Kennedy et al., 1988) by a prejunctional action (Sonders et al., 1988). The cell bodies of neurones innervating the vas deferens lie in the hypogastric ganglion. The aim of this study was to determine the effects of a proposed sigma receptor agonist, (+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine ((+)3-PPP), on the electrophysiological properties of the cells in the mouse hypogastric ganglion in vitro.

Mice (DBA/1A strain) were killed by cervical dislocation. The hypogastric ganglion was dissected free and pinned to the base of a recording chamber lined with silicon resin. The preparation was superfused with oxygenated Krebs solution maintained at 37°C in the current-clamp experiments and at 30°C in the voltage-clamp experiments. Drugs were applied in the superfusate. Intracellular recordings were made from ganglion cells using 3M KCl-filled microelectrodes.

In neurones studied under current clamp (+)3-PPP (10μM-300μM) evoked a concentration-dependent depolarisation of between and 3 and 13mV. The depolarisation took 5-6min to reach a maximum, was maintained for at least 15 min in the presence of (+)3-PPP and was reversible on washout of the drug. The depolarisation was associated with an increase in membrane input resistance when membrane potential was manually reclamped to its initial level. In two cells the depolarisation was also associated with the appearance of spontaneous action potentials. At membrane potentials less negative than -65mV the amplitude of the depolarisation evoked by (+)3-PPP (30μM) was inversely related to resting membrane potential. (+)3-PPP had no effect on membrane potential at potentials negative to -65mV.

These results suggest that (+)3-PPP produces depolarisation by inhibiting an outward current which is active at membrane potentials less negative than -65mV. Therefore, the actions of (+)3-PPP were further studied using voltage-clamp. In the presence of tetrodotoxin (1μM) hyperpolarising step-commands from a holding potential of -35mV evoked slow inward current relaxations which became outward negative to -90mV. Repolarisation evoked slow outward current relaxations. Inward and outward current relaxations were still seen in the presence of 0mM Ca^{2+}/20mM Mg^{2+} or tetraethylammonium (5mM) but were inhibited by external Ba^{2+} (1mM) and carbachol (30μM). (+)3-PPP (10-300μM) applied at a holding potential of -35mV, produced a steady inward current and reversibly inhibited the slow inward and outward current relaxations evoked by hyperpolarising step-commands to -65mV.

Thus, (+)3-PPP produces depolarisation by inhibiting the M-current present in the postganglionic sympathetic neurones which innervate the mouse vas deferens. This M-current inhibition may be the mechanism underlying the potentiation of neurogenic twitch contractions produced by (+)3-PPP.

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IN VITRO STUDIES ON HOMOLOGOUS AND HETEROLOGOUS DESENSITIZATION OF HUMAN LYMPHOCYTE β_2-ADRENOCEPTORS

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Desensitization appears to be a general mechanism of cellular adaptation to agonist stimulation with time. Two major types of desensitization have been described: "homologous" desensitization results in a decreased cellular response only to the desensitizing agonist, while "heterologous" desensitization results in a blunted cellular response also to additional agonists (Lefkowitz & Caron, 1986). Little is known about homologous and heterologous desensitization of human β-adrenoceptors (AR). Circulating lymphocytes containing a homogeneous population of β_2-AR coupled to the adenylate cyclase/cyclic AMP system are a frequently used model for studies on β-AR in humans (Brodde et al., 1987). Therefore, we studied in vitro the effects of catecholamines, prostaglandin E_1 (PGE_1) and the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on lymphocyte β_2-AR density and cyclic AMP responses to isoprenaline (IPN), PGE_1 and forskolin.

Lymphocytes were isolated from EDTA-blood (obtained from healthy volunteers) by the method of Böyum (1968) and incubated at 37°C in autologous plasma for 60 min with the agonists. Incubation was stopped by the addition of 10 ml ice-cold phosphate-buffered saline (PBS); the lymphocytes were washed three times with ice-cold PBS and β_2-AR density (by (-)-[^{125}I]-iodocyanopindolol binding) and cyclic AMP response to IPN, PGE_1 and forskolin were determined in intact cells as recently described (Brodde et al., 1985).

Incubation of lymphocytes with catecholamines resulted in a time- and concentration-dependent decrease in β_2-AR density and cyclic AMP response to 10 µM IPN; the order of potency was IPN > adrenaline > noradrenaline. The 10µM IPN-induced decrease in lymphocyte β_2-AR function was completely abolished by the β_2-AR antagonist ICI 118,551 (50nM), but not affected by the β_1-AR antagonist bisoprolol (500 nM). Incubation of lymphocytes with 10µM PGE_1 did not affect lymphocyte β_2-AR function, but did significantly attenuate the PGE_1-evoked increase in cyclic AMP in lymphocytes. Incubation of lymphocytes with 10µM TPA did not affect lymphocyte β_2-AR density, but did markedly decrease lymphocyte cyclic AMP response to 10µM PGE_1 or 10µM IPN; this effect was completely suppressed by the protein kinase C inhibitor H-7 (10µM). In contrast, 10 µM forskolin-induced increase in lymphocyte cyclic AMP content was enhanced following TPA incubation.

It is concluded that human lymphocyte β_2-AR can undergo homologous and heterologous desensitization. TPA-induced heterologous desensitization seems to involve activation of protein kinase C that presumably leads to inactivation of the stimulatory as well as inhibitory G-proteins.

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NO EVIDENCE FOR DIFFERENCES BETWEEN PRE AND POSTJUNCTIONAL α_2-ADRENOCEPTORS IN THE PERIPHERY

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We have recently reported that yohimbine and SK & F 104078 (see Ruffolo et al., 1987) have similar effects at pre- and postjunctional alpha$_2$-adrenoceptors both in vivo in the pithed rat and in vitro in the rat atrium and human saphenous vein (Connaughton et al., 1988). We now present further evidence that SK & F 104078 does not distinguish between pre- and postjunctional alpha$_2$-adrenoceptors by examining the prejunctional alpha$_2$-adrenoceptors of the rat vas deferens and the postjunctional alpha$_2$-adrenoceptors of the human platelet.

In prostatic portions of rat vas deferens, the alpha$_2$-adrenoceptor agonist xylazine produces a concentration-dependent inhibition of the isometric contraction induced by a single electrical stimulus. Apparent pA_2 values of antagonists were obtained from the effects of a fixed concentration of antagonist against the inhibition produced by xylazine.

In human platelet membranes, K_i values were obtained for antagonists from their ability to displace (^{3}H)-yohimbine binding.

In rat vas deferens, prejunctional pA_2 values (-logM) of 7.50 (95% confidence limits of 7.16-7.84) and 6.39 (5.98-6.65) were obtained for yohimbine and SK & F 104078, respectively (n=4, each). These compare with prejunctional EC_{30} values (concentration producing 30% potentiation of stimulation-evoked overflow of tritium in tissues pre-incubated with ^{3}H-noradrenaline) of 7.89 and 6.68 obtained for yohimbine and SK & F 104078, respectively, in the rat atrium.

At the postjunctional alpha$_2$-adrenoceptors of the human platelet, K_i values (-log M) of 8.00 (7.68-8.32) and 6.73 (6.21-7.25) were obtained for yohimbine and SK & F 104078, respectively (n=4, each). These compare with postjunctional pA_2 values obtained in the human saphenous vein of 7.40 and 6.33 for yohimbine and SK & F 104078, respectively (n=4, each).

Hence, both yohimbine and SK & F 104078 failed to distinguish between pre- and postjunctional alpha$_2$-adrenoceptors, and yohimbine was approximately 10 times more potent than SK & F 104078, in the tissues examined (see also Bertie et al., 1988; Connaughton et al., 1988). Our results lend no support to the suggestion that pre- and postjunctional alpha$_2$-adrenoceptors may differ (Ruffolo et al., 1987).

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FREEZING OF HUMAN PLATELETS CAUSES PREFERENTIAL LOSS OF AGONIST BINDING AT α_2-ADRENOCEPTORS

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Platelet α_2-adrenoceptors have been monitored as putative biochemical markers for depression and antidepressant drug action in man. Studies using antagonist radioligands have consistently reported no change whereas those using ^{3}H-clonidine indicate increased binding in depressed patients which is reduced by antidepressant treatment (Elliott 1984; Kafka & Paul, 1986). Platelet tissue employed in such studies may be analysed immediately after venepuncture or stored frozen until assay. We have therefore investigated the effects of freezing platelets on agonist and antagonist binding to α_2-adrenoceptors.

Blood (30-60 ml) was obtained from the anticubital vein of healthy drug-free subjects and anticoagulated by addition of 1/10 vol 1% EDTA. The platelets were isolated by differential centrifugation and either stored frozen (-20°C) for 2-12 days or used immediately. Membranes were prepared by homogenisation in cold hypotonic buffer (5mM Tris; 5mM EDTA; pH7.5) and washed by repeated centrifugation and homogenisation prior to resuspension in the assay buffer (25mM glycylglycine; 10mM $MgCl_2$; 1mM EGTA; pH7.5). α_2-Adrenoceptor binding characteristics were determined using the antagonist ligand ^{3}H-yohimbine and the agonist ligand ^{3}H-UK 14304.

	Binding Capacity (fmol/mg protein)	
	Fresh tissue	Frozen tissue
^{3}H-Yohimbine	240 ± 23	331 ± 34 *
^{3}H-UK 14304	77 ± 6	51 ± 8 *

Table 1: α_2-adrenoceptor binding capacity in fresh and frozen human platelet tissue. Mean + sem (n=7). *P<0.05 Student's paired t-test v Fresh tissue.

Freezing of the platelets caused a significant decrease in the binding capacity of ^{3}H-UK 14304 but a significant increase in the binding capacity of ^{3}H-yohimbine, when expressed in terms of unit protein content. However when expressed in terms of sites per cell the binding capacity of both ligands were reduced by freezing. The binding affinity of each ligand was unchanged by freezing. Displacement of ^{3}H-yohimbine binding by unlabelled UK 14304 in fresh and frozen tissue indicated a substantial decrease in inhibitory potency associated with freezing (IC_{50} (fresh) = 290nM; IC_{50} (frozen) = 1702nM). Similar analysis using the antagonist idazoxan indicated no such change in affinity after freezing (IC_{50} (fresh) = 1.5nM; IC_{50} (frozen) = 1.0nM).

We conclude that freezing of human platelet tissue causes a preferential loss of agonist high-affinity binding at the α_2-adrenoceptor. This effect is apparent both as a decrease in agonist radioligand binding capacity and as a decrease in the competitive affinity of agonists.

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BINDING SITES FOR [^{3}H]-NECA ON NG108-15 CELL MEMBRANES ARE NOT ADENOSINE A_2 RECEPTORS

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The neuroblastoma x glioma hybrid cell line NG108-15 expresses adenosine A_2 receptors which mediate stimulation of adenylate cyclase activity. In a previous study (Keen et al. 1988), we have shown that these cells possess a specific binding site for [^{3}H]-N-ethylcarboxamidoadenosine (NECA), an A_2 receptor agonist, which exhibits many of the properties expected for an adenosine A_2 receptor. In this study we have carried out a detailed comparison of the binding properties of the [^{3}H]-NECA site with the pharmacology of the A_2 receptor mediated stimulation of adenylate cyclase activity, to determine whether or not the [^{3}H]-NECA site is really an A_2 receptor.

NG108-15 cells (passage 16-25) were grown to confluency in 80 cm^2 flasks. In pretreatment studies cells were then cultured for a further 24h in the presence of 10μM NECA, 100μM (L-2-phenylisopropyl)-adenosine (PIA) or the appropriate vehicle. Cells were harvested, washed three times to remove any drug and then frozen at -80°C. Adenylate cyclase activity and [^{3}H]-NECA binding activity were measured in homogenates of these cells as described by Keen et al. (1988).

In NG108-15 cell homogenates adenosine analogues stimulate adenylate cyclase activity with the following order of potency (EC_{50} values are shown in brackets): NECA (0.5μM) 2-chloroadenosine (3μM) PIA = cyclohexyladenosine = 2-phenylaminoadenosine (30μM). The adenosine receptor antagonists 3-isobutyl-1-methylxanthine (IBMX), theophylline and caffeine inhibit NECA stimulated adenylate cyclase activity, with Ki's of ~40, 100 and 500μM respectively.

As previously reported (Keen et al. 1988) [^{3}H]-NECA binds to an apparently homogeneous population of saturable sites in NG108-15 cell homogenates, with an affinity of ~1μM and a Bmax of 10pmol/mg protein. Specific ^{3}H-NECA binding is displaced by unlabelled NECA, 2-chloroadenosine, IBMX and theophylline, with IC_{50}s of ~1,10,100 μM and 300 μM respectively. However, PIA, cyclohexyladenosine and 2-phenylaminoadenosine produce no significant inhibition of ^{3}H-NECA binding at concentrations (1mM) that produce a maximal stimulation of adenylate cyclase activity.

Pretreatment of NG108-15 cells with either NECA or PIA results in a pronounced homologous desensitization; subsequent responses to all the adenosine analogues are reduced by up to 90%, while responses to iloprost and NaF are unaffected. This is strong evidence that all the adenosine analogues examined act via activation of the same receptor.

[^{3}H]-NECA binding is not inhibited by all the adenosine agonists used here. However, from the desensitization studies it is clear that all these agonists do act at the same receptor. Therefore the [^{3}H]-NECA binding site cannot represent the A_2 receptor.

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NG108-15 AND PC12 CELLS CONTAIN AN ATYPICAL MUSCARINIC RECEPTOR

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Ligand binding studies indicate the existence of 3 muscarinic receptor subtypes, termed M1, M2 and M3 (De Jonge et al., 1986). M1 receptors are present in cerebral cortex and have high affinity for pirenzepine. M2 receptors are present in heart and have low pirenzepine affinity and high affinity for methoctramine and AF-DX 116. The M3 receptor occurs in gland tissue and displays low affinity for AF-DX 116 and methoctramine. Previously we have identified an atypical receptor present in the PC12 rat pheochromocytoma cell line (Michel et al., 1988). In the present study we report that this atypical muscarinic receptor is also present in the NG108-15 neuroblastoma x mouse glioma cell line.

Muscarinic receptors in PC12 and NG108-15 cell membranes were labeled with [^{3}H]N-methylscopolamine (0.1 nM) by incubation in 1 ml of Tris-EDTA buffer (Michel & Whiting, 1988) for 2 h at 32^{o}C. Incubations were terminated by vacuum filtration using a Brandel cell harvester. Non specific binding was determined using 1 uM atropine. Data were analysed using iterative curve fitting techniques. Affinity estimates for the M1, M2 and M3 receptor were obtained as described in Michel & Whiting (1988).

LIGAND	CORTEX (M1)	HEART (M2)	GLAND (M3)	PC 12	NG108-15
AF-DX 116	6.5 (1.0)	6.7 (0.9)	5.6 (0.9)*	6.5 (1.1)	6.6 (0.9)
4-DAMP	9.2 (1.0)	8.4 (1.0)*	9.0 (1.1)	9.0 (0.9)	9.0 (1.0)
METHOCTRAMINE	7.6 (1.0)*	8.7 (0.9)*	6.8 (1.0)*	8.0 (0.9)	8.2 (0.9)
PIRENZEPINE	8.3 (0.9)*	6.6 (1.0)*	7.0 (0.9)	7.1 (1.0)	7.2 (0.9)

The ligand was [^{3}H]NMS except in the M1 assay where [^{3}H]pir was used.
Values are pKi (nH). SEM < 7%. n=4. nH values not different ($p<0.05$) to 1.
* pKi value different ($p<0.05$) to that obtained in the NG108-15 cells.

Antagonist affinity estimates in the NG108-15 cells were not consistent with the presence of either an M1, M2 or M3 muscarinic receptor. The affinity estimates in the NG108-15 cells were however similar to those obtained in PC12 cells. Furthermore the muscarinic receptor in both the PC12 and NG108-15 cell lines was capable of interacting with a pertussis toxin substrate. Thus, in control PC12 cells carbachol produced pKI (5.9$\pm$0.05) and nH (0.5$\pm$0.03) values that differed significantly from the pKi (5.2$\pm$0.07) and nH (0.8$\pm$0.04) values obtained in PC12 cells exposed to 30 ng/ml of pertussis toxin for 18 hr. Pertussis toxin produces similar effects in the NG108-15 cell line (Evans et al., 1985). These data suggest the presence of an atypical muscarinic receptor in both the PC12 and NG108-15 cell lines. Whether the receptor corresponds to that expressed by the m4 receptor gene described by Bonner et al., (1987) awaits further study.

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GUANINE NUCLEOTIDES MODULATE MUSCARINIC STIMULATION OF PHOSPHOINOSITIDE METABOLISM IN PERMEABILIZED NEUROBLASTOMA (SH-SY5Y) CELLS

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Several lines of evidence indicate that a guanine nucleotide-binding (G) protein, termed Gp, is involved in coupling activated receptors to phospholipase C and thus to increased phosphoinositide breakdown. In intact cells, the binding of GTP to Gp is thought to be an essential part of this process. We describe here, in a neuronal cell line, the effects of permeabilization on activation of phospholipase C by muscarinic agonists and the effects of GTPγS, a hydrolysis-resistant analogue of GTP, on the muscarinic responses. Suspensions of [^{3}H]-inositol-labelled SH-SY5Y cells were permeabilized ('electroporated') at 4°C in a cytosol-like medium containing 2 mM ATP, 0.5 mg/ml bovine serum albumin and 0.1 mM EGTA by exposure to 12 discharges of a 3 μF capacator (field strength, 3.75 KV/cm; time constant, 0.1 msec). Intact or permeabilized cells were then incubated with various stimuli in the same medium at 37°C for 5 min.

In intact cells, [^{3}H]-inositol phosphate production (a measure of phosphoinositide breakdown) was stimulated by carbachol and, to a lesser extent, by arecoline (Table 1). Electroporation reduced the maximal effects of both agonists and significantly ($P < 0.001$) altered the EC_{50} of the response to carbachol but not to arecoline (Table 1). Consistent with a role for Gp in receptor-mediated activation of phospholipase C, GTPγS markedly enhanced the effects of both agonists (Table 1). However, the maximal response to carbachol was enhanced 2.8 fold and that to arecoline 5.4 fold by GTPγS. Furthermore, the EC_{50} for carbachol was significantly decreased ($P < 0.001$) from 46 ± 15 μM to 4 ± 1 μM by GTPγS whereas that for arecoline was not significantly altered. Only GTP analogues and GTP supported the effect of carbachol; GDP and UTP were without effect and GDPβS inhibited the muscarinic response.

Thus, in comparison to intact cells, permeabilized cells in the presence of GTPγS are more responsive to muscarinic agonists, manifested in the case of carbachol (an apparent full agonist) by a lower EC_{50} and a small (1.4 fold) increase in maximal response and in the case of arecoline (an apparent partial agonist) by no significant change in EC_{50} and a large (2.3 fold) increase in maximal response. These effects suggest that the relationship between receptor occupation and phosphoinositide hydrolysis can be markedly influenced by the level of Gp activity and that this may subserve the receptor-reserve phenomena noted in certain tissues.

Table 1 Agonist-stimulated inositol phosphate production in the absence or presence of 100 μM GTPγS (> 3 independent experiments; mean ± SD)

	Carbachol				Arecoline			
	-GTPγS		+GTPγS		-GTPγS		+GTPγS	
	Max.	EC_{50} (μM)	Max.	EC_{50} (μM)	Max.	EC_{50} (μM)	Max.	EC_{50} (μM)
Intact cells	100	17±6	-	-	45±5	8±2	-	-
Permeabilized cells	49±4	46±15	139±14	4±1	19±4	5±2	103±12	3±1

Max., maximum effect of agonist (in %) normalized to maximal stimulation by carbachol in intact cells in the absence of GTPγS.

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MUSCARINIC RECEPTORS COUPLED TO PHOSPHOINOSITIDE HYDROLYSIS AND ELEVATED CYTOSOLIC CALCIUM IN A HUMAN NEUROBLASTOMA CELL SH-SY5Y

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Muscarinic receptor occupation leads to a multiplicity of intracellular events with end-organ responses as diverse as secretion and muscle contraction. The present study reports phosphoinositide hydrolysis and elevated cytosolic calcium coupled to M_3 muscarinic receptors expressed by a human neuroblastoma cell line SH-SY5Y.

[^{3}H]-N-methylscopolamine ([^{3}H]-NMS) binding to suspension cultures of whole SH-SY5Y cells were performed in Krebs/Henseleit (KH) buffer at 37°C over 20 min periods. Bound and free ligand were separated by vacuum filtration. Non-specific binding was defined in the presence of 1 µM atropine. Inositol phosphate (InsP) production was measured at 37°C in KH using cells either preincubated for 24 hr with 4 µCi/ml [^{3}H]-inositol in the absence of lithium (individual phosphates), or preincubated for 1 hr and subsequently stimulated in 4 µCi/ml [^{3}H]-inositol in the presence of 5 mM lithium (total phosphates). InsPs were separated by Dowex anion exchange chromatography. Intracellular calcium measurements were made at 37°C in SH-SY5Y cells incubated in KH for 45 min with 5 µM Fura 2AM and calculated according to Grynkiewicz et al. (1985).

The binding of [^{3}H]-NMS to SH-SY5Y cells was saturable with a K_d of 0.203 ± 0.026 nM and a B_{max} of 145.98 ± 32.86 fmol/mg protein (mean ± SEM, n = 3). [^{3}H]-NMS binding was displaced by pirenzepine and AFDX-116 with low affinity. 4-DAMP displaced with high affinity and all slope factors were unity. These data coupled with the absence of any high affinity specific [^{3}H]-pirenzepine binding suggests these cells may express an homogenous M_3 receptor population. Carbachol increased total [^{3}H]-InsP production dose-dependently with an EC_{50} of 7.5 µmol/l. Maximum stimulation of some 34-fold (116 ± 5 to 4059 ± 278 dpm, n = 3) occurred in response to 1 mM carbachol. Carbachol increases [^{3}H]-inositol mono-, bis-, tris- and tetrakis-phosphates by 139.3 ± 23.1, 56.1 ± 14.7, 75.0 ± 6.2 and 46.3 ± 6.3% above basal (n = 3) respectively after 5 min. [^{3}H]-$InsP_3$ accumulation reached maximum after 5-10 sec. Carbachol (1 mM) produces a muscarinic receptor-mediated biphasic increase in intracellular calcium (69.1 ± 8.2 to 614.9 ± 62.5 nM, n = 3) spiking at 8.67 ± 1.20 sec (n = 3) then declining to a maintained plateau phase (203.1 ± 6.9 nM, n = 3) some 30-60 sec later which persists for at least 6 min. The EC_{50} values for carbachol stimulated spike and plateau were 9.4 and 0.8 µM respectively. The plateau phase was dependent upon extracellular calcium and replacing extracellular calcium with 0.1 mM Mn^{2+} causes a carbachol-stimulated quench at both 340 and 380 excitation wavelengths at time points corresponding to the plateau phase.

These data show that M_3 muscarinic receptor stimulation leads to a rapid increase in $InsP_3$, intracellular calcium mobilisation and calcium entry across the plasma membrane of a neuronal cell line. Differences in the potency of carbachol to induce the two components of calcium homeostasis could suggest separate controlling mechanisms.

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PHORBOL ESTERS RELAX ILEAL SMOOTH MUSCLE THROUGH ACTIVATION OF THE SODIUM/POTASSIUM PUMP

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The role of protein kinase C (PKC) in agonist-induced contractions of guinea-pig ileum longitudinal smooth muscle has been investigated.

The phorbol esters, phorbol 12,13-dibutyrate (PDBu), phorbol 12,13-diacetate (PDA) and phorbol 12-myristate 13-acetate (PMA), relaxed tissues pre-contracted by sub-maximal concentrations of carbachol. The EC50 values for PDBu, PDA or PMA were 0.086 μM, 0.530 μM and 3.53 μM, respectively. These phorbol esters also induced a similar inhibition of contractions induced by sub-maximal concentrations of histamine and substance P. 4-Alpha phorbol produced little relaxation.

The addition of ouabain (1 μM), an inhibitor of Na^+-K^+-ATPase, after phorbol ester-induced relaxation caused a rapid reversal of this inhibition and the tension quickly rose to a level exceeding that in the control tissues. Higher concentrations of ouabain (3 - 10 μM) produced a more rapid reversal of the relaxation. Similarly, pre-treatment with ouabain inhibited the ability of phorbol esters to relax tissues pre-contracted by the above agonists. The slow relaxation of the tonic component of contraction induced by sub-maximal concentrations of carbachol and histamine, and all concentrations of substance P, was abolished in the presence of ouabain.

Since the above data suggest the involvement of the Na^+-K^+-ATPase pump in the inhibition of the tonic component of contractions by phorbol esters, we decided to investigate the action of PDBu on Na^+-K^+-ATPase analysing ouabain-sensitive $^{86}Rb^+$-uptake. Preliminary experiments revealed, however, that more than 90 % of the basal uptake was insensitive to ouabain. To increase the size of the ouabain-sensitive component, tissues were loaded with Na^+ by pre-incubation in K^+-free solution for 3 h. This resulted in almost total loss of tissue K^+ (as measured by atomic absorption spectrophotometry). The extracellular K^+ concentration during the uptake was maintained at 1 mM to avoid maximal pump activation.

PDBu caused a concentration-dependent increase in ouabain-sensitive $^{86}Rb^+$-uptake over a period of 4 min. A maximally effective concentration of PDBu (3 μM) stimulated the uptake by 23.9 ± 3.8 % above basal ($p < 0.01$). The EC50 for this action of PDBu was 225 nM which is in good agreement with the value for inhibition of the tonic component of contraction described above. 4-Alpha phorbol had no significant effect on the uptake. Na^+-extrusion from the Na^+-loaded tissues during 4 min exposure to 1 mM-K^+ was significantly enhanced by PDBu (3 μM).

Carbachol (0.1 mM) also increased the pump activity of these Na^+-loaded tissues by 16.2 ± 3.8 % above basal ($p < 0.01$), although lower concentrations had no significant effect. When activated by 3 μM-PDBu (a maximally effective concentration), the Na^+-K^+-pump was no longer stimulated by the addition of carbachol (0.1 mM).

We conclude that phorbol esters inhibit the tonic component of contractions induced by sub-maximal concentrations of the agonists through activation of Na^+-K^+-ATPase, and suggest that PKC may exert a feedback control over the tonic component of agonist contractions through the stimulation of this pump.

PHOSPHATIDYL-INOSITOL HYDROLYSIS BY PHOSPHOLIPASE C FROM HUMAN LUNG

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We have described a phospholipase activity in cell-free extracts of human lung capable of hydrolysing phosphatidyl-inositol (PI), labelled with ^{14}C-arachidonic acid (AA) (Bakhle & Yeats, 1986). Two metabolites, free AA and labelled diglyceride (DG), were formed from this substrate and not from similarly labelled phosphatidyl-choline or -ethanolamine, suggesting the presence of a PI-selective phospholipase C (PLC). The highest specific activity for PI hydrolysis was found in the microsomal fraction and we have further studied the effects of varying concentrations of H^+ and other cations on the PLC-like activity in this microsomal fraction.

Under standard incubation conditions (substrate PI, 15.5µM, 0.09µCi; microsomal protein, 200µg/ml; 5mM Ca^{++}, 10mM Tris-HCl buffer, pH 7.4; 40 min incubation), about 60pmoles AA and 250pmoles DG were formed. The sum of the two products was taken as representing total hydrolysis of PI and the ratio of AA:DG was also recorded. The effect of each condition was assayed at least six times using three lung preparations. Over a range of pH (4-9), total hydrolysis increased to a maximum, at pH 6, of nearly 3-fold standard values (i.e., at pH 7.4) and a minimum at pH 9 of 49±6% of standard values. The AA:DG ratio was essentially unchanged over this range except at pH 9 when it increased to almost twice its standard value.

Hydrolysis of PI required Ca^{++}: at low concentrations of Ca^{++}, i.e., addition of 25mM Na_2EDTA to the incubation mixture, hydrolysis was only 11±3% of standard values, but at 2.5 and 9.5mM Ca^{++}, hydrolysis was almost identical to that at 5mM Ca^{++}. However, addition of CaEDTA (5mM) increased PI breakdown by 35%. Three other chelating agents, o-phenanthroline, 8-hydroxyquinoline and 2,2-dipyridyl, at much lower concentrations (10µM-1mM), gave much larger increases in hydrolysis, up to 120%. No inhibition of PLC activity was observed at higher concentrations, 25mM 2,2-dipyridyl giving a 22% increase in PI hydrolysis. Addition of $ZnCl_2$ inhibits macrophage PLA_2 (Finnen et al 1986). In our human lung microsomes, the addition of $ZnCl_2$ (final concentration, 10µM-1mM) strongly inhibited total hydrolysis of PI, to 26±3% of standard values at 1mM, and increased the AA:DG ratios almost 3-fold.

We conclude from these results that, although human lung PLC requires Ca^{++} for activity, other cations including Zn^{++} have important effects on PI hydrolysis. This suggests an additional ionic control mechanism for the production of free AA and of DG in human lung and perhaps in other tissues.

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THE ADENOSINE RECEPTORS MODULATING THE ACCUMULATION OF INOSITOL PHOSPHATES AND CYCLIC AMP IN BRAIN SLICES ARE DIFFERENT

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We have shown previously that adenosine, although ineffective alone, can augment histamine-stimulated inositol phospholipid hydrolysis in guinea-pig cerebral cortical slices (Hill & Kendall, 1987). Adenosine can also markedly stimulate cyclic AMP accumulation in this tissue (Sattin & Rall, 1970). The aim of the present study was to investigate whether there were differences between the receptors mediating these two effects.

Agonist-induced accumulation of [^{3}H]-cyclic AMP and [^{3}H]-inositol phosphates was measured in slices of guinea-pig or mouse cerebral cortex as we have described previously (Donaldson et al., 1988, Hill & Kendall, 1987). Antagonists were added 20 - 40 min prior to the addition of agonist.

2-Chloroadenosine (2CA), a stable analogue of adenosine, stimulated [^{3}H]-cyclic AMP accumulation in guinea-pig cerebral cortical slices with an EC_{50} of 39 $\pm$ 4 μM (n=7). Five xanthine-based compounds antagonised this 2CA-mediated cyclic AMP response yielding the following apparent dissociation constants (K_D, μM, n=3): 0.082 $\pm$ 0.005 (8-cyclopentyl-1,3-dipropylxanthine, DPCPX), 0.20 $\pm$ 0.03 (1,3-diethyl-8-phenylxanthine, DPX), 0.40 $\pm$ 0.20 (1,3-dimethyl-8-phenylxanthine, 8PT), 2.3 $\pm$ 0.2 (8-cyclopropyl-1,3-dimethylxanthine, CPT), and 40 $\pm$ 8 (7-benzyl-3-(2-methylpropyl)-xanthine, BMPX). These compounds also antagonised the augmentation of the histamine-stimulated accumulation of [^{3}H]-inositol phosphates produced by 2CA (EC_{50} 9.9 $\pm$ 2.5 μM, n=10) with K_D values (μM, n=3) of: 0.010 $\pm$ 0.002 (DPCPX), 0.5 $\pm$ 0.1 (CPT), 0.6 $\pm$ 0.2 (DPX), 0.8 $\pm$ 0.1 (8PT), 5.0 $\pm$ 1.6 (BMPX). Thus, while DPX and 8PT were slightly more potent as antagonists of the cyclic AMP response, DPCPX and BMPX were almost an order of magnitude more potent as antagonists of the inositol phosphate response. Altering the assays to standardise conditions (i.e. agonist incubation times of 40-45 min, histamine (0.3 mM), cimetidine (0.3 mM), and adenosine deaminase (1 U/ml) present in the incubation medium, testing for effects of LiCl) led to no alteration in the selectivity of the latter two compounds. These compounds also showed a similar selectivity in mouse cerebral cortical slices for the inositol-phosphate related response, through which adenosine selectively inhibits histamine-stimulated [^{3}H]-inositol phosphate accumulation (Kendall & Hill, 1988).

Other agents that stimulated cyclic AMP accumulation (VIP, 1μM, or forskolin, 1 μM, n=3) were virtually inactive compared to 2CA when assayed for effects on histamine-stimulated [^{3}H]-inositol phosphate accumulation in both mouse and guinea-pig cerebral cortex, as was 8-bromo-cyclic AMP (1mM, n=3).

The results of this study therefore suggest that the adenosine receptor mediating the stimulation of cyclic AMP accumulation in guinea-pig or mouse brain slices is different from those mediating augmentation or inhibition of histamine-stimulated inositol phosphate accumulation.

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ALCOHOLS ACTIVATE PHOSPHOINOSITIDASE C IN TURKEY ERYTHROCYTE MEMBRANES

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It is now generally accepted that phosphoinositidase C plays a pivotal role, via a guanine-nucleotide sensitive mechanism, in the production of the two second messengers inositol 1,4,5-triphosphate and diacylglycerol. We have shown previously in intact hepatocytes that ethanol stimulates the formation of inositol 1,4,5-triphosphate and the mobilization of intracellular calcium (Hoek et al. 1987). In the present study we have examined the effects of alcohols on phosphoinositidase C activity in turkey erythrocytes.

Turkey erythrocyte ghosts were prepared by cell lysis of erythrocytes which were prelabelled with [^{3}H] inositol (Harden et al. 1987). Incubations were performed in the absence of ATP or a nucleotide regenerating system and terminated by addition of perchloric acid (7%). Both [^{3}H] inositol phosphates (InsP) and deacylated inositol phospholipids were separated by anion exchange chromatography methods.

Guanosine 5' - [γ-thio]triphosphate (GTP[S]) (300 μM) stimulated the production of both $InsP_2$ (16 fold) and $InsP_3$ (6 fold) in this system. No stimulations of $InsP_1$ or $InsP_4$ were observed. The accumulation of $InsP_2$ and $InsP_3$ were linear for 10 min. with an initial 2 min. lag period. Ethanol (300 mM) reduced the lag period for InsP formation at submaximal GTP[S], and caused a shift to the left (3 fold) in the dose response curve. In the absence of guanine-nucleotides ethanol failed to produce any stimulation of InsP formation. Higher alcohols 1-propanol (200 mM) and 1-butanol (50 mM) also enhanced the accumulation of InsPs produced by submaximal GTP[S] concentrations. Receptor activation by adenosine 5' [β-thio]diphosphate (ADPβS) (10 μM) shifted the GTP[S] dose response to the left (10 fold) and increased the maximal response (2.3 fold). None of the alcohols tested affected the response to receptor activation in the presence of GTP[S]. The formation of InsPs was associated with a concomitant decreased labelling in both phosphatidylinositol monophosphate and phosphatidylinositol bisphosphate, but no decrease in phosphatidylinositol was observed.

Similarly, the dose response to guanosine 5-[$\beta\gamma$-imido]triphosphate (GppNHp) for InsP formation was displaced to the left by ethanol (300 mM) and ADPβS (10 μM) (2 and 6 fold) respectively. ADPβS also enhanced (1.6 fold) the maximal response to GppNHp. The response to ADPβS in the presence of GppNHp was also unaffected by alcohol addition. Significantly, the InsP formation produced in response to sodium fluoride (1-30 mM) was unaffected by either ethanol or receptor activation.

It is clear from these results that ethanol initiates an activation of phosphoinositidase C which is dependent upon the maintenance of a functional guanine nucleotide-binding protein. The precise mechanism by which this is achieved remains to be established.

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NITRENDIPINE DECREASES THE ELECTROPHYSIOLOGICAL CHANGES IN VITRO WHICH FOLLOW CHRONIC TREATMENT WITH ETHANOL IN VIVO

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The dihydropyridine calcium channel antagonist, nitrendipine, given concurrently with ethanol in chronic treatment, prevented the ethanol withdrawal syndrome and development of tolerance to ethanol (Whittington & Little, 1988; Little & Dolin, 1987). The increase in dihydropyridine binding sites found after chronic ethanol administration was also prevented, suggesting a causal relationship between the binding site changes and dependence on ethanol. (Dolin & Little, 1988). Acute administration of calcium channel antagonists also prevented the ethanol withdrawal syndrome (Little *et al.*, 1986). We have now studied the effects of nitrendipine, given during the chronic ethanol treatment or added to the perfusion medium, on the changes in field potentials in the hippocampal slice seen following chronic ethanol treatment (Whittington & Little, this meeting).

Male mice, C57 strain, 25 - 30g, were given ethanol, 24% v/v, as sole fluid, for twelve weeks. For the chronic nitrendipine treatment the compound was dissolved in the drinking fluid at 1.77mM; this did not alter the ethanol intake. Nitrendipine intake was 50-100 mg/kg/day. Controls drank water only. In separate experiments, nitrendipine was included in the perfusion medium at 10μM, throughout the recordings, after being dissolved in DMSO (the final DMSO concentration, 0.02%, did not alter the field potentials). Hippocampal slices were made between 9 and 10am (animals were not withdrawn from the ethanol) and perfused with standard Ringer solution. Extracellular recordings from CA1 area were started 30 min later, using orthodromic stimulation of Schaffer collateral fibres. Thresholds for production of first and multiple population spikes and paired pulse potentiation (70 msec interval at 1.25 x threshold stimulation) were measured every 15 min and input/output curves determined at 1, 3, 5, and 7h from start of recording. Results were recorded on videtape and the areas of the population spikes measured by computer analysis. Results, expressed as mean ± s.e.m. were compared by analysis of variance (n=5 each group).

The decreases in the thresholds for elicitation of single and multiple population spikes, seen in slices from animals treated with ethanol alone, from 3h onwards, compared with controls, were reduced, but not abolished, after both chronic nitrendipine treatment and nitrendipine in the perfusion medium ("Perf.nitr."). The increase in paired pulse potentiation, seen in slices from ethanol treated animals, between 2 and 4h from start of recording only, was also decreased by chronic nitrendipine administration and by nitrendipine in the medium.

(h=from tissue removal)	Controls	Ethanol	Eth + Chr. nitr	Eth + Perf.nitr.
Threshold single spike, μA (6h)	21.6±4.7	15.5±1.4	19.4±1.7	17.7±1.1
Threshold mult. spike, μA (6h)	231±7	186±4	212±7	202±5
Paired pulse potentiation (2 h) (% second spike/first spike)	204±8	363±48	268±25	309±16

P values:	control v. eth.	eth. v. eth.+ chr. nitr.	eth. v. eth.+ perf. nitr.
Single spikes (4-7h):	<0.01	<0.02	<0.02
Mult. spikes (4-7h):	<0.002	<0.002	<0.01
Paired pulse potn. (1-4h):	<0.002	<0.002	<0.01

These results show that nitrendipine, when given chronically during ethanol administration or acutely on withdrawal, can decrease, but not prevent, the changes seen in the field potentials in the hippocampal slice. This may indicate that changes such as these may be partially responsible for the ethanol withdrawal syndrome.

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NIFEDIPINE AND NIMODIPINE ARE EFFECTIVE AGAINST PENTYLENETETRAZOLE-INDUCED SEIZURES IN MICE

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The search for non-sedative anticonvulsants may lead to calcium antagonists. Initiation of seizures is dependent on calcium influx (Wong & Prince, 1979) and is inhibited by these drugs (Witte et al, 1987). Limited patient trials show promising results with flunarizine (Overweg et al, 1986) and nifedipine (Larkin et al, 1988) despite doubts over the latter's penetration of the blood-brain barrier. We have studied nifedipine and nimodipine in pentylenetetrazole (PTZ)-induced seizures in mice to support projected clinical trials.

Methods: CF1 mice weighing 20g-35g were used. Brain and blood levels of nifedipine were measured in 8 mice (20g) after 6mg/kg i.p. For tests of efficacy, PTZ (0.85%, 0.1ml/10g body weight) was injected subcutaneously and animals monitored. Time to first clonic jerk was noted as "seizure latency" (SL). Exp.1: the PTZ test was performed in 30g mice 10 min after i.p. nifedipine (0.1 mg), nimodipine (0.1mg), carbamazepine (0.2mg) or control solvent 50:50 ethanol:water. Carbamazepine + nifedipine vs monotherapy was tested separately. Exp.2: animals (30-35g) were fed added nifedipine or nimodipine (1mg, 2mg or 4mg daily - approximately 30, 60, 120 mg/kg) or normal feed alone for 2 weeks before PTZ test was performed. Statistics: Krustal-Wallis and Mann-Whitney U test.

Results: Nifedipine entered mouse brain and a correlation between blood and brain levels was found ($r = 0.8$, $n = 8$). Both oral and parenteral antagonists increased seizure latency as did parenteral carbamazepine (Table 1). Nifedipine + carbamazepine (SL $\pm$ s.e., mean 240 $\pm$ 35s) was no better than nifedipine (325 $\pm$ 16s) or carbamazepine (263 $\pm$ 17s) alone. The highest oral dose of dihydropyridine - 4mg/day (183 $\pm$ 17s) - was less effective than 2mg (330 $\pm$ 71s, $p<0.05$) or 1mg (392 $\pm$ 57s, $P<0.002$).

Table 1: PTZ seizure latency in seconds (mean $\pm$ s.e. mean) after i.p. or chronic oral dosing

	Control	Nifedipine	Nimodipine	Carbamazepine
Parenteral Administration	185 $\pm$ 12 (n=22)	318 $\pm$ 23 *** (n=18)	276 $\pm$ 32 * (n=15)	294 $\pm$ 18 *** (n=20)
Oral Administration	96 $\pm$ 16 (n=10)	277 $\pm$ 47 ** (n=14)	322 $\pm$ 49 *** (n=15)	-

vs Controls *P<0.02 ** P<0.002 *** P<0.001

This study confirms the efficacy of oral and parenteral dihydropyridine calcium antagonists in an epilepsy model responsive to carbamazepine. Over-dosage may diminish effectiveness. Controlled trials of both these drugs are underway in epileptic patients.

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THE ACTIONS OF THE NON-STEROIDAL ANTI-INFLAMMATORY DRUG, MEFENAMIC ACID WITH THE $GABA_A$ RECEPTOR

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Mefenamic acid is a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of dysmenorrhoea. Whilst therapeutic doses of the drug may produce CNS effects which include drowsiness, overdosage is associated with generalised convulsions (Balali-Mood, et al., 1981), occasionally preceeded by coma (Gössinger et al., 1982). Convulsions can be induced by antagonists of the $GABA_A$-benzodiazepine receptor complex, such as bicuculline and picrotoxin, whereas sedation may occur to drugs which potentiate the actions of GABA on this site. In the present study, we have evaluated the effects of mefenamic acid on $GABA_A$ receptor mediated chloride currents recorded from bovine adrenomedullary chromaffin cells maintained in cell culture (Cottrell et al., 1985).

Currents evoked by locally applied GABA (100 µM, 10-50 ms, 1.4×10^5 Pa) were recorded at a holding potential of -60 mV, and ambient temperature (20-22°C), from isolated chromaffin cells held in vitro for 1-7 days (Cottrell et al., 1987). Mefenamic acid, bath applied for 10 min, depressed the amplitude of GABA-evoked currents in a reversible and concentration-dependent manner, 3, 10 & 100 µM of the drug reducing the response to 47.2 ± 8.3% (n=5), 26.5 ± 5.4% (n=10) and 0.0 ± 0.0% (n=4) of control respectively. In 6 of 10 cells challenged with 10 µM mefenamic acid, the reduction in the GABA current was preceeded by a small potentiation of its amplitude (to 130.9 ± 10.7% of control), this effect occurring shortly (2-3 min) after the introduction of the drug to the bath. Throughout the application of the drug the duration of the GABA-evoked current was substantially prolonged, the time to half decay being increased to 277.0 ± 39.0% (n=5) and 376.1 ± 30.0% (n=9) of control after a 10 min incubation with 3 and 10 µM mefenamic acid respectively. The relative changes in amplitude and time course of the GABA-evoked current were such that the total charge passed during a response was increased to 121.1 ± 21.1% (n=5) of control in the presence of 3 µM mefenamic acid, but depressed to 76.3 ± 16.4% (n=9) of control by a higher concentration (10 µM) of the drug. The effects of mefenamic acid did not involve a change in the reversal potential of the GABA response and were not blocked by the benzodiazepine receptor antagonist flumazenil (1 µM). In contrast to the complex actions of mefenamic acid, the convulsants bicuculline (3 µM) and picrotoxin (3 µM) reversibly depressed the amplitude of GABA-evoked currents to 9.2 ± 1.9% (n=10) and 26.1 ± 4.4% (n=4) of control respectively with no evidence of a transient potentiation or increase in their duration.

Unlike mefenamic acid, the structurally distinct NSAID fenbufen (100 µM) had little influence upon currents evoked by GABA, producing only a small suppression of response amplitude (to 92.9 ± 5.5% (n=5) of control). However, indomethacin (100 µM) reversibly reduced the amplitude of the GABA-evoked current to 44.3 ± 9.1% (n=4) of control in the absence of an obvious effect upon its time course.

In conclusion, the modulatory actions of mefenamic acid on the $GABA_A$ receptor may contribute to the reported sedative and convulsive properties of the drug.

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DIFFERENTIAL DISTRIBUTION IN BOVINE BRAIN OF MRNAS ENCODING DISTINCT $GABA_A$ RECEPTOR α SUBUNITS

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Following the elucidation of the primary structure of the α and β subunits of the $GABA_A$ receptor (Schofield *et al*, 1987) recent work has revealed the presence, in bovine brain, of two additional mRNA species (designated α2 and α3, collaboration with P R Schofield and P H Seeburg), with sequences distinct from, but closely related to, that encoding the α-subunit. Complementary 45 base oligonucleotides were constructed to unique sequences from each of these α-subunit mRNAs. These were used to localise the corresponding mRNA in bovine brain by *in situ* hybridisation histochemistry. The oligonucleotide probes were labelled using ^{32}P-dATP or ^{35}S-dATP and terminal transferase. Cryostat sections (12μm) were processed according to standard procedures (Brandt *et al*, 1987; Morris *et al*, 1988) and hybridised overnight with the probe in buffer containing 50% formamide at 42°C. After stringent washing, the sections were exposed to x-ray film or dipped in photographic emulsion. Sections were obtained from at least three different animals.

All three α-subunit probes yielded a strong hybridisation signal in the cerebral cortex. Whereas the α1- and α2- transcripts were located predominantly in layers II to IV, the α3- transcript was found mainly in layers V and VI. In the hippocampal complex, the levels of α1 mRNA were much lower in the dentate gyrus and Ammon's horn than in the subiculum, while α2mRNA was found to be concentrated in the dentate gyrus and hilus. Levels of α3 mRNA in the hippocampus appeared to be low. In all the brain regions, a non-complementary oligonucleotide, used under identical conditions, showed no hybrisation signal.

Since there is evidence that the various α subunits confer functional differences on the $GABA_A$ receptor (Levitan *et al*, 1988), this differential distribution suggests that there are different subtypes of the $GABA_A$ receptor in different regions of bovine brain.

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CHARACTERISATION OF 3-AMINOPROPYL PHOSPHINIC ACID (3-APPA) AS A $GABA_B$ AGONIST IN RAT BRAIN TISSUE

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$GABA_B$ sites are present in mammalian brain and appear to be of physiological importance (Dutar & Nicoll, 1988). These sites can be readily detected in the central nervous system using conventional radioligand binding techniques and receptor autoradiography (Bowery et al., 1983, 1987). Moreover, functional responses to $GABA_B$ site activation can be assessed by examining changes in cellular cAMP. The most selective agonist used so far to define $GABA_B$ sites is (-)-B-p-chlorophenyl GABA (baclofen) but recent reports by Dingwall et al. (1987) and Bittiger et al. (1988) indicate that 3-APPA may be an agonist with higher affinity for $GABA_B$ sites. The present study was performed to establish and characterise this interaction.

A comparison of the relative potencies of both baclofen and 3-APPA in displacing [^{3}H]GABA binding to both $GABA_A$ and $GABA_B$ sites was assessed in rat brain crude synaptic membranes as well as autoradiographically in 10um rat brain slices (Hill & Bowery, 1981; Bowery et al., 1987). $GABA_B$ receptor-mediated modulation of forskolin- and noradrenaline-stimulated cAMP production was determined as described by Hill (1985) with the modifications of Watling & Bristow (1986). Test drugs were preincubated with the tissue for 5 min at 37^{o}C prior to the initiation of the reaction with 10uM forskolin or 100uM noradrenaline.

3-APPA displaced the binding of [^{3}H]GABA to $GABA_B$ sites in synaptic membranes and brain slices with relative potencies of 65 times (IC_{50} = 1nM) and 10 times (IC_{50} = 3nM) that achieved with baclofen, respectively. Moreover, 3-APPA was selective for $GABA_B$ sites; IC_{50} values for displacement at $GABA_A$ sites were 420nM and 20uM in membranes and slices, respectively (n=3-5). However, functional studies involving cAMP production showed 3-APPA and (-)-baclofen to be equieffective at inhibiting forskolin-stimulated cAMP production (respective EC_{50} values of 3.1$\pm$1.2uM and 2.2$\pm$0.9uM, mean$\pm$sem) but weaker in potentiating noradrenaline-stimulated cAMP production. In fact, 3-APPA appeared to be a partial agonist with respect to (-)-baclofen. The maximal potentiation produced by 3-APPA was only 60% of the maximal response to (-)-baclofen (EC_{50} values 6.0$\pm$1.5uM and 25.4$\pm$7.3uM ($p<0.05$ Student 't' test) (-)-baclofen and 3-APPA, respectively; n=5).

In summary, these results support the contention that 3-APPA is a more potent displacer of [^{3}H]GABA binding than baclofen at $GABA_B$ sites. However, although it acted as a full agonist to inhibit the forskolin-stimulated cAMP production, being equiactive with (-)-baclofen, it appeared to act as a partial agonist on the noradrenaline-stimulated system. The possibility exists that these two effects on cAMP production may be mediated through separate $GABA_B$ receptor subtypes.

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EFFECT OF BACLOFEN ON THE RELEASE OF GABA AND OTHER AMINO ACIDS FROM RAT CORTEX, SPINAL CORD AND RETINA

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In the vertebrate retina, GABA is utilized as a transmitter substance by a substantial subpopulation of amacrine cells. In the rabbit retina the release of ACh is modulated by GABAergic drugs (Cunningham & Neal, 1983). Thus, muscimol and GABA depress ACh release but baclofen increases the light evoked release of ACh. It seemed possible that the effects of baclofen in the retina might involve a reduction in the release of GABA, perhaps by an action on presynaptic $GABA_B$ autoreceptors.

The present experiments were undertaken to see whether GABAergic amacrine cells in the rat retina possess $GABA_B$ autoreceptors. The effect of baclofen on GABA release from the rat retina, and for comparison, from cortex and spinal cord was examined.

Slices of rat cerebral cortex, spinal cord, or entire isolated retinas were placed in Krebs bicarbonate medium in a small chamber (volume 0.5ml) and gently agitated with a stream of 5% CO_2 in oxygen. At 10 min intervals the medium was removed and subjected to amino acid analysis using HPLC. In most experiments, the rats were injected with γ-vinyl-GABA (250mg/kg I.P.) (GVG), an irreversible GABA-T inhibitor, 18h before death.

Exposure of cortical slices to KCl (50mM) produced a large increase in the release of GABA but no increase in GABA release was detectable from spinal cord or retina. However, when animals were pretreated with GVG the spont aneous resting release of GABA was increased, and more importantly, KCl evoked a striking release of GABA from all three tissues. (-)Baclofen (5-20μM) significantly depressed the K-evoked release of GABA from cortical slices, a concentration of 20μM inhibiting release by 80%. This depressant effect was blocked by phaclofen (500μM), a $GABA_B$ antagonist. In spinal cord slices from GVG treated rats (-)baclofen (10μM) almost abolished the K-evoked release of GABA. In contrast, (-)baclofen (10μM) or (±) baclofen (1mM) did not affect the K-evoked release of GABA from retinas of GVG treated rats.

In the cerebral cortex, baclofen also reduced the K-evoked release of aspartate, glutamate, glycine and taurine.

These experiments suggest that GABAergic amacrine cells in the rat retina do not possess autoreceptors. However, baclofen had clear depressant effects on the release of GABA and other amino acids from the cerebral cortex and spinal cord

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HOMOCYSTEIC ACID MAY BE A BIPOLAR CELL TRANSMITTER IN THE RABBIT RETINA

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Studies of drugs that affect the release of acetylcholine (ACh) from the rabbit retina suggest that the transmitter released from bipolar cells onto cholinergic amacrine cells is an excitatory amino acid (Cunningham & Neal, 1985a). Thus, glutamate, aspartate, kainate and quisqualate (but not NMDA) increase the spontaneous resting release of ACh from the retina and suppress the light evoked release. Furthermore, Cis-2, 3-piperidine dicarboxylic acid (PDA) blocks both the light evoked release of ACh and the release caused by glutamate, aspartate, kainate and quisqualate. However, it seems unlikely that glutamate or aspartate can be the bipolar cell transmitter because NMDA, which acts as an antagonist at some retinal synapses (Cunningham & Neal, 1985b), blocks the light evoked release of ACh but does not antagonise the effects of glutamate or aspartate (Cunningham & Neal, 1985a).

In the present experiments, we have examined the possibilty that the sulphur containing amino acid, homocysteic acid (HCA), might be the bipolar cell transmitter released onto cholinergic amacrine cells because this compound is known to excite many inner retinal neurones (Miller, personal communication).

Analysis of retinal tissue using HPLC revealed that HCA is present in the rabbit and ox retina at a concentration of 1 to 2 picomoles/mg wet wt. Since the retina possesses HCA we proceeded to examine its effects on ACh release from the rabbit retina *in vivo* using methods described previously (Cunningham & Neal, 1985a).

The local application to the retina of HCA (0.5-5mM) glutamate (5mM), kainate and quisqualate (8μM) suppressed the light evoked release of ACh and increased the spontaneous resting release three to four fold. None of the compounds significantly decreased the amplitude of the e.r.g. b-wave at these concentrations indicating that their action was not at the outer plexiform layer.

The effects of all the drugs, and also flashes of light (3Hz), on the release of ACh from the retina were blocked by PDA (2mM) but NMDA (5mM) blocked only the effects of HCA, kainate and light flashes.

These results suggest that in the rabbit retina, HCA (or a related sulphur containing amino acid) may be the transmitter released at the bipolar cell/cholinergic amacrine cell synapse.

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REGIONAL HAEMODYNAMIC EFFECTS OF 8-OH-DPAT IN CONSCIOUS, LONG EVANS AND BRATTLEBORO RATS

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Although the regional haemodynamic effects of 8-OH-DPAT* have been described in anaesthetized cats (Dreteler et al., 1988), the data were obtained with the microsphere technique and changes were measured at only one time point. We have made continuous measurements of changes in regional haemodynamics in conscious, Long Evans (LE) and Brattleboro (B) rats in response to i.v. injection of 8-OH-DPAT (50 ug kg^{-1}). Under sodium methohexitone anaesthesia (60 mg kg^{-1} i.p.), pulsed Doppler probes (Haywood et al., 1981) were sutured around left renal (R) and superior mesenteric (M) arteries and the distal abdominal aorta (H; representing hindquarters flow). At least 7 days later animals were briefly re-anaesthetized (sodium methohexitone, 40 mg kg^{-1} i.p.) for the implantation of intravascular catheters. The following day continuous measurements were made of mean BP, instantaneous heart rate (HR) and R, M and H Doppler shift (DS) signals; % changes in vascular resistance were calculated from mean BP and DS signals (Haywood et al., 1981). The results are summarized in the table.

Table 1 Cardiovascular changes following administration of 8-OH-DPAT in LE or B rats (n=7; values are mean (s.e.m.))

Time after injection	1 min		5 min	
	LE	B	LE	B
HR (b min^{-1})	-15(8)	-45(8)*	-46(11)*	-46(10)*
mean BP (mmHg)	-12(2)*	-17(3)*	-18(3)*	-22(2)*
RDS (%)	- 1(4)	- 5(1)*	-14(5)*	- 9(3)
MDS (%)	-24(3)*	-17(5)*	-17(5)*	-20(5)*
HDS (%)	44(6)*	10(4)*	4(4)	2(4)
RVR (%)	-13(4)*	-10(2)*	- 4(5)	-10(4)
MVR (%)	17(7)*	5(7)	1(7)	4(6)
HVR (%)	-38(3)*	-22(2)*	-21(4)*	-20(3)*

* P <0.05 versus baseline (Friedman's test)

The initial marked hindquarters vasodilatation in LE rats was associated with motor activity, and hence could have been a secondary phenomenon; B rats showed a smaller increase in HDS 1 min following 8-OH-DPAT consistent with the lesser hyperactivity in this strain. However, both LE and B rats showed similar H vasodilatations 5 min after 8-OH-DPAT when hyperactivity was not present, indicating these changes may have been independent of behavioural effects. The increase in MVR in LE rats 1 min after 8-OH-DPAT was absent in B rats, perhaps indicating an involvement of vasopressin in the regional haemodynamic profile of 8-OH-DPAT in the former strain. The possible contribution of central 5-HT_{1A} receptors to the results obtained remains to be established.

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*8-hydroxy-2-(di-n-propylamino)tetralin

REVERSAL OF INJURY INDUCED REDUCTION IN BAROREFLEX SENSITIVITY BY NALOXONE IN THE CONSCIOUS RAT

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It has recently been shown that the reduction in baroreflex sensitivity which accompanies injury (Redfern et al, 1984) may be mimicked by injection of the enkephalin analogue D-Ala^2-Met^5-enkephalinamide into the lateral cerebral ventricle (icv) of the conscious rat (Little et al, 1988). Since the endogenous opioid system has been implicated in the cardiovascular response to injury (Holaday, 1983) it was of interest to determine the effects of the opiate antagonist naloxone on this response. Experiments were performed on conscious male Wistar rats. The baroreflex was assessed as the slope of the relationship between heart period and mean arterial blood pressure during phenylephrine induced pressor responses (Little & Redfern, 1981). Injury was induced by the application of rubber-band tourniquets to both hindlimbs under a brief period of ether anaesthesia (Redfern et al, 1984). The baroreflex was assessed before and again 30 min after the application of tourniquets, by which time the animals had regained consciousness.

In each of 8 control, untreated, animals there was a significant ($P<0.05$) reduction in baroreflex sensitivity after the application of tourniquets from a group mean control value of 1.44 ± 0.12 to 0.52 ± 0.13 ms.mmHg^{-1} (mean ± SD). In 6 animals treated with naloxone (96μg in 20 μl 0.9% saline icv) there was no significant reduction in the sensitivity of the baroreflex after the application of tourniquets, group mean baroreflex sensitivity being 1.67 ± 0.18 before and 1.82 ± 0.13 ms.mmHg^{-1} during bilateral hindlimb ischaemia. The same dose of naloxone did not consistently affect the sensitivity of the baroreflex when given to uninjured animals.

It is concluded that naloxone treatment can reverse the reduction in baroreflex sensitivity produced by injury, and implicates the endogenous opioid system in this response. The site of action of naloxone cannot be deduced from the present study. However, a likely candidate is the periaqueductal grey region of the midbrain which contains enkephalinergic neurones (Murakami et al, 1987), and has been implicated in the cardiovascular response to injury (Little et al, 1988)

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8-BROMO CYCLIC GMP INHIBITS MYOCARDIAL INOTROPIC RESPONSE TO THE DIHYDROPYRIDINE AGONIST BAY K 8644

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The myocardial actions of cyclic GMP are not established. It is reported to reduce isometric force and block cardiac calcium slow channels in some species, particularly in beta adrenergic stimulated preparations. We investigated the effects of 8-bromo cyclic GMP on the inotropic responses of isolated ferret cardiac papillary muscles (n = 24) to isoprenaline and the calcium channel agonist Bay K 8644. Muscles were mounted at lmax in organ baths containing Krebs Ringer solution (2 mM calcium; pH 7.4; 29^{o}C), attached to a length-tension transducer and stimulated electrically at 0.2 Hz (Chappell et al., 1985).

8-bromo cGMP (0.1 mM) significantly ($p < 0.01$) decreased peak isometric twitch tension (TT) by 12%, peak rate of tension development (+dT/dt) by 6%, peak isotonic shortening (PS) by 8% and time to peak isometric tension (tTT) by 9%. Bay K 8644 (0.01 to 10 μM) produced concentration-dependent positive inotropic effects, at 10 μM increasing TT by 30%, +dT/dt by 39%, PS by 19%, tTT by 10% and half isometric relaxation time (RT 1/2) by 13% ($p < 0.02$). Isoprenaline (0.1 μM) increased TT by 40%, +dT/dt by 54% and PS by 18% ($p < 0.03$), but decreased tTT by 15% and RT 1/2 by 12% ($p < 0.01$). In the presence of 0.1 mM 8-bromo cGMP (30 minutes pretreatment) inotropic responses to Bay K 8644 were significantly reduced, TT increasing at 10 μM Bay K 8644 by only 8%, +dT/dt by 9%, PS by 4% and RT 1/2 by 6% ($p < 0.05$ compared to control values). Responses to isoprenaline, however, were not significantly altered by 8-bromo cGMP pretreatment.

The results of this study show that (a) the effects of 8-bromo cGMP were similar to those of endocardial removal (Brutsaert et al., 1988), (b) the patterns of positive inotropic response to isoprenaline and Bay K 8644 were different, reflecting differing mechanisms of action at the level of calcium slow channels, sarcoplasmic reticulum and/or contractile proteins (Tsien, 1977; Tsien et al., 1986), (c) 8-bromo cGMP does not influence the inotropic effects of elevating intracellular cyclic AMP levels by beta-receptor stimulation in ferret cardiac muscle, (d) 8-bromo cGMP reduces the inotropic effect of the calcium channel agonist Bay K 8644.

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THE EFFECT OF NIFEDIPINE AND MIOFLAZINE ON ISOLATED HEARTS SUBJECTED TO LOW-FLOW ISCHAEMIA (LFI)

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Calcium antagonists have proved to be effective in reducing cardiac damage due to ischaemia. This effect appears to be associated with a reduction of the adenine nucleotide (AN) catabolism (Harmsen et al., 1983; Hugtenburg et al., 1988b) and increased ATP levels after reperfusion (Watts et al., 1986; Hugtenburg et al.,1988a). Isolated paced (5 Hz) hearts of rats and guinea pigs (GPs) were perfused with Tyrode (Ca^{2+}: 1.8mM). In rat and GP hearts LFI was induced by reducing the coronary flow from 7.5 to 2.0 ml/min and from 11.0 to 2.5 ml/min for 15 min, respectively. After LFI the hearts were reperfused for 25 min. The effect of treatment (15 min before and during LFI) with nifedipine (N) and mioflazine (M) on creatine phosphate (PCr) and AN levels was determined spectrophotometrically after 15 min of LFI and after reperfusion. The data (µmol/g dry weight) are presented in table I as means ± S.E.M. (n=6-7). Statistical significance ($p < 0.05$) was evaluated using Student's tailed t-test.

Table I:

Rat	PCr	ATP	Total AN	PCr	ATP	Total AN
Normoxia	21.0±1.2	20.5±1.2	28.3±1.3			
ibid. GP	24.5±1.2	20.4±1.0	25.5±0.5			
	LFI			LFI and reperfusion		
Control rat	7.5±1.1	14.0±1.5	27.2±0.9	18.1±1.5	12.0±1.0	21.8±0.9
N (80 nM)	8.5±0.9	14.6±0.3	26.1±0.7	18.4±2.2	16.3±0.2	25.2±0.3
M (3000 nM)	10.1±1.5	13.8±0.9	25.0±0.5	21.0±1.9	16.5±0.9	26.3±0.6
Control GP	10.1±1.9	10.7±1.4	18.3±1.0	19.3±2.7	15.1±1.1	18.9±1.0
N (8 nM)	20.5±0.3	14.9±0.3	20.6±0.1	20.6±1.3	16.9±0.4	21.5±0.4
M (300 nM)	17.2±2.5	14.8±0.6	19.8±1.1	20.6±1.3	17.4±1.0	21.3±0.9

In rat and GP hearts ATP decreased during LFI. After reperfusion in rats ATP was further reduced, whereas in GPs ATP was enhanced. In addition, in GPs, but not in rats, total AN was markedly decreased by LFI. Reperfusion reduced total AN in rats, but not in GPs. In rat heart N and M reduced AN metabolism during reperfusion, whereas in GPs N and M inhibited ATP breakdown during LFI. The nucleoside release (NCR) during LFI and during the first 3 min of reperfusion in the coronary perfusate was determined by means of HPLC. In rats and GPs LFI increased NCR from 0.6 ±0.2 to 2.4±0.3 and from 0.3±0.1 to 2.2±0.7 µmol/g dry weight (g dw)/15 min. During the first 3 min of reperfusion NCR in rats amounted to 1.9±0.4 µmol/g dw and in GPs to 0.4±0.05 µmol/g dw. During LFI in GPs, but not in rats, N and M effectively suppressed NCR. In contrast, during reperfusion N and M proved more effective in reducing NCR in rats than in GPs. It is concluded that rat and GP hearts are differentially sensitive to LFI and reperfusion. N and M, in the concentrations applied, retarded AN metabolism in the GP heart during LFI, but not during reperfusion, whereas in the rat heart N and M appeared to be effective in reducing AN catabolism during reperfusion only.

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DISCREPANCIES BETWEEN AGONIST-INDUCED RESPONSES AFTER CA^{++} ENTRY BLOCKADE AND UNDER CA^{++}-FREE CONDITIONS IN A RAT VEIN AND ARTERY

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In the present study we investigated the characteristics of the agonist-sensitive Ca^{++}-pools in the jugular vein and aorta of the rat in vitro. After calculation of the agonist-sensitive pools we compared these data with values obtained after pretreatment with the calcium entry blockers (CEB's) nifedipine and diltiazem.

Contractions were measured in isolated rings of jugular vein and helically cut strips of aorta of male Wistar rats (300-400 g) under isometric conditions in Krebs-Henseleit solution ($CaCl_2$ 1.25 mM) at 37°C. Every 20 min the bath fluid was replaced. After changing the Ca^{++}-containing medium for a Ca^{++}-free Krebs-Henseleit solution (t=0) serotonin (5-HT, 10^{-5} M, aorta and jugular vein) and noradrenaline (NA, 10^{-5} M, aorta) were added at t=5, 10, 20, 30, 50, 70, 90 and 120 min. Addition of EGTA (2 mM) to the Ca^{++}-free medium was also investigated. Each preparation was exposed to the agonists only once. Pretreatment with CEB's was performed 25 min before addition of the agonist in the Ca^{++}-containing medium.

In Ca^{++}-free medium the KCl (100 mM)-induced contraction has completely disappeared after 5 min, indicating the absence of extracellular Ca^{++} under these conditions. The 5-HT- and NA-induced responses still present after a 5 min exposure to Ca^{++}-free medium decreased dependent on the time of incubation. Addition of EGTA (2 mM) to Ca^{++}-free medium accelerated the decline in contractility which was more pronounced for 5-HT than for NA. A biexponential curve was obtained which was compatible with a two compartment model. In the jugular vein the total size of the intracellular compartment amounted to 35% of the total 5-HT response. In the aorta these values were 42 and 47% for 5-HT and NA, respectively. After maximal contraction of the aorta by 5-HT or NA, addition of the other agonist could still increase the contraction.

After maximally effective concentrations of CEB's only a slight inhibition of the 5-HT and NA-induced responses could be observed. In the jugular vein 79.7 $\pm$ 3.8% (n=6) and 82.9 $\pm$ 5.8% (n=5) of the 5-HT response remained unaffected after pretreatment with nifedipine (10^{-8} M) and diltiazem (10^{-5} M), respectively. In the aorta after pretreatment with nifedipine (10^{-7} M) and diltiazem (10^{-6} M) 74.0 + 2.5% (n=5) and 70.3 $\pm$ 2.0% (n=5), respectively, of the 5-HT- and 83.0 $\pm$ 2.1% (n=5) and 81.2 $\pm$ 1.0% (n=6), respectively, of the NA-induced response persisted.

These data suggest that after maximally effective concentrations of CEB, an influx component in the 5-HT- and NA-induced contractions remains unaffected. These data are supported by studying the original tracings which revealed that whereas responses in Ca^{++}-free medium consist of a rapid transient phase only, after CEB pretreatment a similar response as the control response, including a slow sustained component, was obtained. The Ca^{++}-influx component not affected by maximally effective concentrations CEB's seems to represent an influx of extracellular Ca^{++} direct into the cytosol and not into a intracellular Ca^{++}-store.

EFFECTS OF R 56865 AND R 70608 ON THE VERATRIDINE-INDUCED ^{45}CA-UPTAKE IN RAT AORTIC STRIPS

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Toxic concentrations of cardiac glycosides result in an increased Na- and Ca-uptake of the heart. It was suggested that at least part of the enhanced Ca-uptake was due to an activation of the Na/Ca-exchanger. R 56865 (N-[1-[4-(4-fluorophenoxy)-butyl]-4-piperidinyl]--N-methyl-2-benzothiazolamine) and R 70608 (4-[(2-benzothiazolyl)-methylamino]-α-[2-(4-fluorophenoxy)ethyl]-1-piperidine-ethanol) are compounds which decrease toxic effects of cardiac glycosides without influencing positive inotropic effects (J.Schneider et al., 1988; J.Schneider et al., 1989). We studied the effects of R 56865 and R 70608 on the ^{45}Ca-movements in de-endothelialized rat aortic strips elicited by a veratridine-induced increase in intracellular Na-concentration. For the measurement of ^{45}Ca-movements we used the method described by Wermelskirchen et al.(1988). In brief, rat isolated aortic strips were equilibrated in Tyrode-solution (37°C) gassed with 95% O_2 and 5% CO_2 at a resting tension of 0.33 g. After pretreatment for 60 minutes, the strips were labelled for 5 minutes (K-induced uptake) or 30 minutes (veratridine-induced uptake) in ^{45}Ca (1 µCi/ml) containing Tyrode-solution in the presence or absence of the stimulus. Thereafter, the strips were washed for 45 minutes with a Tyrode-solution of 4°C and the residual ^{45}Ca was detected. Veratridine (10^{-7}-10^{-4} M) showed a concentration-dependent increase in ^{45}Ca-uptake. The maximal increase in veratridine-induced ^{45}Ca uptake amounted to 20-30% of the unstimulated values. Nifedipine (10^{-8}-10^{-6} M) and verapamil (10^{-7}-10^{-5} M) diminished the veratridine (10^{-4} M)-induced ^{45}Ca-uptake maximally by 60%, whereas the K-induced ^{45}Ca-uptake was completely blocked in a concentration range of 10^{-9}-10^{-6} M and 10^{-8}-10^{-5} M, respectively. R 56865 inhibited the veratridine (10^{-4} M)-induced ^{45}Ca-uptake in the concentration range 10^{-8} to 10^{-5} M maximally by 70%. The K-induced ^{45}Ca-uptake was attenuated at higher concentrations (10^{-7}-10^{-5} M). R 70608 (10^{-8} to 10^{-5} M) blocked the veratridine (10^{-4} M)-induced ^{45}Ca-uptake completely, although the K-induced ^{45}Ca-uptake was diminished in the concentration range 10^{-7} to 10^{-5} M with a maximal effect of 70%.

In conclusion, in the rat aorta the veratridine-induced ^{45}Ca-uptake seems to result from activation of Na-channels and is antagonized less efficiently and at higher concentrations by nifedipine and verapamil than the K-induced ^{45}Ca-uptake. R 56865 and R 70608 however, showed a higher affinity for the veratridine (10^{-4} M)-induced ^{45}Ca-uptake, R 70608 causing a complete and R 56865 a partial inhibition. The veratridine-induced ^{45}Ca-uptake seems to enter the cell via a pathway distinct from the potential operated channels. Whether the agents studied interact with the Na-uptake or the subsequent Ca-uptake cannot be decided by these experiments.

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IMPLICATION OF CALCIUM IN ENDOTOXIN-INDUCED IMPAIRMENT OF RAT AORTIC CONTRACTION EX VIVO

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For unknown reasons, the positive inotropic and vasoactive properties of catecholamines are impaired in septic or endotoxic shock. This cardiovascular hyporesponsiveness has been well documented for an extensive range of compounds both in experimental endotoxic animals (Parratt, 1973; Auclair et al., 1986), and in *ex vivo* vascular preparations (Wakabayashi et al., 1987). The mechanism involved is unclear, but a disorder in calcium utilization has been proposed (Wakabayashi et al., 1987). This study is an attempt to verify such an hypothesis.

Male Wistar rats (300g) were given E. Coli endotoxin 055:B5 (20mg/kg, i.p.) or the same volume (0.5ml) of saline. After 4 h, the animals were killed by vertebral dislocation and rings of aorta were set up in 20 ml organ baths, under 2 g tension. The Krebs'solution contained (mM): NaCl 117, KCl 5, $MgSO_4$ 1.26, $CaCl_2$ 1.25, KH_2PO_4 1, $NaHCO_3$ 2.5, glucose 10.

Tissues removed from shocked rats showed, both in presence and in absence of endothelium (which was removed by rubbing), a marked hyporesponsiveness to α-adrenoceptor agonists (Table). This occurred also with changes in extracellular calcium (Ca_o) and to a greater extent with calcium agonists (see Table), whatever the level of depolarisation used.

Table : Effect of endotoxin pretreatment on *ex vivo* rat aorta responsiveness in absence of endothelium.

	Controls		Treated	
	EC50 (M)	Emax (g tension/mg tissue)	EC50 (M)	Emax (g tension/mg tissue)
Noradrenaline	$1.20\pm0.30\times10^{-8}$	1.54±0.17	$1.66\pm0.70\times10^{-7}$*	0.96±0.23*
Phenylephrine	$2.00\pm0.50\times10^{-8}$	1.47±0.10	$1.28\pm0.44\times10^{-7}$*	0.93±0.17*
Sgd 101-75	$5.28\pm0.56\times10^{-7}$	1.04±0.06	$1.99\pm0.60\times10^{-6}$*	0.22±0.14*
Ca_O (in 40mM K^+)	$2.23\pm0.26\times10^{-4}$	1.43±0.18	$8.98\pm0.43\times10^{-4}$*	1.32±0.14
In 12mM K^+:				
(-)BAY K 8644	$7.90\pm0.32\times10^{-9}$	1.34±0.19	$1.63\pm0.51\times10^{-8}$*	0.45±0.23*
(+)S 202-791	$4.70\pm0.36\times10^{-8}$	1.13±0.10	$3.02\pm0.47\times10^{-6}$*	0.12±0.06*

* $P<0.05$ for difference between treated and controls. n=5 for all values.

These results suggest that endotoxin-induced aortic hyporesponsiveness is due to an impairment of smooth muscle but not endothelial cell function. Because of the marked diminution of the effects of calcium agonists, calcium channel damage could be involved.

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CALCIUM OVERLOAD FOLLOWING ADMINISTRATION OF VITAMIN D_3 AND NICOTINE IN THE RAT

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Fleckenstein et al, (1985) have proposed a rat model of cardiovascular calcium overload induced by administration of vitamin D_3 (single dose, 300,000 IU/kg, im) plus nicotine (25 mg/kg, twice daily, po) (Vit D + nic). We have studied the selectivity of this calcium overload following 4 days' treatment plus 1 (day 5) or 13 days' recovery (day 17). The cardiovascular changes observed in these groups are reported elsewhere (Chillon et al, and Thorin-Trescases et al, this meeting). Calcium and magnesium levels were measured by atomic absorption. Plasma urea and creatinine were measured with standard colorimetric methods.

Group	Controls	Day 5	Day 17
Body weight (g)	232±5 (18)	167±3* (13)	232±7 (22)
Plasma			
Creatinine (mg/ l)	5.8±0.6 (4)	8.7±1.0* (4)	7.5±0.6* (4)
Urea (mg/ml)	0.43±0.02 (4)	0.89±0.17* (4)	0.43±0.02 (4)
Calcium (mmol/l)	2.37±0.02 (5)	3.93±0.11* (4)	2.58±0.05* (5)
Tissue calcium (µmol/g dry weight)			
Myocardium	6.6±0.6 (18)	10.3±1.6* (16)	26.5±6.1* (13)
Aorta	10.1±1.0 (18)	39.7±7.9* (16)	331±68* (13)
Tail artery	5.9±0.3 (6)	10.2±0.3* (5)	73.8±31.1* (5)

Means ± SEM. * = $P < 0.05$, t-test comparison with controls.

Vit D + nic produced a fall in body weight (day 5); rats recover once the treatment is stopped (day 17). Renal function is modified during treatment but, once again when treatment is stopped this tends to return to normal (fall in plasma urea on day 17). Circulating calcium levels increase substantially during treatment (presumably reflecting the action of Vit D), then return towards normal (day 17). Circulating magnesium levels were also slightly increased (controls: 0.81±0.03, day 5: 1.05±0.05 and day 17: 1.08±0.03 mmol/l, n=4 per group). Vit D + nic increased calcium levels in cardiovascular tissues, but not in other tissues such as brain and liver. The sensitivity of the tissues to this effect was: aorta > tail artery > myocardium. Calcium levels continued to increase after stopping the treatment. Tissue magnesium levels were not changed.
In conclusion, Vit D + nic produces a selective overload of calcium in cardiovascular tissues. Vessels seem to be more sensitive than myocardium. Use of the original model (day 5) proposed by Fleckenstein et al, (1985) for the development of cardiovascular active agents may be complicated by other changes such as weight loss and a modification of renal function. If animals are allowed to recover (day 17) this extended model could be useful in studies on cardiovascular changes associated with calcium overload.

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EFFECTS OF VARIOUS CALCIUM ENTRY BLOCKING COMPOUNDS ON THE ANGIOTENSIN II-INDUCED INCREASE IN SLOWLY EXCHANGING $^{45}CA^{++}$ IN RAT AORTA

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In the present study we investigated the increase in slowly exchangeable $^{45}Ca^{++}$ induced by angiotensin II (A II) in the rat aorta, since in this preparation it was demonstrated that the classical calcium entry blockers (CEB) nifedipine, verapamil and diltiazem strongly inhibited the contraction elicited by A II (Van Heiningen & Van Zwieten, 1988). The experiments were performed using the method for measuring $^{45}Ca^{++}$-influx described by Wermelskirchen et al. (1988). In addition to the classical CEB, other compounds affecting calcium entry like TMB-8 and $CoCl_2$ were studied.

Male Wistar rats (300-350 g) were sacrificed and the thoracic aorta was excised and cleaned. For the measurement of slowly exchanging $^{45}Ca^{++}$ aortas were cut longitudinally and subsequently transversally into two parts. The strips were suspended in a gassed (95% O_2 + 5% CO_2) Krebs-Henseleit (K-H) solution (1.25 mM Ca^{++}, 37°C, pH=7.3) under a resting tension of 0.33 g for 60-90 minutes. The strips were pretreated with the CEB for 45 minutes and incubated for 5 minutes in a $^{45}Ca^{++}$-containing K-H solution (1 µCi/ml) without or with A II (10^{-7} M). After washing the strips for 45 minutes with a cold (4°C) K-H solution, they were dried completely, weighed and dissolved. The residual $^{45}Ca^{++}$ tissue content was measured by means of liquid scintillation counting. For contraction studies helically cut strips were suspended in the K-H solution under a resting tension of 0.8 g. Strips were pretreated with the CEB for 30 minutes or incubated in Ca^{++}-"free" medium for 5 minutes.

A II elicited an increase in slowly exchangeable $^{45}Ca^{++}$ by 48.5 ± 2.9% (n=58) compared to the control experiments. This effect could be antagonized using different compounds with calcium entry blocking properties. An average inhibition of 69.5% could be observed after treatment with the classical CEB in concentrations that maximally reduced A II-induced contractions of rat aortic strips (nifedipine, 10^{-6} M; verapamil and diltiazem, 10^{-5} M). The inorganic CEB $CoCl_2$ ($3x10^{-4}$ M) did not show any additional activity compared to the classical organic CEB. The percentage inhibition amounted to 66.9 ± 9.4%. TMB-8 (10^{-4} M), however, completely abolished the A II-induced increase in slowly exchanging $^{45}Ca^{++}$. The basal $^{45}Ca^{++}$-uptake was not affected by the agents under study.
In functional experiments the classical CEB (Van Heiningen & Van Zwieten, 1988) and $CoCl_2$ proved equieffective in inhibiting A II-induced contractions (75-80%). Concomitantly, experiments in Ca^{++}-"free" medium showed that A II still exerts a constrictor effect (21.2 ± 3.8%). TMB-8 completely suppressed the vasoconstriction elicited by A II.
The experimental findings show that part of the A II-induced increase in cellular $^{45}Ca^{++}$-content is insensitive to the classical CEB or to $CoCl_2$. However, TMB-8 did suppress the nifedipine-insensitive increase in slowly exchanging $^{45}Ca^{++}$, probably by the blockade of an additional Ca^{++}-influx pathway or by an interaction with the storage of Ca^{++} in the slowly exchanging compartments. These data are in good agreement with functional experiments in which the major part of the contraction elicited by A II is inhibited by all compounds studied. The additional inhibitory effect of TMB-8 may be explained by its intracellular action. A possible additional Ca^{++}-influx component, insensitive to calcium entry blockade with nifedipine is apparently not sufficient to elicit a contraction.

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EFFECTS OF NITRENDIPINE AND WB 4101 ON DIFFERENT COMPONENTS OF THE RESPONSES OF ISOLATED RAT AORTA TO α-ADRENOCEPTOR AGONISTS

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It has recently been suggested that α1-adrenoceptor subtypes could be characterized in smooth muscle by their differential affinity for WB 4101 and by their ability to induce either mobilization of calcium from intracellular sources or entry of calcium through dihydropyrine-sensitive calcium channels (Han et al., 1987). However there are discrepancies between different proposed subclassifications (McGrath & Wilson, 1988). In the present study we have examined the influence of a maximally active concentration of the calcium entry blocker nitrendipine (Nitr) on concentration-effect relationships of α-adrenoceptor agonists and of the antagonist WB 4101 on the rat isolated aorta.

The experiments were performed on thoracic aortic rings from male Wistar rats (12-13 weeks old). The endothelium was removed and the rings were mounted under a tension of 2 g in a physiological salt solution (composition mM : NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5) maintained at 37°C and bubbled with 95% O_2, 5% CO_2. Cumulative concentration-effect curves were elicited in absence or 20 min after addition of Nitr (1 M). When present, WB 4101 was added 30 min before the first dose of agonist.

Nitr decreased the maximal responses (Emax) to the 3 agonists, decreased the half maximally effective concentration (EC50) of the full agonists (noradrenaline and phenylephrine), but did not modify the sensitivity to the partial agonist Sgd 101-75. Two different pA2 values with and without Nitr could be distinguished for WB 4101 antagonism of noradrenaline evoked responses, but not for the other two agonists.
The results obtained with noradrenaline are consistent with the hypothesis of 2 receptors with high and low affinity for the agonist and for WB 4101, differenciating the Nitr-sensitive and resistant components of the response. However WB 4101 does not discriminate between the 2 components of the response to phenylephrine and it presents an apparently high affinity for the Nitr-resistant response to Sgd 101-75. Taken together, these results show that, in the isolated rat aorta, α-adrenoceptor subtypes cannot be simply discriminated by the use of WB 4101 in the absence and presence of Nitr.

Table Effect of nitrendipine (Nitr) on the responses of rat aortic rings without endothelium to α-adrenergic agonists and on their inhibition by WB 4101 (n=4)

Agonist		-logEC50 (M)	Emax % control	WB 4101 pA2	WB 4101 slope
Noradrenaline	control	7.90 ± 0.16	100	9.42 ± 0.07	1.02
	+Nitr	7.59 ± 0.15*	66.8 ± 12.5*	8.70 ± 0.8*	0.99
Phenylephrine	control	7.61 ± 0.12	100	9.47 ± 0.16	0.98
	+Nitr	6.74 ± 0.13*	42.4 ± 7.7*	9.37 ± 0.17	0.94
Sgd 101-75	control	6.51 ± 0.20	100	-	0.57
	+Nitr	6.69 ± 0.17	44.8 ± 6.2*	9.63 ± 0.12	0.97*

*significantly different from control values in absence of Nitr, $P<0.05$

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α_1-ADRENOCEPTOR SUBTYPES IN TISSUES OF THE RAT AND GUINEA-PIG

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Contraction of rat and guinea-pig aorta (RA,GPA) is mediated by α_1-adrenoceptors with distinct qualities. A major difference comprises 50-fold higher pA_2 values for prazosin in RA than in GPA (Beckeringh et al., 1984). α_1-Adrenoceptors in RA appear to resemble those of the α_{1A}-subtype whereas those present in GPA seem to be of the α_{1B}-subtype (Han et al., 1987). In the present study we have examined α_1-adrenoceptors in several tissues of male Black-Hooded rats and Pirbright White guinea-pigs.

Segments from RA and GPA and vasa deferentia (RVD,GVD) were suspended in Krebs-Henseleit solution containing 1 µM dl-propranolol, 10 µM cocaine and 20 µM cortisol at 37°C. Isometric tension was expressed as percentage of the maximal response to 100 µM noradrenaline (NA). Concentration-response curves (CRCs) were constructed cumulatively (RA,GPA) or noncumulatively (RVD,GVD) in the absence or presence of prazosin. Turnover of phosphatidylinositols, measured as the accumulation of [^{3}H]-inositolphosphates (IPs) was deteremined as described by Minneman and Johnson (1984) using 2-3 mm tissue segments or 1000x350x350 µm slices of cerebral (RCC,GCC) and renal (RRC) cortex. Accumulation of IPs by 100 µM NA was considered 100%.

Table I.	EC_{50} (µM) contraction	Antagonism by prazosin	EC_{50} (µM) acc. of IPs	Antagonism by prazosin
RA	0.21 ± 0.34	pA_2:9.36 ± 0.15 slope 0.57	0.65 ± 0.18	pK_B:9.54 ± 0.18
RVD	0.57 ± 0.12	pA_2:8.66 ± 0.09 slope 0.93	1.3 ± 1.6	pK_i:8.43 ± 0.12
RCC	---	---	5.2 ± 1.4	pK_B:9.04 ± 0.04
RRC	---	---	6.1 ± 1.9	pK_i:7.85 ± 0.12
GPA	6.6 ± 1.2	pK_B:7.82 ± 0.07	8.1 ± 2.8	pK_B:7.91 ± 0.09
GVD	2.2 ± 0.5	pK_B:7.95 ± 0.11	42.1 ± 0.7	pK_i:7.68 ± 0.14
GCC	---	---	4.6 ± 2.3	pK_B:8.28 ± 0.08

Data are expressed as means ± S.E.M..

In contrast to CRCs for contraction in GPA and GVD, in RA and RVD CRCs to NA were shallow and stretched over 4 to 5 decades. With initial responses in the nM range, NA was about 10-fold more potent in RA and RVD than in GPA and GVD (initial concentrations in the µM range). Increasing concentrations of prazosin gradually decreased the slope of CRCs in RA which obtained a shape similar to those in GPA and GVD. In RVD, GPA and GVD prazosin shifted CRCs in a parallel fashion to the right. CRCs for NA induced accumulation of IPs in RA and RVD were shallow and biphasic. In RCC, RRC and GVD CRCs were steep and monophasic. A higher potency of NA, notably at initial concentrations, was associated with a higher affinity of prazosin.

The results of the present study suggest that in several tissues of the rat and guinea-pig α_{1A}- and α_{1B}-adrenoceptors functionally coexist. α_{1A}-adrenoceptors seem to predominate in RA, whereas α_{1B}-adrenoceptors are predominantly found in RRC, GPA and GVD. RVD seems to contain equally effective amounts of both subtypes and in RCC and GCC the majority of the α_1-adrenoceptors is of the α_{1B}-subtype although small numbers of the α_{1A}-subtype also seem to be present.

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THE EFFECT OF ISOPRENALINE ON BLOOD VESSLES FROM DOGS WITH PACING-INDUCED HEART FAILURE

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The dorsal pedal artery (dpa) and saphenous vein (sv) from dogs with pacing-induced heart failure (HF) have been shown to be more reactive to α_1-agonists compared to sham-operated controls (Forster et al, 1988). A decrease in ß-receptor function may be responsible for this and therefore, this study examines isoprenaline (IP) on dpa and sv in vitro before and at peak HF.

The dpa and sv were removed before and at peak HF in 9 dogs as outlined previously (Forster et al, 1988). Two types of experiments were performed: 1) IP was examined on 2 dpa and 2 sv rings from each of 4 dogs before and at peak HF. In the absence of α and ß-blockade, concentration effect curves (CE-C) were constructed to IP in endothelium denuded (D) rings following precontraction with $PGF_2\alpha$. Thereafter, a second CE-C was constructed in which 1 ring of each pair received phentolamine (phent; 10^{-6}M). 2) Five animals were used, yielding 6 vascular rings for each vessel of which alternate rings were D and 3 rings were endotheliumintact(I). Propranolol was present throughout and CE-C to IP were constructed.

Table 1A % Relaxation (-) and % Contraction (+) of $PGF_2\alpha$ response before and at peak HF to IP in the absence and presence of phent

	Before HF		Peak HF	
	no phent	phent	no phent	phent
dpa	-18±9	-40±10	-25±5 *	-8±12 ++
sv	+147±20	-94±6 ++	-39±19	-76±6 ++

++P< 0.05 no phent vs phent and * P<0.05 before vs peak HF.

Table 1B Maximum tension and EC_{50} in endothelial-intact and -denuded before and at peak CHF

	Max(g tension)	EC_{50}(uM)	Max(g tension)	EC_{50}(uM)
dpa I	3.3±0.6	8.8(3.3-11.6)	1.6±0.5 *	13.8(5.7-33.1)
dpa D	2.5±0.9	6.4(2.6- 9.6)	1.7±0.4 *	6.1(2.3-16.0) *
sv I	3.0±0.6	0.5(0.2- 0.6)	1.4±0.4	4.9(3.0-9.0) *
sv D	3.0±0.7	0.3(0.1- 0.5)	1.8±0.4	1.6(1.2-2.4)

*p<0.05 before vs peak CHF; +P<0.05 d vs i; n 6-12 rings, 2-5 dogs

The table shows maximum (x±se) and EC50 (geometric x with 95% cl). The results to 1) show that in the dpa the response to IP was not modified by either phent or HF. However, before HF, a contraction was seen in sv which was attenuated by phent. At peak HF, IP caused only a relaxation which was enhanced by phent. The results to 2) show that, IP caused contractions before HF which were endothelium-independent. At peak HF, contractions to IP in dpa I and sv I were lower compared to before HF. In sv I and D, the EC_{50} was significantly higher than before HF and also the EC_{50} in sv I was higher than sv D. We conclude that IP elicits an α-mediated effect which is attenuated at HF. These results support the concept of heterogeneous α-receptors in dpa and sv and imply an abnormality in vascular α-receptor function at HF.

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EOSINOPHILIA AND HYPER-REACTIVITY OF LUNG PARENCHYMAL STRIPS IN RATS TREATED WITH SEPHADEX PARTICLES INTRAVENOUSLY

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Injection of Sephadex G200 particles intravenously into rats (0.5mg/ml) on days 0, 2 and 5 induced a blood eosinophilia which peaked on day 7. Lung parenchymal strips prepared from Sephadex treated rats showed an hyper-reactive response (ie. an increase in the maximal response) compared with lung parenchymal strips prepared from control rats to 5-hydroxytryptamine, carbachol and potassium chloride.

Blood samples and lung parenchymal strips were taken from rats at various times after Sephadex or saline administration (1,2). Cumulative dose response curves were obtained for the lung parenchymal strips to 5-hydroxytryptamine and carbachol using a concentration range from 1ng/ml to 20.8µg/ml in the tissue bath. The maximum response of the lung strips to increasing concentrations of potassium chloride was also determined. The area under the dose-response curves was calculated by computer.

Treatment		No of Eosinophils in the blood $x10^6$/ml	Area under Curve 5HT	Carbachol	Max. Tension (mg) developed to KCl
Sephadex	day 6	0.77±0.09***	2.9±0.2***	3.1±0.2***	165±21
	day 7	0.92±0.07***	2.6±0.2***	3.3±0.2***	196±18**
	day 8	0.99±0.07***	3.0±0.2***	4.7±0.3***	288±41**
	day 50	0.14±0.01	2.5±0.3***	4.5±0.4***	317±30***
Saline		0.11±0.01	0.2±0.04	1.4±0.1	126±7

Values are mean ± SEM, n=6 or more. **p=<0.01, ***p=<0.001 compared to the saline treated group.

The hyper-reactivity which developed to 5-hydroxytryptamine reached a maximum earlier than that to carbachol and potassium chloride. The hyper-reactivity to all three mediators persisted for a least 50 days. The blood eosinophil counts returned to control values by day 21.

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INHIBITION OF REFLEX BRONCHOCONSTRICTION BY THE OPIOID PEPTIDE BW443C IN THE ANAESTHETISED CAT

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A variety of vagally mediated reflexes, including cough and reflex bronchoconstriction, occur as a result of stimulation of sensory nerve endings in the respiratory tract (Coleridge & Coleridge, 1986). The opioid peptide H.Tyr.D-Arg.Gly.Phe(4-NO_2).Pro.NH_2 (BW443C) produces antitussive effects, mediated peripherally, by an inhibitory action on the discharges in vagal Aδ and C-fibre afferents (Adcock et al, 1987; Adcock & Smith, 1987). In the present study, these observations have been extended by examining the effects of BW443C on reflex bronchoconstriction in the anaesthetised cat.

Male cats (2.8-4.4kg) were anaesthetised with chloralose (60-80mg.kg^{-1}) following induction with 5% halothane, paralysed with dimethyl-tubocurarine and artificially ventilated. Dynamic compliance (C_D) and airway resistance (R_A) were monitored with an online analogue computer (Buxco, Model 4). Pressure (TBP) in a region of the trachea distal to the endotracheal cannula and below the larynx was recorded with an air filled balloon (Statham P23 pressure transducer). This area of the trachea was not accessible to aerosols. Cardiovascular parameters were also recorded. Drugs were administered either i.v or by aerosol, atropine and BW443C dissolved in saline and capsaicin in an ethanol, Tween 80 and saline mixture. Aerosols were generated using an ultrasonic nebuliser (DeVilbiss).

Capsaicin aerosol (100μg.ml^{-1}, n=5) delivered in 3 breaths produced bronchoconstriction (reproducible at least 5 times) as measured by a decrease in C_D (20 ± 4%), and increases in R_A (79 ± 13%) and TBP (3 ± 1 cmH_2O).Bilateral vagotomy (n=4), and atropine (0.1mg.kg^{-1} i.v bolus, n=4), abolished capsaicin-induced changes in C_D, R_A and TBP. BW443C (1mg.kg^{-1} i.v., n=4) significantly ($p<0.01$) inhibited capsaicin-induced decreases in C_D by 86 ± 11% and increases in TBP and R_A by 73 ± 8% and 91 ± 5% respectively. BW443C administered by aerosol (1mg.ml^{-1}, 20 breaths, n=8) also significantly ($p<0.01$) reduced capsaicin-induced bronchoconstriction; TBP 58 ± 9%, C_D 94 ± 6% and R_A 97 ± 2%. Similarly, atropine (20 breaths 1mg.ml^{-1}, n=4) significantly ($p<0.01$) inhibited capsaicin-induced increases in TBP by 83 ± 8% and abolished changes in C_D and R_A. Inhibitory effects of BW443C (i.v. and aerosol) were reversed by N-methyl nalorphine (0.3mg.kg^{-1} i.v., n=8), a peripherally acting opioid antagonist.

Capsaicin-induced bronchoconstriction, by aerosol, was thus mediated solely by a vagal reflex pathway, likely to be due to stimulation of "capsaicin sensitive" sensory nerve endings in the respiratory tract. BW443C (i.v. and aerosol) inhibited reflex bronchoconstriction by an action on opioid receptors. This effect of BW443C, in contrast to atropine, may be due to its inhibitory action on the activity of "capsaicin sensitive" sensory nerve endings. These observations with BW443C, together with its documented antitussive effects may have important implications for the therapeutic use of such a compound in non-specific airway hyperreactivity if vagal reflex mechanisms are involved.

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INHIBITORS OF ACE AND ENKEPHALINASE POTENTIATE IRRITANT INDUCED BRONCHOCONSTRICTION, BUT NOT COUGH, IN CONSCIOUS GUINEA-PIG

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Cough and reflex bronchoconstriction (Bc) are provoked in conscious guinea-pigs by inhaled irritants acting on rapidly adapting receptors or C-fiber endings (Forsberg et al 1988). Tachykinin peptides contained in C-fibers seem to mediate the Bc produced by capsaicin (Caps) and partly that by citric acid (CA). These peptides are catabolized by ACE (Angiotensin converting enzyme) and enkephalinase. We have now examined whether inhibition of ACE (by captopril) or enkephalinase (by thiorphan) alters airway responsiveness to inhaled Caps and CA.

Thiorphan (0.5 mg in 10% DMSO) or vehicle was injected iv. 10 min before exposure of guinea-pigs (350-500 g) to Caps and CA. Captopril (10 mg/kg ip.) or saline were administered to guinea-pigs 1 h before exposure to inhaled irritants. Conscious guinea-pigs (n=8-10 in each group) were challenged with ultrasonically nebulized CA (0.40 M) or Caps (30 μM) and the number of coughs (during 3 min) and the time to onset of Bc (development of slow laboured breathing, cf Forsberg et al 1988) recorded. Statistical differences were calculated by use of Student's t-test for unpaired observations.

Neither thiorphan nor captopril caused any respiratory effects in conscious guinea-pigs. Captopril enhanced the Bc produced by inhaled Caps but tended to inhibit coughing to both Caps and CA (Table 1). Thiorphan enhanced the Bc response to both Caps and CA but was without effect on cough (Table 1).

Table 1. Effect of treatment with inhibitors of ACE (captopril, Capt) or enkephalinase (thiorphan; Thi) on cough and time to bronchoconstriction (Bc) in conscious guinea-pigs (mean ± SEM, n=8-10 in each group).

	Cough		Bc (sec)			Cough		Bc (sec)	
	Sal	Capt	Sal	Capt		Veh	Thi	Veh	Thi**
Caps	2.1±0.6	0.9±0.4	100±8	73±12	Caps	1.3±0.9	1.1±0.5	168±26	61±6
CA	6.3±1.2	3.9±1.6	80±10	113±22	CA	5.3±0.7	5.9±1.7	155±37	103±14

** = P<0.01

The shortening of the time to onset of Bc by thiorphan supports the view that tachykinins, released by inhaled Caps and CA, mediate this response. The Bc produced by CA was potentiated to a lesser extent than that to Caps, which is in accordance with data showing CA also to involve cholinergic mechanisms (Forsberg et al 1986). Substance P (SP) is metabolized by both ACE and enkephalinase whereas neurokinin A (NKA) seems to be catabolized by the latter only. The observation that the Bc responses were enhanced significantly more by thiorphan than by captopril thus suggests that NKA rather than SP mediates this response. The lack of effect of both enzyme inhibitors on cough indicate that the cough reflex is evoked by the irritants acting directly on the afferent nerve ending and that it is not secondary to release of tachykinins.

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NPY-MEDIATED POTENTIATION OF VASOCONSTRICTOR RESPONSES IN RAT KIDNEY IS NOT DEPENDENT ON A FUNCTIONAL ENDOTHELIUM

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NPY is a tyrosine-rich 36 amino acid peptide associated with vascular sympathetic nerves that presynaptically inhibits transmitter release and postsynaptically causes direct vasoconstriction or potentiates the actions of other vasoconstrictor agents in isolated blood vessels (Edvinsson et al,1987;Carter & Halliday,1988). It has been reported that in the rabbit ear artery the NPY-mediated potentiation of the responses to field stimulation is dependent on the presence of endothelium (Daly & Hieble,1987). NPY is known to be present in nerves in the rat kidney and can cause direct vasoconstriction (Allen et al,1985). In the current experiments we have investigated whether NPY administered via the vasculature can potentiate agonist- and nerve-evoked vasoconstrictor responses in the isolated, perfused rat kidney and whether such potentiation is dependent on an intact endothelium.

Kidneys were taken from male Wistar rats weighing 200 - 350g and cannulated via the renal artery and perfused at a constant flow rate of 4 ml/min with oxygenated (O_2/5%CO_2) Krebs solution at 37°C, giving basal perfusion pressures of 50-80 mmHg. The renal sympathetic nerves were stimulated (10Hz,0.3ms pulse width,20V,20s) by electrodes placed around the renal artery. Responses were measured as changes in inflow perfusion pressure.

A 100pmol bolus injection of NPY caused little or no vasoconstriction in all the kidneys studied (n=10), but when given one minute prior to phenylephrine (PE) caused a marked potentiation of the PE response, giving a three fold shift to the left of the dose response curve, whilst leaving the amplitude of response to a maximal dose of phenylephrine unaffected. The same dose of NPY also caused potentiation of the response to nerve stimulation (37 ±7%;n=6).

The endothelium was then removed by perfusion with the detergent CHAPS,4.5mg/ml for 30 seconds, absence of functional endothelium being taken by lack of vasodilator response to carbachol. After CHAPS treatment the slope and maximum of the PE dose response curve was depressed [maximum by ≈60%], however NPY still potentiated submaximal PE responses. Potentiation of the nerve-evoked response was also present after the CHAPS treatment.

Thus in the isolated perfused rat kidney preparation NPY-mediated potentiation of either agonist- or nerve-evoked vasoconstrictor responses does not depend absolutely on a functional endothelium.

D.V.C. is an MRC scholar.

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THE EFFECTS OF ADENOSINE AND ADENINE NUCLEOTIDES UPON PORCINE ISOLATED TRACHEAL SMOOTH MUSCLE

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Adenosine and the adenine nucleotides are known to induce relaxation of tracheal smooth muscle in a variety of species. In this study the pharmacology of these compounds on porcine tracheal smooth muscle was investigated, as was their degradation by ectoenzymes present on the tissue.

Pharmacological studies were performed in 10ml tissue baths, on strips of smooth muscle (2mm by 10mm) in which the tension had been raised with methacholine (0.1μM; EC_{50}); dose-response curves were produced non-cumulatively. The enzymic degradation of each of the compounds was studied by incubating pieces of tissue, identical to those used in the pharmacological studies, in 100μM solutions of the purines made in Krebs solution. Aliquots were removed at intervals for assay by HPLC, as described previously (Welford & Anderson, 1988).

Adenosine (EC_{50}=104μM) and ATP (EC_{50}=356μM) were more effective in relaxing porcine tracheal muscle than the other purine and pyrimidine nucleosides and 5'-triphosphates which were tested (cytidine, CTP, guanosine, GTP, inosine, ITP, uridine, UTP; none of which produced greater than a 40% relaxation when used at 1mM). AMP (EC_{50}=419μM) and ADP (EC_{50}=407μM) were approximately equipotent with ATP, and about 4 times less potent than adenosine.

ATP, ADP and AMP were each sequentially dephosphorylated, by ectonucleotidase enzymes present on the porcine trachea, to adenosine which itself was then degraded (by adenosine deaminase on the tissue) to inosine which had very little activity. We therefore considered the possibility that there may only be adenosine (P_1) receptors on the tissue and that ATP, ADP and AMP may first have to be dephosphorylated to adenosine in order to relax the tissue.

The addition of adenosine deaminase (1 unit/ml) to the tissue bath reduced the potency of adenosine 6.9 times (EC_{50}=715μM) but did not greatly inhibit AMP (EC_{50}=501μM), ADP (EC_{50}=599μM) or ATP (EC_{50}=541μM). This enzyme should have instantly degraded any adenosine formed as a breakdown product of the nucleotides. These results suggested that the nucleotides themselves can induce relaxation, without first having to be dephosphorylated to adenosine.

The phosphorothioate analogues of AMP, ADP and ATP (AMPS, ADP-β-S and ATP-τ-S) were each found to be resistant to dephosphorylation by ectonucleotidases on porcine trachea, but nevertheless at concentrations of 3-100μM they had similar potency to their naturally-occurring equivalents.

We conclude that there exist on porcine tracheal smooth muscle P_1 (adenosine) and P_2 (nucleotide) purinoceptor subtypes (Burnstock & Brown, 1981) and that, as on guinea-pig trachea (Welford & Anderson, 1988), degradation to adenosine is not a necessary prerequisite for ATP to induce relaxation.

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MECHANICAL, ELECTRICAL AND BIOCHEMICAL EFFECTS OF AH 21-132 IN GUINEA-PIG ISOLATED TRACHEALIS

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AH 21-132 (Markstein et al, 1984) caused concentration-dependent (0.25-8μM) suppression of the spontaneous tone of guinea-pig isolated trachealis and was not antagonised by propranolol (1μM). In tissue treated both with indomethacin (2.8μM) and acetylcholine (ACh; 1mM) removal of tracheal epithelium potentiated AH 21-132 but did not potentiate theophylline. Similar observations have been made by Bewley & Chapman (1988).

Intracellular electrophysiological recording showed that the relaxant effects of AH 21-132 (8μM) were accompanied by suppression of spontaneous electrical slow waves and by mild hyperpolarisation. In tissue treated with indomethacin (2.8μM) and either histamine (200μM), K^+-rich (120mM) Krebs solution or ACh (1mM), log concentration/relaxation curves for isoprenaline, theophylline and AH 21-132 were moved to the right, particularly in the case of ACh (Table 1). Whereas the maximal effects of isoprenaline were reduced by the K^+-rich medium or by ACh, those of theophylline and AH 21-132 were unaffected by the spasmogens.

In tissue treated with indomethacin (2.8μM) and maintained at 12°C, theophylline (0.1-12.8mM) caused spasm attributable (Small et al, 1988) to the release of Ca^{2+} from intracellular stores. This effect was not shared by AH 21-132.

Table 1. (a) Tracheal relaxation; pD_2 (mean ± s.e.m.)

	Spontaneous tone	Histamine 200μM	KCl 120mM	ACh 1mM
Isoprenaline	7.69±0.05	7.66±0.06	6.64±0.07	6.37±0.08
Theophylline	4.15±0.05	3.48±0.03	3.49±0.04	2.93±0.07
AH 21-132	5.55±0.07	5.39±0.09	4.64±0.05	4.09±0.04

(b) Phosphodiesterase (PDE) inhibition; - log molar IC_{50} (mean ± s.e.m.)

	cAMP-PDE	cGMP-PDE
Theophylline	3.44±0.09	3.35±0.24
AH 21-132	5.40±0.20	4.16±0.11

Both AH 21-132 and theophylline inhibited cAMP-dependent and cGMP-dependent PDE derived from the trachea. AH 21-132 was the more potent inhibitor in each case (Table 1) and, in contrast to theophylline, exhibited selectivity for cAMP-PDE.

We conclude that the relaxant action of AH 21-132 more closely resembles that of theophylline than that of isoprenaline. AH 21-132 may, however, be discriminated from theophylline in that (a) its relaxant action is potentiated by epithelial removal (b) it does not release Ca^{2+} from intracellular stores and (c) it exhibits selectivity as an inhibitor of cAMP-PDE as opposed to cGMP-PDE.

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EICOSANOIDS AND PITUITARY FUNCTION: A ROLE FOR PHOSPHOLIPASE A_2 IN THE STIMULATION OF ACTH SECRETION FROM ISOLATED PITUITARY CELLS

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It has been proposed that glucocorticoids exert their effects on a variety of cell types by induction of the phospholipase A_2 (PLA_2) inhibitory protein, lipocortin (Flower, 1988). The aim of this study was to investigate the role of PLA_2 activity in the secretion of ACTH by the pituitary gland.

Rat anterior pituitary cells were grown in primary culture as previously reported (Carey & Stone, 1987). We previously demonstrated inhibition of CRF stimulated ACTH release by mepacrine and now show that the irreversible PLA_2 inhibitor p-bromophenacyl bromide (100uM) attenuates the release of immunoreactive ACTH induced by 10nM CRF-41 or hypothalamic extract (HE) by 100% and 50% respectively (n=8 $p<0.005$). To determine if these effects are due to inhibition of the production of arachidonic acid metabolites the effects of a range of cyclo-oxygenase and 5-lipoxygenase inhibitors on ACTH release were investigated.

Whilst dexamethasone (1uM) inhibited CRF-41 induced ACTH release (80% n=8 $p<0.005$), the cyclo-oxygenase inhibitors aspirin (100uM), indomethacin (5uM) and flurbiprofen (5uM) and the putative 5-lipoxygenase inhibitors phenidone (100uM), BW 755C (100uM) and AA 861 (10uM) were all without effect. NDGA (50uM) produced a significant attenuation of ACTH release ($p<0.005$) but the mechanism by which this non-specific agent exerts this effect is unclear. As reported previously (Buckingham et al, 1987) arachidonic acid (100uM) mimicked CRF-41 and HE and thus elicited release of ACTH (60% stimulation over basal: n=8 $p<0.01$) whilst PGE_2 (0.1-10uM) and LTD_4 (0.01-1.0uM) were without effect. Furthermore, in dispersed pituitary cells, no immunoreactive PGE_2, 6-oxo$PGF_{1\alpha}$ or LTB_4 could be detected in basal or stimulated fractions.

These results indicate a possible role for PLA_2 in the release of ACTH. This effect may be brought about by the generation of arachidonic acid but does not appear to be due to the secondary generation of cyclo-oxygenase or 5-lipoxygenase products. Interestingly a role for cis-unsaturated fatty acids in the fusion of secretory vesicles has been shown (Drust & Creutz, 1988), furthermore a link between PLA_2 activation and GTP-binding proteins has recently been made (Burch et al, 1986) opening up potential mechanisms by which ACTH secretion could be regulated.

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DETECTION OF LIPOCORTIN 1 EXPRESSION BY WESTERN BLOTTING

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It has long been recognised that the anti-inflammatory glucocorticoids inhibit the release of arachidonate from membrane phospholipids and the subsequent formation of prostaglandins, leukotrienes and PAF (Flower 1988 for review). Recently, a glucocorticoid inducible protein, lipocortin 1, was cloned and expressed in E coli (Wallner et al 1986) and was shown to inhibit phospholipase A_2; the enzyme considered to be the rate determining step in eicosanoid biosynthesis. Later this protein was shown to possess anti-inflammatory activity in the carrageenin paw oedema model (Flower 1988).

We were interested in studying the conditions necessary for expression of this protein and raised polyclonal antibodies to specific N-terminal epitopes in order to measure levels in immunoreactive lipocortin 1 in U937 and rat mononuclear cells (Carey et al 1988). Antibodies were raised against amino acid residues 24-37 and 38-50 of the human lipocortin 1 sequence; referred to as epitope A and B respectively. Whilst antibodies to eptitope A recognised only human lipocortin, antibodies to epitope B recognised both rat and mononuclear and U937 protein.

Particulate and cytosolic fractions of rat and human cells were detergent solubilised and samples subject to PAGE. Using duplicate gels, protein was located by staining with Coomassie Blue and lipocortin 1 by Western blotting using anti-lipocortin antibodies. Detergent solubilised cells contained a protein of approx 35.5×10^3 Mr as determined by Western blotting, for which, antibody binding was a linear function of the log of mass of protein (range 5-50μg). We conservatively estimate by densitometric scanning of Coomassie Blue stained gels that lipocortin 1 constituted less than 2% of total cellular protein in U937 cells reflecting a limit of detection of approx <10 pmole. U937 cells grown in the presence of 1.25% DMSO differentiate into mature phagocytes and this was accompanied by an approx 2-fold increase in the amount of lipocortin (mean 1.76; n=3) when expressed as a function of protein concn.

When cells were disrupted in low calcium buffer (<100uM), or in the presence of 2mM EGTA immunoreactive lipocortin was associated with the cytosolic fraction but in the presence of 1mM calcium immunoreactivity was recovered almost exclusively in the particulate fraction. The readdition of calcium (0.3-2mM) to samples prepared in low calcium promoted the appearance of immunoreactive protein bands of lower Mr. Since this also resulted in loss of immunoreactive protein detected by antibodies raised against epitope A we concluded that proteolysis was directed at the N-terminus of lipocortin 1. We conclude that these antibodies will be useful in understanding the regulation of lipocortin 1 expression and in elucidating the mechanism(s) of the anti-inflammatory properties of glucocorticoids.

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THE LONG LASTING EFFECT OF CGRP IN POTENTIATING NEUTROPHIL ACCUMULATION IN RABBIT SKIN

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Vasodilator mediators and chemoattractants act synergistically to induce neutrophil accumulation and oedema formation in vivo (Rampart & Williams, 1987). C5a induces protracted neutrophil accumulation when injected intradermally (id) in the rabbit. The neuropeptide, CGRP, induces protracted vasodilation in skin whereas PGI_2 induces a shorter effect (Brain & Williams, 1985). We have therefore studied synergism between C5a and these two vasodilators to investigate possible differences in the time courses of their effects.

Neutrophil accumulation, in response to intradermally (id)-injected agents was measured over 30 mins, by the accumulation of iv.-injected ^{111}In-neutrophil which were prepared from donor rabbits (Rampart & Williams, 1988). CGRP acted in a dose-dependent way to potentiate neutrophil accumulation induced by C5a ($3x10^{-11}$mol/site, in the form of zymosan activated plasma). A significant effect ($p<0.01$ paired t-test) was observed with 10^{-11}mol/site CGRP (sal 554±85, C5a 2529±412, C5a+CGRP 6965±1627, CGRP 796±105, ^{111}In-neutrophils/site, mean±SEM, n=12 experiments). CGRP when injected alone at doses up to 10^{-10}mol/site did not induce neutrophil accumulation.

In further experiments, ^{111}In-neutrophils were injected iv. followed by id. injection of mediators. Thirty minutes later mediators were injected id. into other sites. Animals were killed after a further 30 minutes. Thus responses were measured over 0-30 and 0-60 minute periods, and a 30-60 minute period by subtraction. Doses of PGI_2 ($3x10^{-10}$mol/site) and CGRP (10^{-11}mol/site) were chosen to give matched responses over a 0-30 minute period. Results, expressed as a percentage (%) of the 0-60 minute C5a+CGRP response are shown in the table.

	% Neutrophil Accumulation			
	0-30 min	0-60 min	30-60 min	n
Control	ND	8.3±1.8	-	7
C5a	21.5±4.0	51.0±6.5	29.5±5.5	7
C5a+CGRP	50.5±3.9*	100*	49.6±3.9*	7
C5a+PGI_2	52.1±3.9*	85.8±3.8*	33.6±4.9	7
CGRP	ND	7.9±1.7	-	7
PGI_2	ND	9.2±1.9	-	7

*$p<0.05$ when compared with C5a alone. ND = not determined.

Thus, synergism between C5a and PGI_2 was only apparent over the first 30 min period, whereas marked synergism was seen between C5a and CGRP over 60 minutes.

These results suggest that interactions between a peptide derived from tissue fluid, C5a and a neuropeptide, CGRP may be important in inducing protracted responses in inflammatory reactions.

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THE ACTIONS OF ENDOTHELIN ON THE HEPATIC ARTERIAL AND PORTAL VASCULAR BEDS OF THE ANAESTHETISED DOG

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Endothelin (ET) is a 21-residue peptide recently discovered in culture supernatant of porcine aortic endothelial cells and shown to possess potent pressor activity in the rat (Yanagisawa et al., 1988). To establish the effects of ET on a peripheral vascular bed, synthetic ET was injected into the separately perfused arterial and portal vascular beds of the liver of anaesthetized dogs.

In 5 experiments, dogs (18-26 kg body weight) were anaesthetized (chloralose 50 mg kg^{-1}, urethane 500 mg kg^{-1}, i.v.) and the hepatic artery was cannulated and perfused with arterial blood taken from the femoral artery (Corder & Withrington, 1988). Mean hepatic arterial blood flow (HABF) and mean perfusion pressure were monitored continuously for changes in vascular resistance. Endothelin (porcine) was injected intra-arterially over the dose range 1 pmol - 1 nmol.

At the lower dose range (1-50 pmol) ET caused dose-dependent increases in HABF indicating a fall in hepatic arterial vascular resistance. This vasodilator response to ET lasted slightly longer than that following a maximally effective dose of isoprenaline. At the higher dose range (100 pmol - 1 nmol) the initial vasodilator responses were succeeded by marked and prolonged vasoconstrictions. After ET (100 pmol) the mean time from injection to the maximum vasodilator effect (increase in flow of 37.1 $\pm$ 18%) was less than 1 min whereas the mean time to the peak vasoconstrictor response (mean reduction in flow 12.9 $\pm$ 4.3%) was 16.4 $\pm$ 1.6 min. The vasodilator or vasoconstrictor phases of the vascular responses to ET (100 pmol) were not significantly affected by treatment with indomethacin (2 mg kg^{-1} i.v. or 1 mg kg^{-1} i.a.); the mean changes in hepatic arterial vascular resistance being -27.8 $\pm$ 9% and +18.8 $\pm$ 5.7% before and -31.8 $\pm$ 9% and +13.45 $\pm$ 0.95% after (n=4).

In contrast to the biphasic responses to ET observed in the hepatic arterial vascular bed, intraportal injections of ET evoked only dose-dependent increases in portal perfusion pressure. These portal vasoconstrictor responses to ET lasted 4-5 times longer than those induced by equi-effective doses of noradrenaline.

Endothelin releases eicosanoids from guinea-pig isolated lungs (Antunes et al., 1988) and EDRF from the rat isolated perfused mesentery (Warner et al., 1988). The finding that the increase in the HABF was not affected by indomethacin suggests that ET does not release vasodilator cyclo-oxygenase products in this preparation. Thus, the vasodilatation observed is probably due to release of EDRF.

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EFFECTS OF ENDOTHELIN ON CARDIAC OUTPUT AND REGIONAL BLOOD FLOW IN THE PITHED RAT

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Endothelin (ET), a vasoactive peptide isolated from culture fluid of endothelial cells is a potent pressor agent in chemically denervated rat preparations (Yanagisawa *et al.*, 1988). The mode of action proposed for the peptide is via activation of calcium channels in vascular smooth muscle, but little is known about the selectivity of ET on individual vascular beds. We have investigated the effects of ET on cardiovascular parameters including blood flow and its distribution in the pithed rat.

Male Wistar rats (280-330g) pithed under halothane anaesthesia and respired with air were used. The right femoral artery was cannulated for the measurement of systemic blood pressure and the left jugular vein for the administration of drugs. A polythene cannula was introduced via the right common carotid artery into the left ventricle. A stabilization period of 15 min was allowed after the operative procedures. Endothelin (1 nmole/kg) was administered and during a stable pressor response of 45 $\pm$ 4 mmHg, 60,000 - 80,000 ^{113}Sn labelled microspheres 15 $\pm$ 3μm diameter, suspended by ultrasonication in 0.3 ml 0.9% saline containing 0.01% Tween 80, were injected into the left ventricle over a period of 20 seconds. Concurrently, blood was withdrawn from the left femoral artery at a rate of 0.5 ml/min during the introduction of the microspheres and for 70 s afterwards. The animals were then killed and the tissues dissected out, weighed and placed in vials for counting.

In addition to a sustained pressor response ET caused a doubling of left ventricular $dp/dt_{(max)}$ and a 118% increase in total peripheral resistance (TPR), the latter being partially reversed by indomethacin (15 μmoles/kg; i.v.). However, this effect of indomethacin on TPR was compensated for by an increase in cardiac output (CO). A decrease in blood flow was observed in kidney (-23%), spleen (-62%), small intestine (-28%), large intestine (-45%), pancreas/mesentery (-85%) and pectoral skeletal muscle (-70%). Concomitantly, in these tissues the falls in blood flow were accompanied by increases in vascular resistance. Indomethacin reduced the ET-induced fall in blood flow to the skeletal muscle. Endothelin alone had no effect on gastric blood flow but in the presence of indomethacin this was decreased by -21%. Endothelin caused an increase in blood flow to the liver (hepatic artery supply; 102%), coronary bed (94%), and the lung (bronchial artery supply; 610%).

Thus, ET causes a decrease in blood flow by increasing vascular resistance in a wide range of vascular beds via a mechanism independant of cyclo-oxygenase products. However, in certain organs eicosanoids seem to play a role in the modulation or potentiation of the response to ET. Taking the proportion of the CO received by the individual beds into account the kidneys, gastro-intestinal tract and skeletal muscle were the major contributors to the pressor effects of ET.

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DIFFERENTIAL INHIBITION OF COLLAGEN AND ADP INDUCED PLATELET AGGREGATION BY PROSTACYCLIN IN VIVO

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Prostacyclin (PGI_2) inhibits platelet aggregation induced by most agents in vitro (Whittle & Moncada, 1985) by elevating adenosine 3', 5'- monophosphate (cyclic AMP) levels (Tateson et al, 1977). However, the effect of PGI_2 on platelet aggregation in vivo has been less extensively characterised. We have examined the antiaggregatory activity of PGI_2 against collagen and adenosine diphosphate (ADP) in an in vivo model (Page et al, 1982) in which accumulation of radiolabelled platelets in the lungs of anaesthetised guinea pigs is monitored externally following intravenous (i.v.) administration of the aggregating agent.

Male guinea pigs (Dunkin-Hartley, 500-700g) were anaesthetised with urethane (1.5 $g.kg^{-1}$ i.p.). The trachea was cannulated and artificial ventilation applied (56 strokes.min^{-1}, 8 $ml.kg^{-1}$). Both jugular veins were cannulated for platelet and drug administration. Collimated gamma scintillation probes were positioned to record counts from the thorax (C1) and abdomen (C2). Platelets, obtained from citrated blood from donor guinea pigs, were radiolabelled with 50 µCi ^{111}Indium oxine and administered to the surgically prepared recipient. Aggregatory responses were then obtained to ADP (0.3-300 $\mu g.kg^{-1}$ i.v.) or collagen (10-1000 $\mu g.kg^{-1}$ i.v.) during i.v. infusion (0.01 $ml.min^{-1}$) of PGI_2 (5, 50 or 500 $ng.kg^{-1}.min^{-1}$) or vehicle (glycine buffer, pH 10.5).

Both ADP and collagen produced dose related aggregatory responses measured as increases in the ratio C1/C2. Doses of each agent giving a 25% (ED_{25}) increase in C1/C2 during either vehicle or PGI_2 infusion are shown in table 1. Infusion of PGI_2 at 50 and 500 $ng.kg^{-1}.min^{-1}$ produced marked dose dependent inhibition of ADP induced aggregation. Aggregatory responses to collagen were more resistant to PGI_2 and although significant inhibition was produced by 50 $ng.kg^{-1}.min^{-1}$ a ten-fold increment in the PGI_2 dose gave little further inhibition.

Table 1 Inhibition of collagen and ADP induced aggregation by PGI_2

PGI_2 ($ng.kg^{-1}.min^{-1}$)	Collagen ED_{25} ($\mu g.kg^{-1}$)	ADP ED_{25} ($\mu g.kg^{-1}$)
0 (vehicle)	33 ± 4	1.5 ± 0.5
5	47 ± 11	1.8 ± 1.0
50	* 180 ± 23	* 121 ± 71
500	* 300 ± 87	* >300

Values are means ± s.e.m. from 4-5 experiments. * $p<0.05$ c.f. vehicle (unpaired t-test)

Collagen and ADP invoke quite different transduction pathways in stimulating platelets (Rink & Hallam, 1984). Our results show that the collagen pathway in vivo is less susceptible to inhibition by PGI_2 and are consistent with the demonstration (Rink & Sanchez, 1984) of a PGI_2- , forskolin- and, by implication, cyclic AMP- resistant component of collagen induced activation of human platelets in vitro.

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MENSTRUALLY-RELATED VARIATIONS IN RESPONSIVENESS OF HUMAN ISOLATED UTERINE VASCULATURE TO PROSTAGLANDINS

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Prostaglandins (PGs) have been widely implicated in cyclic endometrial shedding and in dysmenorrhoea. Their precise role remains unclear (Pickles, 1957; Eglington et al, 1963) but may involve interactions with uterine vasculature. Accordingly we have studied the effects of PGs E_2 and $F_{2\alpha}$ on the contractility of human uterine arteries and veins obtained fresh from surgical hysterectomies. Individual vessels were threaded over a glass rod and cut spirally to give predominantly circular muscle strips 2-3cm x 2-4 mm which were suspended in isolated organ baths in Krebs' solution at 37°C, gassed with 5% CO_2 in O_2 (pH 7.4). Up to 6 strips from 1 patient were studied simultaneously, recording muscle activity isotonically. Responses were magnified 5-375 times.

In 71 artery and 54 vein strips from 72 patients (mean age 40 years, range 23-53) doses-related contractions were evoked by (-)noradrenaline bitartrate (NA, 10^{-8}-10^{-5} gml^{-1}, dissolved in 0.9% NaCl containing 0.57mM ascorbic acid and PGs E_2 or $F_{2\alpha}$ (10^{-9}-10^{-5} gml^{-1}, dissolved in 0.2M phosphate buffer, pH 6.7). However, in a few vein strips PGE_2 in concentrations < 10^{-8} gml^{-1} caused small relaxations. The responsiveness of vessels from different patients to NA was relatively constant but sensitivity and amplitude of contraction to the PGs varied greatly. Thus to quantify the responsiveness to the PGs for comparisons between patients, the ratios PGE_2 or $PGF_{2\alpha}$:NA of the amplitudes of contractions induced by PG and NA in equal concentrations (0.5 or 1.0 x 10^{-6} gml^{-1}, at which NA regularly caused large but submaximal contractions) were calculated. Ratios for strips taken at different stages of the menstrual cycle are shown in table 1.

Table 1 Responsiveness to PGs expressed as ratio of PGE_2 or $PGF_{2\alpha}$:NA response

	Menstrual (days 1-4)		Follicular (days 5-14)		Luteal (days 15-28)	
	n	median (quartiles)	n	median (quartiles)	n	median (quartiles)
Artery PGE_2	9	0.2 (0.1-0.5)	16	0.5 (0.1-0.7)	9	0.1 (0.1-0.2)
Artery $PGF_{2\alpha}$	10	0.7 (0.1-0.9)	14	0.5 (0.3-0.6)	13	0.8 (0.2-1.0)
Vein PGE_2	9	1.3 (1.0-1.4)	11	1.2 (0.6-1.5)	5	2.0 (0.7-2.3)
Vein $PGF_{2\alpha}$	6	0.4*(0.2-0.9)	14	1.1*(0.9-1.4)	9	1.0 (0.5-1.3)

* Significantly different from each other, P <0.001, Mann Whitney-U-Test

Arterial strips were clearly less responsive to PGs than to NA. Their activity did not vary significantly during the menstrual cycle. Venous strips were more responsive to the PGs, although in the menstrual phase reduced contractility to $PGF_{2\alpha}$ was apparent. This may represent a protective mechanism preventing excessive venospasm resulting from elevated endometrial $PGF_{2\alpha}$ production at this stage (Singh et al, 1975). The role of venous tone and contractility in uterine blood flow and disorders thereof, however, awaits investigation.

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EXPRESSION OF FUNCTIONAL ADENOSINE A_2 RECEPTORS ON BOVINE AORTIC ENDOTHELIAL CELLS

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Adenosine reduces vascular tone by interaction with A2 purinoceptors on arterial smooth muscle. The response is mediated by activation of adenylate cyclase, and is longer lasting than responses to adenine nucleotides (e.g. ATP or ADP at P2 purinoceptors). Adenosine also inhibits noradrenaline release from perivascular sympathetic nerves. The response augments the dilator effects of adenosine on arterial smooth muscle, and is also mediated by A2 receptors, (for review see Burnstock, 1987). We have now examined A2 responsiveness in arterial endothelial cells.

AG4762 bovine aortic endothelial cells were obtained from the cell repository of the institute of aging (USA). They were grown to confluency on monolayer surfaces in Dulbecco's modified Eagle's minimim medium. Adenylate cyclase activity was measured in homogenates of these cells in the absence or presence of 5'-(N-ethylcarboxamido)-adenosine (NECA), 2-chloroadenosine and adenosine at selected concentrations. All three compounds increased enzyme activity, and the ligand concentrations required for half-maximum activation (Kact) were 0.53 ± 0.07, 2.46 ± 0.24, and 11.8 ± 0.80 µM respectively. NECA-dependent enzyme activation was inhibited by 3-isobutyl-1-methylxanthine, theophylline and caffeine, with Ki values of 9.36 ± 0.79, 35.2 ± 8.0, 526 ± 138 µM respectively (values are means $\pm$ SEM, n = 3-5). These results confirm the presence of A2-dependent responses to adenosine (for review see Daly, 1982).

Prolonged exposure of AG4762 cells to 0.5µM NECA resulted in the slow loss of subsequent responses to 4 µM NECA; 50% desensitization occuring in about 1h under these conditions. Cells were then exposed for 19h to selected concentrations of NECA between 0 and 100 nM. Desensitization was dose dependent, with half-maximum desensitization at 45nM under these conditions. Finally, cells were cultured for 24h in cytosine arabinofuranoside, to inhibit further cell division, and then desensitized with 0.5 µM NECA for 19h. Thereafter, the NECA was removed, and the cells cultured for selected times between 0 and 48h. The stimulatory effect of 4µM NECA was then measured in AG4762 homogenates, and A2 responsiveness was seen to increase slowly back to control levels (T 1/2 = 8h).

These results confirm the presence of functional adenosine A2 receptors in these cells, and demonstrate regulation of their function by prolonged exposure to agonist. The role *in vivo* of endothelial A2 purinoceptors remains obscure, but is the subject of current research in our laboratory.

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THROMBOXANE TRIGGERS RELEASE OF PROSTACYCLIN FROM CULTURED AORTIC ENDOTHELIAL CELLS

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Thromboxane A2 (TXA2) is a potent vasconstrictor, whose effects are mediated by activation of smooth muscle receptors. However, numerous constrictor substances, including ATP and ADP, activate endothelial cells to release endothelium derived relaxant factor (NO) and prostacyclin, both of which are vasodilators. The probable significance of this dual role of certain vasoconstrictors has been reviewed (Burnstock 1987). In this study we have examined the effects of the TXA2 mimetic, U46619, on calcium mobilization and prostacyclin release from bovine aortic endothelial cells.

The AG4762 bovine endothelial cell line was obtained from the cell repository of the National Institute of Aging (USA). Cells (passage 16-22) were grown as monolayers in flasks and then transferred to microcarrier beads. The release of prostacyclin from perfused cells was measured as described by Crossman et al. (1987). Prostacyclin release was measured on a 2ml column, and 6-oxo-PGF1α (the stable hydrolysis produce of prostacyclin) measured in the eluate by radioimmunoassay. Due to the cross reactivity of the antibody with U46619, 6-oxo-PGF1α was extracted prior to analysis. Calcium mobilization was measured in monolayers of the AG4762 cells using fura-2, as described by Hallam et al. (1988).

Basal levels of 6-oxo-PGF1α at the beginning of each assay were within the range 100-200pg/ml per million cells. Cells were perfused with bradykinin or thrombin to act as positive controls. The drugs were perfused in the order U46619 (1μM), thrombin (1/2 unit/ml), followed by bradykinin (1μM). U46619 produced a peak concentration of 853 $\pm$ 140pg 6-oxo-PGF1α/ml (mean $\pm$ SD, n = 3). Thrombin produced a peak of 1780 $\pm$ 330pg 6-oxo-PGF1α/ml (mean $\pm$ SD, n = 3), which was twice that produced by U46619. Bradykinin was found to stimulate production of 6-oxo-PGF1α to 1867 $\pm$ 416 pg/ml (mean $\pm$ SD, n = 3). The effect of Bradykinin was delayed relative to the responses to the other agonists, possibly due to its retardation on the column.

Calcium fluorimetry studies showed that U46619 and bradykinin both produced prompt increases in intracellular calcium in these cells.

In conclusion, both U46619 and bradykinin initiate prostacyclin release from endothelial cells,and trigger a transient increase in intracellular calcium. It seems likely that prostacyclin release occurs as a consequence of a calcium-dependent activation of phospholipase A2. These results provide compelling evidence for the release of vasodilator substances from endothelium, following binding of a thromboxane-like mimetic. The identity of the receptor will be further characterized by an examination of the inhibitory effects of known TXA2 antagonists.

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DIFFERENTIAL DRUG ACTIONS ON OEDEMA FORMATION AND LEUKOCYTE ACCUMULATION IN RAT IMMUNOLOGICAL INFLAMMATION

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Cytokines have been reported to induce vascular and cellular changes in the rat and may mediate delayed-type hypersensitivity (Issekutz, et al. 1988; Martin et al. 1988). In the present study we report the actions of several agents on oedema formation and leukocyte accumulation in a rat model of immunological inflammation.

Male CFHB rats (130-150g, Interfauna) were sensitised using a modification of our previous method (Hambleton & Miller, 1988). Methylated bovine serum albumen (MBSA, 10mg/ml in saline) was emulsified with an equal volume of Freund's complete adjuvant and rats were injected s.c. in the tail base with 1mg MBSA in 0.2ml emulsion. In separate experiments rats were challenged 7 days later either into a hind paw (0.5mg MBSA in 0.2ml saline) or 6 day s.c. air pouch (1mg in 5ml saline). Paw oedema was expressed as increase in paw weight (mg) or diameter ($mm.10^2$), and air pouch inflammation was expressed as leukocyte count ($x10^6$) and exudate volume (ml) following harvesting of pouch contents. Extended time courses typical of delayed-type hypersensitivity were observed for both sites. In the drug studies the agents were administered p.o. at -1h and +6h relative to challenge and their effects on 24h paw oedema and air pouch leukocyte accumulation are shown below.

Compound	Dose (mg/kg)	% Change Oedema (mg)	% Change Leukocytes ($x10^6$)
Tiaprofenic Acid	2 x 5	-8	+33*
Prednisolone	2 x 50	-43*	-92**
Cyclosporine	2 x 20	-81**	+45*
HWA 486	2 x 10	-42**	+15

*P<0.05 **P<0.005 (t test)

The non-steroidal anti-inflammatory agent tiaprofenic acid had little effect on oedema and increased leukocyte accumulation whereas the steroid prednisolone inhibited both parameters with a greater effect on leukocytes. The immunomodulatory agents cyclosporine and HWA 486 (Bartlett, 1986) both inhibited oedema, and cyclosporine increased the leukocyte number.

These data indicate the possibility of differential modulation of aspects of immunoinflammatory effector mechanisms and support previously reported observations for cyclosporine in mice (Dunn & Miller, 1986; Pereira et al. 1988).

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A PHORBOL ESTER INHIBITS SUBSTANCE P-INDUCED EDRF RELEASE FROM CULTURED PIG AORTIC ENDOTHELIAL CELLS

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Agents that release endothelium-derived relaxing factor (EDRF) are known to elevate the cyclic GMP content of pig aortic endothelial cells, and this can be used as a measure of EDRF release (Martin et al., 1988). Phorbol-12,13-dibutyrate (PDB) has been shown to inhibit EDRF release by acetylcholine or substance P in rings of rabbit aorta (Lewis and Henderson, 1987). This study investigates the effects of PDB on substance P-induced EDRF release from cultured pig aortic endothelium, using endothelial cell cyclic GMP content as the measure of EDRF release.

Endothelial cells were isolated from pig aorta using collagenase as described by Gordon and Martin (1983), seeded into 9.6 cm^2 multiwell plates and grown to confluence. The culture medium was replaced with Krebs solution, and the cells incubated at 37°C for 90 min prior to addition of drugs. Cyclic GMP was measured by radioimmunoassay and DNA assayed by the method of Kissane and Robins (1958).

Substance P (10^{-8}M, for $1^1/_2$ min) induced a 5 fold increase in the cyclic GMP content of these primary cultures of pig aortic endothelial cells, from a resting level of 14.5 ± 0.8 fmol/µg DNA (n=12) to 74.7 ± 10.2 fmol/µg DNA (n=10, $P<0.001$). Pretreatment for 20 min with the EDRF inhibitor haemoglobin (Hb, 10^{-5}M) reduced the resting level of cyclic GMP (6.5 ± 1.9 fmol/µg DNA, n=6, $P<0.001$) and prevented the substance P-induced increase (10.2 ± 2.5 fmol/µg DNA, n=6, $P>0.05$). Pretreatment with PDB ($3x10^{-7}$M), but not the inactive phorbol didecanoate (PDD, $3x10^{-7}$M) inhibited the substance P-induced increases in cyclic GMP. The extent of the inhibition by PDB ($3x10^{-7}$M) increased with increasing pretreatment time, reaching a maximum at 5 min when 10^{-8}M substance P no longer produced a significant elevation of cyclic GMP levels. The effect of PDB was also concentration-related, as the substance P-induced increase in cyclic GMP levels was inhibited $22.4\pm10.1\%$ ($P>0.4$) by $3x10^{-8}$M PDB, $76.4\pm5.3\%$ ($P<0.005$) by $3x10^{-7}$M PDB and $81.1\pm10.7\%$ ($P<0.005$) by $3x10^{-6}$M PDB (n=6, all 5 min preincubations with PDB). Sodium nitroprusside (SNP, 10^{-5}M for 3 min) induced a 6.7 fold increase in endothelial cell cyclic GMP content from a resting level of 15.6 ± 1.9 fmol/µg DNA (n=12) to 100.4 ± 9.2 fmol/µg DNA (n=6, $P<0.001$), and this was prevented by 20 min pretreatment with 10^{-5}M Hb (12.1 ± 2.5 fmol/µg DNA, n=6, $P>0.2$). Pretreatment for 5 min with $3x10^{-7}$M PDB or PDD did not inhibit the SNP-induced increases in cyclic GMP (86 ± 10.7 fmol/µg DNA, n=6 and 75.6 ± 3.8 fmol/µg DNA, n=6 respectively) when compared with solvent (ethanol) controls (81.6 ± 5.3 fmol/µg DNA, n=6, $P>0.8$ and >0.3 respectively).

It is concluded from these results that activation of protein kinase C can inhibit receptor-mediated release and/or production of EDRF induced by substance P.

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PROTECTION MEDIATED BY INHIBITION OF THROMBOXANE SYNTHESIS IS NOT ENHANCED BY ACE INHIBITION IN CYCLOSPORIN A TREATED ANIMALS

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Cyclosporin A nephrotoxicity involves a reduction in glomerular filtration rate (GFR) which may be due to thromboxane A2 induced vasoconstriction. Thromboxane synthetase inhibition (TSI) has been shown to protect partially against CSA nephrotoxicity in rats (Whiting et al., 1986; Smeesters et al., 1988). Inhibition of angiotensin converting enzyme (ACE) also results in an improved GFR in patients with compromised renal function (de Zeeuw et al., 1983). The aim of this study was to test the hypothesis that administration of an ACE inhibitor (ACEI) in combination with TSI would further protect against the CSA induced decrease in GFR.

Three groups of rats weighing between 300-400g (n=6) were treated for 14 days with CSA (50mg/kg p.o.). Group A received CSA only whilst group B additionally received TSI (10mg/kg p.o. of 8-[3-methyl-2-(3-pyridyl)-1-indoyl]-octanoic acid, CGS 12970). Group C received both TSI (as above) and ACEI (10mg/kg p.o. of CGS 16617). The control group D received the CSA and inhibitor vehicles, ethanol/olive oil and 0.9% saline respectively. Creatinine clearance (CCR) and urinary N-Acetyl-β-D-glucosaminidase (NAG) excretion were measured at 0,7, and 14 days of treatment. Results were compared by one way ANOVA with significances assigned using the Neuman-Keuls test.

Table 1 Creatinine clearance and NAG exretion in control and treated animals

	Creatinine Clearance (ml/h / kg)			NAG(nmol 4MU/h/mmol creatinine)		
	Days			Days		
	0	7	14	0	7	14
Groups						
A. CSA	343 ± 93	260 ± 113	134 ± 54	65 ± 11	121 ± 79	185 ± 65
B. CSA + TSI	341 ± 93	280 ± 166	269 ± 78*	60 ± 7	117 ± 54	107 ± 39
C. CSA+TSI+ACEI	468 ± 23	311 ± 44	163 ± 55	56 ± 17	124 ± 41	119 ± 113
D. Control	341 ± 96	427 ± 66	446 ± 47†#Ω	64 ± 11	62 ± 9	54 ± 6†

Values are given as means # SD. Significant differences between groups $p<0.05$ at day 14 are denoted † for D vs A; # D vs B; Ω D vs C; * A vs B.

These data confirm the involvement of vasoconstrictor eicosanoids, such as thromboxane A_2, in the development of CSA nephrotoxicity. In this study CGS 12970 partially protected against CSA induced changes in both GFR and tubulopathy, in contrast to an earlier suggestion that TSI selectively improves GFR. Although TSI and ACEI may be expected to have a synergistic effect, the ACEI could also be acting to remove the constrictor effect of angiotension II at the efferent renal arterioles, resulting in a decrease in GFR.

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INVOLVEMENT OF LTB_4 IN THE NEUTROPHIL CHEMOATTRACTANT ACTIVITY RELEASED FROM ANAPHYLACTIC GUINEA-PIG LUNG

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Recent work has revealed the accumulation of neutrophils and eosinophils in guinea-pig lung fluid after aerosol antigen challenge, (Hutson et al, 1988). The involvement of LTB_4 in the immediate neutropenic response following iv antigen challenge of sensitised guinea-pig has been indicated, (Walker and Woodhouse, to be published). Schenkel et al, (1982) reported the presence of neutrophil chemoattractant activity, (NCA), in the high MW component of the supernatant from guinea-pig lung fragments after in vitro antigen challenge. This present study aimed to investigate the role of LTB_4 in the NCA released in vitro from anaphylactic challenge of guinea-pig lung fragments using the selective LTB_4 receptor antagonist LY223980, 5-(3-carboxybenzoyl)-2-((6-(methoxyphenyl)hexoyl)oxy)benzeneproprionic acid).

The method used for obtaining supernatant was similar to that described by Schenkel et al., (1982). Detection of NCA was carried out using guinea-pig peritoneal neutrophils in an in vitro chemotaxis assay.

Unchallenged, sensitised lung fragments were found to release small amounts of NCA spontaneously into the supernatant. This release was greatly increased after the lung fragments had been incubated with antigen, (ovalbumin, 200ug/ml) for 20 mins at 37°C, $p<0.001$. NCA was also detected in the high MW fraction of challenged supernatant after ultrafiltration through an Amicon filter, (retentates >10,000MW, filtrates <10,000MW). The activity, again, was greater in challenged supernatant than in unchallenged controls, $p<0.001$, and was similar to the activity in unfiltered, challenged supernatants. Little NCA was observed in low MW fractions of challenged and unchallenged material.

The cellular response to the unfiltered, challenged supernatant was reduced by 92% following pre-incubation of the neutrophil suspension with LY223980 10^{-5}M, 5 mins before commencement of the chemotaxis assay, $p<0.001$. This level of LY223980 has previously been shown to be selective for the LTB_4 receptor, (Walker et al, 1988). The cellular response to the high MW fraction of challenged supernatant was also reduced by 92%, $p<0.001$, on pre-incubation of the cells with the LTB_4 receptor antagonist. This work provides evidence for involvement of LTB_4 in the NCA released from guinea-pig lung fragments after antigen challenge in vitro.

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PROSTANOID RECEPTOR PROFILE OF ENPROSTIL AND ISOMERS IN VITRO

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Enprostil is an allenic E_2 prostaglandin analogue shown to be a potent inhibitor of gastric acid secretion in both animal and human studies. We have assessed the action of enprostil and its individual 4 isomers (denoted RS-86505-007, RS-86812-007, RS-86505-008 and RS-86812-008) at prostanoid receptors as defined by Coleman et al., (1985). The methods used have been reported previously (Eglen and Whiting, 1988). Tissues were suspended under 1.0 g tension in physiological salt solution containing indomethacin and atropine (1 μM) and maintained at pH 7.4, 37°C.

Table 1: Enprostil -log EC_{50} values at prostanoid receptors

Compound	EP_1 (GPI)	EP_2 (GPT)	EP_3 (GPVD)	FP (RC)	TP (GPA)
Enprostil (racemate)	nr	nr	8.30	7.34	6.54
RS-86505-007 (natural R)	nr	nr	8.86	7.41	7.43
RS-86812-007 (natural S)	nr	nr	8.18	7.57	6.86
RS-86505-008 (unnatural S)	nr	nr	nr	6.76	6.46
RS-86812-008 (unnatural R)	nr	nr	>6.0	nr	5.39
PGE_1	7.81	6.57	8.46	6.23	nr
PGE_2	6.84	7.43	7.70	7.21	>5.0
16,16 dimethyl PGE_2	8.96	5.45	8.84	6.93	6.44
$PGF_{2\alpha}$	6.21	nr	6.16	8.02	6.20
U46619	nr	nr	nr	nr	8.44

Values are mean SEM <5%, n=8; nr - no response. GPI, GPT, GPVD, GPA - guinea-pig ileum, trachea, (relaxation), vas deferens and aorta respectively; RC - rat colon. All compounds were studied between $1x10^{-10}$ - $3.2x10^{-5}$M.

Enprostil acted as an agonist at EP_3, FP and TP receptors whilst no agonism was observed at EP_2 receptors at the concentrations studied (Table 1). The EP_1 effects of enprostil, RS-86505-007 and RS-86512-007 in the ileum differed from those observed with either PGE_1 or PGE_2 in that a general increase in spontaneous activity was observed in contrast to a clear contractile response.

At EP_3, FP and TP receptors, the rank order of potency was RS-86505-007 > RS-86812-007 ≥ Enprostil > RS-86505-008 > RS-86812-008. These data suggest that the R form of the 'natural' epimer is optimal for EP_3, FP and TP agonism. It should be noted that the major forms of enprostil in human plasma, after oral administration, are the RS-86505-008 and RS-86812-008 epimers, which possess the least effect at EP_3, FP and TP receptors.

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α-ADRENOCEPTOR SUBTYPE MEDIATING THE TUBULAR ACTIONS OF THE RENAL NERVES IN DOCA-SALT AND 2K 1C GOLDBLATT HYPERTENSION IN RATS

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Activation of the renal nerves causes an increase in tubular sodium reabsorption which is mediated by α_1-adrenoceptors on the epithelial cells of the proximal tubules and thick ascending limb of the loop of Henle. Radioligand binding studies have shown a proponderance of α_2-adrenoceptors in the renal cortex which increase in density in genetic hypertension and salt loading. The aim of this study was to functionally define the subtype of α-adrenoceptors involved in the neural control of sodium reabsorption in salt induced and renovascular models of hypertension.

Rats (170-200g) were unilaterally right nephrectomised and one week later given twice weekly injections of DOCA in arachis oil, 15mg/kg s.c., and saline (0.9% NaCl) to drink. Goldblatt hypertension was induced by placing a silver clip (0.25mm diameter) on the renal artery of rats (160-200g). All animals were used 5-6 weeks later when they were anaesthetised (60mg/kg i.p., sodium pentobarbitone), the carotid artery and jugular vein cannulated, the left kidney exposed, its artery cleared and a flow probe fitted, its ureter cannulated and the renal nerves isolated and sectioned. Saline containing inulin was infused at 6.0ml/h i.v. and experiments begun 2h later. Fifteen minute clearance periods were taken, two before and two following a period of renal nerve stimulation (15v, 0.2ms) at a frequency to cause minimal changes in renal blood flow. Prazosin (α_1-antagonist) or idazoxan (α_2-antagonist), 250μg/kg plus 50μg/kg/h was given and 30 min later the sequence of clearance periods were repeated.

The blood pressure (BP) of DOCA-salt treated rats was elevated compared with unilaterally nephrectomised vehicle-treated control rats (174±2 versus 148±2mmHg, $p<0.001$). During the first and second renal nerve stimulation periods renal blood flow (RBF), at 25.2±1.3ml/min/kg, decreased by 9% ($p<0.001$) each time, glomerular filtration rate (GFR), at 2.96±0.11, fell by 9% ($p<0.001$) and 5% ($p<0.01$) respectively, urine flow, at 98.0±13.8μl/min/kg, was reduced by 56% and 57% (both $p<0.001$) respectively, absolute ($U_{Na}V$) and fractional sodium (FE_{Na}) excretions, at 15.6±2.1μmol/min/kg and 3.5±0.6% respectively, decreased by between 61% to 65% (all $p<0.001$). A similar pattern of renal haemodynamic and excretory responses to renal nerve stimulation were obtained in DOCA-salt hypertensive rats given idazoxan in the second half of the experiment whereas they were abolished following administration of prazosin. BP in the Goldblatt hypertensive rats, at 179±3mmHg, was significantly ($p<0.001$) higher than the sham operated controls, at 163±2mmHg. Stimulation of the renal nerves in the first and second periods reduced RBF, at 20.1±1.0ml/min/kg, by 9% (both $p<0.001$) each time, GFR, at 2.83±0.18ml/min/kg, by 4% and 5% (both $p<0.05$) respectively, UV, at 115.9±18.1μl/min/kg, by 53% and 48% (both $p<0.001$) respectively, $U_{Na}V$, at 19.7±3.9μmol/min/kg, by 65% and 62% (both $p<0.001$) respectively and FE_{Na}, at 4.73±0.86%, by 63% and 60% (both $p<0.001$) respectively. In the group of Goldblatt hypertensive rats given idazoxan in the second half of the experiment an identical pattern of renal excretory responses were obtained whereas they were abolished following administration of prazosin.

These results show that in both DOCA-salt and 2K 1C Goldblatt hypertensive rats the renal nerves stimulate tubular sodium reabsorption via α_1- but not α_2-adrenoceptors. These findings are similar to those obtained in normotensive controls and indicate that neither DOCA-salt treatment nor renovascular degeneration affects the composition of the α-adrenoceptors mediating the tubular actions of the renal nerves.

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DIFFERENTIAL REGULATION OF [^{3}H] YOHIMBINE AND [^{3}H] IDAZOXAN BINDING IN THE RABBIT

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Recently we have reported differences in [^{3}H] yohimbine and [^{3}H] idazoxan binding in rabbits (Hamilton et al, 1988) with more [^{3}H] yohimbine than [^{3}H] idazoxan binding sites in the brain and more [^{3}H] idazoxan than [^{3}H] yohimbine binding in kidney. In 'in vitro' agonist and antagonist displacement studies [^{3}H] yohimbine binding was consistent with binding to an alpha$_2$-adrenoceptor but [^{3}H] idazoxan appeared to bind preferentially to a non-adrenergic site. These findings have been confirmed by by others (Coupry et al, 1987). We have now compared the effects of in vivo treatment with an alpha$_2$ agonist, antagonist and an uptake blocker on the [^{3}H] yohimbine and idazoxan binding sites.

Groups of rabbits (2.5-3.0 kg, n = 6 - 12) received noradrenaline (1.1 mg/kg/day i.v.) for 10 days, idazoxan (4.9 mg/kg/day i.v) for 5 days or amitriptyline (30 mg/kg/day i.p.) for 21 days. At the end of the experiments rabbits were killed, brain and kidney removed, and membranes prepared by centrifugation and homogenisation. Tissue homogenates were incubated with [^{3}H] idazoxan (0.5-20 nM) or [^{3}H] yohimbine (1.25-37.5 nM) for 25 min at 25^{o}C. The maximum number of binding sites (Bmax) and the dissociation constant (K_D) were estimated from Scatchard plots. The treatments resulted in tissue and ligand specific changes in Bmax (Table 1) with no changes in K_D.

Table: Effect of treatment on [^{3}H] yohimbine and [^{3}H] idazoxan binding

Treatment	Bmax [^{3}H] Yohimbine			Bmax [^{3}H] Idazoxan		
	Forebrain	Hindbrain	Kidney	Forebrain	Hindbrain	Kidney
Control	100 ± 40	132 ± 56	56 ± 18	72 ± 20	45 ± 17	216 ± 77
Idazoxan	201 ± 48*	106 ± 34	66 ± 17	81 ± 47	35 ± 15	23 ± 16**
Control	147 ± 38	93 ± 23	-	52 ± 16	34 ± 8	-
Amitriptyline	116 ± 44	41 ± 11*	-	49 ± 26	29 ± 13	-
Control	-	-	90 ± 20	-	-	200 ± 60
Noradrenaline	-	-	32 ± 14*	-	-	260 ± 40

Results are mean ± SD, Bmax (fmoles/mg protein),
* $p < 0.05$ ** $p < 0.01$ using unpaired t-test.

Chronic amitriptyline and noradrenaline treatments significantly reduced [^{3}H] yohimbine binding to hindbrain and kidney respectively with no changes in [^{3}H] idazoxan binding sites. In contrast idazoxan infusion significantly increased [^{3}H] yohimbine binding to forebrain but decreased [^{3}H] idazoxan binding to kidney. The reduction in [^{3}H] idazoxan binding is not consistent with effects of an antagonist on receptors, although idazoxan has been reported to have partial agonist properties (Hannah et al, 1983). However, the differential regulation of idazoxan and yohimbine binding observed in these studies during chronic drug treatment is consistent with their binding at distinct sites, and it is possible that idazoxan is acting as an agonist at its binding site.

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A PHYSIOLOGICAL ROLE FOR ADRENOCEPTOR DESENSITISATION?

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Desensitisation of alpha$_2$- and beta-adrenoceptor responses without a concomitant decrease in receptor number has been observed within minutes of agonist exposure in vitro and in vivo in animal studies (Lefkowitz et al 1983; Hamilton et al 1987; Strasser et al 1985). However, high non-physiological doses of agonist have generally been used to demonstrate this phenomenon. In the present study the effects of moderate increases in adrenaline (A) and noradrenaline (N) on alpha$_1$-, alpha$_2$- and beta-adrenoceptor mediated responses were examined in the conscious rabbit to determine the minimum exposure required to cause desensitisation. Conscious animals received intravenous infusions of A (0.05 and 1.5 um/kg/hr); N (0.09 and 0.5 um/kg/hr) or vehicle through an ear vein. Cannulae were also inserted in the central ear artery for blood pressure measurements and blood sampling and in another ear vein for administration of bolus doses of drugs as previously described (Hamilton et al 1987). Responses to alpha methylnoradrenaline, phenylephrine and isoprenaline were examined before and during infusion.

Ten minutes into the infusion mean arterial pressure was increased by 14 ± 5, 21 ± 13, 8 ± 4, 19 ± 8 and 1 ± 4 mmHg for 0.05 and 1.5 um/kg/hr A, 0.09 and 0.5 um/kg/hr N and saline infusions respectively. Plasma A levels rose from 1.1 ± 0.3 to 12.7 ± 2.1 and 30.6 ± 9.1 nM during A infusion while N levels rose from 2.1 ± 1.1 to 14 ± 5 and 29 ± 11 nM during N infusions. No change in responses to phenylephrine were observed during any infusion. Alpha methylnoradrenaline responses were attenuated within 2.5 mins of commencing the 1.5 um/kg/hr A infusion but only after 60 mins with the lower dose of A; the higher dose of N caused attenuation within 10 mins while the lower dose caused no changes. Isoprenaline responses were only attenuated by A, significant reductions occurring within 2.5 and 60 min infusion with the higher and lower doses respectively.

Thus we were able to show attenuation of alpha$_2$- and beta but not alpha$_1$-adrenoceptor mediated responses during infusion with the endogenous agonists N and A. However rapid desensitisation was only observed using the higher doses of agonists and although the increases in blood pressure were within the range observed in man, plasma catecholamine levels were generally higher than those reported during exercise or laboratory stress tests (Bramnert and Hokfelt 1985; Eisenhofer et al 1985). Thus, these studies in the rabbit did not provide evidence for a physiological role for adrenoceptor desensitisation in short-term cardiovascular regulation.

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THE EFFECT OF ANTIHYPERTENSIVE TREATMENT ON PLATELET INTRACELLULAR FREE CALCIUM CONCENTRATION IN RABBIT PERINEPHRITIS HYPERTENSION

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Intracellular free calcium concentration ($[Ca^{2+}]_i$) in platelets has been reported to be elevated in human essential hypertension, to be positively correlated with blood pressure and to decrease in response to treatment (Erne et al, 1984). However some groups have been unable to confirm these findings in humans (Lenz et al, 1985) or in spontaneously hypertensive rats (Zimlichman et al, 1986). We have examined the relationship between platelet $[Ca^{2+}]_i$ and blood pressure in the perinephritis model of hypertension in the rabbit. In addition, the acute effects of prazosin (0.1 mg/kg/i.v.) or verapamil (0.2 mg/kg/i.v.) were examined as were the effects of chronic six day infusions of prazosin (0.1 mg/kg/day) or verapamil (3.5 mg/kg/day). Rabbits were studied at least six weeks after surgery. A cannula was inserted in the ear artery for mean arterial pressure (MAP) measurement and withdrawal of blood for platelet $[Ca^{2+}]_i$ levels before and after treatment. Platelet $[Ca^{2+}]_i$ was estimated using the Quin 2 fluorescence technique. Each treatment group contained 7 hypertensive animals and 7 sham operated normotensive animals. Results are expressed as mean $\pm$ SEM.

Pretreatment values showed that MAP was elevated in hypertensive animals (123 ± 3 mmHg) compared to normotensive animals (89 ± 1 mmHg, $p < 0.01$). In contrast, platelet $[Ca^{2+}]_i$ was similar for both groups with 237 ± 27 nM for normotensive and 249 ± 26 nM for hypertensive animals. There was no significant correlation between pretreatment MAP and platelet $[Ca^{2+}]_i$ measurements ($r=0.19$, $p=0.17$, $n=56$). After treatment with prazosin or verapamil, a significant reduction in MAP occurred in all animals with the exception of normotensive animals on chronic treatments. No differences in platelet $[Ca^{2+}]_i$ were observed between normotensive and hypertensive rabbits in any treatment group after drug administration. Chronic verapamil treatment produced an elevation in platelet $[Ca^{2+}]_i$ in both normotensive and hypertensive rabbits. Normotensive rabbits in this treatment group had mean $[Ca^{2+}]_i$ levels of 128 ± 27 nM which rose to 217 ± 27 nM post treatment, and the mean $[Ca^{2+}]_i$ levels for hypertensive rabbits were 167 ± 37 nM pretreatment and 233 ± 46 nM post treatment ($p<0.01$ for both groups). No alterations in platelet $[Ca^{2+}]_i$ were observed after acute verapamil and acute and chronic prazosin treatments.

The elevation in platelet $[Ca^{2+}]_i$ after chronic verapamil treatment is difficult to interpret. Further investigations are required to elucidate the different mechanisms of prazosin and verapamil in the control of platelet $[Ca^{2+}]_i$ in hypertension.

In conclusion, platelet $[Ca^{2+}]_i$ was not elevated in the perinephritis model of hypertension, nor was it reduced by antihypertensive therapy. Platelet $[Ca^{2+}]_i$ is unlikely to be a univerally useful index of vascular tone.

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EFFECTS OF BRL 34915 (CROMAKALIM) ON ADRENERGIC RESPONSES IN VITRO AND IN VIVO

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BRL 34915 (BRL) is a recently developed vasodilator which appears to act by opening K^+ channels in smooth muscle. This results in hyperpolarisation of the cell membrane, thereby functionally antagonising the effects of agonists (Hamilton et al, 1986). The aim of the present study was to investigate the interactions between BRL and adrenergic responses in vitro and in vivo.

Rings of canine saphenous vein or rat thoracic aorta were bathed in normal Krebs' buffer at 37°C and at 1.5g and 1.0g resting tensions respectively. Transmural nerve stimulation (TNS) parameters were 12V, 2.0ms pulse width, 2Hz (20 pulses) every 100sec. Contractile responses to TNS were abolished by phentolamine ($3x10^{-5}$M) (n=4) suggesting that they were of sympathetic origin. Female Alderley Park Wistar rats were pithed and ventilated with 40% O_2–60%N_2 to produce normal arterial blood gas tensions. Dose–response curves were obtained with phenylephrine and xylazine (i.v. boluses) ± BRL (0.01 & 0.1mg/kg i.v.). Only one dose of BRL was tested per rat.

In canine saphenous veins pretreated with propranolol (1μM), BRL (0.01μM, 0.1μM & 1μM) attenuated the responses to TNS by 14±4%, 47±3% and 42±3% respectively (n=4). BRL (0.1μM & 1.0μM) did not shift the noradrenaline dose response curve but decreased the maximum by 42±7% and 47±6% respectively (n=4).

In the pithed rat, BRL (0.1mg/kg) shifted the dose–response curves to phenylephrine and xylazine to the right:

Dose BRL	Dose Ratios (at ED_{30}mmHg level)	
mg/kg	phenylephrine (α_1)	xylazine (α_2)
0.01	1.9 ± 0.3 (n=5)	0.8 ± 0.2 (n=4)
0.1	5.9 ± 1.8 (n=3)	4.5 ± 1.2 (n=4)

In rat aorta, BRL (0.01μM–1.0μM) did not shift the phenylephrine dose–response curves but decreased the maximum responses to phenylephrine reaching 54% inhibition at 1μM. BRL (0.01μM–0.5μM) produced rightward shifts of the methoxamine dose response curve whilst BRL (1μM) apparently decreased the maximum by 74%.

In conclusion, the results show that in saphenous vein BRL attenuated responses to both sympathetic nerve stimulation and noradrenaline suggesting that at least part of the effect of BRL may be of postjunctional origin. Also, BRL attenuated responses to α–agonists in the pithed rat, irrespective of the α–adrenoceptor type involved. In contrast to the findings of Buckingham (1988) in vivo, BRL attenuated responses to both phenylephrine and methoxamine in this in vitro study.

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K^+ CHANNEL OPENING DOES NOT ALONE EXPLAIN THE VASODILATOR ACTIVITY OF PINACIDIL AND ITS ENANTIOMERS

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It has recently been shown that pinacidil can open $^{86}Rb^+$ permeable K^+ channels in vascular smooth muscle in a manner similar to cromakalim (Bray et al., 1987; Cook et al., 1988a). The present study looks at the stereoselectivity of this effect for (+) and (-) pinacidil and at the relative contribution of K^+ channel opening to the vasodilator activity of these enantiomers in isolated vascular tissues. The methods used to study effects of these drugs on $^{86}Rb^+$ efflux and tone of the rat portal vein (rpv) and antivasoconstrictor effects on the rabbit aorta have been described previously (Quast, 1987; Cook et al., 1988b).

Both (+) and (-) pinacidil stimulated $^{86}Rb^+$ efflux from the rpv concentration-dependently, a 20% increase in flux being seen with 20 and 2 μM respectively. Spontaneous contractile activity was inhibited by approximately 40-fold lower concentrations of each enantiomer (IC_{50}'s; (+)pinacidil 0.51 ± 0.12 μM, (-)pinacidil 0.042 ± 0.003 μM). There is thus an approximately 10-fold stereoselectivity in the ability of the enantiomers of pinacidil to open K^+ channels in and to inhibit spontaneous activity of the rpv. In the rat aorta, the K^+ channel blocker glibenclamide inhibited $^{86}Rb^+$ efflux stimulated by 10 μM (±) pinacidil with an IC_{50} value of 0.17 ± 0.09 μM. This value is similar to the inhibitory activity of glibenclamide against 3 μM cromakalim (Quast, 1988), suggesting that pinacidil and cromakalim may activate the same K^+ channel(s) in this vessel.

On the rabbit aorta, (±)pinacidil causes a greater non-competitive inhibition of the angiotensin II concentration response curve than does cromakalim (Cook et al., 1988a & b). Pinacidil (> 10 μM) also has some inhibitory activity against high KCl contractions, whereas cromakalim does not. The sustained contraction elicited by 1 μM noradrenaline was concentration-dependently inhibited by (+) and (-) pinacidil, yielding IC_{50} values of 16 and 2.5 μM. 100% inhibition was seen with the highest doses tested whereas cromakalim inhibited only 70% of this contraction at saturating concentrations (Cook et al., 1988b). In the presence of 35mM KCl and 0.1μM isradipine, conditions which abolish vasodilator activity of cromakalim, (+) and (-)pinacidil still inhibited the 1μM noradrenaline contraction, with IC_{50}'s of 40 and 25 μM respectively. Hence this additional vasodilator activity does not show the stereoselectivity which characterizes the K^+ channel opening activity of (+) and (-)pinacidil. This effect constitutes a major part of the vasodilator activity of (+)pinacidil and may contribute to the in vitro and in vivo effects seen with higher doses of (±)pinacidil. K^+ channel opening is not likely to be involved in this effect (since it is present in depolarized vessels) and it cannot be explained by (dihydropyridine-sensitive) Ca^{2+} channel blockade. An effect on receptor-operated Ca^{2+} channels or on cyclic nucleotide levels may however explain these effects of (+) and (-) pinacidil.

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DECREASED NORADRENALINE INDUCED TURNOVER OF PHOSPHATIDYLINOSITOLS IN RENAL CORTEX OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

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α_1-Adrenoceptors of the α_{1A}-subtype, characterised by a higher affinity for noradrenaline (NA) and prazosin, seem to be predominantly present in rat aorta. On the other hand, in rat renal cortex the majority of the α_1-adrenoceptor population appears to be of the α_{1B}-subtype (Beckeringh and Brodde, this Meeting; Han et al., 1987; Neylon and Summers, 1987). We have previously shown that in aortic segments from SHR basal and maximal NA induced turnover of phosphatidylinositols (TPI) was considerably enhanced as compared to normotensive WKY rats (Beckeringh, 1988). α_1-Adrenoceptors have been related to various physiological processes in the kidney (Neylon and Summers, 1987). Some of these, such as the α_1-adrenoceptor mediated increase of sodium reabsorption and the release of prostanoids, may be important factors in the development of hypertension in SHR. In the present study we have examined α_1-adrenoceptor mediated TPI in renal cortex from SHR and WKY rats.

TPI, measured as the accumulation of [^{3}H]-inositolphosphates (IPs), was determined as described by Minnenman and Johnson (1984) using 1000x350x350 µm slices of renal cortex from 20-22 weeks old SHR and WKY. Accumulation of IPs was expressed as percentage of the effect induced by 100 µM NA. The basal accumulation of IPs was similar in slices from SHR and WKY (SHR: 8510 ± 631 cpm, WKY: 8989 ± 507 cpm (n=5). In SHR 300 µM NA maximally increased the accumulation of IPs by 137.0 ± 5.4%. In WKY NA induced accumulation of IPs was significantly ($p < 0.05$) higher and amounted to 176.1 ± 16.3% of the basal value. In SHR and WKY concentration-response (CR) curves to NA were shallow. In SHR an EC_{50} of 10.1 ± 2.2 µM was determined, in WKY a value of 2.5 ± 1.3 µM was obtained. With respect to the incorporation of [^{3}H]-inositol (0.1 µCi/assay) into phospholipids, the basal and maximally NA (300 µM) induced incorporation was also lower in SHR than in WKY (basal-SHR: 43898 ± 4132 cpm, WKY: 59673 ± 4115 cpm; maximal SHR: 49284 ± 5373 cpm, WKY: 59621 ± 2438 cpm).

In conclusion, the shallow CR curves and the intermediate EC_{50} values for NA in renal cortex of SHR and WKY suggests that NA induced TPI is also mediated by α_1-adrenoceptors of the α_{1A}-subtype. In renal cortex of SHR α_{1B}-adrenoceptor function is impaired as suggested by a decreased TPI. The decreased α_{1B}-adrenoceptor mediated TPI in renal cortex sharply contrasts with an enhanced α_{1A}-adrenoceptor mediated TPI in aorta (Beckeringh, 1988). The opposite state of the α_1-adrenoceptor mediated TPI in SHR with fully developed hypertension might reflect differential processes of desensitisation of α_{1A}- and α_{1B}-subtypes.

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EFFECT ON BAROREFLEX FUNCTION AND DURATION OF ACTION OF THE ANTI-ARRHYTHMIC UK-52,046 IN CONSCIOUS DOGS

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UK-52,046 (4-amino-6,7-dimethoxy-2-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl) quinoline methanesulphonate) is an α_1-adrenoceptor antagonist which is thought to act predominantly on myocardial α_1-adrenoceptors, abolishing arrhythmias in experimental models with little effect on blood pressure (BP) and heart rate (HR) (Uprichard et al., 1988). The present study investigated the effects of UK-52,046 on baroreflex function and its duration of action on peripheral α_1-adrenoceptors during acute and chronic administration.

Five dogs (weight 29.0 ± 1.2 kg mean ± SEM) were restrained in the supine position; arterial BP was measured using an intra-arterial catheter connected to a pressure transducer and HR recorded from a direct writing electrocardiogram (Lead II). Following 30 min quiet rest increasing doses of phenylephrine (PE) (1-128 μg/kg i.v.) were administered at intervals to progressively increase systolic BP (SBP) by >50 mmHg; this was repeated at 2, 4, 8, 12 and 24h on day 1 and day 8 after administration of UK-52,046 (25 μg/kg) or placebo as single oral doses daily for 8 days. Treatments were randomised and 7 days apart. Comparisons between placebo and active drug were analysed non-parametrically using Friedman's analysis of variance, Mann-Whitney U Test and Wilcoxon's signed rank tests for matched pairs.

The results indicate that following placebo no change occurred in SBP and diastolic BP (DBP) or HR on day 1 and day 8 (8h: Day 1 SBP 156.5 ± 5.6 mmHg; DBP 79.5 ± 7.1; HR 62.3 ± 8.1; Day 8 SBP 160.7 ± 7.9 mmHg; DBP 76.8 ± 6.9; HR 64.1 ± 10.7). These values were not significantly different from those following treatment with UK-52,046 at any time interval (8h; Day 1 SBP 161.8 ± 6.9; DBP 76.5 ± 6.2; HR 61.6 ± 10.2; Day 8 SBP 157.4 ± 6.7; DBP 89.2 ± 7.4; HR 65.0 ± 9.7).

Baroreflex sensitivity (ΔR-R ms/mmHg) calculated from the slopes of the regression lines of R-R interval vs SBP using PE to increase BP, was not significantly different at any time interval between placebo and UK-52,046 (day 1, 24.4 ± 5.8 (placebo) vs 30.76 ± 12.74 (UK-52,046); day 8, 21.59 ± 4.4 (placebo) vs 26.35 ± 7.9 (UK-52,046) (values 8h)). The intercepts of the regression lines were not changed indicating no resetting of the baroreflex function line. The duration of action of UK-52,046 was assessed from the PE dose response curves by calculating that dose of PE required to increase the SBP by 50 mmHg (PE_{50}). Following placebo there was no change in PE_{50} but this was increased ($P<0.05$) during UK-52,046 administration at 2, 4, 8 and 24h on day 1 (maximum at 4h; 22.6 ± 2.4 μg/kg) and at 2, 4, 8 and 12h on day 8 (maximum at 8h; 44.6 ± 7.9 μg/kg). The PE_{50} during UK-52,046 administration on day 8 was greater ($P<0.05$) than day 1 at 0, 8 and 12h suggesting accumulation of the drug with chronic administration.

In conclusion, UK-52,046 at doses which have an antiarrhythmic action in experimental models does not alter BP, HR or baroreflex function. Peripheral action on α_1-adrenoceptors assessed using PE indicates ($P<0.05$) long-lasting α_1-adrenoceptor blockade which increases ($P<0.05$) following chronic administration.

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ANTIHYPERTENSIVE EFFECT OF A NANOCAPSULAR FORM OF THE CALCIUM ENTRY BLOCKER, DARODIPINE

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Utilisation of dihydropyridine calcium entry blockers is limited by their short half-lives and their initial hypotensive effects (Doyle, 1988). Such disadvantages may be overcome by incorporation into isobutylcyanoacrylate nanocapsules. Such forms are absorbed after oral administration by a paracellular route (Damgé et al, 1987). We prepared a nanocapsular form of darodipine (Al Khouri et al, 1986). This preparation was administered either p.o. or i.m. to 2 kidney, 1 clip renovascular hypertensive rats with chronically implanted dorsal aorta cannula (NANO). Controls (CON) received nanocapsules without darodipine. A third group of rats (PEG) received darodipine (20 mg/kg) dissolved in polyethylene glycol 400 (a sustained release form; darodipine is insoluble in water). Mean arterial pressure was recorded in awake rats (6 per group) before and for 24 hours following injections. Plasma darodipine levels were determined by gas chromatography.

	Mean arterial pressure (mm Hg)				Plasma darodipine (ng/ml)		
(h)	0	+ 0.5	+ 8	+ 24	+ 0.5	+ 8	+ 24
After oral administration:							
CON	164±11	159±6	158±7	158±7	-	-	-
NANO	156±5	120±5*	122±11*	142±6	90±17	43±10	34±6
PEG	150±5	96±4*°	116±2*	140±6	282±63°	44±7	25±6
After intramuscular administration:							
CON	168±8	160±6	164±6	162±10	-	-	-
NANO	166±9	103±2*	122±7*	129±12	243±57	41±3	20±4
PEG	158±4	95±3*	91±5*°	118±5	338±49	171±30°	53±4°

Results are expressed as means±SEM. * = $P < 0.05$ NANO or PEG versus CON; ° = $P < 0.05$ NANO versus PEG.
In group PEG there is an initial rapid absorption of darodipine and a large fall in blood pressure. Both of these phenomena are attenuated when the same dose of darodipine is administered in a nanocapsular form (NANO). This latter preparation presents the same sustained release characteristics as the polyethylene glycol mixture as values at 24 h are similar in groups NANO and PEG.

In conclusion a nanocapsular form of darodipine is a sustained release form producing an antihypertensive effect which is slow in onset.

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ANTIARRHYTHMIC AND ELECTROPHYSIOLOGICAL EFFECTS OF THE AMINOSTEROID, ORG 7797, A NEW CLASS IC DRUG

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Org 7797, currently undergoing clinical evaluation, was developed from steroidal compounds known to possess local anaesthetic activity (Bucket, et al., 1967). This compound has previously been shown to possess potent activity against aconitine and ischaemia-induced arrhythmias in rodents and to inhibit the fast inward sodium current in cardiac muscle (Campbell, et al., 1986). The present study was designed to profile this compound in greater detail.

Results obtained showed that Org 7797 (0.5 mg/kg i.v.) completely prevented early ischaemia-induced ventricular fibrillation (VF) in anaesthetised greyhound (incidence of VF was 50% in controls). Org 7797 was 10-20 times more potent than disopyramide (DP) in this respect. In beagles with 5 to 6 day old myocardial infarcts sustained ventricular tachycardia (VT) or VF was more difficult to induce (by programmed electrical stimulation: PES) in 3 of 5 dogs, given Org 7797 (0.5 mg/kg i.v.) and in 2 of 5 dogs given the same dose of propafenone (P), the most potent clinical antiarrhythmic known. Org 7797 additionally slowed the rate of PES induced VT. In cats, Org 7797 (0.5 mg/kg i.v.) completely suppressed aconitine-induced atrial fibrillation (which developed in 39% of the animals). Higher i.v. doses of Org 7797 (1+2 mg/kg) and DP (2+5 mg/kg i.v.) suppressed late ischaemia-induced VT in conscious beagles. This effect of DP, but not that of Org 7797, was accompanied by a sinus tachycardia.

Chronic oral administration of Org 7797 (10 mg/kg b.i.d. for 10 days) to rats reduced the expected number of arrhythmias and the incidence of VF (from 2350±451 to 439±175, $p<0.05$, and from 50 to 20% respectively) following coronary artery ligation (CAL) performed 6 hours after receipt of the last dose. One hour after completion of the dosing regime, ventricular fibrillation thresholds (VFT) were increased compared to controls (382±49 vs 575±54 μA, $p<0.05$) and the expected fall in VFT (to 220±24 μA in controls, $p<0.05$) following CAL was reduced (to only 474±96 μA, NS).

Electrophysiological studies in vivo in infarcted beagles showed that Org 7797 (0.5 mg/kg i.v.) increased A-H and HV conduction times by 17±1 ($p<0.01$) and 33±7 ($p<0.05$) %, respectively and prolonged the PR interval by 14±2% ($p<0.01$). Atrial and ventricular refractory periods were only marginally increased (by 7 and 3%, respectively) whilst QT_c was unchanged. The class 1c drug, propafenone, exerted qualitatively similar effects but was less potent. Studies in vitro (in porcine ventricular tissue) on the rate of development and decay of sodium channel block (using the rate of rise of the monophasic action potential as an index of the sodium current) and comparison with P, lignocaine (L) and DP confirmed the classification of Org 7797 as a 1c drug. Rate constants (AP^{-1}) for onset of block were 0.076±0.003, 0.092±0.007 and 0.181±0.120 for Org 7797, P and DP (class 1a) respectively. Block in response to L (class 1b) was too fast too measure.

It was concluded that Org 7797 is a potent, orally active, broad spectum antiarrhythmic agent belonging to Class 1c.

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THE BIOCHEMICAL EFFECTS OF ORG 30029 IN CARDIAC AND VASCULAR TISSUES

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Org 30029 (N-hydroxy-5,6-dimethoxy-benzo[b]thiophene-2-carboximidamide HCl) is a novel cardiotonic agent with positive inotropic and vascular relaxant actions (Shahid, et al., 1988). In this study, the mechanism of action of Org 30029 has been examined.

Developed tension and cyclic nucleotide measurements were performed in rabbit papillary muscles and KCl (40 mM) pre-contracted aortic strips (Rodger and Shahid, 1984). Cyclic AMP-dependent protein kinase (cAMP-PrK) activity ratio in rabbit papillary muscles, was determined using the assay procedure of Roskoski (1983) with histone type IIA as substrate. Cyclic nucleotide phosphodiesterase (PDE) isoenzymes from rabbit ventricle were separated by IEC using DEAE-Sepharose or Mono Q on a FPLC system and assayed according to the procedure of Methven et al., (1980). Results are presented in Table 1.

Table 1 Effects of Org 30029 on developed tension, cyclic nucleotide levels and cAMP-PrK activity ratio (means±s.e. mean n>4)

Treatment	Conc. (μM)	Tension (mg)	cyclic AMP (pmol/mg tissue)	cyclic GMP	cAMP-PrK activity ratio
Control	-	338±54	0.76±0.08	0.025±0.007	0.26±0.04
Org 30029	692	1013±128	1.14±0.12*	0.087±0.017*	0.44±0.09*
Isoprenaline	2	1894±176	1.45±0.02**	N.D.	0.85±0.18*

* $p<0.05$ (Student's t-test compared to control) N.D. - not determined

The positive inotropic effect of Org 30029 at a near maximally effective concentration, was associated with increases in tissue content of cyclic AMP and cyclic GMP and activation of cAMP-PrK. The significance of the increase in cyclic GMP to the positive inotropic response, is not clear. PDE from rabbit ventricle were resolved into four main activity peaks. Regulatory characteristics were essentially as described by Reeves, et al., (1987) for guinea pig cardiac PDEs. Org 30029 produced concentration-dependent inhibition of cyclic AMP hydrolysis by PDE III and PDE IV with IC_{50} values of 37 μM and 20 μM respectively. In contrast, the cyclic AMP hydrolytic activities of PDE I and PDE II were weakly inhibited (IC_{50}>250 μM). Org 30029-induced (300 μM) relaxation of KCl pre-contracted rabbit aortic strips was also associated with increases in both cyclic AMP and cyclic GMP content (cyclic AMP:- control:0.17±0.01, Org 30029 0.23±0.03*; cyclic GMP:- control:0.005±0.001, Org 30029 0.017±0.002* *$p<0.05$ Mann-Whitney U-test).

The results indicate that selective inhibition of type III and IV PDE, with a consequent increase in cyclic nucleotide levels, is involved in the cardiac and vascular effects of Org 30029. The selective inhibition of types III and IV PDE is a novel profile for a cardiotonic which thus merits further evaluation in the clinic as an agent for the treatment of congestive heart failure.

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α_1-ADRENOCEPTOR-MEDIATED PRESSOR RESPONSES AFTER HAEMORRHAGE IN THE ANAESTHETISED RAT

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Using a rat model for haemorrhagic shock, we have previously observed an increase in the pressor response to the α_1-adrenoceptor agonist amidephrine following haemorrhage (MacLean *et al.*, 1989). Here we determined the pressor responses to other α_1-adrenoceptor agonists after haemorrhage and the effect of a calcium channel blocker, nifedipine, on these responses.

Male Wistar rats (250-300 g; Bantin & Kingman) were anaesthetised with sodium thiobutabarbitone (120 mg/kg i.p., Inactin, BYK). The right common carotid artery, left jugular vein and left femoral artery were cannulated for blood pressure monitoring, drug administration and blood removal respectively. Agonists were administered as a bolus injection followed immediately by an infusion: phenylephrine (Sigma, Dorset) 5 µg (bolus), 1 µg/min (infusion); amidephrine (Mead Johnson & Co., Indiana) 6 µg, 0.5 µg/min; cirazoline (Synthelabo, Paris) 1 µg, 1 µg/min. After control pressor responses to the agonists were established, blood was slowly withdrawn from the femoral artery until a mean arterial blood pressure (MAP) of 50 mmHg was achieved and this was maintained for 30 min. prior to further administration of the agonist. In the nifedipine study groups, after the initial dose of agonist, 0.3 mg/kg nifedipine (Bayer, UK Ltd) was given 10 min. prior to a repeated agonist dose. Haemorrhage was induced as above except that 10 min. prior to further agonist administration, nifedipine (0.3 mg/kg) was administered. Table 1 shows the effect of haemorrhage on MAP and the changes in MAP induced by the agonists.

Table 1. Pre- and post-haemorrhage pressor responses to α_1-adrenoceptor agonists.

	Phenylephrine (n=7)	Cirazoline (n=4)	Amidephrine (n=7)
Pre-haemorrhage			
Basal MAP	112 ± 5	104 ± 9	112 ± 7
Increase in MAP-Bolus	45 ± 4	58 ± 3	46 ± 4
Increase in MAP-Infusion	34 ± 4	42 ± 3	32 ± 2
Post-haemorrhage			
Basal MAP	49 ± 2	50 ± 2	51 ± 1
Increase in MAP-Bolus	83 ± 4 ***	80 ± 7 *	70 ± 5 **
Increase in MAP-Infusion	58 ± 1 **	64 ± 9 *	62 ± 4 ***

Mean ± s.e. mean; Statistical comparisons between pre-and post-haemorrhage values within groups, Students' paired t test *P<0.05;**P<0.01;***P<0.001.

Following haemorrhage, the basal MAP values were not significantly different between the groups. Table 1 shows that haemorrhage potentiated the pressor responses to the bolus injections of phenylephrine, cirazoline and amidephrine by 84%, 38% and 53%, and to the infusions by 71%, 52% and 94% respectively. The effect of nifedipine on the pre- and post-haemorrhage pressor responses to phenylephrine (n=6) and cirazoline (n=3) was studied. From a basal MAP of 116±8mmHg, phenylephrine induced a pressor response of 53±3mmHg(bolus) and 30±5mmHg (infusion). Nifedipine induced a transient fall in MAP to 77±2mmHg, but did not significantly affect the pressor responses to phenylephrine which were 55±4mmHg (bolus) and 28±3mmHg (infusion) after nifedipine. After haemorrhage (MAP=48±1mmHg) and in the presence of nifedipine, the pressor responses to phenylephrine were no longer potentiated and were 54±4mmHg (bolus) and 28±5mmHg (infusion). From a basal MAP of 120±2mmHg, cirazoline induced a pressor response of 55±8mmHg(bolus) and 45±5mmHg (infusion). Nifedipine induced a transient fall in MAP to 78±3mmHg, but did not significantly affect the pressor responses to cirazoline which were 68±4mmHg (bolus) and 38±7mmHg (infusion) after nifedipine. After haemorrhage (MAP=48±2) and in the presence of nifedipine, the pressor responses to cirazoline were no longer potentiated and were 70±3mmHg (bolus) and 45±6mmHg (infusion).

These results demonstrate that the pressor responses to α_1-adrenoceptor agonists are potentiated after haemorrhage. The effect of nifedipine indicates that this potentiation calcium-dependent. There are reports of increases in circulating angiotensin II and neuropeptide Y after haemorrhage (Scornik & Paladini, 1964; Morris *et al.*, 1987) and the vasoconstrictor effects of these peptides is calcium-dependent. In the haemorrhaged rat, α_1-adrenoceptor stimulation increased blood pressure through an increase in cardiac stroke volume, although there was evidence of potentiated α_1-adrenoceptor-mediated vasoconstriction in several vascular beds (MacLean *et al.*, 1989). It is possible that, following haemorrhage, increased circulating vasoconstricting peptides potentiate α_1-adrenoceptor-mediated inotropic and/or vasoconstrictor effects.

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CO-TRANSMISSION IN THE RABBIT SAPHENOUS ARTERY

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Field stimulation of intramural sympathetic nerves to blood vessels, evokes electrical and mechanical responses, which are mediated in many cases by two transmitters, one noradrenaline (NA) and the other adenosine triphosphate (ATP) or a related substance. The extent to which the mechanical response to each transmitter depends upon a change in membrane potential varies among different smooth muscles. In most cases, evoked release of the nonadrenergic (ATP) transmitter, is responsible for the electrical change (e.j.p.). This may (rabbit mesenteric, Lim et al., 1986) or may not (rabbit ear artery, Allcorn et al., 1985) be involved in the contraction. Similarly neuronally-released NA acts on α_1-receptors to elicit a contraction, which may (rabbit ear artery, Allcorn et al., 1985) or may not (Lim et al., 1985) be accompanied by a subsequent membrane depolarisation. In contrast to the lack of electrical change in these tissues, neuronally released NA produces e.j.p.s in some sympathetically innervated smooth muscles (anococcygeus, Creed et al., 1975, retractor penis muscle, Byrne & Muir, 1984) and exogenously added NA produces a depolarisation in others (McKenzie et al., 1988). This apparent anomaly led, in the present investigation, to a reexamination of the responses to nerve stimulation and exogenously added NA and ATP in a tissue, the rabbit saphenous artery, in which both NA and ATP act as co-transmitters (Burnstock & Warland, 1987). Normetanephrine (NMN, 10^{-6}M) and desmethylimipramine (DMI, 10^{-6}M), which respectively block uptake of NA into neuronal and non-neuronal tissues were present in those experiments which examined the NA response, in order to maximise any NA-mediated electrical changes. The responses were measured using intracellular microelectrodes simultaneously with mechanical recordings.

Field stimulation (100V, 1-64Hz for up to 1 minute) produced e.j.p.s, which summated and facilitated, and contractions. There was no accompanying adrenergic membrane depolarisation as in the rabbit ear artery. The evoked e.j.p.s were abolished by α β Methylene ATP (α β Me ATP, 10^{-6}M) but unaffected by prazosin (10^{-6}M). Each drug reduced by some 50% and together abolished the mechanical response to field stimulation. Alteration in the external environment showed that the evoked e.j.p.s were mediated by Na^+ and K^+, and addition to the bathing fluid of TEA (10^{-3}M) and diltiazem (10^{-4}M) demonstrated that Ca^{2+} mediated action potentials were aborted in this tissue by a fast rectifying K^+ current.

NA (10^{-2}M) was ineffective, but ATP (10^{-2}M) produced a dose-dependent depolarisation when applied by hydrostatic pressure (Picospritzer, General Valve Corp., N.J.). Addition of NA by infusion (10^{-6}-10^{-4}M) or bolus injection (10^{-6}-10^{-3}M) was likewise ineffective. α β Me ATP (10^{-6}M) had no effect on the contraction to exogenously added NA (10^{-6}-10^{-4}M).

The results indicate that of the two transmitters released by field stimulation of the rabbit saphenous artery, both are involved in the contraction, whereas only the non-adrenergic component produces an electrical change. Thus the NA released may act through a separate, voltage independent mechanism.

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VASODILATORY ACTIONS OF FLOSEQUINAN ON HUMAN SUBCUTANEOUS RESISTANCE VESSELS

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Flosequinan (7-Fluoro-1-Methyl-3-Methylsulphinyl-4-Quinolone) is a new vasodilator for use in heart failure and hypertension. Its exact mechanism of action is uncertain but it is associated with increased levels of cyclic GMP.

Resistance vessels (internal diameter 114-280 μm, mean 202± 9.3 μm, n=30) were isolated from biopsies of subcutaneous fat from 26 normotensive subjects on no medication, undergoing routine surgery (mean blood pressure 128/80 ± 5/2, mean age 48±2 years, 15 females). The vessels were dissected free of surrounding tissue and mounted as ring preparations on a myograph capable of measuring isometric tension. They were allowed to equilibrate for 1 hour in a physiological salt solution. Passive tension-internal circumference characteristics were obtained. The vessels were held at an internal circumference equivalent to 90% of that they would have had *in situ* when relaxed and under a transmural pressure of 100mmHg.

Cumulative dose response relationships were obtained to noradrenaline (0.02-10μM) and potassium (12-125mM) alone and in the presence of flosequinan 100μM. In the presence of flosequinan there was a shift to the right of the dose response curves for both noradrenaline and potassium. The pD_2 ($-\log[ED_{50}(M)]$) for noradrenaline falling from 6.89±0.09 to 6.29±0.07 ($P<0.01$) and for potassium from 1.54±0.03 to 1.34±0.03 ($P<0.001$). Cumulative dose response characteristics were obtained for flosequinan (10^{-5}-10^{-2} M), acetylcholine (10^{-8}-10^{-4} M) and sodium nitroprusside (10^{-9}-10^{-5} M), in random order, against contractions produced by noradrenaline (3μM) or potassium (66mM). Flosequinan produced dose dependent relaxation in vessels contracted by noradrenaline or potassium. Similar relaxation was obtained with acetylcholine and with sodium nitroprusside. Noradrenaline induced contractions were more readily relaxed by flosequinan and acetylcholine than were those induced by potassium (see table).

	Noradrenaline pD_2	Potassium pD_2	
Flosequinan	4.04±0.16	3.43±0.08	P<0.005
Acetylcholine	7.18±0.14	6.22±0.19	P<0.001
Sodium nitroprusside	6.78±0.31	6.42±0.12	NS

Flosequinan has been shown to produce dose dependent relaxation of human subcutaneous resistance vessels. The concentrations of Flosequinan used are of the same order as those achieved therapeutically in patients receiving the agent.

EFFECTS OF MATURATION AND AGEING ON THE RESPONSE TO VASODILATORS IN THE RAT AORTA

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It is well established that the relaxation to vasodilator agents can be altered in maturation and ageing (for references, see Sawyer and Docherty, 1987). Since the rat aorta has been widely employed in ageing studies, we have chosen to investigate the effects of maturation and ageing on endothelial-dependent and endothelial-independent relaxations of the rat aorta.

Aortic rings were obtained from male Sprague-Dawley rats of 3 age groups: 1.5 month (immature); 6 month (adult) and 22 month (aged) rats. Tissues were contracted with KCl 40mM (KCl 80 mM for relaxations to nifedipine), and cumulative concentration-response curves were obtained for the relaxation to various agents.

In maturation, there was a significant reduction in the vasodilator potency of atriopeptin II with IC_{50} values of 0.50 nM (95% confidence limits of 0.30-0.85nM) and 1.86 nM (0.62-5.6 nM) in 1.5 month and 6 month animals, respectively (Student's t-test:$P<0.01$). However, the vasodilator potency of histamine was unaltered during maturation, with IC_{50} values of 6.9 μM (4.0-12.0 μM) and 7.6 μM (1.8-31.6 μM) in 1.5 month and 6 month animals, respectively. Potency of sodium nitroprusside was unaltered in maturation.

In ageing, the potency of acetylcholine was significantly increased from 0.56 μM (0.26-1.48 μM) in 6 month to 0.19 μM (0.10-0.37 μM) in 22 month animals ($P<0.05$) (see also Hynes and Duckles, 1987) although the potency of atriopeptin II does not increase (see Emmick and Cohen, 1986). Potency of sodium nitroprusside was unaltered by ageing. However, the potency of the calcium entry blocker nifedipine showed a small but significant increase in ageing, with IC_{50} values of 30.2 nM (20.4-44.7 nM) in 6 month and 13.8 nM (9.8-19.5 nM) in 22 month animals ($P<0.05$).

In summary, both maturation and ageing can differentially affect the response to vasodilators in the rat aorta. Even endothelial-dependent relaxations can be differentially affected, suggesting that age-related alterations may be at the receptor level in this tissue.

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THE EFFECTS OF BROMOACETYLALPRENOLOLMENTANE (BAAM) ON THE RESPONSES OF THE RAT RIGHT VENTRICLE AND LEFT ATRIA TO ISOPRENALINE

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Bromoacetylalprenololmentane (BAAM) is an irreversible β-adrenoceptor antagonist (Posner et al., 1984). I have studied the effects of BAAM on the contractile responses of the electrically driven rat right ventricle to isoprenaline and on the contractile responses of the rat left atria to electrical stimulation and isoprenaline.

A maximal contractile response of the electrically driven rat right ventricle or left atria to isoprenaline was obtained. Tissues were then washed prior to some being treated with BAAM for 30 min while others were treated with vehicle (ethanol). Following a rapid 5 min wash, tissues were electrically driven and isoprenaline response curves determined.

BAAM treatment of the ventricle at 10^{-7}M for 30 min caused a small rightward shift of the isoprenaline response curve and a depression of the isoprenaline maximum response. Higher concentrations of BAAM (3×10^{-7} - 10^{-6}M) for 30 min caused nonparallel rightward shifts of the isoprenaline response curves and a marked depression of the maximum response of the ventricle to isoprenaline. The data obtained with BAAM at 10^{-6}M was used to determine the dissociation constant (k_A) for isoprenaline according to the method of Furchgott and Bursztyn (1967). The k_A for isoprenaline is 4×10^{-7}M.

BAAM treatment of the atria at 10^{-6} - 10^{-5}M for 30 min had no effect on the response to electrical stimulation, caused parallel rightward shifts of the isoprenaline response curves and had no effect on the isoprenaline maximum responses. BAAM treatment at 3×10^{-5}M (30 min) had no effect on the responses to electrical stimulation, and caused a parallel rightward shift of the isoprenaline response curves with a small depression of the isoprenaline maximum response. Higher concentrations of BAAM ($6 \times 10^{-5}/10^{-4}$M, 30 min) depressed the responses of the left atria to electrical stimulation and isoprenaline. This data obtained with BAAM on the atria was unsuitable for use in k_A determination.

The fractional β-adrenoceptor occupancy by isoprenaline was calculated using the k_A obtained from the interaction of isoprenaline with ventricular β-adrenoceptors according to the procedure of Ruffolo (1982). Isoprenaline will produce a half maximal response in the left atria and right ventricle by occupying <1% and 8% of the available β-adrenoceptors, respectively. In order to produce a maximum response of the atria and ventricle, isoprenaline occupies 20 and 70% of the available β-adrenoceptors, respectively.

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DOPEXAMINE HYDROCHLORIDE IS AN INHIBITOR OF THE UPTAKE-1 MECHANISM IN RABBIT AORTA

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Dopexamine hydrochloride is an agonist at peripheral dopamine receptors and beta$_2$-adrenoceptors (Brown et al, 1985). Although dopexamine possesses only weak beta-1 adrenoceptor agonist activity, it had positive inotropic and chronotropic effects on the myocardium (Bass et al, 1987; Mitchell et al, 1987). These effects were considered to be due to an inhibition of the uptake-1 mechanism. This view is supported by the finding that dopexamine inhibited the accumulation of tritium by rabbit brain synaptosomes incubated with [^{3}H]-noradrenaline (Mitchell et al, 1987).

In vascular tissues, noradrenaline is removed from the synaptic cleft by neuronal and extraneuronal uptake mechanisms. The aim of the present work was to study the ability of dopexamine to interfere with uptake-1 and uptake-2 in the rabbit isolated aorta. The effects of dopexamine were compared with some other myocardial stimulants and well-known uptake inhibitors.

The method described by Nedergaard & Abrahamsen (1987) was used. Rings of rabbit aorta were incubated with [^{3}H]-noradrenaline (10^{-8}M) for 1 hr. Monoamine oxidase and catechol-O-methyltransferase were blocked by pargyline and U-0521 (3',4'-dihydroxy-2-methylpropiophenone). For experiments designed to examine the ability of a drug to alter the uptake of [^{3}H]-noradrenaline, the drug was added to the bath 30 min before [^{3}H]-noradrenaline and remained in the bath throughout the experiment.

Dopexamine (3×10^{-9}-10^{-5}M), desipramine (3×10^{-9}-10^{-4}M), cocaine (10^{-7}-10^{-4}M), dopamine (3×10^{-8}-10^{-4}M), dobutamine (3×10^{-7}-3×10^{-4}M), ADTN ((±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphtalene; 3×10^{-7}-10^{-4}M), and ouabain (3×10^{-6}-10^{-4}M) reduced the accumulation of tritium by aorta incubated with [^{3}H]-noradrenaline. Dopexamine (3×10^{-9}-10^{-7}M) caused the same degree of inhibition, irrespective of whether corticosterone (4×10^{-5}M) was present or not. The order of inhibitory potency was: desipramine > dopexamine > dobutamine ≥ dopamine > ADTN ≥ cocaine > ouabain. In the presence of desipramine (10^{-6}M), corticosterone (10^{-6}-10^{-4}M), but not dopexamine (10^{-6}-10^{-4}M), reduced the ^{3}H-accumulation.

It is concluded that dopexamine is a potent inhibitor of uptake-1 in rabbit aorta. Dopexamine has no affinity for the uptake-2 mechanism in this tissue.

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LACK OF EFFECT WITH ICI 169,369 AT THE 5-HT_3 RECEPTOR IN THE ISOLATED PERFUSED RABBIT HEART

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We have previously shown that ICI 169369 possesses potent 5-HT_2 antagonist activity (Blackburn et al 1987), has low affinity for the 5-HT receptor in the dog basilar artery and little or no effect for the 5-HT_1-like receptor in the dog saphenous vein (Growcott et al 1987). However, we have not previously reported the effects of this compound at the 5-HT_3 receptor mediating release of noradrenaline in the rabbit heart. We therefore decided to evaluate the effects of ICI 169369, together with a number of the known standard compounds, on the 5-HT-induced tachycardia in the isolated perfused rabbit heart - a system demonstrated to be sensitive to the effects of 5-HT_3 antagonists (Humphrey 1984).

The isolated perfused rabbit heart was set up as described by Fozard and Mwaluko (1976). 5-HT-induced tachycardia was evaluated in the absence and presence of ICI 169369 (10^{-6}M), ketanserin (10^{-7} to 10^{-6}M), methiothepin, (5 x 10^{-8}M), ICS 205-930, MDL 72222 and GR38032 (5 x 10^{-10} to 5 x 10^{-8}M). All compounds were allowed a 15 min contact time prior to addition of 5-HT.

ICS 205-930, MDL 72222 and GR38032 all produced significant antagonism of the 5-HT-induced tachycardia. These compounds have previously been reported to be effective and selective antagonists at the 5-HT receptor in this tissue (Humphrey 1984). Methiothepin had no signficant effect on rate. Ketanserin appeared to have membrane-stabilising effects at the higher concentrations, causing dysrhythmia and a failure of the heart to respond to 5-HT. ICI 169369 possessed no significant effect on the 5-HT induced tachycardia, moreover, the force of contraction of the heart was largely unaffected.

These results demonstrate that ICI 169369 has no significant 5-HT_3 antagonist activity in the rabbit heart. Moreover, at these concentrations, no significant antagonism of 5-HT at the "M" receptor in the methysergide-treated guinea-pig ileum could be demonstrated with ICI 169369 (Growcott, unpublished observations).

Thus, ICI 169369 appears to be a selective compound which could prove to be an invaluable tool for probing functional 5-HT receptors.

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BRADYCARDIC AND TACHYCARDIC EFFECTS OF MUSCARINIC ANTAGONISTS IN CONSCIOUS RABBITS

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Muscarinic antagonists can increase heart rate through blockade of postsynaptically located cardiac (M_2) receptors. Accordingly, cardioselective antagonists like AF-DX 116 (11-[[2-[(diethylamino) methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4] benzodiazepine-6-one) (Engel et al. 1987) have been synthesized as potential antibradycardic drugs. It has been reported that M_1-selective antagonists can cause bradycardia in humans, probably due to the existence of presynaptically located M_1-receptors mediating acetylcholine release (Pitschner et al. 1988). It was the aim of the present study to compare the effects on heart rate of antagonists possessing high affinity for the M_1-receptors (Pirenzepine, PZ, and UH-AH 37; 6-Chloro-5,10-dihydro-5-[(1-methyl-4-piperidinyl)acetyl]-11H-dibenzo[b,e] [1,4]diazepine-11-one) with those of the M_2-selective antagonists AF-DX 116 and AF-DX 384 (5,11-Dihydro-11-[[[2-[2-[(dipropylamino)methyl]-1-piperidinyl] ethyl]amino]carbonyl]-6H-pyrido[2,3-b][1,4]benzodiazepine-6-one). Ipratropium was used as a reference compound. Heart rate was measured in conscious rabbits (Chinchilla; 2-3 kg, ♂) via ECG.

PZ and UH-AH 37 produced paradoxical effects on the heart rate. Low doses (3-300 µg/kg; i.v.) decreased whereas higher doses increased heart rate. A dose of 100 µg/kg (i.v.) PZ produced a fall in heart rate of 42 ± 6 beats/min (n = 6) from an initial value of 197 ± 9 beats/min. The bradycardic effect lasted approximately 1 hour. When PZ (0.1 mg/kg) was given following an infusion of 10-20 µg/kg/min AF-DX 384 the bradycardic effect was abolished. On the contrary, AF-DX 116, AF-DX 384 and ipratropium produced only tachycardic effects. The pED_{25} values, -log of the dose that increased or decreased heart rate by 25 beats, are given in table 1.

Table 1 pED_{25} values for the bradycardic and tachycardic effects of muscarinic antagonist (n = 4-8).

	pED_{25} (mol/kg)	
	Bradycardia	Tachycardia
Pirenzepine	7.49	5.80
UH-AH 37	7.26	5.37
AF-DX 116	-	6.60
AF-DX 384	-	7.07
Ipratropium	-	7.91

In conclusion: M_1-antagonists show different effects on heart rate in conscious rabbits compared to M_2- or non selective antagonists. At low, selective, doses PZ and UH-AH 37 produce bradycardic effects presumably due to blockade of autoreceptors. At higher doses PZ and UH-AH 37 do not differ from AF-DX 116 and AF-DX 384, namely the tachycardic response due to blockade of postsynaptically located M_2-receptors predominate.

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COMPARISON OF THE MUSCARINIC RECEPTORS IN PORCINE CORONARY ARTERY, ATRIUM AND ILEUM

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It has been suggested that the parasympathetic nervous system is involved in the triggering of attacks in patients with Prinzmetal's angina (Yasue et al., 1986). The aim of the present study was to investigate the nature of the muscarinic receptor subtype involved in the contraction of isolated porcine coronary arteries with respect to the proposed subdivision into M_1 (neuronal tissue), M_2 (heart and M_3 (glandular tissue) (De Jonge et al., 1986; Doods et al., 1987), extended to M_4 (ileal smooth muscle) (Batink et al., 1987). In vitro experiments were performed on endothelium-denuded rings of the left anterior descending coronary artery and for comparison also on preparations of porcine atrium and ileal smooth muscle. After 3 h of equilibration in oxygenated (95% O_2 + 5% CO_2) Tyrode solution (pH=7.4, 37° C) cumulative concentration-response curves (CRC's) to MCh were made. Responses on the coronary artery and ileum were expressed as a percentage of the preceding maximal response to KCl (104 mM), those on the atrium as a percentage of the initial inotropy. MCh induced a concentration-dependent contraction of the coronary resp. ileal preparations, and a concentration-dependent negative inotropic response of the atrium. Pretreatment for 2 h with the muscarinic antagonists used in this study shifted the CRC's of MCh to the right in a competitive manner.

Table 1 Order of affinity of the antagonists studied for the muscarinic receptors in the coronary artery (M_{CA}), atrium (M_2) and ileum (M_4) of the pig

M_{CA}: atropine ≈ ipra ≈ 4-DAMP > HHSiD > PZ > AF-DX 116 ≈ methoc
M_2: atropine ≈ ipra > 4-DAMP > methoc ≈ HHSiD ≈ AF-DX 116 > PZ
M_4 : atropine ≈ ipra > 4-DAMP ≈ HHSiD > PZ > AF-DX 116 ≈ methoc

Abbreviations:
ipra = ipratropium; 4-DAMP = 4-diphenylacetoxy-N-methylpiperidine; HHSiD = hexahydrosiladifenidol; PZ = pirenzepine; AF-DX 116 = 11-2[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one; methoc = methoctramine.

A comparison of the relative orders of affinity of the antagonists studied reveals that the muscarinic receptor responsible for the coronary contraction does not belong to the M_2 subtype, in agreement with our previous report (Van Charldorp et al., 1988). Furthermore, the low affinity of pirenzepine excludes the M_1 receptor subtype. However, there is a close resemblance between the porcine coronary and ileal smooth muscle receptors (M_4). Therefore we conclude that the muscarinic receptor mediating coronary contraction in the pig adheres to the M_3 or M_4 receptor subtype.

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THE CARDIOPROTECTIVE EFFECT OF DIFFERENT CALCIUM ANTAGONISTS DURING NORMOTHERMIC GLOBAL ISCHAEMIA

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The guinea-pig heart-lung preparation (HLP) was adapted to study the influence of different calcium antagonists (CA) on ischemic damage. Two concentrations of the following CA were studied: nifedipine, verapamil, lidoflazine, bepridil and the novel drug CERM 11956 (a structural analogue of bepridil). One concentration reduced contractile force (dP/dt_{max})by approximately 15% (EC_{15}), whereas the other one did not influence this parameter (EC_0). After 15 min of equilibration and pretreatment for 15 min with the drugs at EC_{15} and EC_0 levels, respectively, global ischemia was induced by simultaneously clamping of the vena cava inferior and aortic cannula, while hearts were allowed to unload via the right atrium and a needle inserted in the left ventricular cavity. Subsequently, hearts were subjected to 60 min of ischemia. Temperature during ischemia was controlled and maintained at 37°C. After 10 min of retrograde reperfusion with fresh blood, hearts were converted into the working mode again. Subsequently, blood in the reservoir and the extra-corporal circulation was replaced by fresh blood, while the same concentration of drug prior to ischemia was added. After a further 20 min of reperfusion the recovery of functional (LVP_{max}, dP/dt_{max}, AoP and CO) and biochemical (ATP and CrP) parameters was monitored and calculated as percentage of the corresponding values before and after pretreatment with the CA. After ischemia all CA-pretreated hearts proved in a significantly better condition compared with controls. With respect to dP/dt_{max}, hearts pretreated with verapamil, lidoflazine and bepridil at EC_{15}-levels, respectively, showed higher ($p < 0.05$) recoveries compared with the hearts pretreated at EC_0 concentrations of the same CA (93.8 $\pm$ 3.1 vs 77 $\pm$ 0.4%; 91.9 $\pm$ 4.2 vs 76.0 $\pm$ 3.6%; 83.5 $\pm$ 7.6 vs 66.4 $\pm$ 3.6%, respectively). A possible explanation may be offered by intracellular effects in addition to calcium entry blockade. CERM 11956 showed similar protective effects at low and high concentrations. Hearts pretreated with CA showed an overshoot of CrP after 30 min of reperfusion (> 100%, except for nifedipine (EC_0) and CERM 11956 (EC_0)). All groups of preparations showed a marked depression of ATP levels. CrP tissue contents correlated better with functional recovery than those of ATP.
In conclusion, the heart-lung preparation of the guinea pig appears to be a suitable model to study anti-ischemic drugs during normothermic ischemia. CA afforded protective cardiac effects even at concentrations which did not influence contractile force (dP/dt_{max}). CrP levels correlated with functional recovery, but those of ATP did not.

EFFECTS OF NIFEDIPINE AND MIOFLAZINE UPON ELECTROMICROSCOPICALLY DETERMINED CALCIUM OVERLOAD INDUCED BY ISCHAEMIA AND REPERFUSION

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The effect of nifedipine (N) and mioflazine (M) on the recovery of cardiac functional parameters (aortic dP/dt and cardiac output) after global ischaemia (I) and reperfusion (R) was investigated in relation to the influence of N and M on calcium overload (COL) after I as well as after R. Guinea-pig isolated working hearts (paced at 5 Hz) were perfused with Tyrode (Ca^{2+}: 1.8 mM) at 37°C. The hearts were pretreated with N or M for 15 min, after which global I (37°C) was maintained for 45 min. Subsequently the hearts were reperfused for 10 min according to Langendorff after which they were switched into the working mode for 15 min. N and M were applied at concentrations that reduced aortic dP/dt by 10% (EC_{10}) and 30% (EC_{30}), respectively. These values were 8 and 20 nM for N and 0.3 and 1 µM for M, respectively. Table I shows the effect of N and M on the recovery of the aortic dP/dt and cardiac output in percentage of the initial values (means ± S.E.M.; n=6-7).

Table I:

	Aortic dP/dt	Cardiac output
Normoxia	100%	100%
I + R	31.5 ± 10.4	30.1 ± 10.5
N (EC_{10})	46.7 ± 13.8	54.7 ± 11.0
N (EC_{30})	52.1 ± 10.2	66.3 ± 11.4
M (EC_{10})	56.8 ± 8.4	65.5 ± 9.9
M (EC_{30})	65.9 ± 11.3	83.3 ± 13.8

N and M, applied at the EC_{10} and EC_{30} levels improved the recovery of the mechanical function of the heart. N (EC_{10}) was slightly less effective than M, whereas N (EC_{30}) was similarly active as M. Therefore, we studied the effect of N (EC_{30}) and M (EC_{10}) on COL. In separate experiments the hearts were fixated either after I or after R. Primary perfusion fixation occurred according to Borgers et al. (1984) with glutaraldehyde 3% in 0.09 mM oxalic acid (pH 7.4) in order to fixate cardiac tissue and calcium. After postfixation with OsO_4 1% and K-pyroantimonate 2%, the tissue was embedded in LX 112. Ultrathin (70 nm) sections were made. Calcium in the mitochondria was determined in 50-55 pictures (16400x) of each preparation and quantified as "none, little or much". Statistical significance was evaluated using chi square test. Data are presented as percentage of the total of photographs:

Table II:

	Calcium		
	None	Little	Much
Normoxia	4	96	0
I	0	32	68
I + N	54	46	0
I + M	30	70	0
I + R	5	46	49
I + R + N	45	55	0
I + R + M	54	46	0

The results show that in the guinea-pig heart mitochondrial COL observed after I and R develops during I, but is not influenced by R. N and M strongly reduce COL during I. The N- and M-induced improved recovery of cardiac function during R appears to originate in a reduced COL during I.

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DIFFERENTIAL EFFECTS OF CALCIUM ANTAGONISTS AND A LOW CALCIUM MEDIUM ON THE EXTRACELLULAR POTASSIUM ACCUMULATION DURING ISCHAEMIA

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The influence of cardiodepressant concentrations of diltiazem (D), nifedipine (N) and the influence of a low calcium medium (LCM) on the time course of accumulation of extracellular potassium ($([K^+]_{out}$) was measured during global ischemia in the isolated rat heart. Rat hearts were perfused according to Langendorff, and stimulated at 5Hz. In separate experiments the inotropic effects of D, N and LCM were studied by means of an intraventricular balloon. For the measurements of K^+ three ([D], [N] and $[Ca^{2+}]$) were chosen that induced similar negative inotropic effects (IC_0, IC_{20} and IC_{50}). Global ischemia was induced during three consecutive periods of 10 min, which were separated by 25 min periods of reperfusion. $[K^+]_{out}$ was measured by means of K^+-sensitive electrodes of the valinomycin PVC-matrix type (Coronel, 1988). Measurements of K^+-electrodes were accepted only if they had a calculated response of 55 to 61.5 mV per decade change in $[K^+]$ during in situ calibration. Measurements of the $[K^+]_{out}$ obtained during a second period of global ischemia served as a reference to those obtained during the third period, which was induced after 15 min of drug pretreatment with D, N or LCM.

During the second period of ischemia, $[K^+]_{out}$ increased from a preocclusion value of 4.0 mM at t=0 to 8.8 ± 0.5 mM at t=3 min to 10.1 ± 0.6 mM at t=5 min and 10.2 ± 0.8 mM at t=10 min (n=8, plateau level). D and N slowed the initial rate of $[K^+]_{out}$ increase in a concentration-dependent manner without affecting the final plateau value reached at t=5 to t=10 min.
At t=3 min in the presence of N ($3x10^{-8}$, 10^{-7} to $1.5x10^{-7}$M) $[K^+]_{out}$ reached values of 7.9 ± 0.5 mM (n=6), 6.5 ± 0.6 mM (n=5) and 6.4 ± 0.5 mM (n=5), respectively. After 3 min in the presence of D ($2x10^{-6}$, $3x10^{-6}$ and 10^{-5}M) $[K^+]_{out}$ ranged from 7.4 ± 0.6 mM (n=8), 6.7 ± 0.5 mM (n=7) to 6.3 ± 0.8 mM (n=8), respectively. Preperfusion with LCM ($[Ca^{2+}]$ from 1.3 to 0.9 mM) had no effect on the time course and magnitude of extracellulair K^+ accumulation, although the inotropic effects of LCM were similar to those produced by N and D.

The results suggest that there is no direct relation between the contractile state of the heart before ischemia and the potassium release during ischemia. The inotropic effects induced by LCM are not identical to those induced by the calcium antagonists.

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THE CARDIOPROTECTIVE EFFECT OF TRIMETAZIDINE AND NIFEDIPINE IN GUINEA-PIG HEARTS SUBJECTED TO GLOBAL ISCHAEMIA

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Trimetazidine (T) (1-(2,3,4) piperazine dihydrochloride) is an anti-anginal compound that reduced the deleterious effect of ischaemia (I) in several models of experimental ischaemia (Lavanchy et al., 1986). The chemical structure of the drug is unrelated to that of the classical calcium antagonists. In the present study we compared the cardioprotective effect of T and nifedipine (N) in the guinea-pig heart-lung preparation subjected to global I.

Drugs were studied at the concentration that reduced dP/dt by 0 and 20% respectively. These concentrations amounted for N to 8 and 20 nM and for T to 10 and 200 µM, respectively. After 15 min of equilibration and 15 min of pretreatment with the drug, hearts were brought into a state of global ischaemia (37°C) for 60 min, by clamping both the venous and the aortic cannulae. Subsequently, the hearts were reperfused for 10 min according to Langendorff, whereafter they were switched into the working mode for 20 min. The recoveries of the dP/dt and cardiac output (CO), expressed as the percentage of the initial values as well as phosphocreatine (PCr) and ATP (µmol/g dry weight) are shown in table I. Statistical significance was evaluated using Student's two tailed t-test ($p < 0.05$).

Table I.

	dP/dt	CO	PCr	ATP
Normoxia	100%	100%	27.4 ± 4.4	17.7 ± 1.6
I	29.6 ± 10.7	40.4 ± 13.7	19.2 ± 1.3	7.0 ± 1.6
N 8 nM	59.1 ± 5.3*	89.3 ± 6.3*	27.0 ± 0.4*	8.3 ± 0.7
N 20 nM	62.9 ± 2.0*	85.1 ± 2.5*	30.3 ± 0.5*	9.0 ± 1.0
T 10 µM	68.3 ± 3.6*	91.2 ± 1.6*	29.7 ± 2.7*	6.6 ± 0.5
T 200 µM	75.0 ± 5.5*	86.2 ± 2.4*	33.8 ± 0.5*	8.6 ± 1.0

T was slightly more active than N in improving the recovery of dP/dt, however, CO was similarly enhanced by both drugs. PCr but not ATP was markedly enhanced in hearts perfused with T and N, applied at both concentrations mentioned.
To determine whether α-adrenoceptor antagonism or the blockade of calcium entry may be involved in the anti-ischaemic action of T, in the pithed rat the influence of the drug on vasoconstriction induced by the selective α_1- and α_2-adrenoceptor agonists cirazoline and B-HT 920 was evaluated. α_2-Adrenoceptor mediated vasoconstriction is highly dependent on the influx of extracellular calcium (Van Meel et al., 1983).
In contrast to N, T did not influence vasoconstriction induced by both cirazoline and B-HT 920. It is concluded that the cardioprotective effect of T is not related to calcium entry blockade or α-adrenoceptor antagonism, but is rather caused by unknown mechanisms at a cellular level.

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200P

EFFECTS OF BENZODIAZEPINE DERIVATIVES ON AN ISOLATED HEART PREPARATION

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Acute toxicity of benzodiazepines is very low. Cardiovascular and respiratory effects in humans occur in relative high dosis, particularly in combination with other central depressants (Goodman et al., 1985). In a previous report (see Beusenberg et al., 1988) we have shown effects of benzodiazepines on cardiac and respiratory parameters in vivo in rats under anesthesia, i.e. respiratory rate, heart rate, diastolic and systolic blood presure. The four drugs studied were diazepam, chlordiazepoxide, the anti-epileptic drug clonazepam and the 1,5-benzodiazepine clobazam. All four mentioned benzodiazepines were subsequently tested as to actions on a modification of the isolated rat Langendorff heart. Male rats (CU Wb:WI), 300-350 g were used throughout. After dissection, the heart was quickly placed in an organ bath with Tyrode solution (37°C). For perfusion with Tyrode a cannula was inserted in the aorta. Perfused fluid was collected via the coronary arteries in order to get an estimation of the flow rate when drugs were added. The perfusion rate was 8 ml/min. A balloon connected to a pressure transducer was placed in the left ventricle in order to measure the contraction force. Equilibration time was 10 min, after which an experiment was performed with subsequent increasing concentrations of a drug. Perfusion time for each concentration was 10 min. Ventricular contraction force was registrated on a Kipp (BD 41) or a Gould recorder (C 8188.22.01.06). The results can be summarized as follows.

Control experiments with saline or other solvents showed no effects on the contraction force. It was noted however, that the flow rate was markedly reduced (by 30%) during the course of the experiments.

Using diazepam in concentrations of 0.058 to 0.43 mmol/l a biphasic effect on the contraction force was observed. At the lower concentrations diazepam produced a slight short lasting stimulation of the contraction force, whereas at higher concentrations either stimulation or inhibition followed by stimulation was seen. The flow rate was decreased concentration dependently. In the case of chlordiazepoxide (0.058 to 0.60 mmol/l), in nine experiments several events occurred. In three experiments no effects were observed on the contraction force. In four experiments chlordiazepoxide increased the contraction force, whereas the preparation was inhibited to 70% in two experiments, at the higher concentrations. Effects on the flow rate were barely observed, apart from the fact that at the lowest concentration a small drop was seen.

Clonazepam (0.058 to 0.86 mmol/l) was only effective in very high concentrations. At 0.78 and 0.86 mmol/l it produced a decrease of the contraction force. The flow rate was not affected. Clobazam (0.058 to 0.60 mmol/l) had neither effect on the contraction force nor on the flow rate, in any concentration used.

In conclusion. Benzodiazepines may have direct effects upon functions in the isolated heart; effects of the drugs tested differ both in quality and quantity. Moreover the results have indicated that the possibility must be considered that receptors for the benzodiazepines may be present peripherally.

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EFFECTS OF TRANDOLAPRIL, A NEW ANGIOTENSIN CONVERTING ENZYME INHIBITOR, ON CARDIAC HYPERTROPHY IN 20 WEEK- AND 2 YEAR-OLD SHR

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Left ventricular hypertrophy is a major complication of essential hypertension and is considered to be an independent risk factor in mortality. Several evidences are in favour of an implication of the renin–angiotensin system in the development of cardiac hypertrophy and dysfunction in hypertension (DEVEREUX et al. 1987). Indeed, angiotensin converting enzyme (ACE) inhibitors have been shown to decrease cardiac hypertrophy in animal models of hypertension (SEN et al. 1980 ; PFEFFER et al. 1982) as well as in human (MUIESAN et al. 1988).
The effects on cardiac hypertrophy of the new potent angiotensin converting enzyme inhibitor trandolapril (BROWN et al. 1987) were studied in 20 week- and 2 year-old genetically hypertensive rats (SHR).
In 20 week-old SHR, oral treatment, at 3, 0.3, 0.03 mg/kg/day, during 15 days, induced a decrease in mean blood pressure of 36%, 22% and 16% respectively. At the same dosage, serum ACE activity was inhibited by 84%, 69% and 56%. The heart weight/body weight ratio was also decreased from a value of 4.07 ± 0.08 mg/g (control group, $n = 20$) to 3.32 ± 0.06 mg/g (3 mg/kg, $n = 10$, $p < 0.01$), 3.64 ± 0.05 mg/g (0.3 mg/kg, $n = 20$, $p < 0.01$) and 3.81 ± 0.07 mg/g (0.03 mg/kg, $n = 10$, $p < 0.01$).
In 2 year-old SHR, oral treatment at 3 mg/kg/day during 1 month, induced a decrease in mean blood pressure of 18% ; the ventricles weight/body weight ratio was also markedly decreased from 5.56 ± 0.44 mg/g in control group ($n = 7$) to 3.69 ± 0.08 mg/g ($n = 10$) ($p < 0.01$). This observed regression of cardiac hypertrophy was still significant (-19%) 2 weeks after the end of treatment.
Therefore our results demonstrate that trandolapril is able to induce in SHR a dose-dependent regression in cardiac hypertrophy, even after a short period of treatment and at a low threshold dosage. In addition this beneficial effect is also evidenced in very old SHR in which hypertension and cardiac alterations have been established for a long period of time.

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POTENTIATION, BY ENOXIMONE, OF SELECTIVE β-1 AND β-2-ADRENOCEPTOR STIMULATION IN HUMAN ATRIAL MYOCARDIUM

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Atrial myocardium from patients treated with atenolol is more sensitive to β-2 adrenoreceptor (B2R) mediated inotropic effects of adrenaline than tissues from non β-blocker treated patients (Hall et al 1988). In tissues from such patients B2R stimulation leads to maximal effects, subsequent stimulation of β-1 adrenoreceptors (B1R) produces no further increase in contractile force. This leads us to question whether the pathways of the two receptors are linked through a common pool of cyclic AMP (cAMP), and also whether altered phosphodiesterase (PDE) activity accounts for the increased sensitivity of β-blocker treated patients to adrenaline. We studied the effects of the selective PDE-III inhibitor enoximone (apparent Ki 1.3μM)(Kariya et al 1982) on selective B2R and B1R inotropic stimulation of human atrial myocardium.

Strips of right atrial myocardium from patients undergoing routine cardiac surgery were mounted in an organ bath at 37°C, paced at 0.5 Hz and incubated for 2 hours with phenoxybenzamine 5μM.(Gille et al 1985) All patients were receiving β-1 selective blockade (atenolol or metoprolol) prior to surgery. Selective stimulation of B1R was with noradrenaline (NA) in the presence of 50nM ICI 118551 and B2R was with adrenaline (AD) in the presence of 300nM CGP 20712A. Contractile responses were measured in response to cumulative concentrations of agonist, two successive concentration-response curves were determined for each tissue, the second was in the presence or absence of 2μM enoximone (E).

	-logEC50 M		maximum response (as % of first maximum)	
	NA+ICI (n=5)	AD+CGP (n=6)	NA+ICI (n=5)	AD+CGP (n=6)
without E	6.7±0.2	7.4±0.1	67±14	76±5
	p<0.05	p<0.02		
with E	7.3±0.2	8.1±0.1	90±7	89±6

Results are mean ± s.e.m. EC50's were corrected for desensitisation and compared by Student's t test.

Enoximone produced an increase of 18±4%, of catecholamine maximum response, above baseline force.(table) Stimulation of both B1R and B2R mediated responses were similarly potentiated by enoximone. Enoximone produced no significant increase in the maximum responses to B1R or B2R stimulation. The -log EC50 values of adrenaline were significantly lower than those of noradrenaline both in the untreated ($p<0.02$) and the enoximone treated ($p<0.02$) tissues.

CONCLUSIONS: 1.The potentiation of the effects of catecholamines by enoximone is consistent with inhibition of cAMP hydrolysis and greater availability for cAMP dependent protein kinase activation and contraction (Kaumann et al 1988). 2.The preservation of increased B2R responsiveness following enoximone suggests that altered PDE III activity is unlikely to account for the increased responsiveness of β-blocker treated patients to adrenaline. 3.B1R and B2R may generate a common pool of cAMP leading to contraction.

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Kariya et al J.Cardiovasc.Pharmacol. 1982 4:509-514
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THE α_1-ADRENOCEPTOR ANTAGONISTIC PROPERTIES OF R 56865 IN THE PITHED RAT

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R 56865 (N-[1-[4-(4-fluorophenoxy)-butyl]-4-piperidinyl]-N-methyl-2-benzothiazolamine) is an agent which shows antiarrhythmic and antiischaemic properties. In rat and guinea-pig aorta, R 56865 in a concentration of 10^{-8} - 10^{-5} M displayed a strong, limited and saturable antagonism of the noradrenaline-(NA) induced vasoconstriction, which was attributed to a site of action distinct from the NA-binding-site (Koch et al., 1988). The aim of the present study was to assess the α-adrenoceptor antagonistic properties of R 56865 in the pithed rat as an in vivo model.

Male, normotensive Wistar rats (250-300 g) were anaesthetised with hexobarbitone sodium (250 mg/kg, i.p.). A tracheal cannula was inserted, the rats were pithed through one orbit with a steel rod and immediately thereafter artificially respirated. The left carotid artery and the right jugular vein were cannulated for recording of diastolic BP and for the administration of drugs, respectively. Heart rate was derived from the BP pulse in the carotid artery. Intravenous treatment with antagonists or saline (control) was carried out 15 min before administration of the agonists or before electrical stimulation. The dose-response curves to NA and tyramine were obtained after α_2-adrenoceptor blockade with rauwolscine (10^{-6} mol/kg, i.v.). Electrical stimulation was performed after bilateral adrenalectomy and vagotomy and administration of atropine (1 mg/kg, s.c.) and tubocurarine (0.5 mg/kg, i.v.).

Neither prazosin nor R 56865 influenced the basal diastolic BP (37 ± 1.5 mm Hg, mean ± s.e. mean; n = 40), whereas the basal heart rate was reduced by R 56865 (10^{-5} mol/kg; 315 ± 8.8 vs. 227 ± 6.5 beats/min, mean ± s.e. mean; n = 18). The dose-response curves for the increase in diastolic BP to the α_1-adrenoceptor-mediated response of the endogenous neurotransmitter NA and to the selective α_1-adrenoceptor agonists methoxamine and ST 587 were shifted approximately 10-fold to the right by R 56865 (10^{-5} mol/kg). R 56865 (10^{-5} mol/kg) caused only a modest but significant ($P < 0.05$) depression of the maximum of the dose-response curve of the α_2-adrenoceptor agonist B-HT 920. The increase in diastolic BP elicited by α_1-adrenoceptor stimulation by the indirectly acting sympathomimetic tyramine and electrical stimulation were dose-dependently antagonized by R 56865 (10^{-6} - 10^{-5} mol/kg). The increase in heart rate elicited by NA, tyramine and electrical stimulation was not antagonized by R 56865. For comparison, prazosin was applied in a concentration of 6×10^{-8} mol/kg, which caused a similar antagonism as R 56865 (10^{-5} mol/kg) on the NA-, tyramine- and electrical stimulation-induced increase in diastolic BP.

In conclusion, in the pithed rat R 56865 in high doses exhibits a competitive antagonism of α_1-adrenoceptor-mediated vasoconstrictions. The absence of a depression of the maximum of the dose-response curve of ST 587 and the very moderate attenuation of the maximal B-HT 920-induced increase in diastolic BP confirms the lack of calcium entry blocking properties of R 56865 for α-adrenoceptor activated calcium channels in vitro (Koch et al., 1988). In doses up to 10^{-5} mol/kg the interaction of R 56865 with the sympathetic neurotransmission can solely be explained by α_1-adrenoceptor blockade. In contrast to the isolated rat aorta, where R 56865 (10^{-8} - 10^{-5} M) seems to interact allosterically with the NA-binding-site on the α_1-adrenoceptor, R 56865 (10^{-6} - 10^{-5} mol/kg) acts like an α_1-adrenoceptor antagonist of the competitive type in vivo.

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CARDIAC IN VITRO PHARMACOLOGY OF R 70608

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R 56865 (N-[1-[4-(4-fluorophenoxy)-butyl]-4-piperidinyl]-N-methyl-2-benzothiazolamine) reduces ouabain toxicity in the isolated guinea-pig papillary muscle (Vollmer et al., 1987) and in the guinea-pig heart-lung preparation (Schneider et al., 1988). The present investigations were conducted in order to evaluate the cardiac effects of a hydroxy-derivative, R 70608 (4-[(2-benzothiazolyl)-methylamino]-α-[2-(4-fluorophenoxy)ethyl]-1-piperidine-ethanol).

R 70608 (3×10^{-9} - 1×10^{-6} M) did not change contractile force of isolated rat or guinea-pig left atria. Frequency of spontaneously beating rat atria was impaired in the concentration range of 10^{-6} to 10^{-5} M and amounted to 50% at 10^{-5} M. The compound's influence on cardiac glycoside intoxication was tested in isolated rat left atria and guinea-pig papillary muscles.

Isolated left rat atria were submitted to a graded intoxication by a stepwise increase of stimulation rate allowing an evaluation of the compound's influence on both positive inotropic and toxic effects of ouabain. The positive inotropic response to ouabain was not impaired by R 70608 (10^{-6} M). The toxic response to ouabain consisting of an increase in diastolic tension and a decline in systolic tension was prevented by the compound. Potassium loss occuring during ouabain intoxication (77 ± 9 mmol/kg cell; mean ± s.d., n = 6) was reduced by R 70608 (99 ± 14 mmol/kg cell). The increase in cellular sodium content amounting to 61 ± 13 mmol/kg cell was reduced (41 ± 6 mmol/kg cell). The ouabain-induced calcium gain (4.6 ± 1.2 mmol/kg cell) was prevented by pretreatment with R 70608 (2.5 ± 0.3 mmol/kg cell).

In order to get more insight into the mechanism of action, electrophysiologic studies were done on isolated guinea-pig papillary muscles. The action potential duration at 90% of repolarization (APD_{90}) was slightly prolonged (15 ± 8 ms) by R 70608 in concentrations from 3×10^{-7} M onwards. Resting membrane potential (RMP) and the rate of rise were unaffected. R 70608, even in high concentrations (10^{-6} - 10^{-5} M) had no influence on isoprenaline-induced Ca^{2+}-dependent slow action potentials in depolarized guinea-pig papillary muscle.
Ouabain (8×10^{-7} M) induced electrophysiologic changes resulting in a pronounced shortening of the APD, a decrease in RMP and amplitude, and the occurrence of delayed afterdepolarizations (DAD). Pretreatment with R 70608 (10^{-8} - 10^{-6} M) prevented the occurrence of DAD while it attenuated the shortening of the APD and the decrease in RMP and APA.

In conclusion, R 70608 has only minor effects on cardiac contractile force and frequency. It appears that R 70608 is very potent in preventing cardiac glycoside toxicity. The specific inhibition of the occurrence of DAD favours the speculation that R 70608 prevents either the oscillatory release of calcium from the sarcoplasmic reticulum or the occurrence of a transient inward current.

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PROTECTIVE EFFECTS OF R 70608 AGAINST DIGITALIS-INDUCED TOXICITY IN THE GUINEA-PIG HEART-LUNG PREPARATION

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First observations indicated that R 70608 (4-[(2-benzothiazolyl)methylamino]-α-[2-(4-fluorophenoxy)ethyl]-1-piperidine-ethanol) antagonizes digitalis-induced changes of action potentials in guinea-pig papillary muscle (Peters et al., 1989). Therefore, the effects of R 70608 on cardiac contractile function and output were investigated in blood perfused guinea-pig hearts during cumulative digitoxin application.

The experimental model was an autoperfusing heart-lung-preparation (according to Starling) modified for small rodents. The experiments were performed in guinea-pigs of either sex, wheighing 300-350 grams. The animals were respirated with 95% O_2 and 5% CO_2. During the surgical procedure the respiration gas was mixed with halothane (2-3%). The hearts were perfused with whole blood at 37°C. Parameters measured were LVP, $LVdP/dt_{max}$ and cardiac output. The hearts were paced at a constant rate of 320 beats/min.

In intoxication experiments the addition of digitoxin (2×10^{-6} M; single dose) led to ventricular arrhythmias after 7.6 ± 0.5 min; mean ± s.e. mean. Pretreatment with R 70608 (-30 min, 10^{-6} M) significantly ($p < 0.01$; Mann-Whitney U test) delayed the onset of arrhythmias due to digitoxin overdosage to 12.3 ± 1.1 min; mean ± s.e. mean.

In separate experiments cumulative dose-response curves of digitoxin could be influenced by pretreatment with various doses of R 70608. Control experiments ($n = 10$) were performed by applying increasing concentrations of digitoxin (10^{-7} - 3×10^{-6} M) to the hearts after an equilibration period of 75 min. Each dose of digitoxin was allowed to equilibrate for 20 min. The pretreated hearts received R 70608 (10^{-6}, 10^{-7}, 10^{-8} M) 30 min prior to the addition of digitoxin. Under these conditions pretreatment with R 70608 had no effect on either LVP, $LVdP/dt_{max}$ or cardiac output. The addition of digitoxin up to a concentration of 10^{-6} M caused a clear positive inotropic effect both in pre-treated and control hearts. The development of the positive inotropic effect in the therapeutic dose range (3×10^{-7} - 10^{-6} M) was not affected by pretreatment with R 70608. In control hearts concentrations of digitoxin above 10^{-6} M led to a marked loss of mechanical function whereas pretreated hearts still showed an increase in contractility upon digitoxin addition. Digitoxin (3×10^{-6} M) nearly abolished cardiac function in control hearts. Pretreated hearts nevertheless exhibited a dose-dependent preservation of cardiac mechanical function.

The antiarrhythmic properties of R 70608 as well as the protection against mechanical dysfunction after digitalis overdosage may result from a stabilization of the physiological ion homeostasis within the myocardial cell by R 70608. This putative mode of action is also reflected by the suppression of oscillatory afterpotentials due to digitalis intoxication as found in guinea-pig papillary muscle preparations (Peters et al., 1989). A comparable type of interaction with the cardiac glycoside intoxication was observed for R 56865, a structural analogue of R 70608 (Vollmer et al., 1987; Schneider et al., 1988). It remains subject to future experiments whether or not a specific blockade of distinct ion channels which are open under pathological conditions is responsible for the protective effects of R 70608.

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CENTRAL CARDIOVASCULAR ACTIONS OF CALCIUM CHANNEL ANTAGONISTS IN DOGS

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Several pharmacological and clinical studies have suggested that calcium channel antagonists (CCA) can exert central actions . The aim of the present study was to investigate their putative central cardiovascular properties in dogs.

In normotensive pentobarbital anesthetized dogs, intravenous (i.v.) nicardipine (0.3 to 10 μg/kg), nifedipine (0.01 to 10 μg/kg), verapamil (0.003 to 0.1 mg/kg) or diltiazem (1 to 300 μg/kg) induced a dose-dependent decrease in systolic and diastolic blood pressures followed by a tachycardia due to baroreflex activation. By contrast, central (intracisternal: i.c.) administration of the same CCA (at doses ineffective by i.v. route) induced an increase in both blood pressure and heart rate.

These pressor and positive chronotropic responses are due to sympathetic activation and involves brain alpha 1 (and not alpha 2) adrenoceptors, since they are suppressed by pretreatment with guanethidine (15 mg/kg i.v.), chlorisondamine (2 mg.kg i.v.) or ARC 239 (an alpha 1 blocking agent, 10 μg/kg i.c.) but not by yohimbine (50 μg/kg i.c.). A functional interaction between central cholinergic systems and central dihydropyridine binding sites was also demonstrated: i.c. methylatropine reduced the nicardipine-induced increases in blood pressure and heart rate.

Two kinds of neurogenic hypertensive sinoaortic denervated (SAD) dogs were used to study whether the central sympathoexcitatory effects of CCA depend on the level of the sympathetic tone.

In acute SAD dogs (where the sympathetic tone is nearly maximum), i.v. administration of nicardipine (50 μg/kg), nifedipine (5 μg/kg), verapamil (200 μg/kg) or diltiazem (300 μg/kg) significantly reduced the SAD-induced increase in blood pressure and heart rate, thus demonstrating the sympathoinhibitory properties of CCA when sympathetic tone is maximum.

In chronic SAD awake dogs (where the sympathetic tone is high but not maximum), i.v. nicardipine (50, 100 or 200 μg/kg) significantly decreased arterial blood pressure but also induced both a marked tachycardia and a significant increase in plasma noradrenaline and adrenaline release. However, this direct central activation was not observed with verapamil (200μg/kg i.v.) under the same experimental conditions (chronic SAD awake dogs).

The present data shows that CCA could exert central cardiovascular actions in dogs.The positive chronotropic effects of CCA not only result from a baroreflex activation (following the decrease in blood pressure) but also from a direct centrally-mediated effect on sympathetic tone. Moreover, the magnitude of this sympathoexcitatory effect seems to vary according to the CCA group (phenylakylamines being less potent than dihydropyridines) and depend on the level of sympathetic tone: CCA exert stimulant effects when the sympathetic tone is low (normal dogs) and depressor actions when it is high (SAD dogs).

EFFECT OF RAISED INTRACELLULAR SODIUM ON INTRAPLATELET CALCIUM AND CYCLIC AMP CONCENTRATION

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It has been suggested that changes in the cytosolic concentration of sodium ions ($[Na^+]_i$) can alter platelet responses to aggregating agents (Motulsky & Insel, 1983). We have shown previously that the increased sensitivity to collagen of ouabain-treated platelets is associated with increased basal and stimulated cytosolic calcium levels ($[Ca^{2+}]_i$) (Lees et al, 1988). The effects of raised $[Na^+]_i$ on platelet sensitivity to stimuli and $[Ca^{2+}]_i$ have been investigated further using the sodium ionophore monensin. As it has been reported that adenylate cyclase is inhibited by sodium (Steer & Wood, 1981), and the inhibitory mediator cyclic AMP (cAMP) can alter basal $[Ca^{2+}]_i$ (Erne et al, 1983), we have also investigated the effects of raised $[Na^+]_i$ on the platelet cAMP.

PRP was prepared from citrated whole blood and incubated (30 min, 37°C) with either monensin (20uM), ouabain (30uM) or an equal volume of buffer. Aggregation to collagen was recorded as a change in light transmission. Basal $[Ca^{2+}]_i$ was measured using the calcium sensitive fluorescent probe fura 2 (Pollock et al, 1986). Platelet basal and prostacyclin-stimulated cAMP were measured by radioimmunoassay.

Monensin sensitized platelets to collagen such that the concentration of collagen to induce a 30% increase in light transmission (measured at 3 min) was significantly reduced from 1.078 ± 0.30 ug ml^{-1} (mean$\pm$s.e.mean)for control platelets to 0.196 ± 0.6 ug ml^{-1} (n=6, $P< 0.02$) for the monensin-treated platelets. Although the monensin-treated platelets aggregated when stirred this could not account for the increase in collagen sensitivity. Monensin also induced a 230% rise in basal $[Ca^{2+}]_i$ from 76.1 ± 4.1nM to 251.6 ± 19.9nM (n=5, $P< 0.005$).Neither ouabain nor monensin inhibited basal or prostacyclin (1- 30nM) stimulated platelet cAMP.

These results support our earlier findings that raised $[Ca^{2+}]_i$ is associated with and possibly mediates the increased sensitivity of platelets to aggregating agents following exposure to agents which raise $[Na^+]_i$. Our results also suggest that this effect does not appear to be caused by raised $[Na^+]_i$ leading to inhibition of platelet adenylate cyclase.

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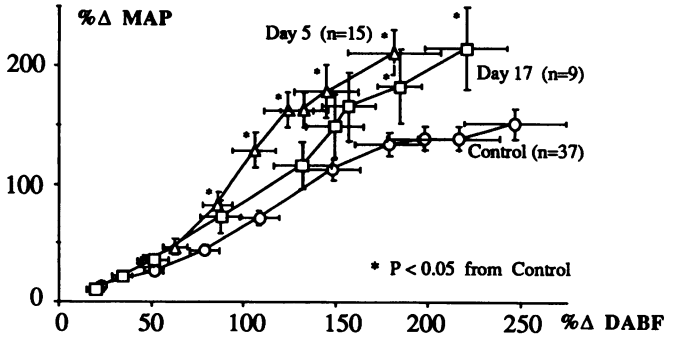
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HAEMODYNAMIC EFFECTS OF BAY K-8644 IN RAT MODEL OF CALCIUM OVERLOAD

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Ca^{2+} overload may be a phenomenon common to hypertension, atherosclerosis and ageing (Fleckenstein et al; 1985). In the Fleckenstein rat model, (vitamin D_3 and 4-5 b.i.d. doses of nicotine; vit D + nic), large increases in myocardial and arterial [Ca^{2+}] have been obtained. We now describe the hemodynamic effects of Bay K-8644 in day 5 and day 17 (recovery) calcinotic pithed rats.
Male Wistar rats (220 g) were treated with vit D (day 1:300,000 IU,i.m) and nic (25 mg/kg p.o; b.i.d. for 4 days). Hemodynamic studies were made in rats anesthetised with pentobarbitone (55 mg/kg i.p) on day 5 (at least 15 h after the last dose of nic) and on day 17. Descending aortic blood flow (DABF) was measured with an electromagnetic flow probe on the aorta below the diaphragm with both carotid arteries ligated. Total peripheral resistance (TPR) was calculated by dividing mean blood pressure (MAP) by DABF. Dose-response curves to Bay (0.1-1000 ug/kg, i.v) were determined in pithed rats.The increases in myocardial and aortic Ca^{2+} on days 5 and 17 are reported elsewhere (Oster et al; this meeting).
In intact rats, MAP was significantly elevated (140+6 mmHg;n=16) at day 5 when compared with controls (119+3;n=37) but was not different on day 17 (121+6 mmHg; n=12). The increase in MAP at day 5 was due to an increase in TPR (22+1.4 vs cont: 17+1.2 mmHg/ml/min/100g;p 0.05), with no change in DABF (day 5: 7.5+0.4 vs cont: 7.1+0.6 ml/min/100g). Heart rate was not changed by calcinosis (day 5: 400+9 vs cont: 395+6 b/min). In pithed day 5 rats the increase in DABF (3.8 + 0.4 vs cont: 2.9+0.2 ml/min/100 g;p 0.05) was particularly associated with higher resting systolic BP (58 + 3 vs cont:43+ 2 mmHg), but no change in HR.
In day 5 rats pressor responses to Bay were markedly enhanced over controls, particularly at E max (fig). These effects were due to vascular changes, since TPR-response curves to Bay were also enhanced, while the DABF-response curves were decreased (fig). Although base-line hemodynamic values had returned to control levels at day 17, the maximum increase in MAP after Bay remained elevated.

Hemodynamic effects of BAY-K in calcinotic pithed rats



This extended calcinosis model could be useful in studies on cardiovascular changes associated with tissue Ca^{2+}-overload.

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CHANGES IN VASCULAR REACTIVITY IN A RAT MODEL OF CALCIUM OVERLOAD

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Fleckenstein et al, (1985) proposed that vitamin D_3 (single dose, 300,000 IU/kg, im) plus nicotine (25 mg/kg, twice daily, po) produce cardiovascular calcium overload. We studied the changes in vascular reactivity following 4 days' treatment plus 1 (day 5) or 13 days' recovery (day 17). Hemodynamic changes and increases in calcium levels are reported elsewhere (Chillon et al, and Oster et al, this meeting). Tail arteries (1 cm) were perfused (2 ml/min) with Krebs bicarbonate. Noradrenaline, NA, cirazoline, CIR, UK 14304, UK, or serotonin, 5HT, was injected as bolus (0.1 ml, 0.1 µM to 1 mM, 5 - 10 minutes between each bolus, 1 artery per agonist). Relaxation time is the time from the maximal increase in perfusion pressure to 50% of this value. In separate experiments, passive (hydrodynamic resistance estimated from basal perfusion pressures at perfusion rates of 1 to 9 ml/min), and active pressure (maximum increase in perfusion pressure following 0.1 mM NA at various perfusion rates) were determined.

Group	Controls	Day 5	Day 17
NA	17	16	16
Δ maximum (mm Hg)	168±25	185±9	206±18
Δ mm Hg at 30µM	59±7	81±8*	92±16*
relaxation (sec)	28.6±5.3	39.0±12.2	222.0±40.6*
CIR	5	6	14
Δ maximum (mm Hg)	170±28	173±8	173±13
Δ mm Hg at 30µM	126±26	129±22	106±20
relaxation (sec)	130.3±41.1	221.±22.6	431.0±57.5*
UK	6	7	14
Δ mm Hg at 1 mM	3±2	14±7	5±2
5HT	6	7	6
Δ maximum (mm Hg)	179±30	120±15	145±27
Δ mm Hg at 30µM	113±18	53±13*	95±22
relaxation	108.8±43.8	58.0±31.1	308.0±32.3*
Passive pressure	5	-	5
Δ mm Hg at 2 ml/min	43±1	-	34±1*
Δ mm Hg at 9 ml/min	126±13	-	77±1*
Active pressure	5	-	5
Δ mm Hg at 2 ml/min	96±14	-	136±6*
Δ mm Hg at 9 ml/min	99±3	-	88±6

Means ± SEM. * = $P < 0.05$, t-test comparison with controls. Excepting an increase in midrange sensitivity to NA (days 5 and 17) and a decrease in response to a mid-range concentration of 5HT (day 5), vasoconstrictor responses to various agonists were similar. At 17 days relaxation times increased and passive pressures decreased. In conclusion, this extended calcium overload model (day 17) is characterized by an increase in vascular rigidity with little change in sensitivity.

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A DELAYED TONOTROPIC EFFECT OF ISOPRENALINE ON ISOLATED RAT RIGHT ATRIUM

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Positive chronotropic and inotropic effects of β-agonists on isolated mammalian right atrial preparations are well known. We report here an additional effect, a slowly developing but sustained rise in the resting tension (tonotropic effect) in rat isolated right atrium, produced by isoprenaline and by the indirect sympathomimetic tyramine.

Adult male Wistar rats (200-300 g) were killed by decapitation; the right atrium excised and set up in a 2 ml organ bath containing Tyrode solution aerated with a mixture of 95% O_2 + 5% CO_2 at 30°C, in order to record the resting tension and the amplitude of the spontaneous beats.

Addition of isoprenaline 25 nM produced almost immediately positive chronotropic and inotropic effects. A slight rise in the resting tone also occurred (peak time 2.2 ± 0.2 min., n = 9) which then showed signs of decline. Subsequently the decline was arrested and the resting tension started to rise again (Figure 1A), peaking at 13.7 ± 1.0 min. after initial introduction of isoprenaline (maximum increase in resting tension: mean ± SEM = 426 ± 97 mg, n = 9). Tyramine 10 μM, produced results qualitatively similar to isoprenaline. Phentolamine 5 μM, did not antagonise the effects of isoprenaline or tyramine. Tyramine, but not isoprenaline, was ineffective in atria from reserpine-treated animals (2.5 $mg.kg^{-1}$ I.P.). The effects of isoprenaline and tyramine were completely blocked in the presence of propranolol, 5 μM. In preparations from animals treated with 6OH-DA (4 x 50 $mg.kg^{-1}$ I.P.) the maximum increase in the resting tension evoked by isoprenaline 25 nM, was significantly smaller than in controls (mean ± SEM = 136 ± 28 mg, n = 5; $P < 0.05$ t-test).

Exposure to capsaicin 10 μM, for 10 min. led to a total block of the delayed tonotropic effect of isoprenaline (Figure 1B) but only a modest reduction of the inotropic effect. The effect of capsaicin was reversible on washing.

A possible explanation for the tonotropic effect of isoprenaline, its resistance to reserpinisation, its reduction after 6OH-DA pretreatment, and its abolition by capsaicin may be that it originates in β-adrenoceptor-mediated release of a non-adrenergic mediator(s) from the nervous elements in the rat right atrium.

Fig. 1

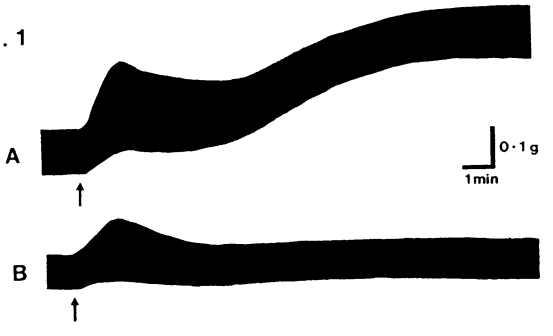


Figure 1. Effect of isoprenaline on the isolated spontaneously beating rat right atrium. Isoprenaline 25 nM was injected at the arrows. A) Control B) 10 min. exposure to capsaicin preceded isoprenaline injection.

ENDOTHELIUM-DEPENDENT ACTION POTENTIALS WITH NORADRENALINE IN THE RABBIT ISOLATED BASILAR ARTERY

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In the rabbit basilar artery, noradrenaline produces smooth muscle depolarization and contraction. The majority of contraction occurs with concentrations of noradrenaline greater than 10^{-4} M. . These high concentrations of noradrenaline also generate action potentials (Garland and McPherson, 1988). Recently, the endothelium-derived peptide endothelin has been isolated and sequenced. Endothelin is a potent vasoconstrictor, which shares certain structural similarities with neurotoxins which act on voltage-dependent Na^+ channels (Yanagisawa et al, 1988). This suggests that action potentials produced by high concentrations of noradrenaline may reflect the release of endothelin.

Membrane and contractile responses to noradrenaline were measured simultaneously in isolated cylindrical (1-2 mm.) segments of the basilar artery (Garland, 1987). 10^{-6} - 10^{-4} M. noradrenaline produced concentration-dependent depolarization and contraction. Concentrations greater than 10^{-4} M. produced at least one action potential, followed by constant membrane depolarization. Separate experiments were performed on arteries whose endothelial cells had been removed, either by perfusion with 0.1% Triton X-100 in PSS or by gentle rubbing of the intimal surface. Noradrenaline produced similar-sized responses in these arteries, compared to vessels with a functional endothelium. However, in the absence of endothelial cells, noradrenaline failed to initiate action potentials. With 10^{-3} M. noradrenaline the magnitude of contraction was not significantly reduced in these arteries (2082±295 compared with 2098±222mg, n=12), although when action potentials did not occur the speed at which contraction developed was reduced. In segments with an intact endothelium the time required to produce 50% of the contraction with 10^{-3} M. noradrenaline was 53.0 ± 5.1 seconds (n=16), compared with 84.8 ± 11.0 seconds (n=19) in segments without an endothelium ($p<0.05$%).

The results indicate an endothelium-dependence of the action potentials produced by high concentrations of noradrenaline, a dependence which may reflect the release of endothelin. If this is so, then the released endothelin does not significantly increase the magnitude of contraction with noradrenaline.

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AUTORADIOGRAPHIC LOCALISATION OF [^{3}H]5-HT BINDING SITES WITHIN INTACT AND DENERVATED CAROTID BODIES OF THE CAT

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Injection of 5HT into the carotid sinus evokes a chemosensory discharge by activating $5HT_2$ and $5HT_3$ receptors (Kirby and McQueen, 1984). The present study was undertaken in order to localize [^{3}H]5HT binding sites within the carotid bodies of normal cats and to assess the effects of carotid sinus nerve (CSN) section.

Two groups of cats were used (i) normal and (ii) animals which had the CSN sectioned under Ketamine (50mg/kg i.m.) and Xylazine (1mg/kg i.m.) anaesthesia and which were allowed an 8 day postoperative recovery period. Chemoreceptor reflexes in this group were obtained from the innervated, but not the denervated, carotid body on intracarotid injection of Na CN. Animals from both groups were anaesthetized (42mg/kg sodium pentobarbitone i.p.) and tissue removed for in vitro autoradiography. Sections of tissue were incubated in 2nM [^{3}H]5HT (16-20 Ci/mmol, Amersham) essentially as described by Young and Kuhar (1980). The degree of non-specific binding was established by incubating in the presence of 1μM unlabelled 5HT creatinine SO4.

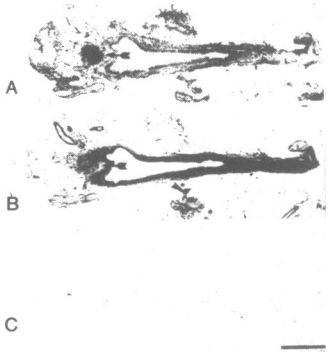


Figure 1. [^{3}H]5HT binding to cat carotid body.

A. Autoradiograph of [^{3}H]5HT binding.
B. Haemotoxylin and Eosin stained tissue underlying A.
C. Section adjacent to A indicating non-specific binding.

Arrows indicate carotid body.
Scale bar = 2mm.

Strong binding of [^{3}H]5HT was seen in the carotid artery and the carotid body (Figure 1). There was a marked increase in this binding to the ipsilateral carotid body following CSN denervation.

These results confirm the presence of [^{3}H]5HT binding sites in the carotid body of the cat. Moreover it appears that 5HT receptors are associated with elements other than sensory terminals within the carotid body, probably type I or type II cells or blood vessels. The fact that [^{3}H]5HT binding increases following denervation suggests that an upregulation of 5HT receptors in the carotid body may take place.

We are grateful to the British Heart Foundation and Servier Pharmaceuticals for support.

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THE EFFECT OF ICI 169,369 ON THE NEUROGENIC AND EXOGENOUS 5-HT RESPONSES OF THE RABBIT BASILAR ARTERY

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Results from our previous study on transmitter release (Holly & Tahir, 1988) provide evidence in support of both noradrenaline and 5-HT being released on transmural nerve stimulation of the rabbit basilar artery but in the rabbit internal carotid artery only 5-HT was released. However, some of our unpublished studies have shown that in the rabbit internal carotid artery, noradrenaline release does occur but only at lower frequencies of stimulation.

We have now shown that in the RBA and RICA, responses to exogenous 5-HT (EC_{50}: $2.54 \pm 0.82 \times 10^{-8}$ M; $5.19 \pm 0.41 \times 10^{-8}$ M, respectively) and TNS were antagonised by ICI, 169,369, probably through an action on 5-HT_2-type receptors. In both vessels, ICI, 169,369 (10^{-9} M to 10^{-6} M), was a competitive antagonist, with the pA_2 ± s.e.m. being, 8.54 ± 0.09, RBA; 8.45 ± 0.11, RICA. In the RBA, the pA_2 value for ICI, 169, 369, was similar to that obtained for cinanserin (8.35 ± 0.12). In addition, in the same vessel, ICI, 169,369, was found to be ineffective (up to 5×10^{-6} M), at antagonising responses to 2-methyl-5-HT (selective 5-HT_3 receptor agonist, Richardson et al., 1985; EC_{50}: $2.21 \pm 0.63 \times 10^{-5}$ M), 5-carboxytryptamine (selective 5-HT_1 receptor agonist, Fenuik et al., 1981; EC_{50}: $3.13 \pm 0.85 \times 10^{-5}$ M) and was also without effect (> 10^{-5} M), at α-adrenoceptors and histamine receptors. Due to the non availability of a selective 5-HT_2 agonist, we were unable to examine the effects of ICI, 169, 369 on such an agent.

In both arteries, ICI, 169, 369, caused a dose dependent decrease in the neurogenic vasoconstrictor responses, at 5 Hz and 20 Hz. At a concentration of 4×10^{-10} M, ICI, 169,369, caused a demonstrable reduction of the neurogenic responses in both the RBA (10 ± 2% of control) and RICA (7 ± 1.5%, of control). A maximum reduction was observed at 4×10^{-5}, being 87 ± 3.2%, in the RBA and 79 ± 2.5% in the RICA. The IC_{50} ± s.e.m. of ICI, 169, 369, for the two vessels was $8.9 \pm 1.6 \times 10^{-8}$ M, RBA and $1.9 \pm 2.5 \times 10^{-7}$ M, RICA.

ICI, 169, 369, appears to be selective for 5-HT_2 type receptors in our preparations. It inhibited the neurogenic contractile responses of the RBA and RICA, suggesting that in both vessels, neurally released 5-HT acts predominantly via 5-HT_2 receptors.

Many thanks to Dr. Tom Blackburn for providing the ICI, 169, 369, research compound.

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EFFECT OF CALCITONIN GENE-RELATED PEPTIDE ON CANINE AND PORCINE CORONARY ARTERY PREPARATIONS IN VITRO

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The effect of the calcitonin gene-related peptide (CGRP) was examined on isolated epicardial coronary artery ring preparations of varying internal diameters from pigs and dogs. Porcine hearts were obtained from an abbatoir and transported to the laboratory in cardioplegic solution at 4°C. Canine hearts were obtained form anaesthetized greyhounds at the completion of *in vivo* studies. 4mm rings were prepared from the left anterior descending coronary artery (LAD) and right coronary arteries and their diameters measured. The rings were mounted between two stainless steel hooks and suspended in 5ml baths in Krebs' solution warmed to 37°C and gassed with 95%O_2/5%CO_2. Tension in the rings was adjusted to give a maximal response to 40mM KCl. The rings were contracted with 300nM endothelin and cumulative dose-response curves to CGRP constructed between 0.05 and 150nM. Responses to CGRP were calculated as a percentage of the maximum i.e. a relaxation to baseline.

CGRP caused a dose-related relaxation of endothelin-contracted porcine LAD rings and canine LAD and right coronary artery rings. In the pig, CGRP tended to cause greater relaxation in smaller diameter rings. There was a significant difference between the relaxation caused by 0.05nM CGRP in 1mm and 2mm diameter rings (17.15±3.68%, n=5, and 7.03±2.04%, n=7, respectively, mean±s.e.mean, $p<0.05$, Student's t test for unpaired data) and by 15nM CGRP in 1mm and 2mm diameter rings (70.7±11.76%, n=5, and 30.88±7.95%, n=5, respectively, mean±s.e.mean, $p<0.05$, Student's t test for unpaired data). The maximal relaxation to CGRP was also increased with decreasing vessel diameter (115.51±21.72%, 71.76±11.23% and 67.33±23.49% in 1mm, 1.5mm and 2mm rings respectively, mean±s.e.mean).

In dog LAD and right coronary artery rings no change was observed in the response to CGRP with decrease in vessel diameter, although ring preparations of 1mm and 1.5mm only could be prepared from epicardial vessels.

In conclusion, CGRP dilates dog and pig epicardial coronary artery preparations which have been pre-contracted with endothelin, and in the pig there is a correlation between decreasing vessel diameter and increasing response to CGRP.

STUDIES ON THE MECHANISM OF THE CARDIOVASCULAR EFFECTS OF CALCITONIN GENE-RELATED PEPTIDE IN ANAESTHETISED RATS

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Calcitonin gene-related peptide (CGRP) is a 37 amino-acid neuropeptide. In conscious rats, CGRP produces tachycardia, hypotension, hindquater vasodilatation, mesenteric vasoconstriction and no change in renal resistance (Gardiner *et al.*, 1987). This study attempts to determine the mechanisms of the cardiovascular effects of CGRP in anaesthetised rats.

Male normotensive rats (230-250 g, Sprague-Dawley) were anesthetised with pentobarbitone (70 mg/kg i.p.) and mechanically ventilated. Intravenous administrations were given *via* the femoral vein. Blood pressure (MAP) was measured from a carotid artery and heart rate (HR) obtained with a tachograph. Doppler blood flow probes were placed around the upper abdominal aorta (to estimate cardiac output), left renal artery, superior mesenteric artery and lower abdominal aorta (hindquaters). Vascular resistances were calculated as the ratio of MAP over Doppler shifts. The effects of CGRP (380 ng/kg/min over 15 min) were evaluated in rats pretreated 10 min earlier with i.v. saline (0.25 ml/kg), BW 755C (5 mg/kg), RP 59227 (0.5 mg/kg : antagonist of PAF receptors), propranolol (0.75 mg/kg), quinacrine (5 mg/kg), glibenclamide (20 mg/kg), ranitidine (0.5 mg/kg) plus promethazine (1 mg/kg), S-sulpiride (60 µg/kg) plus SCH 23390 (1 µg/kg), capsaïcine (1 mg/kg), chlorisondamine (0.5 mg/kg) alone or plus vasopressin (7 mIU/kg/min) (n=8/treatment). In pithed rats, dose-pressor response curves to cirazoline, B-HT 920 and angiotensine II were determined 10 min after starting the infusion of either saline or CGRP. Results are given as mean ± S.E.M.

CGRP produced a fall in MAP which attained a maximum (-50 ± 3 mmHg from 127 ± 3 mmHg) 5 min after the start of the administration. This effect persisted until the end of the infusion. CGRP decreased total peripheral resistances (-21 ± 3%) and cardiac output (-25 ± 2%). Hindquater resistance (-46 ± 1%) fell more than renal (-18 ± 2%) or mesenteric resistances (-30 ± 4%). None of the antagonists mentioned above, except chlorisondamine, prevented the hypotensive effects of CGRP. However, in chlorisondamine-pretreated, vasopressin-supported rats, the hypotensive effect of CGRP was restored. CGRP produced a significant increase in heart rate in intact (16 ± 2 beats/min from 419 ± 12) and vagotomised, propranolol-pretreated (41 ± 3 from 356 ± 8 beats/min) rats. In pithed rats, CGRP slightly inhibited the pressor effects to cirazoline and markedly those to angiotensine II and B-HT 920. The doses of these agonists required to increase by 40 mmHg blood pressure were, respectively 2, 5 and 8-fold greater in CGRP than saline-pretreated rats.

These results indicate that the decrease in blood pressure produced by CGRP is the hemodynamic consequence of a fall in total peripheral vascular resistance as well as cardiac output. The latter effect is probably due to a reduction in venous return (due to venodilatation) rather than myocardial depression. Pharmacologically, the hypotension and the rise in heart rate are likely to be mediated by specific receptors for CGRP. Finally, the preferential antagonism by CGRP of the responses to angiotensine II and BH-T 920 suggests that the vasorelaxant effects of CGRP utilize pathways common to the contractile mechanisms shared by the latter vasoconstrictors.

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MECHANISM FOR HUMAN α-CALCITONIN GENE-RELATED PEPTIDE TACHYPHYLAXIS IN RAT ISOLATED RIGHT ATRIUM

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The calcitonin gene-related peptide (CGRP) binds to specific sites in rat atria, stimulates the rate of contraction and activates adenylate cyclase to increase the formation of cyclic AMP (Sigrist *et al.*, 1986). Isoprenaline, which acts via β-adrenoceptors and increases cAMP, also increases atrial rate and this is antagonised by propranolol whereas that of CGRP is not (Marshall *et al.*, 1986). Tachyphylaxis occurs to the positive chronotropic effect of CGRP within 20-30 minutes (Franco-Cereceda & Lundberg, 1985). The aim of the present experiments was to investigate further the CGRP tachyphylaxis in rat atria by comparison of the peptide with isoprenaline and forskolin.

The right atrium of male Sprague-Dawley rats (300-430g) was set up in Krebs solution at 34°C. Human α-CGRP (3×10^{-9} - 10^{-7}M) rat α-CGRP (10^{-9} - 3×10^{-8}M) and isoprenaline (10^{-9} - 3×10^{-8}M) were given cumulatively with doses at 2 min intervals. After one of these drugs a tissue received a 30 minute period either as a control or in the presence of human α-CGRP 3×10^{-7}M or isobutylmethylxanthine (IBMX) 5×10^{-7}M. Subsequently the dose response curve to CGRP or isoprenaline was repeated. In some experiments forskolin was added cumulatively with doses at 15 min intervals.

Dose related increases of up to 170 b. min^{-1} were obtained with isoprenaline and between 70-100 b.min^{-1} with human α-CGRP, rat α-CGRP and forskolin. Similar dose-response curves were obtained after a 30 min resting period. IBMX 5×10^{-7}M did not alter the basal rate although higher concentrations had a positive chronotropic effect. After incubation with IBMX the dose-response curves for human α-CGRP and isoprenaline were both shifted 3-fold to the left. When human α-CGRP 3×10^{-7}M was added for 30 min a peak increase in rate of over 100 b.min^{-1} was reached in about 2 min and then declined for the next 20 min. However it remained above the original basal rate after 30 min (e.g. basal 212±8 b.min^{-1}, mean ± s.e.mean before and 247±11 b.min^{-1}, after 30 min CGRP). At this time there was no positive chronotropic effect to the cumulative doses of human α-CGRP and rat α-CGRP. On the other hand responses to isoprenaline and forskolin were not significantly reduced.

The potentiation by the phosphodiesterase inhibitor IBMX of the positive chronotropic effect of human α-CGRP and isoprenaline is consistent with the reported involvement of cyclic AMP. However cross-tachyphylaxis only developed between human α-CGRP and rat α-CGRP but not with isoprenaline or forskolin suggesting unchanged β-adrenoceptors, guanine nucleotide binding protein and adenylate cyclase. Therefore a change at the level of the CGRP receptors rather than in effector mechanisms might underlie tachyphylaxis to CGRP.

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HUMAN α-CALCITONIN GENE-RELATED PEPTIDE AND CAPSAICIN PRODUCE DIFFERING EFFECTS IN THE RAT MESENTERIC VASCULATURE

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The calcitonin gene-related peptide (CGRP) can increase the rate of beating of rat isolated hearts and atria and is also a potent vasodilator (Marshall *et al.*, 1986*a*,*b*). Capsaicin can selectively stimulate certain sensory nerves and lead to the release of endogenous neuropeptides. Recently CGRP has been suggested to be responsible for the effect of capsaicin in the guinea pig atrium (Franco-Cereceda & Lundberg, 1985). The present experiments studied whether the effect of capsaicin in the mesenteric vasculature might be due to the release of CGRP.

The mesenteric vasculature was removed from male Sprague-Dawley rats (280-370g) and perfused at constant flow (5.8 $ml.min^{-1}$) with Krebs solution containing noradrenaline (10^{-5}M). Human α-CGRP (10^{-11} - 3×10^{-9} mol) and capsaicin (10^{-10} - 10^{-7} mol) were compared with acetylcholine (10^{-11} - 3×10^{-8} mol) and sodium nitroprusside (10^{-9} - 10^{-6} mol) as vasodilators.

All compounds evoked a dose-dependent fall in perfusion pressure unlike the alcohol vehicle (used for capsaicin) which was ineffective. In some preparations the endothelium was 'removed' with sodium deoxycholate which abolished the vasodilator effect of acetylcholine without significantly altering that of sodium nitroprusside, human α-CGRP or capsaicin. Secondly, in some experiments methylene blue (10^{-5}M) was added to the Krebs solution. This procedure significantly shifted the acetylcholine dose-response curve to the right (3-10 times), that of human α-CGRP to the left (3-10 times), without altering the effect of sodium nitroprusside or capsaicin. Thirdly magnesium ions were omitted from the perfusing Krebs solution. This reduced the effects of all vasodilators although to a different extent. For example, while the shift for human α-CGRP was 3-10 fold that for the capsaicin dose-vasodilatation curve was moved between 10 and 100 fold to the right.

The comparison of human α-CGRPP and capsaicin suggests both are endothelial independent in their action as vasodilators. However the quantitative difference in response to omission of Mg^{2+} suggests some difference in action. Finally, a qualitative difference between the drugs was evident as methylene blue potentiated the effect of human α-CGRP without affecting the capsaicin vasodilatation. One explanation for the effect of lack of Mg^{2+} might be an altered potency of capsaicin to release endogenous peptide. However this does not explain the difference seen with methylene blue. In conclusion the results are consistent with the hypothesis that capsaicin vasodilatation is not due solely to the release of CGRP.

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THE EFFECTS OF NEUROPEPTIDE Y (NPY) ON ISOLATED PREPARATION OF RAT RIGHT ATRIUM

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Neuropeptide Y (NPY) coexists with noradrenaline in rat sympathetic cardiac nerves and the atria are richly supplied with NPY containing nerve fibres (Gu et al., 1984). The functional role of NPY, present in cardiac nerves is obscure. NPY has been shown to inhibit the electrical field stimulation (EFS)-evoked noradrenaline (NA) release in the isolated guinea-pig right atrium (Franco-cereceda et al., 1985). It has also been suggested that NPY released from sympathetic nerves inhibits cardiac vagal effects by an action on postganglionic nerve terminals (Potter, 1987). In the present investigation, we have examined the effects of exogenous NPY on the amplitude and the rate of the spontaneous beats of the right atrium of rat and on the positive inotropic and chronotropic effects of NA. Furthermore we have tried to assess indirectly the effects of NPY on acetylcholine (ACh) and NA release by observing its effects on the EFS-evoked +ve inotropic effect of the atrium.

Adult male Wistar rats (200-300 g) were killed by decapitation; the right atrium excised and set up in a 2 ml organ bath containing Tyrode solution aerated with a mixture of 95% O_2 + 5% CO_2 at 30°C, in order to record the amplitude and rate of spontaneous beats. Concentration-response relationships to NA (10^{-11}-10^{-5}M) (n=5) and responses to EFS (0.1 ms pulse duration, 2-64 Hz frequency, 2 s train duration, supramaximal voltage) (n=4) were obtained both in the absence and in the presence of NPY, 0.25 μM. The effect of NPY on responses to EFS was determined both in the absence and in the presence of atropine, 3 μM.

NPY had virtually no effect on the amplitude of the spontaneous beat, nor did it significantly modify the chronotropic and inotropic effects of NA. NPY produced a slight tachycardia (mean beats/min ± SEM: control 140 ± 8, NPY-treated 155 ± 7). EFS produced a +ve inotropic effect, the magnitude of which was proportional to the frequency of stimulation up to 16 Hz. Combined treatment with propranolol, 1 μM and phentolamine 1 μM completely abolished the EFS-evoked +ve inotropic effect, indicating its adrenergic origin. A 10 min incubation with NPY led to an enhancement of the +ve inotropic effect of EFS; the enhancement was most marked at 8 Hz (Mean ± SEM: 138 ± 14% of the control). Treatment with atropine 3 μM, produced an enhancement of the response to EFS at all frequencies studied (mean ± SEM: 283 ± 43% of the control at 8 Hz). Addition of NPY to atropine-treated preparation however produced an inhibition of EFS-evoked +ve inotropic effect (mean ± SEM: 70 ± 7% of the control at 8 Hz). In conclusion, the results indicate that in isolated rat right atrium exogenously applied NPY exerts its main effects prejunctionally by inhibiting the release of both ACh and NA. Of the two, its inhibitory effect on ACh release appears to be more pronounced.

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NEUROPEPTIDE Y (NPY) COUNTERACTS RELAXATION OF RAT MESENTERIC ARTERIOLES INDUCED BY DIFFERENT MECHANISMS

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The mechanism(s) by which neuropeptide Y (NPY) potentiates vascular smooth muscle contraction is still unknown. We recently reported that NPY produced a leftward shift of the concentration-response curves to agonists and to KCl on rat mesenteric arterioles, without any alteration of the maximal response (Andriantsitohaina & Stoclet, 1988). In the present study we investigated the ability of this neuropeptide to reverse relaxation induced by vasodilators which had different mechanisms of action.

Female Wistar rats (11-13 weeks old) were anaesthetized with 60 mg/kg (i.p.) sodium pentobarbitone. The third generation mesenteric arterioles were dissected out and mounted in organ bath in Krebs' solution under 200 mg tension (Mulvany & Halpern, 1977). The arterioles were precontracted with 3×10^{-6} M phenylephrine which elicited 80 % of the maximal contractile response. They were then relaxed by addition of either forskolin, sodium nitroprusside (agents which increase respectively the cyclic AMP and the cyclic GMP levels) or prazosin (an antagonist of α_1-adrenoceptors).

Forskolin and prazosin relaxed the arterioles to the baseline with mean EC_{50} values of $2.0 \pm 0.4 \times 10^{-7}$ M (n=6) and $1.3 \pm 0.2 \times 10^{-9}$ M (n=8) respectively. Sodium nitroprusside produced a maximal relaxation of $83 \pm 6\%$ with an EC_{50} of $6.0 \pm 2.0 \times 10^{-7}$ M (n=6). NPY was added in the presence of concentrations of the relaxant agents producing half maximal relaxation. NPY alone (10^{-10} to 10^{-7} M) had no contractile effect on the arterioles. Both in the absence or in the presence of the relaxing agents, it increased tension to the maximal response of the vessel. The EC_{50} values for NPY as a vasoconstrictor were respectively $4.0 \pm 1.5 \times 10^{-9}$ M (in the presence of forskolin, n=6), $2.5 \pm 0.6 \times 10^{-9}$ M (in the presence of sodium nitroprusside, n=6) and $4.6 \pm 1.2 \times 10^{-9}$ M (in the presence of prazosin, n=6). Whatever the relaxing agent used, the EC_{50} for NPY were not significantly different from each other as compared by two-way analysis of variance.

These results show that NPY is able to counteract the relaxing effects of drugs elevating cyclic AMP or cyclic GMP levels. Also it reverses those of the α_1-adrenoceptor antagonist prazosin, in conditions in which prazosin partially displaces phenylephrine. They therefore suggest that NPY enhances the efficiency of coupling between receptor occupancy and vascular smooth muscle contraction.

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EFFECTS OF K-CHANNEL OPENERS ON THE SPASMOGENIC COMPONENT OF CAFFEINE-INDUCED RESPONSES IN RABBIT AORTA

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We have previously shown that the supply of calcium required for the maintenance of noradrenaline (NA)-induced contractions in rabbit aorta can be reduced by raising the membrane potential with K-channel openers (Bray et al., 1988b). In the present study the effects of cromakalim, pinacidil and diazoxide (Bray et al., 1988a) on caffeine-induced contractions in rabbit aorta have been examined. In this tissue caffeine releases calcium from a cellular site to induce a phasic mechanical response (Karaki et al., 1977).

The thoracic aorta was removed from male half-lop rabbits (2.5-3.5Kg) and divided into segments approximately 1cm in length. Each segment was opened along its longitudinal axis and the endothelium carefully removed by rubbing the intimal surface with a moist cotton bud.

Using the lanthanum technique (Weir & Weston, 1988) a significant increase in the lanthanum resistant calcium fraction (LRCF) was measured after 5 min exposure to NA (300nM) and after 1 min exposure to caffeine (10mM) ($p < 0.05$, $n = 6$). After 5 and 10 min the caffeine-induced calcium fluxes were not significantly different from control levels. Both NA- and caffeine-induced increases in the LRCF were inhibited by preincubation with cromakalim (10μM) or pinacidil (10μM). In calcium-free physiological salt solution (PSS) NA (1μM) produced a phasic contraction, the magnitude of which was taken as an index of intracellular calcium release. Exposure to caffeine (10mM) in calcium free PSS followed by washing prior to exposure to NA, inhibited the NA-induced response by 31±5%, $n = 6$. In addition, both NA (300nM) and caffeine (10mM) produced a rapid increase in ^{86}Rb efflux from aortic strips and this was maintained during exposure to the test drug ($p < 0.05$, $n = 8$).

In separate experiments, sequential concentration-response curves were constructed for mechanical responses to caffeine (1-20mM). Caffeine produced a concentration-dependent increase in peak tension (EC50 value, approximately 2mM), which was inhibited by preincubation with cromakalim (10μM), pinacidil (10μM) and diazoxide (100μM) (caffeine EC50 values, 7.5mM, 9.4mM and 14mM, respectively, $n = 6$).

These data show that some degree of overlap exists between caffeine- and NA-sensitive calcium stores. Furthermore, the K-channel openers cromakalim, pinacidil and diazoxide inhibit the contractions associated with release of calcium from these sites. It is possible that this effect of K-channel openers may contribute to their antihypertensive properties.

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EFFECTS OF CROMAKALIM ON NORADRENALINE INDUCED CONTRACTIONS OF RABBIT AORTA

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Cromakalim can produce hyperpolarisation and myorelaxation in various smooth muscle preparations as a consequence of increased plasmalemmal K^+ conductance. On the basis of this ionic mechanism it was postulated that cromakalim would antagonise the effects of those agonists for which excitation-contraction coupling requires cell membrane depolarisation (Hamilton et al, 1986). The aim of this investigation was to evaluate this suggestion by studying the effects of cromakalim on the non-depolarising contraction evoked by noradrenaline in rabbit aortic strips (Bray et al, 1988; Clapham and Wilson, 1986).

Male New Zealand White rabbits (1.5 - 2.5 kg) were stunned and the thoracic aorta removed, placed in Hepes buffered physiological saline (pH 7.4, 37°C gassed with 100% O_2) cut into transverse strips and mounted for isometric tension recording. The contractile response to noradrenaline (0.01-0.10 µM) was studied in separate tissues previously exposed for 15 min. to La^{+++} (1mM). In separate experiments the effects of pre-treatment with nifedipine (1µM), cromakalim (1-10µM) or vehicle on the cumulative concentration response curves to noradrenaline (0.01 - 10µM) were determined. Furthermore concentration response curves to nifedipine (0.1 - 3µM) or cromakalim (1 - 10µM) were determined in preparations pre-contracted with noradrenaline (0.01 - 10µM) or ionomycin (0.003µM). In different preparations tissues pre-contracted with noradrenaline (0.1µM) were subsequently treated with cromakalim and K^+ (5 - 15mM) in the presence or absence of 1µM nifedipine.

The noradrenaline contraction was resolved into two phases, a rapid initial phase which was reduced by 30% by La^{+++} at 10µM noradrenaline and a maintained phase which was completely abolished by La^{+++} pre-treatment. Pre-treatment with nifedipine 1µM was without effect on the response curve to noradrenaline whereas cromakalim caused a concentration dependent shift to the right. Nifedipine produced no more than a 10% reduction in maximal contraction of the maintained phase of the noradrenaline response whereas cromakalim produced a concentration dependent relaxation with an IC_{50} of 2 ± 0.4 µM (s.e.m., n = 6). No differences were seen in the presence or absence of endothelium. Cromakalim was without effect on contraction induced by ionomycin. Increasing the bath concentration of K^+ from 5 to 15 mM causes a partial depolarisation which is insufficient to change the resting tension of rabbit aorta. Using noradrenaline to contract the tissue, it was found that elevating the bath concentration of K^+ by increments of 2-5mM antagonised in a step wise manner the relaxation induced by 1µM cromakalim, either in the presence or absence of nifedipine.

Thus cromakalim, but not nifedipine, is able to antagonise those components of the noradrenaline contraction which are blockable by La^{+++} pretreatment and hence dependent on Ca^{++} influx across the cell membrane. The influx of Ca^{++} is thus probably via receptor operated channels as would be anticipated of a non depolarising contractile agonist. This action of cromakalim is probably manifest at the level of the plasma membrane, given its inability to relax ionomycin induced contraction, and is secondary to membrane hyperpolarisation.

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COMPARISON OF K^+ CHANNEL ACTIVATORS WITH OTHER VASORELAXANTS AS INHIBITORS OF CAFFEINE-INDUCED ARTERIAL CONTRACTION

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Caffeine can contract isolated arteries by the release of Ca^{2+} from intra-cellular stores (Itoh et al., 1981). The K^+ channel activator, cromakalim (CRK), has been shown to inhibit caffeine-induced contraction of rabbit renal artery (Wilson, 1988) and this action was antagonised by the K^+ channel blocker, glibenclamide (GLIB). The present study examines whether this property of CRK is shared by two other purported K^+ channel activators, pinacidil (PIN) (Southerton & Weston, 1987) and RP49356 (Mondot et al., 1988). For comparison, the dihydropyridine, PY108-068, and the nitro-vasodilator, sodium nitroprusside (SNP), were also examined.

Isometric tension was recorded from isolated rings of rabbit renal artery. Since removal of Ca^{2+} did not influence the inhibitory activity of CRK upon caffeine contractions (Wilson, 1988), experiments were performed in normal (2.5 mM Ca^{2+}) Krebs solution. Tissues were contracted four times with caffeine 10 mM and washed for 30 min after each response. The first contraction served as the pre-treatment control. Subsequent contractions were obtained in the presence of either a) one of three concentrations of vasorelaxant drug, b) vasorelaxant plus GLIB 3 μM, c) GLIB 3 μM or d) vehicle. Vasorelaxants were tested at concentrations which were approx. equieffective for relaxation of a 30 mM KCl contraction.

Table 1. Mean (n=6-18) ± s.e.mean contraction (mg) to caffeine 10 mM.

	Pre-treatment	2nd contraction	3rd contraction	4th contraction
PINACIDIL	1167±126	[0.3] 933±126*	[3.0] 700±84**	[30] 480±101**
PIN+GLIB	1260±169	[0.3]1240±166	[3.0]1273±178	[30] 877±150*
RP49356	747±184	[0.3] 547±103	[3.0] 413±79**	[30] 293±52**
RP+GLIB	720±102	[0.3] 477±45**	[3.0] 613±52	[30] 427±72**
PY108-068	1040±116	[0.01] 940±191	[0.1]1000±206	[1.0]1250±248
SNP	760±141	[0.03] 880±201	[0.3] 760±198	[3.0] 597±147
GLIB control	1057±140	1093±131	1100±133	1103±134
Vehicle con.	898±124	902±113	901±136	862±120

Concentration of drug used shown [in μM]. Sig. diff. from pre-treatment: * $p<0.05$, ** $p<0.01$; Dunnett's test.

As with the vehicle and GLIB controls, PY108-068 and SNP had no significant effect upon caffeine contractions, whereas the K^+ channel activators, PIN and RP produced significant, concentration-related inhibition. The involvement of K^+ channels in the action of PIN is further supported by its antagonism by the specific K^+ channel blocker, GLIB. Antagonism of the RP effect is less clear: the effect of RP 3 μM was antagonised by GLIB yet that to RP 0.3 μM was not. The mechanism whereby K^+ channel activation can inhibit contractions resulting from the presumed release of intra-cellular Ca^{2+} by caffeine remains to be determined.

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COMPARISON OF CROMAKALIM AND THE ADENOSINE ANALOGUE PIA ON ATRIAL AND VENTRICULAR CARDIAC TISSUES

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In cardiac preparations, high concentrations of the K^+ channel agonist cromakalim (CROM) reduce transmembrane action potential duration which decreases the time available for Ca^{2+} influx and consequently exerts negative inotropy (Osterrieder, 1988). In atrial cardiac preparations, adenosine exerts a P1-receptor-mediated negative inotropy due to an increased potassium conductance (Belardinelli and Isenberg, 1983). If the P1-mediated negative inotropy is purely a result of increased potassium conductance, it may be postulated that CROM and a P1-receptor agonist will exhibit similar profiles of action in cardiac tissues. We therefore examined the responses to CROM and the P1-agonist N^6-(L-2-Phenylisopropyl)-adenosine (PIA) in naive and isoprenaline-(ISO) stimulated guinea-pig left atria (LA) and papillary muscles (PM).

Isolated paced LA and PM (2Hz, 5ms, threshold voltage + 50%) were set up in Krebs-bicarbonate solution at 37.5°C, gassed with 5% CO_2 in O_2. Isometric tension was recorded. In each preparation, a single cumulative concentration-response curve for PIA or CROM was constructed either in naive tissues or 2-3 mins after adding a sub-maximal dose of ISO (31nM). Reductions in tension were measured and statistical comparisons between IC_{50} and % reduction values made by Student's unpaired t-test as shown below.

	PIA		CROM	
	NAIVE	ISO	NAIVE	ISO
LA IC_{50} (μM)	0.42	0.29	35.6	68.4*
Max. Red. %	77.25	87.9*	80.1	65.8
PM IC_{50} (μM)	-	0.024†	12.6	39.4*
Max. Red. %	4.44	37.3*	94.9	97.2

Statistical difference from naive: *$p<0.05$ †EC_{50} value

CROM produced dose-dependent negative inotropic responses in PM and LA whether naive or ISO-prestimulated. However, the ISO-prestimulated preparations were less sensitive to CROM, the IC_{50} values being significantly greater than the corresponding values from naive tissues. PIA produced a dose-dependent negative inotropy in both naive and ISO-stimulated LA with no significant difference in the IC_{50} value. The maximum percentage reduction in ISO-stimulated LA was, however, significantly greater than in naive LA. PIA had very little effect upon naive PM but in prestimulated tissues it produced a marked reduction in tension.

The fact that ISO-prestimulation causes a significant increase in CROM IC_{50} suggests a functional antagonism between ISO and potassium channel openers. Because ISO-prestimulation failed to shift the PIA IC_{50} in LA, we suggest that in ISO-stimulated LA PIA is not acting simply as a potassium-channel opener but is exhibiting an additional antiadrenergic effect probably upon cAMP metabolism. The results obtained with PIA in PM are in agreement with the suggestion that a raised cAMP level is a prerequisite for P1-mediated negative inotropy in ventricular preparations (Bohm et al. 1985). The lack of effect of PIA in naive PM is explained by there being no functional link between PI-receptor and potassium channels in ventricular tissue (Nawrath et al. 1985). Supported by an SERC Case Award with SK & F Research Ltd. We also thank Beechams for the generous gift of Cromakalim.

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BLOOD PRESSURE LOWERING AND REGIONAL HAEMODYNAMIC EFFECTS OF SKF-85174 IN THE ANAESTHETISED RAT

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The selective DA_1-receptor agonist fenoldopam and the selective DA_2-receptor agonist quinpirole both lower BP in the rat by reducing total peripheral vascular resistance, but they have differing regional haemodynamic effects. The vasodilator effect of DA_1-receptor stimulation is most pronounced in the superior mesenteric and renal vascular beds, while the vasodilator effect of DA_2-stimulation is most marked in the hindquarters vascular bed (Van der Niepen *et al.*, 1988).

In the present study we investigated the effect of I.V. SKF-85174 (6-chloro-7,8-dihydroxy-1-(4-hydroxyphenyl)-3-(2-propen-1-yl)2,3,4,5-tetrahydro-1H-3-benzazepine), a mixed DA_1/DA_2-receptor agonist (Blumberg *et al.*, 1985) on BP, HR and regional haemodynamics in anaesthetized normotensive male Wistar rats. Superior mesenteric (SM), renal (R) and hindquarters (HQ) blood flow was measured with an electromagnetic flow meter (SKALAR MDL 1401) using perivascular flow sensors. Results were analyzed using Wilcoxon test and analysis of variance.

Increasing doses (1-100 $\mu g.kg^{-1}$, n = 6) produced dose- dependent reductions in BP and HR, which became statistically significant at the $10\mu g.kg^{-1}$ dose. The 30 and 100 $\mu g.kg^{-1}$ doses were used to study the regional haemodynamic effects of SKF-85174 before and after various pretreatments, in separate groups of 6 rats.

Table 1. Percent changes from baseline with SKF-85174 (n = 6, mean ± SEM, *p < 0.05).

DOSE			BLOOD FLOW		
($\mu.kg^{-1}$)	BP	HR	R	SM	HQ
30	-25.3±3.0*	-4.3±2.1*	+12.8±3.3*	+11.9±0.9*	+22.0±7.7*
100	-35.5±3.3*	-4.4±1.3*	+10.8±4.0*	+11.2±2.0*	+29.6±7.4*

SKF-85174 enhanced blood flow in the three vascular beds. This effect was most pronounced in the HQ vascular bed. The calculated regional vascular resistances were significantly and dose-dependently reduced in the three vascular beds. Pretreatment with the selective DA_2-receptor antagonist domperidone (100 $\mu g.kg^{-1}$ I.V., a dose shown to antagonize quinpirole; n = 6) antagonized the vasodilator effect in the HQ vascular bed, but did not affect the vasodilator response of the R and SM vascular beds. The selective DA_1-receptor antagonist SCH-23390 (100 $\mu g.kg^{-1}$ I.V., a dose known to antagonize fenoldopam; n = 6), antagonized the vasodilator effects in the R and SM vascular beds, but did not affect the vasodilator response of the HQ vascular bed. Each antagonist blocked only partially the hypotensive response. The non-selective dopamine receptor antagonist RS-sulpiride (1 $mg.kg^{-1}$ I.V.; n = 6) abolished the hypotensive response and antagonized the vasodilatory effects in three vascular beds. Ganglionic blockade with hexamethonium (20 $mg.kg^{-1}$ I.V.; n = 6) had no effect on the vasodilator response of the R and SM vascular beds, but abolished the vasodilatory response in the HQ vascular bed indicating that the latter response is dependent on an intact sympathetic nervous system; it antagonized only partially the BP lowering effect.

These results suggest that the BP lowering effect of SKF-85174 is due to stimulation of both DA_1- and DA_2-receptors, and is accompanied by a reduction of the vascular resistances in the three vascular beds studied. The vasodilator response of the SM and R vascular beds is mediated through stimulation of postsynaptic DA_1-receptors, while the vasodilator response of the HQ vascular bed is due to stimulation of neuronal DA_2-receptors, and is dependent on an intact sympathetic nervous system.

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THE EFFECTS OF DIFFERENTIALLY SELECTIVE PHOSPHODIESTERASE INHIBITORS ON ANAESTHETISED RAT HINDQUARTER VASCULAR RESISTANCE

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In potassium preconstricted rabbit isolated ear arteries the rank order of potency for vasodilatation of three differentially selective phosphodiesterase (PDE) inhibitors was isobutylmethylxanthine (IBMX) > zaprinast (ZAP) > SK&F 94120. Activators of adenylate and guanylate cyclase, forskolin and sodium nitroprusside (SNP) respectively had similar potencies in this preparation (Owen et al, 1988).

This investigation was undertaken to determine if a similar order of potency was found in a vascular bed, *in vivo*, and to determine the concomittant effects on heart rate and blood pressure. Male SK&F Wistar rats 200-270 g were anaesthetised with a mixture of Inactin (sodium 5-ethyl-5'- (1-methyl- propyl)-2-thio barbiturate) 90 mg/kg I.P. and Sagatal (sodium pentobarbitone) 30 mg/kg I.P. A carotid artery and jugular vein were cannulated for the measurement of blood pressure and the administration of drugs respectively. Heart rate was determined from the blood pressure recording. The trachea was cannulated and the animals breathed spontaneously. The hindquarters were perfused at constant pressure, according to the method of Taylor et al, (1981).

Bolus injection of IBMX, ZAP, SK&F 94120, forskolin and SNP all resulted in a transient increase in hindquarters flow indicating vasodilatation. One rat per dose of compound was used and the dose of compound resulting in a 50% increase in hindquarters flow (ED_{50}) was determined by straight line graphical interpolation between doses (n=5-8 for each ED_{50}). The ED_{50} doses and estimated ranges of accompanying changes in heart rate and blood pressure are shown below.

Compound	ED_{50} ($\mu moles.kg^{-1}$)	95% Confidence intervals	Tachycardia ($beats.min^{-1}$)	Hypotension (mmHg)
IBMX	1.73	1.03 - 2.90	39 - 82	-5 - -40
SK&F 94120	3.20	1.93 - 5.61	52 - 71	-17 - -50
ZAP	65.6	43.2 - 106.2	31 - 83	-9 - -35
FORSKOLIN	0.16	0.1 - 0.26	38 - 102	-4 - -44
SNP	0.07	0.05 - 0.14	-12 - 44	-19 - -31

All compounds were of short duration of action with full recovery from the vasodilatation occuring within 5 minutes. The rank order of potency for the compounds tested was SNP > forskolin > IBMX > SK&F 94120 > ZAP. Using parallel line "bioassay" and Fiellers Theorem (Finney 1987) the ED_{50} were statistically significantly different ($P<0.05$) except IBMX and SK&F 94120 which were similar. All compounds resulted in a concommitant fall in mean blood pressure and rise in heart rate. Further experiments are necessary to determine if the fall in blood pressure is due to vasodilation in other vascular beds, and if the tachycardia is due to a direct action of the drug or cardiovascular reflexes.

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CARDIOVASCULAR EFFECTS OF SELECTIVE CYCLIC GMP PHOSPHODIESTERASE (PDE) INHIBITORS

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M&B 22948, a selective cyclic GMP PDE inhibitor, causes an endothelium-dependent relaxation of vascular smooth muscle by blocking degradation of cyclic GMP whose accumulation is stimulated by endothelium-derived relaxing factor (Martin et al, 1986). Infusion of M&B 22948 into the rabbit ear vein causes pronounced vasodilatation (Owen et al, 1987). 1-Methyl-3-isobutyl-8-(methylamino)xanthine (MIMAX) is also a selective cyclic GMP PDE inhibitor which can induce endothelium-dependent relaxation of rat aorta associated with increased cyclic GMP, but not cyclic AMP (Jordan and Souness, 1988). The in vivo cardiovascular effects of M&B 22948 and MIMAX in rats have been studied to determine whether cyclic GMP PDE inhibitors can lower BP.

Male, Wistar rats (290-360g) were anaesthetised with sodium pentobarbital (60.0 mg/kg i.p.) and artificially ventilated with air (Bioscience Ideal Pump). BP was recorded from a cartoid artery using a physiological pressure transducer (Bell & Howell) and heart rate (HR), derived from the pulse using an instantaneous rate meter (Ormed), was recorded on a chart recorder (Ormed M19). Jugular veins were cannulated for i.v. administration, M&B 22948 and MIMAX being given as infusions over a period of 15 min.

M&B 22948 caused a progressive fall in BP, the maximum effect occurring 10-15 min after the start of the infusion (Table 1). MIMAX also induced a hypotensive response, the maximum effect occurring within 5 min (Table 1). Little recovery of BP was observed up to 30 min after infusion of M&B 22948, whereas recovery from the effects of MIMAX was more rapid. A marked tachycardia resulted from infusion of MIMAX, whereas M&B 22948 had less pronounced effects on HR.

Table 1. Intravenous cardiovascular effects of M&B 22948 and MIMAX in the anaesthetised rat.

	Dose Rate		MBP ± s.e.m. (mmHg)		HR ± s.e.m. (beats.min^{-1})	
	mg.kg^{-1}min^{-1}	n	Initial level	Δmax	Initial level	Δmax
M&B 22948	0.5	6	132±5	-12±4	362±12	- 2±13
	1.0	6	130±6	-28±7	392±12	-10±12
	2.0	4	131±2	-45±2	406±12	-26± 6
MIMAX	0.05	4	135±9	-22±3	389±21	+74±4
	0.1	5	151±2	-39±4	401±6	+85±9
	0.2	5	146±4	-47±2	396±10	+93±8

Results from experiments in rats pre-treated with chlorisondamine (0.5 mg/kg i.v.) or syrosingopine (5mg/kg i.p.) or in adrenalectomized and/or pithed rats were consistent with the hypotensive actions of M&B 22948 and MIMAX resulting from peripheral vasodilation.

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COMPARISON OF THE RELAXANT ACTIONS OF M&B 22948, MY-5445, VINPOCETINE AND 1-METHYL-3-ISOBUTYL-8-(METHYLAMINO)XANTHINE

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The selective cGMP phosphodiesterase (PDE) inhibitor, M&B 22948, induces a relaxation of vascular smooth muscle by inhibiting the breakdown of cGMP whose synthesis is stimulated by endothelium-derived relaxing factor (Martin et al, 1986). Only a few other selective inhibitors of cGMP hydrolysis have been reported. These include MY-5445, vinpocetine and 1-methyl-3-isobutyl-8-(methylamino)xanthine (MIMAX) (Hidaka et al, 1984, Lorenz & Wells, 1983). In the present study, we have compared the effects of these compounds on rat aorta contractility, cyclic nucleotide levels and PDE activities.

Experiments were conducted on aortas from male Wistar rats (250-300g). PDE activities were partially purified from the 100,000xg supernatant fraction of rat aorta by DEAE-trisacryl (IBF) chromatography and measured by a 2-step radioisotope method (Thompson et al, 1979) using substrate ([^{3}H] cGMP and [^{3}H] cAMP) concentrations of 1μM. The smooth muscle relaxant activities of PDE inhibitors were determined in endothelium-preserved aortic strips contracted with 5-hydroxytryptamine (10μM). Tension was measured isometrically using a FT03 Grass Force displacement transducer. Cyclic nucleotides were extracted with 5% trichloroacetic acid and quantified by RIA (NEN).

Three PDEs were separated from the cytosol of rat aorta; these, in eluting order, were designated cGMP PDE, Ca^{2+}PDE, both of which preferentially hydrolysed cGMP, and cAMP PDE. M&B 22948 and MIMAX competitively inhibited cGMP PDE (Kis = 0.1μM and 0.5μM, respectively) and Ca^{2+}PDE (Kis = 7.5μM and 0.4μM, respectively) while only weakly inhibiting cAMP PDE. MY-5445 selectively and competitively inhibited cGMP PDE (Ki = 1.2μM) and vinpocetine selectively and non-competitively inhibited Ca^{2+}PDE (Ki = 10μM). M&B 22948 and MIMAX (1-100μM) induced concentration-dependent increases in cGMP, but not cAMP. These effects were greatly reduced by endothelial denudation and by methylene blue (MB, 5μM). MY-5445 and vinpocetine had no effect on cGMP or cAMP at concentrations up to 100μM. M&B 22948 (EC_{50}=15μM; maximum relaxation, 100% at 100μM; n=4) and MIMAX (EC_{50}=2μM; maximum relaxation, 100% at 10μM; n=4) elicited a relaxation of rat aorta which was almost completely abolished by MB (5μM). MY-5445 (EC_{50}=4μM; maximum relaxation, 95% at 60μM; n=4) and vinpocetine (EC_{50}=1μM; maximum relaxation, 100% at 100μM; n=4) also relaxed aortic strips, MB causing rightward shifts in the concentration-response curves (10-fold and 100 fold, respectively).

The results are consistent with the smooth muscle relaxant actions of M&B 22948 and MIMAX, but not vinpocetine and MY-5445, being mediated through a mechanism involving inhibition of cGMP hydrolysis.

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INFLUENCE OF HEPARIN AND ORG 10172 ON TISSUE-TYPE PLASMINOGEN ACTIVATOR PLASMA ACTIVITY DURING REST AND EXERCISE

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The literature is not unequivocal regarding the effects of heparin and its low molecular weight subfractions on endogenous fibrinolysis (Markwardt & Klöcking, 1977). In earlier studies fibrinolysis was generally evaluated by assays measuring overall fibrinolytic activity. The more recent assay methods for tissue-type plasminogen activator (t-PA) antigen and activity allow more detailed study of these changes. We studied the effects of heparin (H) and Org 10172 (O), a new low molecular weight heparinoid, on plasma t-PA activity both at rest and during active release of t-PA from the endothelium by exercise (Ex).

The study was a double-blind, randomised, placebo-controlled, cross-over trial in six healthy male volunteers (aged 21-25, weight 70-75 kg). Treatments administered at weekly intervals were iv bolus injections of H (5000 IU), O (3250 anti-Xa units) and saline (S) given during supine rest and at another occasion just prior to exercise. Subjects remained supine during the first four hours of each treatment period (except during exercise). Exercise on a bicycle ergometer was individualised to obtain maximal heart rate in 12 minutes. Blood was taken in citrate tubes containing PgE_2 and theophylline to exclude the effects of platelets and collected on ice. Plasma was snap-frozen and kept at -40°C. Plasma t-PA activity was measured by an amidolytic assay (Verheijen J.H. et al, 1982) and the plasma profile of H and O by activated partial thromboplastin time and plasma anti-Xa and antithrombin activity. Differences between the AUC's (IU.min/ml) and C_{max} (IU/ml) of plasma t-PA activity (after subtraction of baseline t-PA activities) during rest and exercise were analysed by non-parametric analysis of variance and differences between individual treatments assessed by Wilcoxon.

H increased the AUC and C_{max} of plasma t-PA activity during exercise compared with O and S. Only the difference between H and O reached statistical significance. There were no effects of H and O on t-PA activity at rest. Anti-Xa activity on H and O treatment were similar.

	AUC t-PA act.	p-value*	C_{max} t-PA act.	p-value
H+Ex	77 (14-206)	0,03	7,2 (1,2-24.7)	0,04
O+Ex	51 (6-140)		4.7 (0,9-15,7)	
S+Ex	41 (4-73)		3.5 (0,6-5,9)	

Results show mean (range). *Friedman Two-way ANOVA.

We conclude that the increase in plasma t-PA activity provoked by exercise is higher after H treatment than after O treatment.Whether this is due to changes in clearance or in release of t-PA from endothelium remains to be investigated. The effects are possibly related to the high molecular weight subfractions of heparin-like drugs (Vinazzer H.et al, 1982).

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MEASUREMENT OF FAECAL PORPHYRINS BY HPLC, A SENSITIVE METHOD FOR MEASUREMENT OF UPPER GASTROINTESTINAL MICROBLEEDING

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Mucosal damage and increased blood loss from the gastrointestinal (GI) tract occurs commonly after the administration of non-steroidal antiinflammatory drugs (NSAID). Although the precise relationship between this and the occurrence of serious GI bleeding is not known, assesment of GI blood loss after different NSAID's might give an indication of their relative enterotoxicity. The detection of [^{51}Cr] labelled red cells in the faeces has been widely used for this but involves administration of considerable amounts of radioactivity. Other methods (gastroscopy, measurement of haemoglobin in gastric washings) can only be applied intermittently and cannot easily be used in long term studies.

In this cross-over study a method based upon the measurement of porphyrins in the faeces was evaluated in healthy volunteers. Six male subjects (aged 20-26) received two doses (2 and 6ml) of autologous heparinised blood or saline(SAL) in an open fashion on three weekly occasions without any dietary constraints. The order of administration was determined by two 3x3 Latin squares. Blood or saline was administered into the stomach through a 6F feeding tube (Vygon) over 30 minutes. Subjects remained supine during the infusion. All faeces produced in the five days following the infusion was collected and stored at -40^{0}.All assays were made blinded to the amount of blood administered. Porphyrins in homogenates of the full feacal mass (FFM) and a random sample(RS) of each collection were analyzed by high performance liquid chromatography (HPLC) with spectrofluorimetric detection.(Beukeveld et al., 1987). Haemoglobin was measured colorimetrically with tetramethyl-benzidine (Standefer & Vanderjagt,1977). Peak heights of coproporphyrin I and III(CI and CIII) pemptoporphyrin (P) and deuteroporphyrin (D) were measured for each sample. Peak heights were normalized by division by the CI peak (CI is not produced in the GI tract)and sum of the P and D peak heights was used. (P and D are produced by the same pathway in the GI tract).Results were analysed as the cumulative normalised peak heights over the five day collection period by multiway analysis of variance and expressed in arbitrary units. Differences between treatments were assessed by t-tests. Significance was taken as $P < 0.05$.The D/P cumulative peak height (but not CIII) was significantly increased by the dose of blood both in the FFH and RS. In the FFH peak height(95%CI) increased from 707 (356-1057) to 933 (582-1283) after 2ml and 1384 (1033-1734) after 6ml; in the RS from 391 (86-696) to 697 (391-1002) and 998 (693-1303) respectively. Peak heights after 6ml were different from SAL for both the FFH and the RS but not after 2ml. A positive benzidine test in the FFH was seen in 2/6 subjects after SAL,3/6 after 2ml and 6/6 after 6ml, demonstrating inadequate specificity.

We conclude that this method (applicable to both FFH and RS) probably has acceptable sensitivity for the detection of upper GI blood loss of as little as 6ml/5days and further evaluation of this method for testing the gastrotoxicity of NSAID's will be performed.

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CONTRACTILE AND RELAXANT ACTIVITY PRODUCED IN GUINEA-PIG TRACHEA BY VERATRIDINE AND ACONITINE

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The alkaloids veratridine (VT) and aconitine (AC) are reported to activate neurones causing transmitter release by an effect on a particular fraction of the voltage-dependent sodium channels (Catterall, 1980). In guinea pig tracheal strips these agents, at 30 μM, cause both marked and sustained contractile and relaxant effects (> 40 min).

The action of the two alkaloids was not identical since, in tissues treated with eserine (0.5 μM), AC caused marked relaxation followed by contraction, whereas VT caused predominantly contraction (n=6).

The *contraction* was abolished in the presence of tetrodotoxin (TTX), 3 μM (n=3), and was atropine-sensitive. Atropine, 1 μM, given before the alkaloids, caused total inhibition (n=3), and when given after, caused 100% reversal of both the VT- and AC-induced contractions, suggesting that the contractile response was entirely due to acetylcholine and that tachykinins and other spasmogens were not involved.

The *relaxation* produced by both agents was not affected by propranolol, 1 μM (n=3), or the cyclooxygenase inhibitors, indomethacin, 3 μM (n=3), and aspirin, 30 μM (n=3), indicating that neither sympathetic neurone transmitters acting at β-adrenoceptors nor relaxant eicosanoids were involved. In three experiments pretreatment with guanethidine (10 μM), had little or no effect on either the VT- or the AC-induced relaxation, indicating that it was unlikely that the alkaloids were causing relaxation by an effect on relaxants co-released with noradrenaline. TTX, 3 μM (n=3), decreased both the rate and degree of relaxation, but did not abolish it; this is in contrast with its effect in totally abolishing the atropine-sensitive contraction produced by both VT and AC in the presence of eserine. Neither VT nor AC had any relaxant effect on the guinea pig lung parenchymal strip (n=3), a model of peripheral airway reactivity (Lulich *et al.*, 1976), which has little, if any, non-adrenergic non-cholinergic (NANC) innervation and virtually no receptors for the postulated NANC relaxant transmitters, vasoactive intestinal peptide and peptide histidine isoleucine (Barnes, 1988).

These results suggest that the alkaloids could be causing release of the *relaxant* NANC transmitter(s) and of acetylcholine, but not of the NANC *contractile* transmitter(s) or of sympathetic transmitter(s).

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INTERACTION OF CAFFEINE WITH 4β-PDBU IN SMOOTH MUSCLE OF RAT AORTA AND GUINEA-PIG LUNG STRIP

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Signal transduction in many cells involves phosphatidylinositol bisphosphate (PIP_2) hydrolysis with production of inositol trisphosphate (IP_3) and diacylglycerol (DAG), which act as second messengers to cause calcium mobilization and activation of protein kinase C (PKC) respectively (Nishizuka, 1984; Berridge and Irvine, 1984). Nishizuka (1984) proposed that many cell responses may involve synergism between the IP_3/calcium and DAG/PKC pathways. There is evidence that in smooth muscles, PKC-activation can result in contraction, as for example in rat aorta (Danthuluri and Deth, 1984) and guinea-pig parenchyma (Dale and Obianime, 1987). PKC-activators and agents which increase $[Ca^{2+}]_i$ synergise in causing contraction in lung strip (Dale and Obianime, 1987; Obianime et al., 1988)
Caffeine can cause contraction in smooth muscles by mobilising intracellular calcium (Leijten and van Breemen, 1984); however, it inhibits phosphodiesterase and can cause relaxation (Small et al., 1988). In the present study, we have examined the interaction between caffeine and the PKC-activator, 4β-PDBu, in both rat aortic rings and guinea-pig parenchymal strips.

Caffeine, (0.1μM - 50mM), caused a biphasic response (i.e contraction followed by relaxation) in the guinea-pig lung strips but in the rat aortic rings it caused a very small transient contraction. 4β-PDBu caused a relatively rapid contractile response and it was possible to obtain a cumulative concentration response curve (CCRC) to 4β-PDBu over the range 10nM-10μM.
Pretreatment with 1mM caffeine before the 4β-PDBu CCRC: In the *rat aorta*, this resulted in a leftward shift of the 4β-PDBu CCRC which was greater in endothelium-denuded rings than endothelium-containing rings, the shift in the ED_{50} being 0.4 and 1.0 log units respectively. In the *guinea-pig lung strip*, caffeine shifted the 4β-PDBu CCRC dextrally, the shift being 0.4 log units. However, an increase of the caffeine concentration to 10mM, caused a dextral shift of the 4β-PDBu CCRCs in both the rat aorta and guinea-pig lung preparations.
Pretreatment with 4β-PDBu, 30nM, before the caffeine CCRC: This potentiated the contractile phase of the caffeine response in both tissues thus shifting the corresponding CCRC's leftwards.
Caffeine effect on tissue precontracted with 4β-PDBu, 10μM: In the *guinea-pig lung strip*, caffeine, 0.1-2mM, caused a dose-dependent relaxation, the relaxation at 2mM being 71 ± 10% of the 4β-PDBu-induced contraction. In the *rat aorta*, caffeine, 10mM, caused relaxation of 0.8 ± 0.21gms.

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EFFECT OF RUBIDIUM ON RELAXANT AGENTS IN GUINEA-PIG TRACHEA

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In the guinea-pig bladder detrusor muscle, the potassium channels activated by cromakalim are unusual in that they are impermeable to Rb (Foster & Brading, 1987). In the present study, we have investigated the role of Rb-impermeable channels in the mechanism of action of cromakalim, salbutamol and verapamil in guinea-pig tracheal spiral strips.

In the first series of experiments, KH_2PO_4 (1.2mM) and KCl (4.8mM) were replaced in the Krebs solution by NaH_2PO_4 and RbCl in the same concentrations (Rb Krebs). In this medium, cromakalim was less potent and less effective at relaxing spontaneous or histamine-induced tone than in normal Krebs. Rb-Krebs had little or no effect on salbutamol-induced relaxations whereas verapamil was significantly more potent and induced greater relaxations (Table).

Table.

		Spontaneous tone *	Histamine(5uM)-induced tone *
Cromakalim	K	0.6(0.5-0.7); 0.90	3.0(1.9-4.9); 0.77
	Rb	2.2(0.9-5.0); 0.84	6.2(5.2-7.5); 0.23
Salbutamol	K	0.02(0.008-0.06); 0.99	0.05(0.03-0.08); 0.99
	Rb	0.04(0.02-0.07); 0.99	0.25(0.16-0.45); 0.87
Verapamil	K	NQ ; 0.20	5.4(3.1-9.7); 0.54
	Rb	1.5(0.6-4.0); 0.79	1.0(0.8-1.2); 0.85

* IC50 (μM); Intrinsic activity @ 10μM relative to isoprenaline 1mM.

In time-course experiments, cromakalim (10μM) induced reproducible transient maximum relaxations of spontaneous (76±8%, n=6) and histamine-induced tone (44±7%, n=6). The relaxations were maximal between 4-6 mins, were not significantly different from controls after 10 mins and were blocked by quinidine (10μM). Salbutamol (100nM) and verapamil (10μM) induced relaxations, on the other hand, were well maintained.

To investigate whether the effect of Rb was dependent upon it replacing intracellular K , tissues bathed in normal Krebs were challenged with RbCl 1, 3 or 5mM. In tissues with an established histamine-induced tone, RbCl caused a further concentration-dependent contraction (RbCl 5mM 19±3%, n=10) which was rapid in onset and plateaued within 10 mins. RbCl also caused a non-competitive concentration-dependent blockade of the cromakalim dose-response experiment which was similar to the effects observed in Rb-Krebs.

These results suggest that rubidium blocks a proportion of K channels present in guinea-pig trachea by a mechanism independent of intracellular K replacement (but see Foster,1988) and makes contraction more dependent on extracellular calcium. The transient effect induced by cromakalim in the presence of rubidium suggests either i) cromakalim has a second mechanism of action independent of K channels or, ii) opens at least two K channels. Blockade of the transient relaxations by quinidine supports the latter explanation.

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CROMAKALIM ACTIVATION OF POTASSIUM CHANNELS IN GUINEA-PIG TRACHEA:EFFECTS OF EXTRACELLULAR RUBIDIUM

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Studies on the K channel opening activity of cromakalim (BRL34915) have routinely used stimulated efflux of 86Rb as a convenient marker for K efflux. However, in the guinea-pig bladder the channel opened by cromakalim is impermeable to 86Rb (Foster and Brading, 1987), and whilst stimulated efflux of 86Rb from guinea-pig trachealis in response to cromakalim has been reported (Allen et al.,1986), the effect was small compared to that in vascular tissues. This led us to investigate further the Rb permeability of the cromakalim activated K channel in this tissue.

Sections of guinea-pig trachealis dissected free of cartilage were loaded with the appropriate isotope(s) by incubation in Krebs solution at 37°c. Efflux was followed by transferring tissues through a sequence of 17 washing samples of Krebs solution at 37°c, with a resident time in each wash of 3 min. When present, cromakalim (10uM) was added to tubes 10-13. Radioactivity in tissues and washing samples was measured by liquid scintillation counting (86Rb and dual label experiments) or -counting(42/43 K experiments). For dual label experiments samples were stored for 10 days to allow decay of 42/43K and recounted. The concentration of 86Rb in the labelling solution for dual label experiments was 50uM. Efflux was calculated as a rate coefficient (fractional loss of radioactivity from the tissue per min., expressed as a percentage) and effects of cromakalim were expressed as peak efflux in the presence of drug relative to the mean efflux in the 3 tubes immediately prior to drug addition.

In single isotope experiments, cromakalim caused a significant stimulation of both 86Rb- and 42/43K- efflux from trachealis, the latter being considerably larger (62 5% v 37 4% (n>20)). The stimulation of 42/43K efflux by cromakalim in dual label experiments was smaller (41 5%,n=6) than that in the single isotope studies whilst the 86Rb efflux response was similar (36 8%,n=6). Possible explanations for this are: i) Rb may modify the permeabilty of the cromakalim activated channel or ii) cromakalim opens two classes of K channel one of which is blocked by Rb. The contribution of Rb impermeable channels to the cromakalim induced 42/43K efflux from trachealis was studied in a K-free Krebs containing 4.8 mM Rb (Morris and Taylor, 1988). Under these conditions cromakalim caused no stimulation of 42/43K efflux, a result which was surprising in view of the 86Rb efflux data. The inhibitory action of extracellular Rb was immediate suggesting that it was not due to replacing of intracellular K. Extracellular Rb itself had no effect on basal 42/43K efflux. The stimulation of 86Rb efflux from guinea-pig trachealis by cromakalim was also inhibited by extracellular Rb.

Whether the effect of extracellular Rb is due to channel blockade or of some other aspect of the action of cromakalim is unclear. Data from in vitro tension studies (Morris and Taylor, 1988) would indicate that blockade is the more likely explanation. The relationship between Rb and the K channel(s) opened in guinea-pig trachealis by cromakalim is complex and requires further study.

I thank Mrs P.Newson for technical assistance and the MRC Cyclotron Unit for supplying the mixed 42K,43K isotope.

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A PRESYNAPTIC INHIBITORY EFFECT OF NEUROPEPTIDE Y (NPY) ON THE CHOLINERGIC MOTOR TRANSMISSION IN ISOLATED RAT URINARY BLADDER

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Postganglionic motor transmission in the urinary bladder of many mammalian species including rat is only partly cholinergic (Ambache & Zar, 1970; Burnstock et al., 1972; Downie & Dean, 1977). A rich presence of neuropeptide Y (NPY) containing nerves in the detrusor of the rat has been demonstrated by several research groups (Mattiasson et al., 1985; Nagata, et al., 1987; Iravani & Zar, 1988). The functional role of NPY in urinary bladder remains uncertain although there is a suggestion that it may serve as a non-cholinergic motor transmitter (Iravani & Zar, 1988). In this investigation we have studied the effects of exogenously applied NPY on the cholinergic transmission.

Male Wistar rats (200-300 g) were killed by decapitation and strips of detrusor 1-1.5 x 0.2 cm were suspended between parallel platinum electrodes in 1 ml organ bath containing Krebs-Henseleit solution and aerated with a mixture of 95% O_2 + 5% CO_2 at 37°C. The Krebs solution contained indomethacin 10 μM. The detrusor was contracted either indirectly by electrical field stimulation (pulse duration 0.1 ms, 1-64 Hz, and supramaximal voltage) using 10 second trains of pulses, or directly by 1 min exposure to acetylcholine 1 μM. The cholinergic transmission was isolated by blocking the non-cholinergic transmission through nifedipine 1 μM. It has been shown that the mechanical response to electrical field stimulation in the presence of nifedipine 1 μM is fully atropine-sensitive and therefore mediated solely through a cholinergic mechanism (Iravani et al., 1988).

Addition of NPY 0.5 μM did not affect the acetylcholine-evoked contractions both in nifedipine-treated and in nifedipine-untreated preparations. In the presence of nifedipine, electrical field stimulation produced a biphasic response; both phases were moderately inhibited almost to the same extent by NPY in the frequency range 2-8 Hz (mean ± SEM % inhibition at 8 Hz: 1st phase 31.4 ± 4.1; 2nd phase 34.2 ± 3.1) (n=6). At other frequencies used, the results were too variable to draw any definite conclusion.

The results may be interpreted to indicate that exogenously applied NPY can inhibit cholinergic transmission in rat urinary bladder by a presynaptic mechanism.

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NPY METABOLISM BY A MEMBRANE BOUND PROCESS IN RAT BUT NOT RABBIT KIDNEY

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In order to investigate whether metabolism of Neuropeptide Y (NPY) could account for the discrepancy between demonstrable pharmacological activity and an apparent absence of NPY binding sites in the rat kidney (Leys et al 1987), 125-I NPY (Amersham) or porcine NPY (pNPY,Sigma) was incubated with cell membrane preparations from rat or rabbit kidneys. Tissues were homogenised in 50mM TrisHCl/0.32 M sucrose/0.1mM PMSF/ 5mM EDTA, pH 7.4 and centrifuged at 16000g for 30 mins at 4°C. The pellet obtained by centrifuging the supernatant at 50000g for 1 h at 4°C was washed in 50 mM TrisHCl/5mM MgCl2 (buffer A), centrifuged and stored at -80°C. Protein estimation was by the Bio-Rad modified Bradford method. 10 fmoles 125-I NPY or 8 μg pNPY were incubated with membranes at a final protein content of 0.1 mg/ml in 0.5 ml buffer A. Incubations were terminated by the addition of 0.05 ml 8% trifluoroacetic acid (TFA) and immediate centrifugation at 50000g for 5 minutes at 4°C. The supernatants were analysed by reverse phase HPLC using 10-80% acetonitrile/ 0.1% TFA. The radioactivity of eluted fractions was counted or absorbance at 212 nm monitored. The percentages of recovered radioactivity from rat renal membrane supernatants that co-eluted with 125-I NPY are shown here:

Duration of incubation.	Temp.	%125-I NPY remaining(±S.E.)	n.
0 minutes.	23°C	94.7±0.9	8
10 minutes.	23°C	42.3±5.6	9
10 minutes.	37°C	29.0±8.8	4
30 minutes.	23°C	13.1±4.1	13
30 minutes.	4°C	64.9±16.3	5

Incubation at 4°C slowed 125-I NPY disappearance significantly ($P<0.01$, Mann Whitney test); at 37°C disappearance accelerated ($P<0.05$). Only one peak of free radioactivity, co-eluting with 125-I NPY was seen in the supernatants of rabbit renal cell membranes, even after incubation for 1 h at 37°C. Pre-heating rat membranes to 65°C for 10 minutes reduced the loss of 125-I NPY in 10 minutes at 23°C to 69.2±4.4% (n=5, $P<0.05$). The following peptidase inhibitors had no effect individually on the rate of 125-I NPY disappearance: phosphoramidon 1μM, 1,10 phenanthroline 1mM, PMSF 200μM, EDTA 5mM, bacitracin 0.1%, leupeptin 1 μM, pepstatin A 1μM or bestatin 10mM. A combination of all these inhibitors reduced 125-I NPY disappearance in 10 minutes at 23°C to 74.2±3.4% (n=3, $P<0.05$) but no smaller group of these agents has yet been shown to be effective. We also found that specific 125-I NPY binding sites were readily demonstrated by autoradiography on 10 μm sections and on filtered cell membranes from rabbit kidney, incubated for 30 mins at 23°C in buffer A, but were not seen in similar preparations of rat kidney, even when the above peptidase inhibitors were included. pNPY was metabolised by membranes from rat but not rabbit kidneys, and its disappearance was associated with the generation of several small peaks eluting between 10-30% acetonitrile. The $t_{\frac{1}{2}}$ for the disappearance of 125-I NPY was not significantly different from that for pNPY (9±1.4 mins, n=9 and 13.7±2.9 mins, n=2, respectively). We are not aware of any published data concerning NPY metabolism. NPY is degraded by a membrane bound process involving one or more enzymes which displays considerable resistance to conventional peptidase inhibitors. Tissue or species differences may account for some of the problems experienced by workers attempting to identify or characterise NPY receptors.

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THE EFFECT OF INHIBITORS OF ENDOPEPTIDASE 24-11 ON ANF BINDING IN RAT KIDNEY

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ANF is a substrate for the very widely distributed enzyme endopeptidase 24-11 (NEP) previously known as encephalinase. Studies of ANF receptor binding in kidney using quantitative autoradiography have localised binding sites predominantly to glomeruli and papilla with much less binding in the tubules. Since several functional studies have suggested a tubular site of action of ANF we postulated that enzymatic degradation of the rat ^{125}I-αANF used might be occurring in the tubules before equilibrium binding could be established. Whole kidney homogenate membrane preparations were used to investigate degradation of ^{125}I-αANF and the effect of such degradation on receptor binding studies.

Breakdown of αANF by kidney membranes has been demonstrated by HPLC. Protease inhibitors are commonly included in receptor binding studies but their efficacy in abolishing degradation of radiolabelled αANF has not been tested. In this study incubation of membranes and ^{125}I-αANF (10-40 pM) was performed at 0°C in the presence of a cocktail of protease inhibitors (bacitracin 1 μg/ml, bestatin 40 μg/ml, aprotinin 1.4 μg/ml, PMSF 100 μg/ml, leupeptin 0.5 μg/ml and pepstatin 0.5 μg/ml). Despite this, degradation of the radioligand was rapid with a $t^{\frac{1}{2}}$ of 8 minutes. Addition of 4 μM phosphoramidone, a metalloendopeptidase inhibitor (K_i of 2nm for NEP) almost completely abolished breakdown of ^{125}I-αANF. Unlabelled αANF 10^{-6}M or the ring-truncated analogue, cANF, did not inhibit degradation. Confirmation of the efficacy of NEP inhibition was obtained using a saturating concentration of DAla2Leu5 encephalin as a substrate for the NEP. The stable degradation product Tyr-DAla-Gly was measured by HPLC and UV detection. Phosphoramidone 4 μM inhibited production of Tyr-DAla-Gly by >99%. cANF 10^{-6}M had no effect on endopeptidase activity.

Receptor binding studies in kidney membrane preparations were performed on ice for 60 minutes and the above cocktail of protease inhibitors was included with or without phosphoramidone 4 μM. Displaceable binding was clearly shown in the presence of phosphoramidone but was often undetectable when the inhibitor was absent. (Figure 1) Despite the inhibition of NEP activity by phosphoramidone, ^{125}I-αANF binding studies in rat kidney using quantitative autoradiography did not reveal further tubular binding sites. The absence of significant tubular binding is therefore not explained on the grounds of enzymatic degradation of radiolabelled ligand.

We conclude that the breakdown of radiolabelled ratαANF by rat kidney membranes is rapid even at 0°C and is not inhibited by non-specific protease inhibitors but is inhibited by phosphoramidone. In the absence of a specific NEP inhibitor equilibrium binding conditions will not exist and this is reflected in the marked decrease in demonstrable displaceable binding. The ANF analogue, cANF does not inhibit enzymatic degradation of radiolabelled ratαANF by rat kidney membranes at a concentration of 10^{-6}M although internalisation of the receptor-ligand complex and subsequent ligand degradation cannot occur in membrane homogenate preparation.

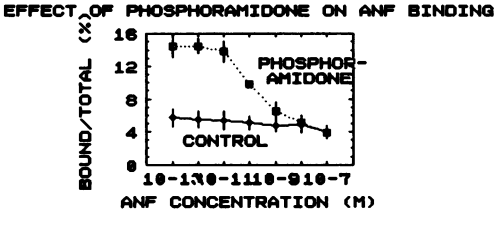


Figure 1

ROLE OF THE α_2-ADRENOCEPTORS IN THE REGULATION OF INSULIN SECRETION IN THE ANAESTHETISED DOG DURING GLUCOSE STIMULATION

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It is well established that stimulation of the sympathetic nerve supply to the pancreatic islets inhibits insulin secretion (Miller, 1981). Activation of α_2-adrenoceptors by endogenously released noradrenaline (NA) appears to be responsible for this inhibition, which is more pronounced for the glucose-stimulated insulin release than for basal insulin secretion (Skoglund et al., 1986).

The present experiments were designed to examine the effects of sympathetic nerve stimulation (SNS) using idazoxan (IDZ) as α_2-antagonist under conditions of glucose-stimulated insulin release.

Fasted (18 hr) dogs were anaesthetized with pentobarbital and artificially ventilated. Pancreatico-duodenal vein was cannulated with a T-shaped catheter allowing blood samples to be determined for immunoreactive insulin (IRI) and NA. The pancreatico-duodenal (P.D.) artery was perfused at a constant flow with heparinized blood withdrawn from a femoral artery. Nerves surrounding the P.D. artery were isolated for SNS (3 Hz, 2 ms, 20-30 V, 3 min). Systemic blood pressure and P.D. artery perfusion pressure (P.D.P.P.) were continuously recorded. An i.v. infusion of D-glucose was initiated at 1 g/kg/hr for 30 min and then maintained at 0.5 g/kg/hr for the duration of the experiments. Chlorisondamine + atropine (1 mg/kg i.v.) were given and some dogs received propranolol (1 mg/kg i.v.). Three baseline samples were drawn from the P.D. vein before SNS. Two samples were taken during SNS, then samples were drawn 2, 10, 20 min after SNS. Three responses to SNS were obtained, saline or IDZ being administered before the second and the third SNS.

SNS resulted in the expected increases in NA release and P.D.P.P., and in a decrease in IRI levels (# 50%). Idazoxan (0.3 and 1 mg/kg i.v.) did not antagonize this inhibitory effect at the level of basal insulin secretion (results from our laboratories). In contrast, the present experiments conducted during the infusion of glucose clearly demonstrate a significant and dose-dependent antagonism of IDZ on the inhibitory effect of SNS on insulin release.

	Control	+IDZ 0.3 mg/kg i.v.	+IDZ 1 mg/kg i.v.
Decrease in IRI levels	329 - 189 ±80 ±82	334 - 279 ±77 ±85	426 - 398 ±112 ±121
% Change	-49 ± 8	-24 ± 11*	-15 ± 9**

Values = means ± S.E.M. (n = 6), * $p < 0.05$ ** $p < 0.01$ when compared against control values

In propranolol treated dogs, IDZ was less effective but it still significantly blocked the effects of SNS on insulin secretion.

In conclusion, our results support the view that endogenous NA, acting through α_2-adrenoceptors is involved in the regulation of glucose-stimulated insulin release.

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P2-PURINERGIC-INDUCED IONISED CALCIUM ELEVATION IN THE HUMAN PROMYELOCYTIC CELL LINE HL60

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HL60 cells are a continuous line of human cells developed from peripheral blood leucocytes of a patient with acute promyelocytic leukemia which consist predominantly of promyelocytes. These cells can be transformed by different agents into cells having most of the characteristics of granulocytes, monocytes or macrophages (for review, see Collins, 1987). To characterize receptor operating calcium mobilization in this model, we have examined the effects of several hormones, transmitters or modulators on calcium mobilization in Indo-1-loaded HL60 cells.

Undifferentiated cells were washed twice with PBS, pH 7.4, suspended at 10^7 cells/ml, incubated for 30 min at 37°C with 5 μM Indo-1 acetomethylester. Loading was terminated by dilution with cold PBS followed by centrifugation. The cell pellet was resuspended to 10^6 cells/ml PBS containing 1 mM calcium. Monitoring of the intracellular free ionized calcium concentration ($[Ca^{2+}]i$) was essentially performed as described by Phaneuf et al, 1987. Briefly, aliquots were transfered to a 1-ml quartz cuvette equipped for temperature control and magnetic stirring. Fluorescence signals were monitored with excitation and emission wavelengths of 332 and 400 nm, respectively using a Kontron SFP 25 fluorimeter. The cells were subsequently lyzed by adding 50 μM digitonin to obtain the signal of the calcium-saturated dye (Fmax). Further addition of EGTA (10 mM, pH 8.2) allowed to record the Fmin signal from the Ca^{2+}-free form. The concentration of $[Ca^{2+}]i$ was calculated by the equation :
$[Ca^{2+}]i = Kd\ (F-Fmin/Fmax-F)$ with Kd = 250 nM according to Tsien et al, 1982.

Arginine vasopressin (0.1 μM), angiotensin II (0.1 μM), carbachol (100 μM), substance P (10 μM) and bradykinin (0.1 nM) were devoid of effect on $[Ca^{2+}]i$. ATP (10 μM) induced an increase in $[Ca^{2+}]i$ from the resting level of 150 nM to a peak reaching 800 nM within 4-5 s. The fluorescence then fell to basal values during the next 2 min. The ATP-induced increase in $[Ca^{2+}]i$ was dose-dependent, with a threshold of about 0.8 μM, a maximal effect at 500 μM and an EC50 value of 10 μM. This dose-response curve was not modified by the absence of Mg^{2+} in the external medium.

The non-hydrolyzable analogue of ATP, ATPγS, also produced a large increase in $[Ca^{2+}]i$ in HL60 cells ; this response practically attained the same intensity as that induced by ATP, at their respective maximal effects, but its EC50 was lower. ADP was also able to induce an increase in $[Ca^{2+}]i$ but its effect was not as potent that of ATP or ATPγS. In contrast, AMP, adenosine and 2-chloro-adenosine only slighly increased $[Ca^{2+}]i$. The ATP-induced increase in $[Ca^{2+}]i$ was inhibited in a dose-dependent fashion by the selective P2 antagonist 6-methoxy-2-phenylsatogen. 5 min preincubation of HL60 cells with protein kinase C activator tetradecanoyl phorbol acetate produced a dose-related inhibition of the ATP-induced elevation of $[Ca^{2+}]i$, whereas phorbol-13 monoacetate was devoid of action.

These results suggest that HL60 cells bear P2 purinergic receptors whose activation causes elevation of $[Ca^{2+}]i$ and that this process is modulated by activation of protein kinase C.

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ENHANCED FMLP-INDUCED PHOSPHOINOSITIDE TURNOVER AND G-PROTEIN EXPRESSION IN DIFFERENTIATED U937 MONOCYTES

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Human U937 cells express phosphoinositidase C (PIC) coupled cell surface receptors for several autacoids, including PAF and leukotrienes but not for the chemotactic peptide FMLP. However, differentiation of U937 cells to mature phagocytes is accompanied by the expression of FMLP receptors on the cell surface (Harris & Ralph, 1985). In common with other signal transduction systems agonist-stimulated PIC requires a guanine nucleotide binding (G) - protein to couple receptor to effector. Using both undifferentiated and differentiated U937 cells we have investigated FMLP induced inositol phosphate (InsP) production, and levels of immunoreactive Gi-2α, a putative PIC coupling G-protein.

U937 cells (10^6/ ml) were incubated for 72h in [medium 199, 1% v/v FCS] containing 2μCi[^{3}H]inositol/ ml and, when required, 1.25% v/v dimethyl sulphoxide to differentiate the cells. Labelled cells were washed and resuspended (5 x 10^6/ ml) in hepes buffered tyrodes (pH 7.4) containing 1mM $CaCl_2$, 10mM LiCl and 1mg BSA/ ml. Following agonist addition reactions were quenched in 6% $HClO_4$, samples neutralised, and [^{3}H]InsPs fractionated by Dowex anion exchange chromatography (Downes et al., 1986). For G-protein studies plasma membranes from differentiated and undifferentiated cells were solubilised and proteins separated by PAGE. Gi-2α was detected by Western blotting using antibody LE2 (1/200) raised against a synthetic decapeptide (Goldsmith et al., 1987).

FMLP up to 10μM failed to cause any stimulation of InsP production in undifferentiated U937 cells in contrast to PAF (1μM) or LTB_4 (1μM) which cause a several fold increase in InsPs. However, in differentiated cells FMLP (0.01- 1μM) caused a concentration dependent elevation in $InsP_1$, $InsP_2$ and $InsP_{3-4}$ with maximum stimulations after 5 min of three, four and two fold respectively. This enhancement of FMLP-induced PIC activation was accompanied by increased expression of Gi-2α. From 50μg of membrane protein immunoreactive Gi-2α was barely detectable in undifferentiated cells but clearly visible in differentiated cells.

These results indicate that differentiation of U937 cells enables FMLP to stimulate phosphoinositide turnover presumably as a consequence of increased FMLP receptor expression. The accompanying increase in Gi-2α expression may be necessary to couple these new receptors to PIC although such a possibility remains to to evaluated.

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INTERACTION OF CYTOTOXIC AGENTS WITH [^{3}H]PN 200-110 BINDING SITES OF RAT CEREBROCORTICAL AND SKELETAL MUSCLE MEMBRANES

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Multidrug drug resistance (mdr), in which tumour cells become resistant to a diverse range of cytotoxic agents, is associated with the expression of high levels of p170 glycoprotein (p170). Recently, the calcium channel blockers verapamil and diltiazem have been found to reverse mdr and to inhibit binding of cytotoxic drugs to p170 (Cornwell et al., 1987). In the present study we have examined if cytotoxic drugs demonstrate any cross-reactivity with the calcium channel.

Dihydropyridine (DHP) binding sites in rat cerebral cortex and skeletal muscle membranes (Gould et al., 1984) were labeled using [^{3}H]PN 200-110 (0.03 - 0.1 nM). Assays were for 2 hr at 25^{o}C in 50 mM Tris HCl (pH 7.4) and were terminated by filtration over polyethyleneimine pretreated glass fibre filters. NSB was defined using 1 micromolar nitrendipine.

Compound	Cortex		Skeletal Muscle	
	pIC50	% Max Inhibition	pIC50	% Max Inhibition
DAUNOMYCIN	5.5 (0.11)	94 (4.6)	5.9 (0.11)	90 (8.2)
DOXORUBICIN	4.5 (0.21)	93 (6.2)	5.1 (0.12)	52 (7.3)
VINBLASTINE	5.6 (0.07)	87 (6.2)	6.5 (0.08)	88 (4.7)
VINCRISTINE	5.0 (0.05)	40 (6.4)	5.8 (0.13)	87 (3.5)
LIDOFLAZINE	6.9 (0.04)	96 (5.5)	6.9 (0.08)	94 (4.8)

n=3. pIC50=-log of the concentration of drug producing 50% of the maximal inhibition of specific binding that could be achieved with that drug.

The cytotoxic agents shown in the Table inhibited [^{3}H]PN 200-110 binding. Vinblastine was the most potent compound and in skeletal muscle, was only 3 fold less active than lidoflazine. Several cytotoxic agents displaced less than 100% of specific binding suggesting a non-competitive interaction. Furthermore, verapmil, which can reverse the inhibition of binding produced by ligands such as prenylamine and tiapamil (Murphy et al., 1986), also reversed the inhibition of binding produced by 100 uM vinblastine (82 $\pm$ 3.5% to 50 $\pm$ 4.2%). This suggests that the cytotoxic drugs interact at a site distinct from the DHP binding site, possibly the same site at which prenylamine, lidoflazine and tiapamil bind.

The rank order of potency of the compounds paralled that described by Cornwell et al., (1987) for interaction with the p170 binding site in an mdr cell line. These data demonstrate a close similarity between the binding sites on the p170 glycoprotein and a binding site on the calcium channel. The site on the calcium channel does not appear to represent the DHP binding site, but rather some site which is capable of allosterically modulating the interaction of ligands with the DHP site. Whether this interaction results in inhibition of calcium entry into cells and contributes to the cardiac side effects associated with the use of cytotoxic agents requires further study.

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(R)-BOK-1 IS A SELECTIVE MUSCARINIC ANTAGONIST AT M1 RECEPTORS

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In a previous study it was demonstrated that the oxotremorine analogue (+)(R)-N-(4-pyrrolidino-2-butinyl)-5-methyl-2-pyrrolidon hydrogenoxalate;(R)-BOK-1, was 105 times more potent at ganglionic (M1) than at ileal ($M2_\beta$) muscarinic receptors (Amstutz et al. 1987). In the present study we provide a more detailed pharmacological profile of (R)-BOK-1 and compare its antagonistic activity with that of the selective M1 receptor antagonist pirenzepine. In in vitro experiments the rat ganglion was used as a model for M1 receptors, rat atria were used to determine $M2_\alpha$ activity and the guinea-pig ileum was used as a model for $M2_\beta$ receptors. In addition the antagonistic activity of (R)-BOK-1 and pirenzepine on both M1 (blood pressure) and $M2_\alpha$ (heart rate) receptors were investigated in the pithed rat model.

Isolated ganglia were superfused at 36°C with oxygenated (95% 02/5% CO2) Krebs solution (1ml/min). Muscarine-induced (pD2=7.4±0.1, n=6) depolarization was recorded differentially via two calomel electrodes, between the ganglion and its post ganglionic trunk. DC potentials were amplified by microvoltmeters. Rat left atria and strips of guinea-pig ileal longitudinal muscle were incubated under 0.5g Krebs solution at 37°C. The atria were paced at 3Hz. Muscarine-induced ileum contractions (pD2=7.3±0.2, n=6) negative inotropic responses in atria (pD2=7.3 ±0.2, n=6) were recorded with force-displacement transducers. PA2 values for pirenzepine and (R)-BOK-1 were calculated from Schild-plot analysis of data obtained using three concentrations of antagonist. Male Wistar rats were pithed according to Wilffert et al. 1983. McNeil-A-343 was used to stimulate ganglionic M1 and oxotremorine to activate cardiac $M2_\alpha$ muscarinic receptors. In the experiments with oxotremorine rats were pretreated with atenolol (10mg/kg i.v.) to exclude catechol-amine induced tachycardia. -Log ID50 values at a dose of 10^{-6}mol/kg Mc Neil-A-343 and 10^{-6}mol/kg oxotremorine were calculated by means of log probit analysis. The pA2 values of pirenzipine and (R)-BOK-1 for ganglionic (M1), cardiac ($M2_\alpha$) and ileal ($M2_\beta$) muscarinic receptors are in the table.

Table 1 Antagonism of the effects of muscarine by pirenzepine and (R)-BOK-1

	ganglion pA2	slope	n	atrium pA2	slope	n	ileum pA2	slope	n
pirenzepine	8.3±0.1	0.9±0.1	6	6.9±0.3	0.9±0.2	6	6.9±0.2	0.9±0.1	6
(R)-BOK-1	9.2±0.2	1.0±0.1	6	7.4±0.1	0.9±0.1	5	7.3±0.3	1.1±0.1	6

The values represent mean values ± s.e.mean.

Intravenous injection of McNeil (10^{-6}mol/kg) increased the blood pressure by 89 ±3mm Hg (n=15). Oxotremorine (10^{-6}mol/kg) reduced the heart rate by 70±2 (n=19). The -log ID50 values for pirenzepine and (R)-BOK-1 for blood pressure change were 7.04±0.06 (n=6) and 7.86±0.08 (n=6), respectively, whereas the -log ID50 values for reducing the heart rate of both compounds were 4.58±0.05, n=6 and 4.66±0.06 respectively. These data indicate that (R)-BOK-1 is a selective M1 receptor antagonist with a higher selectivity for M1 receptors over both $M2_\alpha$ and $M2_\beta$ receptors than pirenzepine in vitro. Similarly a more pronounced selectivity of (R)-BOK-1 compared to pirenzepine was also seen in vivo.

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CHANGES IN NIFEDIPINE SENSITIVITY IN GUINEA-PIG ILEUM FOLLOWING CHRONIC MORPHINE EXPOSURE

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Chronic opioid treatment has been shown to cause an increase in the binding sites for calcium channel blocking drugs in nerve cells (Ramkumar & El-Fakahany, 1984). Furthermore, they have been shown to reduce the naloxone precipitated morphine withdrawal syndrome in rats (Baeyens et al,1987) and attenuate morphine tolerance in mice (Contreras et al, 1988). These findings suggest that calcium channels could play an important role in the adaptive changes that occur following chronic opioid exposure. We have therefore investigated the effect of chronic morphine treatment on the effects of nifedipine in the guinea pig isolated ileum.

Longitudinal muscle strips with adherent myenteric plexus or whole segments of ileum were obtained from guinea pigs (Dunkin-Hartley, 300-500g) 3-4 days after subcutaneous implantation of morphine (5*50 mg) or placebo pellets. Tissues were mounted in morphine free Krebs' solution in tissue baths at 36°C and contractions recorded isometrically. When appropriate, tissues were field stimulated using parallel stainless steel electrodes (supramax V; 1mS; 0.1Hz). Acetylcholine (ACh) release from longitudinal muscle strips was measured using the method of Down & Szerb (1980) in which tissues are loaded with tritiated choline followed by washout in the presence of hemicholinium. Spontaneous and electrically evoked release of label was measured over 10 min periods simultaneous with recordings of isometric contractions.

In control and morphine withdrawn tissues nifedipine (10 μM) inhibited electrically induced contractions by 98.5 ± 1.0 & 89.5 ± 1.5 % ($P<0.01$) respectively. In common with other findings (Kaplita & Triggle, 1983), in control tissues the percentage increase in efflux of tritiated label elicited by electrical stimulation was unaffected by this concentration of nifedipine (318±56 cf 366±68). In contrast, in morphine withdrawn tissues nifedipine significantly attenuated the electrically induced increase in efflux of tritium label, reducing the percentage increase in efflux from 393±60 to 213±38.

In further experiments in control and morphine withdrawn segments of whole ileum, dose response curves to carbachol (CCh) were obtained in the absence and presence of nifedipine. 0.1 μM nifedipine reduced the maximum response to CCh to 84 ± 3 % in withdrawn tissues and to 59 ± 6 % in control tissues ($P < 0.01$). At 0.2 μM the respective changes in the maximum response to CCh were 43 ± 8 & 31 ± 2 ($P < 0.05$).

Thus in the morphine withdrawn ileum the process of ACh release is sensitised to nifedipine whilst the effects of the calcium channel antagonist against CCh induced contractions are diminished. The small but significant reduction in the effect of nifedipine against electrically induced twitches of the morphine dependent ileum is presumably the result of a combination of the above effects. The changes in nifedipine sensitivity that follow chronic morphine treatment may be explained by changes in the number and/or characteristics of voltage sensitive calcium channels in the ileum with the L type channel assuming some importance in the process of excitation secretion coupling.

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BENZODIAZEPINE RECEPTORS REGULATE GABA-ERGIC MODULATION OF 5-HYDROXY-TRYPTAMINE (5-HT) RELEASE FROM GUINEA-PIG INTESTINE

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Experiments from our laboratory showed that the vascularly perfused guinea-pig intestine is an useful in vitro model for studying regulatory mechanisms of the release of 5-HT from enterochromaffin cells, the major source of intestinal 5-HT (Schwörer et al., 1987). Recently, we described complex GABAergic mechanisms involved in the regulation of intestinal 5-HT release. Thus, $GABA_A$ receptor activation directly inhibits 5-HT release from the enterochromaffin cells, and in addition, stimulates the release of 5-HT indirectly via the release of acetylcholine. Endogenous GABA appears to activate both effects simultaneously (Racké et al., 1988). In the present study it was tested whether the effect of endogenous GABA is modulated via benzodiazepine receptors. Therefore, the effects of the benzodiazepine receptor agonist midazolam and of the antagonist flumazenil (Ro 15-1788) on the release of 5-HT were studied.

Male guinea-pigs were anaesthetized with pentobarbitone (40 mg/kg i.p.) and artificially respirated. An intestinal segment with its intact vascular supply was dissected out as described by Schwörer et al. (1987). The isolated segment, suspended in 200 ml Tyrode's solution, was perfused via the cranial mesenteric artery at a constant rate of 1 ml/min. 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined by HPLC with electrochemical detection (Schwörer et al., 1987). The perfusate of the first 90 min was discarded, then it was collected during 8 consecutive 5 min periods. Test substances were added to the perfusion medium after 100 min of perfusion.

The mean spontaneous outflow (determined between 90 and 100 min of perfusion) was 205 $\pm$ 14 pmol/g/5 min for 5-HT and 189 $\pm$ 22 pmol/g/5 min for 5-HIAA (n=43). In control experiments it remained constant during the following 30 min of perfusion. Midazolam (1 nM) increased the outflow of 5-HT and 5-HIAA by about 65 %, whereas at 100 nM midazolam caused a decrease of the release of 5-HT and 5-HIAA by about 35 %. The effect of 1 nM midazolam was prevented by 10 nM flumazenil, present already 40 min before the agonist. Flumazenil (10 nM) alone reduced the release of 5-HT by 35 % and this effect was abolished in the presence of the $GABA_A$ receptor antagonist bicuculline (50 µM). In the presence of tetrodotoxin (1 µM), flumazenil increased the release of 5-HT by about 50 %.

In some experiments the release of 5-HT from the isolated vascularly perfused stomach of the guinea-pig was studied in a similar way as described above for intestinal segments. Flumazenil (10 nM) decreased the release of 5-HT by 40 %.

In conclusion, benzodiazepine receptors may augment the GABAergic modulation of intestinal 5-HT release. Both, the $GABA_A$ receptors located on cholinergic neurones, mediating the indirect stimulation of 5-HT release, and the $GABA_A$ receptor located on the enterochromaffin cells, mediating the direct inhibition of 5-HT release, are regulated by benzodiazepine receptors. The effects of flumazenil on its own suggest that a functionally significant endogenous ligand for the benzodiazepine receptor, probably of non-neuronal origin, may be present in the guinea-pig stomach and intestine.

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AUTORADIOGRAPHIC DEMONSTRATION OF [^{3}H]LOPERAMIDE BINDING TO OPIOID RECEPTORS IN RAT AND HUMAN SMALL INTESTINE

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Loperamide has an antidiarrhoeal action which has been suggested to be via opioid receptors associated with gut motility (van Neuten et al., 1974). However, there is evidence to suggest that the action of loperamide is not confined to its effect on gastrointestinal smooth muscle, but may also involve modification of the intestinal transport of water and electrolytes (Sandhu et al., 1981, 1983). In the present study we have used in vitro autoradiography to localize [^{3}H]loperamide binding sites in both rat and human small intestine and its displacement by mu and delta selective ligands.

Jejunal and ileal tissue removed from rats following anaesthesia (60mg/kg sodium pentobarbitone IP) and human ileal tissue obtained from patients undergoing surgery for intestinal resection were used. These tissues were frozen on solid carbon dioxide, cut in a cryostat at about -15°c and thaw-mounted onto gelatinised microscope slides. Firstly, sections of rat intestine were incubated in increasing concentrations of [^{3}H]loperamide (specific activity 12.6Ci/mmol, Janssen Pharmaceuticals) (1.2 - 19.2nM). In further experiments rat and human tissue was incubated in 9.6nM [^{3}H]loperamide in the presence of 10μM unlabelled loperamide, naloxone, D-Ala^2-Mephe^4-Glyol^5(DAGO) and D-Pen^2, D-Pen^5 (DPDPE). After incubation sections were apposed to [^{3}H]Hyperfilm (Amersham) for 2 - 4 weeks, after which films were processed and autoradiographs examined.

[^{3}H]loperamide exhibited a concentration-dependent increase in the binding to the rat small intestine. Even at 1.2nM there was a significant binding at the tips of the villi. The marked displacement of [^{3}H]loperamide from tissues incubated in the presence of 10μM naloxone is further confirmation that loperamide binds to opioid receptor sites. This binding was also displaced by both DAGO and DPDPE suggesting that [^{3}H]loperamide binds to mu and delta sites respectively.

This study provides autoradiographic evidence that loperamide acts on both mu and delta opioid receptors in the small intestine. The antidiarrhoeal action of loperamide in man may therefore be associated with the modification of intestinal transport of water and electrolytes (delta), as well as its known effects on gut motility (mu).

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EFFECTS OF LACTULOSE AND ITS METABOLITES ON INTESTINAL FLUID TRANSFER

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Colonic bacterial fermentation of lactulose yields predominantly lactic and acetic acids. These have been shown to stimulate intestinal motility and could thus contribute to the laxative effect (Bennett & Eley, 1976). The possibility that lactulose fermentation products directly influence intestinal fluid transfer has now been investigated in everted sacs of rat small intestine following the method of Wilson and Wiseman (1954) using sacs (approx. 20cm long taken between 20 and 80 cm from the gastro-duodenal junction. The prepared sacs were placed in conical flasks containing 25ml unbuffered Krebs' solution to which test substances were added where appropriate. 95% O_2/5% CO_2 was bubbled through the bathing solutions for 1 minute. Then the flasks were sealed, placed on the oscillating platform (80osc/min) of a water bath (37°C) and incubated for 1 hr. Net fluid transfer was measured gravimetrically by weighing the sacs before and after incubation. The experiments were carried out in 3 groups, within which the treatments were randomized between sacs. The results are summarized in table 1 in which positive values indicate net fluid absorption and negative values indicate secretion. Net fluid absorption occurred in control sacs and in those incubated in solutions containing lactic acid, sodium lactate or hydrochloric acid, with no statistically significant differences (P>0.05) between these groups. In contrast, net fluid absorption was converted to secretion by lactulose (5mg/ml approximating the therapeutic concentration) or a mixture of its intermediate breakdown products galactose and fructose.

Table 1 Net fluid transfer (g/hr) across everted sacs of rat small intestine

	n	mean ± se mean	P (t test)
control	7	0.32 ± 0.07	
lactulose 5mg/ml	7	-0.31 ± 0.04	<0.01
lactulose 50mg/ml	7	-0.37 ± 0.05	<0.01
control	7	0.25 ± 0.07	
lactic acid 5mg/ml, pH 3.7	7	0.06 ± 0.02	>0.05
sodium lactate 5mg/ml, pH 7.4	7	0.10 ± 0.03	>0.05
HCl, pH 3.9	7	0.30 ± 0.04	>0.05
control	6	0.19 ± 0.01	
lactulose 5mg/ml	6	-1.20 ± 0.01	<0.001
galactose 2.65mg/ml & fructose 2.65mg/ml	6	-1.30 ± 0.02	<0.001

Thus intestinal fluid transfer, unlike motility, appears to be unaffected by the acid products of lactulose metabolism. However, the monosaccharide components mimic the secretory activity of the parent compound and, when released by colonic bacteria, may contribute to its laxative effect.

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INTERACTION OF DIHYDROALPRENOLOL AND CYANOPINDOLOL WITH ATYPICAL β-ADRENOCEPTORS IN GUINEA-PIG ILEUM

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Bond and Clarke (1988) have described atypical beta-adrenoceptors in guinea-pig ileum which function to inhibit longitudinal smooth muscle tension development. The atypical beta-adrenoceptors respond to isoprenaline and related amines, but are resistant to inhibition by propranolol (5 μM). Recently, we have reported that (±)-alprenolol exhibits a low affinity (pA_2=6.5) for the atypical beta-adrenoceptor (Blue et al, 1988). Thus, the present experiments were undertaken to identify a beta-adrenoceptor antagonist with a higher affinity for the atypical receptor. In this regard, (-)-dihydroalprenolol (DHA) and (±)-cyanopindolol (CYP) were studied as these compounds possess potential as radiochemical probes for the atypical beta-adrenoceptor.

The preparation used was the transmurally stimulated guinea-pig ileum from which the cholinergically-mediated 'twitch' response was recorded. Inhibitory responses to isoprenaline were measured under the same experimental conditions as described previously (Blue et al. 1988).

EDTA-washed membranes were prepared from ileal longitudinal muscle strips according to the method of Michel and Whiting (1988). Competition radioligand binding studies were performed with [^{3}H]DHA at 37°C in a Tris-Krebs buffer. DHA (10 μM) was used to define nonspecific binding.

DHA (0.01-10 μM) antagonized responses to isoprenaline, giving a biphasic Schild regression with a plateau between 0.03 and 0.3 μM DHA. A high affinity site and low affinity site were resolved. The interaction of DHA with the high affinity site (attributable to the $beta_1$-adrenoceptor) obeyed competitive kinetics (pA_2=8.7; slope=1.07; 95% CL: 0.87-1.17), whereas the interaction of DHA with the low affinity site did not (x intercept=6.4; slope=1.39; 95% CL:1.08-1.69). Kinetics inconsistent with competition may have resulted because of multiple pharmacological actions of DHA in ilea. For example, DHA itself inhibited the 'twitch' response. However, ligand binding studies with [^{3}H]DHA failed to identify the atypical adrenoceptor, but instead labelled a non-specific 'lipophilic' site (pIC_{50} DHA=7.4; nH=0.88) which appears similar to that reported by Dax and Partilla (1982) in rat adipocytes. CYP (0.001-1.0 μM) also gave a Schild regression which was the resultant of antagonism at $beta_1$-adrenoceptors and atypical beta-adrenoceptors. However, in the presence of propranolol (5 μM), to saturate $beta_1$-adrenoceptors, CYP gave a Schild regression with a slope of 1 (95% CL:0.8-1.2) and a pA_2 of 7.63. Thus, CYP is the most potent antagonist so far identified for the atypical-beta-adrenoceptor and, in the presence of propranolol, may serve as a useful probe for studies at the atypical receptor.

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5-HYDROXYTRYPTAMINE AND CHOLINERGIC MECHANISMS IN GUINEA-PIG ILEUM

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Gaddum and Picarelli (1957) showed that 5-hydroxytryptamine (5-HT) can activate enteric cholinergic neurons. The experiment was done in guinea-pig ileum pretreated with phenoxybenzamine (PBZ), and the 5-HT receptor involved was termed the M receptor. Evidence exists that the M receptor may be an entity distinct from the 5-HT_3 receptor (Buchheit et al., 1985). However, the mechanism of the interaction of 5-HT with ileal cholinergic neurons remains unclear. We now report on studies with 5-HT utilizing submaximal cholinergically-mediated 'twitch' responses to transmural electrical stimulation.

Longitudinal muscle strips from distal ileum were prepared from reserpine-treated (5 $mg.kg^{-1}$ for 15h) albino guinea-pigs (300-450g). The strips were set-up in Krebs solution and stimulated electrically (0.2 Hz; 1.5 ms pulse duration). The strips were allowed to equilibrate at supramaximal voltage for 30 min before exposure to PBZ (0.3 μM for 20 min). After wash-out of unbound PBZ and challenge with 300 nM 5-HT, the 'twitch' response was decreased (by voltage reduction) to about 50% of that operative at supramaximal voltage. The ability of 5-HT, and related amines, to enhance the 'twitch' response was determined by measuring integrated responses for 1 min following cumulative addition of agonists. Antagonists were preincubated with the muscle for 30 min before testing with agonists.

5-HT (0.3-100 nM) produced a concentration-dependent enhancement of the 'twitch' response (EC_{50}=3-10 nM), which was abolished by atropine (1 μM) and tetrodotoxin (TX, 1 μM). Base- line tension changes were mostly absent, but occurred on occasions at 30 or 100 nM 5-HT or both. In the presence of TX (1 μM), 5-HT failed to enhance the contractile response to exogenously applied acetylcholine. ICS 205-930 (Richardson et al, 1985; 0.3-3 μM) behaved as a competitive antagonist toward 5-HT (pA_2=6.4; slope=1.09; 95% CL: 0.5-1.6). In the presence or absence of ICS 205-930 (1 μM), metergoline (0.1 μM), and N-acetyl-5-hydroxytryptophyl- 5-hydroxytryptophan amide (Mawe et al, 1986; 10 and 30 μM) failed to antagonize 5-HT. 5-Methoxytryptamine (5-MT) and BRL 24924 (Sanger, 1987) acted as agonists, being about 10 and 30 times less potent than 5-HT, respectively. Single point pA_2 values for ICS 205-930 were 6.5 (n=5) and 6.4 (n=2) with 5-MT and BRL 24924, respectively. 2-Methyl-5-HT and 5-hydroxyindalpine were inactive as agonists, and 2-methyl-5-HT, at 10 μM, failed to antagonize 5-HT (n=3). 5-Carboxamidotryptamine (1-30 nM) caused inhibition of the 'twitch' response (which was susceptible to blockade by cyanopindolol, 1 μM, or metergoline, 0.1 μM), followed by an enhancement of the 'twitch' response (0.03 - 10 μM).

It is concluded that the 5-HT receptor under study is not a 5-HT_3 receptor, nor is it the receptor defined by Mawe et al (1986). It may also differ from the "M" receptor described by Buchheit et al (1985), as ICS 205-930 (1 μM) was inactive in their experiments and, in the same model, BRL 24924 acted as a silent competitive antagonist toward 5-HT (Sanger, 1987).

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EFFECT OF LUMINAL ETHANOL ON H+ AND HCO3 SECRETION BY GUINEA-PIG STOMACH IN VITRO

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Rutten and Ito (1986) reported that 10% mucosal ethanol in in vitro guinea-pig stomach inhibited H^+ output and disrupted the surface cell layer. These changes were reversible. It is unclear if these effects were due to inhibition of parietal cell activity, increased H^+ ion flux from or increased HCO^-_3 flux to the lumen. We have measured the effects of luminal ethanol on acid/base output in guinea-pig stomachs in vitro. Base output was measured after suppressing H^+ with SCH28080, 50 μM (Chiu et al, 1983).

Stomachs from fed males (250-350 g) were set up as described by Holton & Spencer (1976). H^+ output was determined by titration with 5 mM NaOH and HCO^-_3 with 5 mM HCl. The serosal solution was buffered with HCO^-_3/5% CO_2 whilst mucosal saline was unbuffered and gassed with 100% O_2. Acid/base output was expressed as μmole cm^{-2} h^{-1}, mean ± sem with n in brackets. Differences between means were assessed by t-test (paired or unpaired as appropriate) and a P of <0.05 taken as significant. Exposure to ethanol (9 vol mucosal saline + 1 vol 100% ethanol: ES) was for 30 min.

Stomachs secreted acid at 0.92 ± 0.08 (5) μmole cm^{-2} h^{-1}. Addition of ES reduced H^+ output to zero with some tissues showing a net alkalinisation. Following washout H^+ output recovered to 0.50 ± 0.08 at 60 min. Alkalinisation was 1.43 ± 0.27 (4) μmole cm^{-2} h^{-1} in SCH28080-treated tissues. ES caused an increase in alkalinisation which was sustained after washout, reaching 2.41 ± 0.3 after 60 min ($P < 0.05$). These experiments were repeated using Hepes/O_2 buffer. H^+ output was reduced by ES from 1.52 ± 0.19 (6) to 0.42 ± 0.08 ($P < 0.05$) and recovered at 60 min to 1.29 ± 0.21. Alkaline output was lower than with HCO^-_3/CO_2; 0.37 ± 0.16 (4), was not altered during ES and 60 min after washout was 0.27 ± 0.04 ($P > 0.05$). Subsequent return to HCO^-_3/CO_2 led to a rapid increase to 2.67 ± 0.55. In a third series in HCO^-_3/CO_2 buffer, ES was added to H^+ secreting stomachs for 30 mins and when washed out, SCH28080 added and subsequent alkalinisation recorded. H^+ output fell to zero during ES, and alkalinisation 60 min after washout was 0.71 ± 0.12 (5).

Luminal 10% ethanol caused a reversible reduction of H^+ output in both HCO^-_3 and Hepes buffered conditions. When acid output was suppressed, however, ethanol caused a sustained increase in luminal alkalinisation, the magnitude of which was not compatible with the recovery of H^+ output seen with tissues which had not received SCH28080. It would seem that acid secreting stomach is more resistant to luminal ethanol than stomachs where H^+ has been suppressed.

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THE EFFECT OF BW443C ON NON-CHOLINERGIC BRONCHOCONSTRICTION IN THE GUINEA-PIG *IN VITRO* AND *IN VIVO*

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Evidence suggests that non-cholinergic bronchoconstriction (NCB) may be due to the release of neuropeptides from sensory nerves (Lundberg *et al*, 1983). Opioids inhibit NCB in guinea-pig airways *in vitro* and *in vivo* (Frossard & Barnes, 1987; Belvisi *et al*, 1987) possibly by an action on sensory nerves to inhibit neuropeptide release. We have shown previously that discharges in vagal afferent Aδ- and C-fibres from the respiratory tract are inhibited by the peripherally acting opioid peptide H.Tyr.D-Arg.Gly.Phe(4-NO_2).Pro.NH_2 (BW443C) (Adcock *et al*, 1987; Adcock & Smith, 1987). We have now investigated the effects of BW443C on NCB in guinea-pig airways *in vitro* and *in vivo*.

Spirally cut main bronchi from male guinea-pigs (Dunkin-Hartley 300-400g) were mounted between 2 platinum electrodes in 20ml organ baths containing Krebs (pH 7.4, 37°C, gassed with 95%O_2/5%CO_2). Contractions, in the presence of atropine (1μM), were induced by electrical field stimulation (EFS: 40V, 0.5 ms, 32Hz for 10 s; Grass S44/S88 stimulators) and measured isometrically. Control EFS responses were obtained before drug treatment and repeated following 20 min incubation with drug. Responses are expressed as % inhibition of control response. In *in vivo* experiments, guinea-pigs (Dunkin-Hartley 300-500g) were anaesthetised with chloralose (100-150mg.kg^{-1} i.v.) following induction with 2.5% halothane and artificially ventilated. Pulmonary inflation pressure (PIP) and cardiovascular parameters were recorded. The peripheral ends of both cervical vagus nerves (sectioned) were placed on silver electrodes and electrically stimulated (10V, 5 ms for 30 s) at various frequencies. Results with morphine are included for comparison.

BW443C (1-100μM, n=3-6) and morphine (1-100μM, n=6) significantly ($p<0.05$) inhibited the NCB response to EFS in a concentration-related manner up to a maximum of 73 ± 6% and 57 ± 5% respectively. In both cases the inhibition was reversed by naloxone (10μM). *In vivo*, bilateral vagal stimulation at 1,3 and 6Hz (n=6) produced frequency-related bronchoconstrictor responses which increased PIP by 12 ± 1.9, 26.5 ± 3.8, 27.3 ± 2.4 cmH_2O respectively. After atropine (1mg.kg^{-1} i.v. bolus, n=6) these responses were reduced to 7.6 ± 1, 15.8 ± 1.8, 19.2 ± 1.8 cmH_2O. In the presence of atropine, both BW443C (100μg.kg^{-1}.min^{-1} i.v., n=5) and morphine (1mg.kg^{-1} i.v. bolus, n=6) caused a significant ($p<0.05$) inhibition of NCB. Maximum inhibition was observed at 3Hz with BW443C (81 ± 4%) and morphine (65 ± 13%). The inhibitory effects of BW443C and morphine were prevented by i.v. naloxone (100μg.kg^{-1}.min^{-1}; 1mg.kg^{-1} respectively).

BW443C, in similar fashion to morphine, inhibited NCB *in vitro* and *in vivo* consistent with its previously established inhibitory action on sensory nerves. The release of neuropeptides from sensory nerves may be involved in local axon reflexes in diseases of the airway such as asthma. BW443C, therefore may represent a novel agent with which to test this hypothesis.

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THE EFFECT OF BW443C AND CODEINE ON CAPSAICIN-INDUCED BRONCHOCONSTRICTION IN THE GUINEA-PIG

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Stimulation of C-fibre endings in the respiratory tract of some species by capsaicin, results in a variety of vagally mediated reflexes, including cough and bronchoconstriction (Coleridge & Coleridge, 1984). However, capsaicin-induced (i.v. or i.a.) bronchoconstriction in guinea-pigs does not involve a vagal reflex pathway but may be due to the localised release of neuropeptides from sensory neurones (Biggs & Goel 1985). We have previously shown that the opioid peptide H.Tyr.D-Arg.Gly.Phe(4-NO_2).Pro.NH_2 (BW443C) inhibits spontaneous and capsaicin-induced discharge in C-fibres arising within the respiratory tract (Adcock & Smith, 1987). We have now investigated whether bronchoconstriction induced in anaesthetised guinea-pigs, by capsaicin aerosol, involves a vagal reflex pathway, and the effect of BW443C and codeine on this response.

Male guinea-pigs (Dunkin-Hartley, 400-450g) were anaesthetised with chloralose 100-150mg kg^{-1} i.v. following induction with 2.5% halothane and artificially ventilated. Pulmonary inflation pressure (PIP) and cardiovascular parameters were recorded. Aerosols of capsaicin (0.9µg.ml^{-1}) were generated with an ultrasonic nebuliser (DeVilbiss). Each animal received a series of aerosols of 5, 10, 15, 20 breaths once only in the presence of saline, vagotomy or drug. Drugs were administered intravenously by infusion (except for atropine) at 0.1ml.min^{-1}, 10minutes prior to and during the capsaicin aerosols. BW443C, codeine and atropine were dissolved in saline; capsaicin in an ethanol, Tween 80 and saline mixture.

Capsaicin 5, 10, 15, 20 breaths produced a dose-related bronchoconstriction that was significantly ($p<0.05$) inhibited by either bilateral vagotomy (n=7; 59 ± 11% reduction of the response to 15 breaths capsaicin) or atropine (0.3mg.kg^{-1} bolus i.v; n=8; 63 ± 10% reduction of the response to 15 breaths capsaicin). BW443C (3, 30, 100µg.kg^{-1} min^{-1} i.v.; n=7/dose) infusion significantly ($p<0.05$) inhibited capsaicin-induced bronchoconstriction in a dose-related manner, (38 ± 24%, 76 ± 12%, and 74 ± 10%, respectively). Inhibition by BW443C (30µg.kg^{-1} min^{-1} i.v.) was prevented by the concomitant infusion of the peripherally acting opioid antagonist N-methyl nalorphine (100µg.kg^{-1} min^{-1} iv; n=6). In contrast to BW443C, codeine (100µg.kg^{-1} min^{-1} i.v. infusion; n=7) did not produce significant inhibition of capsaicin-induced bronchoconstriction.

Bronchoconstriction induced by capsaicin aerosol was mediated in part, by a vagal reflex pathway. BW443C, but not codeine, inhibited capsaicin-induced bronchoconstriction. This effect may be due to inhibition of the vagal reflex component by an action of BW443C on opioid receptors to reduce discharges in C-fibre afferents. However, a similar action on local axon reflexes involving the release of sensory neuropeptides cannot be ruled out. An agent such as BW443C may prove to be of use therapeutically, since both mechanisms may be involved in non-specific airway hyperreactivity that occurs in asthmatics.

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ANTITUSSIVE ACTIVITY ON NEBULIZED BW443C IN THE UNANAESTHETISED GUINEA-PIG

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It has been shown previously that BW443C a novel peripherally-acting opioid pentapeptide H.Tyr.D-Arg.Gly.Phe($4NO_2$).Pro.NH_2 has marked antitussive action by the subcutaneous, intravenous and intranasal routes in the guinea-pig (Adcock et al, 1987, 1988; Schneider & Smith, 1988). As BW443C was relatively inactive by the oral route, the effectiveness of exposing unanaesthetized guinea-pigs to nebulized aqueous solutions of BW443C in suppressing citric acid induced cough was investigated in order to determine its potential usefulness by the inhalation route.

Adult male Dunkin-Hartley strain guinea-pigs were exposed for 27.5 min in a 20L glass exposure chamber, to nebulized solutions of BW443C, or saline, generated by passing compressed air through a Collison inhaler connected via rubber tubing to the inlet of each chamber. Excess vapour passed out through a hole at the top of the chamber. At the end of the exposure period, the Collison inhaler was exchanged for one containing a solution of citric acid 30% w/v in distilled water, and nebulization of the citric acid began 7.5 min later. Coughs were monitored for up to 12.5 min using a microphone connected via a pre-amplifier to a cassette deck and loudspeakers. The mean number of coughs, standard errors, P values and IC_{50} values were determined using computer programs for analysis of variance and regression analysis.

In combined experiments, using a total of 82 animals, nebulized BW443C in a concentration of 30 mg ml^{-1} caused a 72% inhibition of the mean number of coughs induced by nebulized citric acid compared with the number in vehicle pre-treated controls ($P < 0.002$, Mann-Whitney U-test). In the animals exposed to an aerosol prepared from a solution of BW443C (10 mg ml^{-1}) there was a 26% reduction ($P = 0.05$), the approximate IC_{50} value being 18 mg ml^{-1}.

In similar experiments, codeine phosphate nebulized from solutions containing up to 60 mg base ml^{-1} also showed concentration-related antitussive action, the IC_{50} value also being approximately 18 mg base ml^{-1}. However, spontaneous defaecation over a 6h period was inhibited ($P<0.01$) by more than 80% following inhaled codeine (from a solution containing 60 mg base ml^{-1}) suggesting that giving codeine by inhalation to man would not confer any advantages over conventional practice.

These results therefore indicate that BW443C, which in the guinea-pig is relatively inactive orally, is highly effective as an antitussive by inhalation and is thus potentially useful by that route in man.

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EFFECT OF CROMAKALIM ON BRONCHOCONSTRICTION INDUCED BY STIMULATION OF THE VAGUS NERVE IN ISOLATED GUINEA-PIG TRACHEA

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Cromakalim (BRL 34915, (±)6-cyano-3,4-dihydro-2,2dimethyl-trans-4-(2-oxo-1-pyrrolidyl)-2H-benzo[b]pyran-3-ol), a novel smooth muscle relaxant, suppresses spontaneous tone in guinea-pig trachea but has little effect on bronchoconstriction induced by either acetylcholine (ACh) or histamine (Allen et al., 1986). The aim of this study was to examine the effect of cromakalim on bronchoconstriction induced by stimulation of the vagus nerve in isolated guinea-pig trachea. The effects of cromakalim on bronchoconstriction evoked by field stimulation and by applied ACh were studied also for comparison.

The isolated guinea-pig trachea with attendant right vagus nerve and recurrent laryngeal branch was mounted horizontally in an organ bath containing Krebs solution (37°C) and set up for recording intraluminal pressure (ILP) as described previously (Blackman & McCaig, 1983). After a 30 min equilibration period, frequency-response curves to either vagal or field stimulation were obtained before and during exposure to increasing concentrations of cromakalim (10^{-7} - 10^{-5}M). In separate experiments concentration-effect curves to ACh were obtained before and during exposure to cromakalim, 10^{-5}M.

Cromakalim reduced resting ILP and attenuated markedly the increases in ILP elicited by vagal stimulation (which reflect bronchoconstriction) at all frequencies tested (1-50Hz), in a concentration-dependent manner. Responses to vagal stimulation at 20Hz (for 5s), for example, were reduced by 50±15% (mean±s.e.mean,n=3) and 86±5%(n=5) in the presence of cromakalim, 10^{-7}M and 10^{-5}M, respectively. By contrast, increases in ILP evoked by field stimulation were completely unaffected by cromakalim, 10^{-7}-10^{-5}M. Similarly, the concentration-effect curve for applied ACh was unaltered in the presence of cromakalim, 10^{-5}M.

These results confirm that cromakalim reduced spontaneous tone in guinea-pig trachea (Allen et al., 1986). Cromakalim also attenuated markedly responses to stimulation of the vagus nerve, an effect which was concentration-dependent and apparent even at low concentrations of cromakalim. These results are in good agreement with those of Hall and Maclagan (1988). The fact that the response to applied ACh was unaltered by cromakalim indicates that attenuation of vagal responses occurs mainly through presynaptic mechanisms. The fact that responses to field stimulation, which involves direct excitation of the nerve terminals, was unaffected also suggests that cromakalim does not affect ACh release from cholinergic nerve terminals. In this preparation the vagus nerve is stimulated preganglionically therefore cromakalim could be interfering with ganglionic transmission or with the conduction of impulses in the post-ganglionic parasympathetic nerve fibres. Cromakalim may be of benefit in bronchoconstriction where there is a significant reflex component involving vagal efferent fibres.

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LITHIUM TRANSPORT IN HUMAN ILEUM

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Lithium, administered orally, is effective as a prophylactic agent in the control of recurrent affective psychosis: understanding its mode of absorption is important in formulation design (Phillips and Birch, 1988). Absorption of lithium from rodent small intestine has been shown to be passive, probably occurring via a paracellular route, and this is supported by the finding of low tissue uptake of lithium in guinea pig isolated jejunal mucosa (Birch et al, 1985a, 1985b; Phillips et al, 1988). We now report investigations of lithium transport and tissue uptake using a novel technique utilising human ileum.

The method for preparation of isolated guinea pig jejunal mucosa (Lauterbach, 1977) was modified for use with human small intestine (Kmiot et al, 1988). Samples of normal human distal ileum, obtained during total colectomy operations, were dissected to free the absorptive mucosa from the underlying muscle and connective tissue layers. The isolated intestinal mucosal preparations were mounted on nylon gauze and placed in flux chambers as previously described (Birch et al, 1985a, 1985b; Kmiot et al, 1988). Krebs-Hanseleit bicarbonate buffer (pH 7.4) was added to both sides of the flux chamber and gassed with oxygen. Lithium replaced sodium in the mucosal-side buffer over a concentration range from 5 to 50mM, and ^{3}H polyethylene glycol 900 (PEG-900) was also present as a permeability marker. Transport and tissue uptake of lithium were measured after incubation at 37°C for 45 minutes.

Transepithelial lithium transport correlated with lithium concentration on the mucosal side ($r = +0.884$, $p < 0.001$). There was no evidence of saturation up to a mucosal lithium concentration of 50mM. Apparent tissue uptake of lithium was also highly correlated with the mucosal lithium concentration ($r = +0.962$, $p < 0.001$). Calculation of tissue lithium associated with the extracellular space showed that cell uptake of lithium was negligable.

The metabolic inhibitors 2,4 dinitrophenol (0.25mM) and sodium fluoride (5.0mM) significantly reduced transepithelial permeation of 25mM lithium ($p < 0.001$), but had no effect on tissue lithium concentration. Permeation of the paracellular marker PEG-900 was also significantly reduced by these inhibitors ($p < 0.025$) which indicates that maintenance of the integrity of the zonulae occludentes may be energy dependent.

We conclude from these preliminary data that lithium transport in human ileum occurs by a similar process to that in rodent small intestine. Low intracellular lithium concentrations and a dependence on the integrity of the zonulae occludentes support the view that the paracellular pathway is the primary route for passive transepithelial lithium transport in human ileum.

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CROMAKALIM DOES NOT INTERACT DIRECTLY WITH PROTEIN KINASE C

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Cromakalim stimulates rubidium efflux from a range of vascular smooth muscles (Weir and Weston,1986; Coldwell and Howlett,1987) but binding studies (Coldwell and Howlett,1987) and the time course of the response (Coldwell and Howlett,1988) would suggest that this may not be a direct interaction of the drug with the channel. A previous study (Coldwell,1988), examining the possibility of protein kinase C (PKC) being an intermediate step in channel opening, demonstrated antagonism between the effects of cromakalim and phorbol dibutyrate (PDBu), an activator of PKC (Castagna et al,1982). This report examines further this antagonism by investigating the effects of cromakalim on both [^{3}H]-PDBu binding to rat cortex membranes and the activity of a crude preparation of PKC from rat brain.

[^{3}H]-PDBu binding was carried out as described by Worley et al (1986). Phorbol 12-myristate,13-acetate (TPA) displaced the radioligand in a concentration-related manner, with an IC_{50} of approximately 100 nM (n=4). Phorbol 12-monoacetate (which does not activate PKC - Castagna et al, 1982) and cromakalim, at concentrations up to 10 µM, had no effect on [^{3}H]-PDBu binding. Cromakalim (10 µM) was unable to affect the displacement of the ligand by TPA, indicating that it does not modify binding by an allosteric mechanism similar to that described for diltiazem at the dihydropyridine site (Ferry and Glossmann, 1982).

PKC was purified partially (using the first two steps only) and assayed according to the method of Kitano et al (1986), with minor variations. Thus Histone IIIS (Sigma) was used in place of Histone I and phospholipid micelles were prepared in 1% Triton X rather than with diolein. After washing with NaCl-containing buffer had commenced, maximum kinase activity was observed in the fraction eluting between 133-140 ml. Approximately 70-75% of the kinase activity was calcium- and phospholipid-dependent. The PKC activity of the preparation, when stimulated by PDBu (1 µM), was 14.7 pmole phosphate transferred from ATP to histone per minute per µl enzyme at 30°C (mean of two, duplicate experiments). Cromakalim over a concentration range of 0.01 nM to 1 mM had no effect on enzyme activity. However, the PKC inhibitor, H7, inhibited the enzyme with an IC_{50} = 100 µM, a value similar to that obtained against the mouse brain enzyme (Nakadate et al, 1988).

These results show that the interaction between cromakalim and PDBu (Coldwell, 1988) is due neither to an effect of cromakalim at the PDBu binding site, nor at another site on PKC, and suggest that the ability of cromakalim to inhibit PDBu-induced contractions is due to physiological antagonism. However, the present data are insufficient to distinguish between physiological antagonism or a direct phosphorylation of the cromakalim receptor/channel as explanations for the ability of PDBu to inhibit cromakalim-induced rubidium efflux.

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CROMAKALIM AND EXTRACELLULAR Ca^{2+}-DEPENDENT AND Ca^{2+}-INDEPENDENT CONTRACTIONS TO SPASMOGENS

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The K^+ channel activator, cromakalim (CRK) (Hamilton et al, 1986), inhibited contractions to noradrenaline (NA) in rabbit renal artery in normal Krebs solution, but had no further effect following Ca^{2+} removal (Wilson, 1988). However, under these Ca^{2+} free conditions (with 0.1 mM EGTA), contractions to NA were substantially reduced, allowing little scope for an effect of CRK. This problem has now been addressed firstly by using another spasmogen, histamine (HIS), and secondly by using $LaCl_3$, to block spasmogen-induced Ca^{2+} influx, instead of Ca^{2+} free/EGTA.

Rings of rabbit renal artery were set up for isometric tension recording in either Krebs (for subsequent Ca^{2+} free experiments) or HEPES (for subsequent $LaCl_3$ experiments) buffer, both containing 2.5 mM Ca^{2+}. Three point sequential concentration-response curves to HIS 1.0, 3.5 and 23 μM (EC_{30}, EC_{60} and EC_{90} concentrations), were constructed in Krebs buffer. The responses were then repeated after 15 min incubation with or without CRK 1 μM in both normal and Ca^{2+} free/0.1 mM EGTA Krebs. Results are shown in table 1(a). As with NA (Wilson, 1988), the area under the concentration-response curve (AUC) for HIS was greatly reduced in Ca^{2+} free Krebs. However, the further reduction with CRK + Ca^{2+} free was significant ($p<0.05$, t-test).

In separate experiments using HEPES buffer, EC_{30} (0.27 μM), EC_{60} (2.3 μM) & EC_{90} (21 μM) concentrations of NA were tested. Responses were repeated after 15 min incubation with either CRK 1 μM, $LaCl_3$ 1 mM, CRK + $LaCl_3$ or vehicle. Only one concentration of NA was tested per tissue since preparations were not viable after $LaCl_3$ washout. Results are shown in table 1(b). $LaCl_3$ produced a large reduction in the NA AUC and $LaCl_3$ + CRK had no further effect. $LaCl_3$ also produced a large reduction of the HIS EC_{90} response, but $LaCl_3$ + CRK had a significantly ($p<0.05$) greater effect. $LaCl_3$ completely abolished the HIS EC_{30} and EC_{60} responses. Prevention of Ca^{2+} influx by $LaCl_3$ 1 mM was confirmed by the abolition of a 90 mM KCl contraction.

Table 1. Mean ± s.e.mean (n=5) % reduction in AUC or single response.

(a) Krebs	CRK	Ca^{2+}free	CRK + Ca^{2+}free	Control
HIS (AUC)	61.8 ± 6.2	74.5 ± 5.0	86.2 ± 2.6	12.0 ± 3.2
(b) HEPES	CRK	$LaCl_3$	CRK + $LaCl_3$	Control
NA (AUC)	31.8 ± 4.7	74.8 ± 3.2	71.9 ± 2.9	-5.5 ± 6.3
HIS (EC_{90})	46.0 ± 12.3	81.8 ± 7.5	94.6 ± 3.5	-21.3 ± 6.9

Rabbit renal artery, therefore, appears to be highly dependent on extra-cellular Ca^{2+} for contraction to NA and HIS since both Ca^{2+} removal and prevention of Ca^{2+} influx substantially reduced responses. The lack of effect of CRK on contractions to NA resulting from intra-cellular Ca^{2+} release has been confirmed. However, CRK may inhibit a component of the HIS contraction which involves this mechanism.

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DOES CROMAKALIM ACTIVATE AN ATP SENSITIVE POTASSIUM CHANNEL IN VASCULAR SMOOTH MUSCLE CELLS?

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The vasorelaxant effects of the novel antihypertensive agent cromakalim (BRL 34915) are associated with a hyperpolarisation of the smooth muscle cell membrane and an enhancement of 86-rubidium efflux (Hamilton et al, 1986) suggesting that the drug activates potassium channels. Both the relaxation of smooth muscle and the enhancement of rubidium efflux by cromakalim are blocked by the sulphonylurea glibenclamide (GBC) (Quast et al, 1988), thus implicating the opening of an ATP sensitive potassium channel. This study attempts to investigate the involvement of intracellular ATP in this activity.

Mesenteric arteries from New Zealand White rabbits were denuded of endothelium and the smooth muscle cells dispersed using successive papain-collagenase digestion. Cells were grown in Dulbecco's Minimal Essential Medium supplemented with 10% foetal calf serum in a 5% CO_2/95% air incubator at 37°C and were used between passages 4 and 11. For efflux experiments, cells were plated on Thermanox cover slips in 24 well chambers. At confluence, c.10 µCi 86-rubidium was added to each well and following loading (60 min at 37°C), the cover slips were transferred to a superfusion system and the efflux of rubidium studied over the subsequent 60 min. Depletion of intracellular ATP using a HEPES physiological salt solution containing '0' glucose, 1 mM 2-deoxyglucose and 0.25 µg/ml oligomycin (DG buffer) produced an increase in efflux almost identical to that observed with cromakalim in terms of rate of onset, maximum effect and duration of action. In both cases, the stimulation was inhibited by TEA (30 mM) with a stimulation by DG buffer of 76 ± 5% and of 30 ± 8% in the presence of TEA ($p<0.05$; n=12). Similarly cromakalim (10 µM) produced a stimulation of efflux of 71 ± 11% which was reduced to 4 ± 3% in the presence of TEA ($p<0.001$; n=7). Surprisingly, GBC up to 10 µM had little effect on the efflux stimulated by cromakalim or DG buffer. In other experiments, cells were plated directly in 24 well plates and intracellular ATP was measured using luciferin-luciferase, as described by Nguyen et al (1988). Exposure to cromakalim for 120 min decreased ATP levels with a maximum effect at 0.1 µM cromakalim (120 min control 212 ± 16 ngATP/well; cromakalim 158 ± 10; $p<0.02$; n=8). Cromakalim at concentrations greater than 1 µM produced no significant effect on ATP levels. Short exposure (1 min) to cromakalim (0.1-10 µM) produced small non-significant decreases in ATP levels.

These latter results might suggest the existence of an ATP-sensitive potassium channel in vascular smooth muscle which, as in other tissues (see Cook et al, 1988), is highly sensitive to ATP and is opened by small changes in intracellular ATP. The prolonged exposure employed above is obviously far in excess of that required to produce channel opening and was only employed such that cromakalim would decrease ATP sufficiently to allow the detection of a significant effect by the technique employed. Despite the similarities in efflux enhancement by cromakalim and ATP depletion, our original hypothesis of the activation of an ATP sensitive channel is confounded by the unexplainable apparent lack of effect of GBC. Nevertheless, our preliminary findings would suggest that the activation of the channel by cromakalim may be associated with a decrease in intracellular ATP. Whether this decrease is the stimulus to produce potassium channel opening and is the cause of the smooth muscle relaxation requires further investigation.

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ATROPINE DOES NOT INCREASE EVOKED ACETYLCHOLINE RELEASE IN THE ISOLATED FROG SARTORIUS MUSCLE

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Evidence was recently presented (Arenson & Locker, 1988) to the Society which suggested that frog motor nerve terminals possess receptors whose activation by muscarinic agonists depresses quantal release of acetylcholine (ACh). Those experiments, carried out in Mg^{2+}-paralysed muscles, left unanswered the question of whether feedback inhibition is operative at physiologically relevant levels of ACh release. This report describes the results of experiments, undertaken using a wider range of Ca^{2+} and Mg^{2+} concentrations, that were designed to answer this question by examining the effects of atropine on quantal ACh release.

Experiments were performed on isolated sartorius muscles which had been paralysed with Mg^{2+} or treated with glycerol (Eisenberg, Howell and Vaughan, 1971) to disrupt excitation-contraction coupling. Fibres were impaled with 3M KCl-filled microelectrodes and samples of mepps (usually > 100) and epps (120 or 140) evoked at 0.33 or 20 Hz were recorded before (control) and in the presence (test) of atropine. These signals were used to calculate mepp frequency (F) or, after correction for non-linear summation, quantal content (M). Results for F and M are presented (mean $\pm$ s.e.mean) as a ratio test/control and are compared with data from untreated fibres.

In low Ca^{2+}-Ringer solution (Ca^{2+}/Mg^{2+}, 0.5/3.0 mM) atropine (100 nM) did not increase the F-ratio; the value of 1.05 $\pm$ 0.11 (n = 8) was comparable with that of 1.12 $\pm$ 0.10 (n = 8) determined for the untreated group. M-ratios for epps evoked at 0.33 and 20 Hz were 1.04 $\pm$ 0.05 (n = 8) and 1.13 $\pm$ 0.06 (n = 7) respectively for untreated fibres. Atropine (100 nM) did not significantly alter these values (1.07 $\pm$ 0.06, n = 8, 0.33 Hz; 1.00 $\pm$ 0.04, n = 7, 20 Hz).

In glycerol treated fibres (Ca^{2+}/Mg^{2+}, 1.8/0 mM) atropine (100 nM) significantly increased ($P < 0.04$) the F-ratio (1.37 $\pm$ 0.13, n = 12) from the value (0.98 $\pm$ 0.07, n = 12) observed for the untreated group. Raising the atropine concentration (300 nM) produced a smaller increase ($P = 0.24$) in the F-ratio. In this high Ca^{2+}-Ringer solution the M-ratio for epps evoked at 0.33 Hz was 0.97 $\pm$ 0.04 (n = 6) for untreated fibres and 0.99 $\pm$ 0.05 (n = 7) in the presence of 100 nM atropine. The mean M-value of the last ten of a train of 140 epps evoked at 20 Hz was 82 $\pm$ 1.8 % of that of the first epp. Atropine (100 and 300 nM) did not affect M of the first epp or the subsequent rundown. The peaks of epps were at least 15 mV below the reversal potential and paired pulse facilitation was often observed suggesting that neither the asymptotic epp-epc relationship nor the saturation of postsynaptic receptors accounts for these negative results.

In conclusion, the present data extend the results of Beránek & Vyskočil (1968) and do not support the concept that autoinhibitory muscarinic receptors normally modulate the release of ACh during neuromuscular transmission.

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EFFECT OF FLUMAZENIL ON THE BIPHASIC ACTION OF DIAZEPAM ON NEUROMUSCULAR TRANSMISSION OF THE RAT DIAPHRAGM PREPARATION

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Myorelaxation produced by benzodiazepines is thought to be of central origin. Direct effects, however, have also been described. A biphasic action on twitch tension (Driessen et al.,1984) and on tetanic stimulation (van Oeijen et al., 1988) of the phrenic nerve-diaphragm preparation, in vitro, was found. Time-constants of risingphase and decayphase of miniature endplate currents are also affected in a biphasic way and it has been suggested that the potentiating effect of the benzodiazepines is of presynaptic origin, whereas the inhibition is caused by a postsynaptic effect (van Wilgenburg & Leeuwin, 1986). In the present study the effect of flumazenil (Ro 15-1788), a central working antagonist of benzodiazepines, is studied on the biphasic action of diazepam.

Phrenic nerve hemidiaphragm preparations from female Wistar rats (170-200g) were used throughout. The organ bath, containing Krebs solution, was maintained at 35°C $\pm$ 0.5°C and bubbled with 5% CO2 in oxygen, pH kept at 7.4 (7.35-7.45). After 2 hours of incubation the experiment was started. Increasing concentrations of diazepam, 0.02-0.31 mmol/l, were given in a non-cumulative way in six successive sessions. Every session consisted of four periods of tetanic stimulation (6 secs at 30 HZ, stimulusduration 0.1 msec, supramaximal at 5 V) with intervals of five minutes. Diazepam was given at the beginning of a session 1 minute before the first tetanic stimulation. Flumazenil (0.01 and 0.1 mmol/l) was added to the bath 1 minute prior to the administration of diazepam. Between the sessions a drugfree period of at least 10 minutes was maintained.

Diazepam showed a biphasic effect on the tetanic contraction. At concentrations, ranging from 0.02-0.10 mmol/l, there was a significant, time- and dose dependent, enhancement of the tetanic contraction, to 143% of the control value at 0.10 mmol/l diazepam. At higher concentrations (0.17-0.31 mmol/l) there was a time- and dose dependent depression of the tetanus. Flumazenil, 0.1 mmol/l, has a depressive effect on the contraction to 82 % of the control value. Flumazenil, 0.1 mmol/l, given in combination with diazepam antagonizes the potentiating effect caused by diazepam from 143 % to 82 % at a diazepam concentration of 0.10 mmol/l. The depressing effect caused by higher concentrations of diazepam is not prevented by flumazenil.

The results of the present experiment suggest that different mechanisms and different sites of action underlie the biphasic action of the benzodiazepines. The potentation of tetanic contraction by diazepam, which has been proposed to be of presynaptic origin, and which might be caused by an increase in quantum content (Torda & Murphy,1979) as well as by an increase in vesicle size (Leeuwin, et al., 1987), seems to be specifically antagonized by flumazenil. The depression, which has been described as a postsynaptic effect of the benzodiazepines (Torda & Murphy,1979), seems not to be affected by flumazenil.

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DOPAMINE RECEPTORS IN THE MOUSE VAS DEFERENS

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We have investigated the effects of dopamine on the response of the mouse isolated vas deferens to electrical stimulation (3 msec duration pulses at 0.1 Hz) and contrary to previous investigators (Gibson & Samani (1979), Hurst et al. (1979)) have found that there are specific dopamine receptors present in this tissue which inhibit the response to stimulation.

Using vas deferentia from mice of strain MF1/OLA and weight >30 grams we have found that the specific α_2-antagonist RX821002 (Stillings et al. (1986)) at concentrations x10,000 that of the α_2 Ke value, failed to shift the dopamine dose response curve, whereas the noradrenaline dose response curve was shifted x50 times to the right. The addition of 30 nM domperidone, as well as producing a marked shift to the dopamine dose response curve also produced a further shift to the noradrenaline. These results indicate that, in the presence of the RX821002, noradrenaline produces an inhibitory effect via dopamine receptors in this tissue.

A range of dopamine antagonists have been investigated vs. the effects of dopamine (in the presence of 1 μM idazoxan to mask any α_2-agonist effects of dopamine) and the Ke values obtained indicate that the receptor present is of the D_2 subtype. The specific D_1 antagonist SCH23390 (Iorio et al. (1983)) possesses extremely low activity as a dopamine antagonist in this preparation.

Ke values (nM) obtained vs. the various antagonists after a 90 minute contact time were as follows:

Compound	Ke (nM)
Spiperone	0.002
Haloperidol	0.008
Domperidone	0.011
Pimozide	0.041
Fluphenazine	0.17
Fluphenthixol	0.33
Sulpiride	7.6
Tiapride	16.0
Clozapine	414
SCH23390	2054

These results show that in the presence of α_2-antagonists the mouse isolated, electrically stimulated vas deferens is an extremely simple preparation suitable for the rapid screening of compounds for D_2-agonist or antagonist activity.

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EFFECT OF NORADRENALINE ON RUBIDIUM (^{86}Rb) EFFLUX IN THE RAT ISOLATED SEMINAL VESICLE

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Rubidium (^{86} Rb) has been used as a marker for potassium (^{42}K) efflux (Imiazumi & Watanabe,1981), since both isotpes behaved in a similar manner when studied in the smooth muscle preparation (Hamilton,Weir & Weston,1986).
In the present investigation, the effect of noradrenaline (NA) on (^{86}Rb) efflux, in isolated seminal vesicle of the rat,was studied, to see if the NA increased the (^{86}Rb) efflux and enhanced its own contractions in this type of smooth muscle.

Strips of rat seminal vesicle were cut,blotted gently on a filter paper and weighed. They were set up,in separate organ baths containing 20 ml of Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% CO_2 in O_2. The strips were incubated,under initial tension of 0.5 g, in ^{86}Rb isotope for 2 h(radioactivity of 10 $\mu Ci.ml^{-1}$). After the incubation period,the strips (8 in each experiment) were removed,at 30 s time intervals, and the excess radioactivity was washed off.
The effect of NA(123 μM) on ^{86}Rb efflux was studied in the absence and presence of phentolamine(1 μM) or papaverine (5 μM). At the end of the efflux process,the tissues were removed and were digested,using 1 ml of 1 M of Nitric acid.The samples were made up to 5 ml solution,by adding Krebs solution. Measurement of radioactivity of ^{86}Rb efflux was made using an autogamma spectrometer (Hewlett Packard). Flux data were expressed in terms of rate constants per min(k),of the reactive isotope efflux, and the effects of drugs on rate constants were expressed as a percentage difference of the pre-drug value,at the peak effect (Quest,1987).

The control rate constant per min was 0.006±0.001 and this was decreased,with time, reaching a value of 0.004±0.001,in 35±2 min(means±s.e.,n=8,$P < 0.01$). When the NA was added,the efflux of ^{86}Rb was increased immediately,reaching a peak value of 0.0067±0.001,in 7±2 min($P < 0.05$,with respect to the control value).Thereafter,the efflux was reduced with time,reaching a pre-drug value in 30±2 min. Phentolamine (1 μM) reduced the NA-induced increase in ^{86}Rb efflux by 71±3%,of the control value, whereas papaverine (5 μM) enhanced the efflux by 95±3%.

The mechanism of inhibition of the NA-induced increase of ^{86}Rb efflux, by phentolamine,is well understood, whereas the increase in the efflux by papaverine is not clear in the present study, but this could be attributed to an influence on calcium release, possibly via calcium-dependent K^+ efflux (Sidi,Cameron,Duffy & Lange,1986). Furthermore,it has been reported that certain mixtures of phentolamine and papaverine might be useful in relaxing the vascular smooth muscle and increasing the blood flow (Sidi,et.al.,1986; Lue & Tanagho,1987).

In conclusion, noradrenaline enhanced ^{86}Rb efflux,in the rat seminal vesicle,and this effect was antagonised by phentolamine,but potentiated by papaverine.

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MODIFICATION OF THE CONTRACTILE RESPONSES PRODUCED BY ELECTRICAL FIELD STIMULATION IN THE RAT ISOLATED SEMINAL VESICLE

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In the present investigation, the contractile responses produced by electrical field stimulation of the rat isolated seminal vesicle were recorded and the effects of pharmacological agents on these responses were studied. The aim was to further characterise these responses and analyse their mechanisms.

Seminal vesicles, from adult, male, Sprague-Dawley rats, weighing 200-300 g, were dissected and cut spirally into strips (2-3 cm long and 2 mm wide). These were set up, in separate organ baths, containing 20 ml of Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% CO_2 in O_2 . The contractile responses produced by electrical field stimulation, of the isolated seminal vesicle, elicited at 10, 20, 50 and 100 Hz, for 10-20 s duration, with 0.2 ms pulse duration, and 20-25 V (supramaximal), were recorded isometrically.

In the rat isolated seminal vesicle, field stimulation produced biphasic type of contractions, i.e., an initial rapid twitch-like contraction (phasic), followed by a slow (tonic) contraction. At 50 Hz stimulation, the phasic contraction was 1.2± 0.2 g(tension), and the tonic contraction was 0.5±0.1 g tension(mean±s.e., n=6). These contractions were reduced or blocked, by phentolamine (1 μM), α,β-methylene ATP (10 μM) and by verapamil(1 μM). Furthermore, phentolamine specifically and greatly reduced the tonic contraction, α,β-methylene ATP significantly reduced the phasic contraction, and verapamil reduced, unselectively, both types of contractions. Detailed results are shown in Table 1.

Table 1. Contractile responses of rat isolated seminal vesicle produced by electrical field stimulation at 50 Hz, in the presence and absence of phentolamine, α,β-methylene ATP and verapamil (*).

	(1) Control	(2) Phentolamine	(3) α,β-Meth-ATP	(4) Verapamil
Phasic (g)	1.2±0.2	1.1±0.1	0.7±0.2	0.8±0.2
Tonic (g)	0.8±0.2	0.3±0.1	0.6±0.1	0.7±0.2

(*): means±s.e., n=6, $P < 0.05$ (columns 3,4 with column 1 of phasic), $P < 0.001$ (column 2 with column 1 of tonic). Phentolamine (1 μM), α,β-Meth-ATP (10 μM) and verapamil(1 μM) produced selective reductions in the contractile responses produced by electrical field stimulation.

In conclusion, electrical field stimulation, of the rat isolated seminal vesicle, produced a biphasic response, similar to that observed in the vas deferens (Bouquet & Noach, 1971; Meldrum & Burnstock, 1985). In addition, the responses were blocked by selective antagonists, which may also suggest the presence of different types of neurotransmitters (and receptors) in the rat isolated seminal vesicle (see Fedan, Besse, Carpenter & Teague, 1977).

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STIMULATION OF α1- AND β1-ADRENOCEPTORS ENHANCES [^{3}H]-ACETYLCHOLINE RELEASE FROM THE ISOLATED RAT PHRENIC NERVE

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It is generally accepted that sympathomimetic amines can modify neuromuscular transmission of skeletal muscles. It has been shown that stimulation of both α- and ß-adrenoceptors enhances transmitter release from the phrenic nerve (Bowman, 1981; Wessler & Anschütz, 1988). The aim of the present study was to characterize the receptors involved. The effects of isoprenaline (IS), noradrenaline (NA), adrenaline (AD) and phenylephrine (PE) on electrically evoked [^{3}H]-acetylcholine (ACh) release from the rat phrenic nerve were investigated in the absence and presence of different receptor antagonists: CGP 20712 A (1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-2-propanol methanesulfonate), prazosin, propranolol, yohimbine). CGP 20712 A is regarded as a ß1-selective antagonist.

The experimental procedure has been described previously (Wessler & Kilbinger, 1986). Endplate preparations of the left hemidiaphragm of Sprague-Dawley rats were incubated (40 min) with 1 µM [^{3}H]-choline (10 µCi), and the labelling of neuronal transmitter stores was carried out during nerve stimulation at 1 Hz (0.2 ms impulse duration, 8 volts). After a subsequent 60 min washout and the addition of 10 µM hemicholinium-3, to prevent re-uptake of choline, tritium efflux was measured in 2 min samples. Release of ACh was elicited by two periods (S1, S2) of electrical nerve stimulation, 200 pulses at 10 Hz each. S1 was regarded as control and the sympathomimetic amines were added 16 - 24 min before S2. In the respective experiments the antagonists were present from 30 min before S1 onwards.

NA (1 µM) or IS (0.1 µM) enhanced the stimulated ACh release by 92±5 % (n=10) and 83±5% (n=7), respectively. Pretreatment with CGP 20712 A (0.1 µM) prevented the facilitatory effect of both amines. AD (1 µM) enhanced evoked ACh release by about 62±3 % (n=8). This effect was partially blocked by propranolol (0.3 µM) and totally blocked in the presence of both propranolol and yohimbine (1 µM). PE (10 µM) enhanced evoked ACh release by about 47±4% (n=4). This effect maintained in the presence of low concentrations (0.01 and 0.1 µM) of yohimbine; only the high and non-selective concentration of 1 µM yohimbine abolished the facilitatory effect of PE. Prazosin, however, blocked the effect of PE at low, α1-selectice concentrations (0.01 and 0.1 µM).

It is concluded that both presynaptic α1- and ß1-adrenoceptors are present on the motor nerve. Evoked transmitter release from the motor nerve can be enhanced by stimulation of either of these adrenoceptors. Under the present experimental conditions the dominant effect is mediated by stimulation of ß1-adrenoceptors. Circulating NA and AD may facilitate neuromuscular transmission by stimulation of these presynaptic receptors. NA operates mainly via ß1-adrenoceptor stimulation and AD via stimulation of both α- and ß-adrenoceptors.

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PROSTAGLANDINS ARE INVOLVED IN INHIBITION OF ACETYLCHOLINE RELEASE FROM MYENTERIC NEURONES VIA ACTIVATION OF 5-HT_{1A}-RECEPTORS

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Cholinergic neurones of the myenteric plexus preparation (MPP) of the guinea-pig are endowed with 5-HT_3 (ganglionic)- and 5-HT_{1A} (prejunctional)-receptors whose activation causes an increase of the spontaneous and an inhibition of the electrically evoked release of acetylcholine (ACh), respectively (Kilbinger & Pfeuffer-Friederich, 1985; Fozard & Kilbinger, 1985). Prostaglandins (PGs) are involved in the excitatory effect of muscarinic M_1-receptors (ganglionic) on the release of ACh, but not in the inhibitory one mediated via M_2-receptors (Schwörer, 1988). Therefore, the actions of indometacin (Ind), and in addition, that of pertussis toxin (PTx) and AH 21-132, a phosphodiesterase inhibitor (Markstein et al., 1984), on the modulation of ACh release via activation of 5-HT_3- and 5-HT_{1A}-receptors were studied.

MPP of the guinea-pig ileum were incubated with ^{3}H-choline and subsequently superfused with Tyrode's solution. Electrical stimulation (S1, S2, 1 Hz, 180 pulses) was applied with an interval of 45 min.

10 µM 5-HT added 30 min before S2 caused a short-lasting increase of the spontaneous ^{3}H-outflow (total release 2.0±0.22 % of the ^{3}H-tissue content, n=15) and reduced the evoked ^{3}H-outflow during S2 (S2/S1 = 0.67±0.02 vs controls S2/S1 = 0.86±0.03, $P < 0.01$, each n=4). 5-Carboxamidotryptamine (5-CT, 0.3 - 10 µM), a 5-HT_{1A}-receptor agonist, also reduced the evoked ^{3}H-outflow by maximally 33 % ($p < 0.01$ vs control). 1 µM Ind which on its own did not affect the spontaneous or evoked ^{3}H-outflow, completely antagonized the inhibitory effects of 5-HT and 5-CT. The stimulatory effect of 5-HT was not affected by 1 µM Ind. The inhibitory effects of 5-HT and 5-CT on the evoked ^{3}H-outflow were preserved in the presence of 10 µM AH 21-132. The stimulatory effect of 5-HT on the spontaneous ^{3}H-outflow was not altered by 10 µM AH 21-132 or by pretreatment of the guinea-pig with PTx (60-150 µg/kg, i.v., up to 96 h prior to the start of the experiments).

These findings suggest that PGs participate in the 5-HT_{1A}-mediated inhibition of evoked ACh release whereas PTx-sensitive G-proteins or increased cAMP levels are not involved in the effects of 5-HT_3 and 5-HT_{1A}-receptor activation on ACh release from cholinergic neurones of the MPP.

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POSSIBLE ROLE OF α-ADRENOCEPTORS AND ATP-DEPENDENT K^+ CHANNELS IN INSULIN INHIBITION INDUCED BY SYMPATHETIC STIMULATION

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Sympathetic nerve stimulation inhibits basal and stimulated insulin secretion (Miller, 1981). However, this effect does not appear to be exclusively mediated by postsynaptic α_2-adrenoceptors (Ahrén et al., 1987). Glibenclamide, an anti-diabetic drug with antagonist properties towards ATP-dependent K^+ channels, restores the spontaneous electrical activity of rat insulinoma inhibited by galanin, a candidate co-transmitter of noradrenaline (De Weille et al., 1987). This communication reports, in the blood perfused canine pancreas, the effects of idazoxan on the basal or glibenclamide-stimulated insulin secretion during sympathetic nerve stimulation.

Fasted (18h) dogs were anaesthetised with pentobarbitone, artificially ventilated and prepared for aortic blood pressure measurements. Following laparotomy, the pancreatic duodenal artery, its perivascular nerve bundle and the pancreatic vein were dissected. Blood samples were collected from the cannulated vein before and 1 min after initiation of the stimulation of the perivascular nerves (3.0 Hz, 2.0 msec, 20-30 V for 3 min) to determine plasma noradrenaline and insulin levels. After administration of heparin (700 UI/kg i.v. + 100 UI/kg/h throughout the experimental procedure), the pancreas was perfused at a constant flow with blood withdrawn from the femoral artery. Chlorisondamine (1.0 mg/kg), methylatropine (0.3 mg/kg) and propranolol (1.0 mg/kg) were then given i.v. The effects of idazoxan (1.0 mg/kg i.v.) or glibenclamide (1.0 mg/kg i.v.) given alone or in combination were studied on the vascular and humoral responses to sympathetic stimulation.

Glibenclamide did not significantly modify noradrenaline plasma content, but increased slightly perfusion pressure and markedly insulin level in the pancreatic vein (E_{max}: 190 $\pm$ 32 from base-line value of 126 $\pm$ 28 ng/ml). In saline and glibenclamide-pretreated dogs, sympathetic nerve stimulation increased similarly perfusion pressure and venous plasma noradrenaline levels, but markedly reduced insulin plasma levels (E_{max}: -94 $\pm$ 28 from 180 $\pm$ 54 and -166 $\pm$ 25 from 361 $\pm$ 48 ng/ml, respectively). Idazoxan further enhanced in both preparations, the release of noradrenaline in response to nerve stimulation (2 fold) without significantly modifying vascular effects. However, idazoxan inhibited significantly (71%) the decrease in insulin release evoked by sympathetic stimulation only in glibenclamide-pretreated dogs.

Our results suggest that the inhibition of insulin release evoked by sympathetic nerve stimulation is mediated not only by α_2-adrenoceptors, susceptible to blockade by idazoxan, but also by a glibenclamide-sensitive receptor mechanism. It is possible that, in the pancreas, sympathetic nerve stimulation releases noradrenaline as well as a co-transmitter (e.g. galanin) to induce the decrease of insulin secretion. Additional experiments are necessary to confirm this possibility.

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ANTAGONISM OF THE ADENOSINE RECEPTOR AT THE PHRENIC NERVE-DIAPHRAGM AND HIPPOCAMPUS OF THE RAT BY A XANTHINE AMINE CONGENER

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The xanthine amine congener, 1,3-dipropyl-8-(4-((2-aminoethyl)amino)carbonylmethyl oxyphenyl)xanthine (XAC) is a potent antagonist (Ki=23 nM) of the adenosine receptor mediating presynaptic inhibition of transmission at the neuromuscular junction of the frog (Sebastião & Ribeiro, 1989). In the present work, the potency of XAC as antagonist of the adenosine receptor at the neuromuscular junction of the rat as well as at the rat hippocampus was investigated.

The experiments were carried out on the phrenic nerve-diaphragm preparation (see e.g. Sebastião & Ribeiro, 1988) and on hippocampal slices (see e.g. Bartrup & Stone, 1988) of the rat. Nerve-evoked (0.2 Hz) twitch responses of the diaphragm were recorded isometrically at a resting tension of 50 mN; the bathing solution (pH 7.4) was kept at 37°C and contained (mM): NaCl 117, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11; the safety margin of neuromuscular transmission was decreased by tubocurarine (0.7-1.0 µM). Hippocampal slices were 400 µm thick and recordings of orthodromic population spikes in the pyramidal layers of the CA_1 region were made using single glass microelectrodes with 5-10 MΩ resistance. The Schaffer collateral and commissural afferents to the CA_1 pyramids were stimulated (0.1 Hz) by a bipolar electrode placed in the stratum radiatum; the bathing solution (pH 7.4) was kept at 30°C or at room temperature (22-25°C) and contained (mM): NaCl 115, KCl 2.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 2.2, $NaHCO_3$ 25, glucose 10. All solutions were continuously gassed with 95% O_2 + 5% CO_2.

In Table 1 are shown the pA_2 and Ki values for XAC, obtained from the antagonist-induced shifts to the right of the concentration-response curves for the inhibitory effect of 2-chloroadenosine on nerve evoked twitch responses of the diaphragm and on the amplitude of the orthodromically evoked population spikes of the CA_1 pyramids of the hippocampus.

Table 1 Potency of XAC at the innervated diaphragm and hippocampus of the rat

	Diaphragm	Hippocampus	
	37°C (n=10)	30°C (n=5)	22-25°C (n=6)
pA_2	7.97 ± 0.07	7.94 ± 0.04	8.08 ± 0.04
slope*	1.09		1.02
Ki	11 nM	11 nM	8 nM

*slope of the regression line of the Schild plot

Comparing the potency of XAC as antagonist of the adenosine receptor at the rat phrenic nerve-diaphragm preparation and at the rat hippocampal slices no significant differences were observed. This xanthine was about 2-fold less potent as antagonist of the adenosine receptor at the frog neuromuscular junction (Sebastião & Ribeiro, 1989) than at the rat neuromuscular junction or rat hippocampus.

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LACK OF EFFECT OF INDOMETHACIN ON CALCITONIN-INDUCED INHIBITION OF BASAL AND HISTAMINE-STIMULATED GASTRIC ACID SECRETION IN VIVO

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Indomethacin has been shown to cause gastric erosions in the rat (Djahanguiri, 1969), to potentiate histamine-stimulated gastric acid secretion (Reeves & Stables, 1985) and deplete prostaglandin levels in the stomach (Cho et al, 1985). Calcitonin has been shown to inhibit gastric acid secretion when administered either peripherally (Orimo et al, 1973), or centrally (Morley & Levine, 1981). Calcitonin also inhibits indomethacin-induced gastric erosions (Bates et al, 1980), though its action is not necessarily related to gastric acid secretion. The aim of this study is to investigate the effect of salmon calcitonin (sCT) and indomethacin on gastric acid secretion in the basal and histamine-stimulated perfused stomach.

Starved Sprague-Dawley rats (200 - 250g) were anaesthetised and the stomach perfused by cannulation of the oesophagus and duodenum. Drugs were administered via the jugular vein. sCT (10U kg^{-1}) was administered as a bolus injection after basal acid secretion had been established. Histamine infusion (3mg kg^{-1} hr^{-1}) was started at the same time as sCT administration. Indomethacin (0.112mmol kg^{-1}) was administered 45 minutes before sCT. Acid secretion was measured for 110 minutes, and the percentage change in acidity from base rate was then calculated.

The results were analysed by the Mann-Whitney U test. The effect of sCT was significant over the period from 20 to 90 minutes, reaching a peak at approximately 60 minutes. At this time it was found that histamine-stimulated acid secretion was 937% ± 165, and that this was potentiated by indomethacin (1285% ± 84). sCT inhibited histamine-stimulated acid secretion, reducing it to 359% ± 64. sCT also inhibited basal acid secretion, reducing it from 13% ± 8 to -25% ± 7. Furthermore, pretreatment with indomethacin had no effect on the response of sCT on either basal or histamine-stimulated gastric acid secretion (-27% ± 9 and 430% ± 86 respectively).

We therefore conclude that the effects of sCT in inhibiting indomethacin induced gastric erosions may involve a reduction in acid secretion, as well as mechanisms independent of acid secretion.

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M&B 22948, A SPECIFIC cGMP PHOSPHODIESTERASE INHIBITOR DELAYS GASTRIC EMPTYING IN RATS

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M&B 22948 is an inhibitor of cGMP specific phosphodiesterase, with demonstrated anti-asthmatic activity (Rudd et al., 1983). We have previously reported that M&B 22948 relaxes gastrointestinal smooth muscle (Hills et al.,1987). Here we report the effects of M&B 22948 in vivo in the rat gastrointestinal transit model.

A technique for the measurement of gastrointestinal transit as described by Poulakos et al., (1973) was utilised to investigate the effects of oral and intravenous M&B 22948. Intravenous administration and blood sampling was carried out after prior (48 hr) cannulation of femoral and jugular veins respectively. Male Wistar rats (250g) were dosed by gavage with 6uCi ^{51}Cr, as sodium chromate in saline (1ml) which served as a non-absorbable marker of gut transit. Oral dosing of M&B 22948 (5mg/kg) or saline was carried out 10 min prior to the ^{51}Cr in a total of 16 rats. For intravenous administration, M&B 22948 or saline (1ml/h), was infused continuously at 1&5 umol/ml/hr commencing 1h prior to the ^{51}Cr. Plasma concentrations were measured hourly by HPLC. Rats were sacrificed at 2 or 4h after the ^{51}Cr, their intestines removed and divided into ten sections from stomach to colon. Samples were counted in a Packard autogamma spectrometer.

Oral M&B 22948 (5mg/kg) delayed gastric emptying at both 2 and 4h time points (Table 1). Steady state plasma concentrations of approximately 5 and 150uM were attained with infusions of 1 & 5umol/ml/h respectively. Both concentrations of intravenous M&B 22948 also caused a marked delay in gastric emptying (Table 1).

Table 1 Mean ± SE % ^{51}Cr remaining in stomach after oral or intravenous administration of saline or M&B 22948 (n=4 per group)

	2 hours	4 hours
Saline (p.o.)	4.5 ± 2.0	0.7 ± 0.5
M&B 22948 (p.o.) 5mg/kg	25.4 ± 12.0	27.5 ± 11.3*
Saline	8.1 ± 4.1	1.9 ± 0.9
M&B 22948 (i.v.) 1µM/ml/h	33.3 ± 5.2	20.2 ± 2.8*
Saline	2.4 ± 1.5	0.6 ± 0.2
M&B 22948 (i.v.) 5µM/ml/h	44.2 ± 10.8*	42.6 ± 4.1*

* Significant difference, $P < 0.5$, variances were tested for equality and the appropriate un-paired t-test was performed on the data.

We conclude that M&B 22948 given orally or intravenously, delays gastric emptying in the rat. The mechanism of this inhibition is likely to be related to the phosphodiesterase inhibitory activity of this compound.

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THE EFFECT OF ORTHO-IODO SODIUM BENZOATE (OISB) UPON OXYGEN EXTRACTION BY SKELETAL MUSCLE AT REST AND EXERCISE

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In theory an improvement in oxygen supply to an ischaemic muscle could be achieved by lowering the oxygen affinity of haemoglobin. Ortho-iodo sodium benzoate (OISB) is a compound which is reported to reduce the oxygen affinity of haemoglobin, and therefore increase oxygen availability in sheep, dog and human red blood cells (Laver, 1975; Gross et al., 1977). In the present experiment we have investigated whether OISB can increase oxygen extraction at rest and during exercise of a flow restricted skeletal muscle and subsequently reduce muscle fatigue.

Experiments were performed on beagle dogs anaesthetised with sodium pentobarbital (45mg.kg^{-1}) and artificially ventilated. The right gracilis muscle was isolated from the systemic circulation and perfused by a pump at constant flow. Perfusion rate was selected by matching the gracilis perfusion pressure with systemic blood pressure. The right femoral vein was cannulated distal to the gracilis branch for blood sampling. Gracilis muscle tension was measured isometrically at rest and during 20 min of exercise (produced by stimulation of the obturator nerve at 3 Hz, 10V, 0.1msec) and the fatigue calculated by dividing the tension at the end of exercise by that at 1 min. Haemoglobin saturation, pH, pO_2, pCO_2 and oxygen content of arterial and venous blood were measured throughout the experiment. P_{50} (the partial pressure of oxygen required for 50% haemoglobin saturation) was estimated for venous blood from the Severinghaus formula:

$$\text{Estimated } P_{50} = 29 \times pO_2/pO_2 \text{ sat.},$$

where pO_2 sat is the pO_2 on the standard haemoglobin-oxygen dissociation curve for a given haemoglobin saturation (P_{50} of a standard dissociation curve for dogs at pH 7.4 is 29 mmHg).

In five dogs OISB (500mg.kg^{-1}, iv) was administered between two periods of exercise and resulted in changes summarised in Table 1.

TABLE 1

	REST		EXERCISE	
	PRE-OISB	POST-OISB	PRE-OISB	POST-OISB
Est P_{50}(pH 7.4)	34.9±0.7	37.0±1.5	35.3±0.6	35.6±1.1
% O_2 extraction	13.2+1.7(P<.05)	22.0±3.4	53.9±5.0	55.4±2.9
Fatigue (%)	-	-	47.3±8.1	46.1±7.6

Each value is mean ± s.e. of mean, n=5 (statistical analysis by Student t-test for paired data).

The present results show that although OISB was able to increase skeletal muscle oxygen extraction at rest (by reducing haemoglobin oxygen affinity) it failed to do so during exercise of a muscle to which blood flow was restricted. It also failed to alter the development of fatigue. It is likely that changes in the local environment of the muscle during exercise optimally shift the oxygen dissociation curve to the right, maximising oxygen extraction and attenuating the effect of OISB.

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5-HT_3 RECEPTORS MEDIATE DEPOLARISATION OF THE GUINEA-PIG ISOLATED VAGUS NERVE

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Neuronal 5-HT_3 receptors may be heterogenous (Richardson & Engel, 1986). Thus, antagonist pA_2 values vary between 5-HT_3 receptor models such as the rabbit or rat isolated vagus nerve (RVN), the rabbit isolated heart and guinea-pig isolated ileum. However, the latter two preparations have been criticised because responses to 5-HT are indirectly mediated through another transmitter. We now report on the preliminary characterisation of a new direct model of 5-HT_3 receptor-mediated activation, the guinea-pig isolated vagus nerve (GPVN).

Depolarisations of desheathed GPVNs were measured across a silicon grease partition as described by Ireland and Tyers (1987). 5-HT (10^{-6}-3×10^{-4}M) induced rapid concentration-dependent depolarisations, maximum responses were routinely between 800 and 1200μV. At higher concentrations of 5-HT (3×10^{-5}-3×10^{-4}M) depolarisation was followed by a much smaller hyperpolarisation. Non-cumulative concentration-response curves for 5-HT gave an EC_{50} of 12.3±1.3μM (mean ± s.e., n=30). The selective 5-HT_3 agonist, 2-methyl-5-HT (3×10^{-6}-3×10^{-4}M) induced concentration-related depolarisations and after-hyperpolarisations. Maximum depolarisations for 2-methyl-5-HT were 66.3±5.2% of the 5-HT maximum, the EC_{50} was 36.3±9.9μM (mean ± s.e., n=3). Phenylbiguanide, a partial agonist on RVN 5-HT_3 receptors (Ireland & Tyers, 1987) did not induce depolarisations (up to 3×10^{-4}M) or antagonise 5-HT-induced depolarisations (10^{-5}M). α-Methyl-5-HT, 5-CT and 8-OHDPAT did not induce measurable depolarisations or hyperpolarisations (up to 10^{-4}M).

Neither methysergide (10^{-6}M) nor ketanserin (10^{-5}M) had any significant effect on the 5-HT-induced depolarisations or after-hyperpolarisations. 5-HT_3 receptor antagonists caused competitive blockade of 5-HT-induced depolarisations and after-hyperpolarisations. Metoclopramide (10^{-5}-10^{-4}M) caused concentration-dependent parallel rightward shifts of the 5-HT dose-response curve. Schild analysis revealed an apparent pA_2 of 5.4 and slope of 1.13 (n=12). Other 5-HT_3 antagonists were tested at single concentrations and the pA_2 estimated using the equation: $pA_2=\log(\text{dose-ratio}-1)-\log[\text{antagonist}]$. Approximate pA_2 values derived in this way (using 3-5 separate tissues) were as follows: MDL 72222 (10^{-5}M) 6.4, ICS 205-930 (10^{-7}M) 7.6, GR38032F (10^{-6}M) 7.0, BRL 43694 (10^{-7}M) 7.9, GR65630 (10^{-6}M) 7.3, zacopride (10^{-7}M) 7.6, GR67330 (10^{-7}M) 8.2. For the structure of GR65630 and GR67330, see Kilpatrick et al., 1987, 1988.

The antagonist pA_2 values from the GPVN differ from those previously described using the RVN by Ireland & Tyers (1987) e.g. metoclopramide 6.6, MDL 72222 8.2 and ICS 205-930 ~11.0. This taken together with the lack of effect of phenylbiguanide (as an antagonist or agonist) suggests that 5-HT_3 receptors on the GPVN and RVN are different. 5-HT_3 receptors on the GPVN may correspond to those on the guinea-pig isolated ileum described as 5-HT_{3C} by Richardson & Engel (1986).

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POSSIBLE DIFFERENCES IN 5-HT_3-LIKE RECEPTORS IN THE RAT AND THE GUINEA-PIG

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The potencies of 5-HT_3 antagonists when compared in different bioassays has led to the suggestion that subtypes of this receptor may exist (Richardson and Engel, 1986). The receptor mediating 5-hydroxytryptamine (5-HT)-induced contractions of the guinea-pig ileum longitudinal muscle strip (GPI) in the presence of methysergide is one at which 5-HT_3 antagonists have surprisingly low affinity. It has been suggested (Gunning and Humphrey, 1987) that the complex actions of 5-HT in the guinea-pig ileum, not receptor difference, may account for the lower potencies of antagonists in the latter tissue.

We have compared the 5-HT_3-like antagonist actions of zacopride (ZAC, Smith et al., 1988), GR 38032 (GR, Brittain et al., 1987) and ICS 205930 (ICS, Richardson et al., 1985) on the GPI and on the anatomically simpler preparations, the isolated cervical vagus nerves of the rat (RVN) and the guinea-pig (GVN).

The method used for the GPI was that of Buchheit et al., (1985) using Krebs' solution (37°C) containing methysergide (1μM). 5-HT-induced depolarization of the RVN and GPVN was recorded as described by Ireland and Tyers (1987) using an extracellular, 'grease gap' technique, with a Krebs' solution (27°C) perfusion rate of 5 ml min^{-1}. Antagonists were equilibrated with tissues for 60 min.

Schild analysis of 5-HT_3-like antagonism by zacopride, GR 38032 and ICS 205930.

	Rat vagus		Guinea-pig vagus		Guinea-pig ileum	
	pA_2	slope	pA_2	slope	pA_2	slope
ZAC	9.1	1.38	7.6	1.10	7.6	1.24
	(8.9-9.3)	(1.00-1.76)	(7.4-8.1)	(0.78-1.42)	(7.3-8.1)	(0.81-1.67)
GR	8.7	0.96	7.0	1.00	7.1	0.91
	(8.4-9.2)	(0.60-1.32)	(6.8-7.4)	(0.73-1.26)	(6.8-7.4)	(0.64-1.19)
ICS	8.8	1.20	7.6	1.18	7.6	1.26
	(8.6-9.0)	(0.85-1.55)	(7.3-8.1)	(0.82-1.54)	(7.4-8.0)	(0.64-1.47)

95% limits shown in brackets. n = 12-23 tissues per Schild plot. Three or four concentrations (over a 10 to 30 fold range) of each antagonist were tested.

ZAC had a pA_2 value of 7.6 in both the GPI and the GPVN preparations but was some 30 fold more potent in the RVN. ICS and GR had similar profiles of potencies between tissues as ZAC.

The potencies of the 5-HT_3 receptor antagonists in the two tissues from the guinea-pig would be consistent with an action of 5-HT at the same type of receptor in both tissues, their greater potency in the RVN would suggest a different 5-HT_3 receptor subtype in the rat.

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EFFECT OF N-α-BENZOYL -L- ARGININE ETHYL AND METHYL ESTERS ON THE RAT MESENTERIC VASCULAR BED

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The pathway for the biosynthesis of nitric oxide (NO) in vascular endothelial cells is not clear although L-arginine has been proposed as the physiological precursor to NO in bradykinin-challenged cultured aortic endothelial cells (Palmer et al., 1988) and in the rat aorta in vitro (Schmidt et al., 1988). Recently, Thomas & Ramwell (1988) reported that N- α-benzoyl-L-arginine ethyl ester (BAEE) has potent vasodilator activity and proposed that an arginine containing peptide rather than L-arginine itself is the natural precursor to NO. We report here the vasodilator effect of several arginine salts including BAEE and the corresponding methyl ester (BAME) in the perfused rat mesenteric vascular bed.

Rats (male, Sprague-Dawley, 250-400 g) were killed by a blow to the head and exsanguinated. The mesentery was removed to a heated chamber and perfused via the superior mesenteric artery with warmed (37° C), oxygenated (95% O_2 :5% CO_2) Krebs solution containing indomethacin (8 μM). Perfusion pressure was increased to approximately 70-90 mm Hg by addition of noradrenaline (0.06-0.25 mM) to the Krebs perfusate.

Bolus injection of acetylcholine (ED50, 6.1 ± 0.2 nmol, n=5) or nitroprusside (ED50, 8.2 ± 0.9 nmol, n=5) produced transient, dose related falls in perfusion pressure. BAEE and BAME also exhibited vasodilator activity with ED50 values of 1.0 ± 0.01 μmol, (n=5) and 2.9 ± 0.2 μmol, (n=5) respectively. The dose of BAEE required for threshold vasodilator activity in the rat mesentery was 0.1 μmol whilst the maximal fall in perfusion pressure which could be achieved was 37.4 ± 3.1 mm Hg, (n=5, 18.5 μmol). L-arginine hydroxamate (ED50, 3.1 ± 0.7 μmol, n=4) and L-arginine methyl ester (ED50, 2.2 ± 0.3 μmol, n=3) were also vasodilator in this preparation. L-arginine chloride and L-homoarginine chloride were inactive at doses up to 20 μmol. L-arginine base produced small vasoconstrictor responses at high doses (1-20 μmol) but was devoid of vasodilator activity. Responses to acetylcholine, BAEE and BAME were reduced (approx. 65-85%) by addition of gossypol (1 μM) to the Krebs perfusate and abolished following de-endothelialisation with 3,3 cholamidopropyl dimethylammonio 1-propanesulphonate (CHAPS, 4.7 mg/ml, 30 s). Vasodilatation due to nitroprusside was unaffected by gossypol or CHAPS treatment.

Selected arginine salts have been shown to produce dose related vasodilatation of the preconstricted rat perfused mesentery preparation most probably mediated by NO. From the present results it is not possible to determine whether arginine salts cause the release of or act as precursors to NO in this preparation.

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EFFECT OF ARGININE SALTS AND NITRIC OXIDE ON RESISTANCE BLOOD VESSELS OF THE PERFUSED RAT KIDNEY

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The possibility that L-arginine is the physiological precursor for nitric oxide (NO) in vascular endothelial cells has recently been suggested (Palmer et al. 1988; Schmidt et al. 1988). The enzyme(s) which effect this transformation remain unidentified although an arginine deimidase capable of converting L-arginine to nitrite has been demonstrated in macrophages (Hibbs et al. 1987) and may thus be considered a prime candidate for this role in the vascular endothelium. We have now investigated the vasodilator effect of several L-arginine salts and NO in the perfused rat kidney.

Kidneys were removed from male Sprague-Dawley rats (250-350 g) and perfused with warmed Krebs solution containing indomethacin (8μM). Preparations were preconstricted by addition of noradrenaline (0.1-0.5 μM) to the Krebs perfusate. Bolus injection of acetylcholine (ACh), NO, nitroprusside (NP) or L-arginine chloride produced transient and dose related falls in perfusion pressure with ED50s (all n=4-6) of 0.18± 0.05 nmol, 0.66 ± 0.11 nmol, 2.3 ± 0.3 nmol, 14.0 ± 1.2 μmol respectively. L-arginine hydroxamate (ED50 4.4 ± 0.5 μmol, n=5), L-arginine methylester (ED50 2.2 ± 0.4 μmol, n=5) and L-homoarginine chloride (ED50 12.0 ±1.3 μmol, n=5) were also vasodilator whereas D-arginine chloride was inactive at doses up to 170 μmol.

Addition of L-arginine chloride (100 μM) to the Krebs perfusate did not influence vasodilatation due to ACh (ED50 0.21 ± 0.07 nmol, n=5) or NP (ED50 2.6 ± 0.6 nmol, n=5) while at higher concentrations (500μM) responses to both vasodilators were reduced by approximately 40-50%. L-canavanine, a structural analogue of L-arginine, has been reported to inhibit conversion of arginine to nitrite by macrophages (Iyengar et al. 1987) and to inhibit ACh-induced relaxation of rat aortic rings in vitro (Schmidt et al., 1988). In the present experiments, addition of L-canavanine (50 μM) to the Krebs perfusate failed to influence vasodilatation due to ACh (ED50 0.22 ± 0.05 nmol, n=5) or NP (ED50 2.5 ± 0.6 nmol, n=5). At a higher concentration of L-canavanine (150 μM), responses to both drugs were reduced by 50-60%.

L-arginine salts and their putative metabolite NO exhibit vasodilator activity in the perfused rat kidney. NO is approximately 1000 times more potent. Vasodilatation due to ACh or NP were not potentiated by arginine "loading" of perfused rat kidneys. High doses of L-canavanine non-selectively depressed responses to both ACh and NP.

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EFFECTS OF ACTIVATION AND INHIBITION OF PROTEIN KINASE C ON RELEASE OF OXYTOCIN AND VASOPRESSIN FROM RAT NEUROHYPOPHYSIS

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Neurohypophysial release of vasopressin and oxytocin shows a very pronounced frequency-dependent facilitation, i.e. low frequencies (<5 Hz) are markedly less effective than higher frequencies in inducing hormone secretion (Hobbach et al., 1988). In different neuronal tissues it was shown that activation of protein kinase C can facilitate neurotransmitter secretion. Therefore, in the present study the effects of several phorbol esters, known to activate (phorbol 12,13-dibutyrate, PDB) or not to affect (4α-phorbol 12,13-dideconate and phorbol 12-monoacetate) protein kinase C, and of the direct protein kinase C inhibitor 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7) were tested on the frequency-dependent release of vasopressin and oxytocin.

Isolated neural lobes with their stalks fixed to a platinum wire were superfused with Krebs solution. Oxytocin and vasopressin released into the medium were determined by RIAs. After 57 min of superfusion hormone release was evoked by electrical stimulation of the pituitary stalk (450 pulses 0.2 ms, 30 mA at a frequency of 3 or 15 Hz. Test drugs were added to the superfusion medium from the onset of superfusion.

Stimulation at 15 Hz evoked the release of 505±28 µU vasopressin and 557±51 µU oxytocin (means±s.e.m, n=25). Stimulation at 3 Hz induced the release of only 41±5 µU vasopressin and 59±7 µU oxytocin (n=21). PDB (1 µM) increased the release of vasopressin evoked by 15 Hz stimulation maximally by about 40-50 % and that evoked by 3 Hz stimulation by about 150 %. The release of oxytocin evoked by 15 Hz stimulation was increased by about 150 % and that evoked by 3 Hz stimulation by about 400-500 % in the presence of PDB. Both inactive phorbol esters had no effects on the evoked release of vasopressin or oxytocin. The effect of PDB on the release of vasopressin and oxytocin was blocked by H7 (10-30 µM). H7 (30 µM) alone reduced the release of vasopressin evoked by stimulation at 15 Hz by 50 %. The release of oxytocin was not significantly affected by H7. As the release of oxytocin from the isolated neurohypophysis is under inhibitory control of endogenous opioids (Bicknell & Leng, 1982; Racké et al, 1987), some experiments were repeated in the presence of naloxone (1 µM). The release of oxytocin evoked by 3 and 15 Hz stimulation was increased by about 175 and 105 %, respectively. In the presence of naloxone, H7 (30 µM) had no effect on the release of oxytocin evoked by stimulation at 15 Hz and PDB caused an increase of the release of oxytocin similiar to that in the absence of naloxone.

In conclusion, activation of protein kinase C can facilitate impulse-induced hormone secretion from neurosecretory nerve endings. Under the present in vitro conditions, an endogenous activation of protein kinase C appears to be involved in the frequency-dependent facilitation of vasopressin, but not of oxytocin secretion. Inhibition of oxytocin release by endogenous opioids and facilitation by activation of protein kinase C appear to operate independently.

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CONTRASTING GLUCOCORTICOID EFFECTS ON HYPERALGESIA INDUCED BY YEAST AND CARRAGEENAN IN RATS

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We have previously reported that subplantar injection of 1% (w/v) carrageenan (Viscarin, Marine Colloids) induced oedema but not hyperalgesia in rats under conditions in which 20% (w/v) yeast produced both responses (Haworth et al, 1988). In view of published reports of hyperalgesia in response to 1% carrageenan (Winter and Flataker, 1965; Ferreira et al, 1978) we have investigated these findings further, with particular regard to the modulation of hyperalgesia by glucocorticoids. Methods used were as previously described (Carey et al, 1988).

Increasing the concentration of carrageenan injected to 2% (w/v) led to the development of hyperalgesia which was first observed at 3h post-injection and was maximal at 4h (60±9%, mean±s.e.m., n=6). Hyperalgesia was associated with elevation of paw exudate 6oxo$PGF_{1\alpha}$(5.57±4.93ngml^{-1}) and PGE_2(0.79±0.19ngml^{-1}) compared with <0.3 and <0.5ngml^{-1} respectively in saline-injected paws. Rats dosed with 20% yeast at the same time developed maximal hyperalgesia of 59±9% and paw exudate 6oxo$PGF_{1\alpha}$ and PGE_2 were elevated to 2.87±1.05 and 3.17±0.39 ngml^{-1} respectively.

Subplantar injection of 1% carrageenan elevated plasma corticosterone, measured using a specific radioimmunoassay, to 108±19, 109±39 and 357±39ngml^{-1} at 0.5, 1 and 3h post-injection respectively compared with 56±21, 61±11 and 32±5ngml^{-1} in plasma from uninjected rats sampled at the same time. Injection of 20% yeast also elevated plasma corticosterone to 541±45, 300±30 and 345±16ngml^{-1} at 0.5, 2 and 4h respectively compared with 30±4ngml^{-1} in untreated rats. Adrenalectomised rats developed hyperalgesia in response to 1% carrageenan (max. 53±14%) in contrast to sham-operated controls while yeast-induced hyperalgesia was unaffected by adrenalectomy. Dexamethasone (1.0mgkg^{-1}s.c.) abolished hyperalgesia induced by 2% carrageenan while 0.1 and 0.01mgkg^{-1} reduced hyperalgesia by 74±17% and 71±19% respectively. Hyperalgesia in response to 20% yeast was not inhibited by dexamethasone (0.01-1.0mgkg^{-1} s.c.).

These results indicate that hyperalgesia did not develop in response to 1% carrageenan due to high endogenous glucocorticoid levels which probably led to inhibition of the phospholipase responsible for providing the substrate for synthesis of hyperalgesic prostaglandins. In contrast, yeast-induced hyperalgesia was insensitive to glucocorticoid inhibition suggesting that a different phospholipase may be involved in this response.

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INHIBITION OF HISTAMINE RELEASE FROM HUMAN BASOPHILS AND RAT PERITONEAL MAST CELLS BY CYCLOSPORIN-A

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Cyclosporin-A (CS-A) is a cyclic peptide of fungal origin which possesses unique and potent immunosuppressive activities (Borel, 1976), and currently employed widely in prevention of graft rejection in tissue transplants. Early reports suggested that the drug acts selectively on T-cells suppressing their ability to generate interleukin-2 (IL-2), and other lymphokines (Burckhardt & Guggenheim, 1979). However, more recent studies have shown that certain other cell types may in fact be affected to quite a considerable extent (Fan & Lewis, 1982; Pederson *et al.*, 1985). We have therefore studied the effect of CS-A on the release of the allergy mediator - histamine from human basophils and rat peritoneal mast cells (RPMC).

Human leucocytes were isolated from heparinized blood by methylcellulose sedimentation of red cells. RPMC were obtained from Sprague Dawley rats immunized 2-6 weeks previously with 50mg ovalbumin and 10^8 *Bordetella pertussis* organisms per animal given i.m. Cells were washed twice in Ca^{2+}-free Hepes-buffered tyrode solution (pH 7.3) and incubated in Ca^{2+}-containing buffer with antigen or other releasers. Histamine was assayed fluorometrically.

The drug strongly inhibited histamine release from human basophils induced by mite antigen, anti-IgE, Con-A and calcium ionophore A23187. Inhibition was dose-dependent and occurred in the dose range 0.01-10μM (see Table). Maximum inhibition varied widely (52-90%) depending on inducer. The IC_{50} values are 0.03, 0.12, 0.36 and 4.0μM for Con-A, anti-IgE, A23187 and antigen respectively. With Con-A, anti-IgE and A23187, the drug acts promptly when added at the same time as the inducers but with antigen, inhibition increases with time of pre-incubation. The drug is also effective whether present only in the first stage (activation) or only in the 2nd stage (release).

	Mean % inhibition of histamine release ± sem (n=5-8)			
	0.01	0.1	1.0	10.0(Cs-A μM)
Mite (Ag) (0.1μg/ml)	0.0	29.1±9.0	46.9±9.2	56.0±8.0
A-IgE (1:1000)	29.4±6.0	49.2±4.2	62.1±8.7	75.3±8.1
Con-A (5μg/ml)	41.9±16.5	57.9±8.6	82.7±10.9	77.3±5.3
A23187 (1.0μM)	32.1±4.6	41.7±8.8	57.5±9.5	66.2±14.6

The drug also inhibited histamine release from RPMC induced by antigen and various non-immunological releasers, but to a lesser extent, particularly 48/80. The IC_{50} values are 0.3, 23.0, 33.0μM for release induced by Con-A, A23187 and ovalbumin respectively. When histamine release was induced in Ca^{2+} free conditions, by agents such as compound 48/80, substance P and X537A, the drug had little or no effect, thus suggesting an interference with Ca^{2+} influx or utilization.

The results suggest that at therapeutically attainable doses CS-A possesses a potent antiallergic effect especially on human basophils, in addition to its traditional inhibition of T-lymphocyte responses.

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ADENOSINE A_1 RECEPTOR ACTIVATION INHIBITS FUROSEMIDE INDUCED RENIN RELEASE IN BOTH ANAESTHETISED AND CONSCIOUS RATS

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A role for adenosine has been proposed in the regulation of renal haemodynamics and renin release (Tagawa and Vander,1970), and recently Churchill and Bidani (1987) demonstrated in the anaesthetised rat that N^6-cyclohexyladenosine (A_1 agonist) decreased renin release, while N-ethylcarboxamido-adenosine (A_2 agonist) increased renin release. This study examines the effect of another A_1 agonist (L-Phenylisopropyladenosine) on plasma renin concentration (PRC) and its effect on changes in PRC induced by furosemide in anaesthetised and conscious rats.

Male Sprague-Dawley Rats (300-400g) were used throughout the study. Rats were anaesthetised with Inactin (120mg/kg), the trachea was cannulated and the animals were allowed to respire normally. Cannulae were inserted for measurement of blood pressure (BP), heart rate (HR), the withdrawl of blood for PRC determination and drug administration (Furosemide 2.5mg/kg i.v. and L-PIA 10nmole/kg bolus + 6nmole/kg/hr i.v.). Results are expressed as change in plasma renin concentration from pretreatment value and the units of renin are shown as nanograms of angiotensin 1 per millilitre of plasma per hour of incubation with the substrate (ng Al/ml/hr).In conscious rats L-PIA (100ug/kg) and furosemide (25mg/kg) were administered orally and 60 minutes post dose the animals were sacrificed and blood was withdrawn from the vena cava for PRC determination. Results are expressed in absolute values since PRC was only determined at a single time point.

In anaesthetised rats basal plasma renin concentrations did not differ significantly between treatment groups and the mean value was 4.92±0.41 (n=31). In saline treated contol rats PRC fell slighty (-0.39±0.42 ng Al/ml/hr, n=8), whilst treatment with furosemide increased the PRC (+0.74±0.46 ng Al/ml/hr, n=8). In these rats HR and BP remained stable throughout the experiment. L-PIA treatment significantly ($p<0.001$) reduced the PRC (-3.45±0.41 ng Al/ml/hr, n=7); it also reduced HR (-45.7±10.3 BPM) and DBP (-11.4±2.4 mmHg). In the presence of furosemide L-PIA significantly ($p<0.001$) reduced the PRC (-2.0±0.4 ng Al/ml/hr, n=8); it also reduced HR (-65±9.8 BPM) and DBP (-11.9±1.3 mmHg). This fall in PRC was significantly ($p<0.05$) less than that induced by L-PIA alone but the effects on BP and HR were not significantly different. In conscious rats the basal PRC was 3.46±0.7 ng Al/ml/hr (n=13) but in contrast to the study in anaesthetised rats L-PIA did not significantly reduce PRC (3.27 ±0.5 ng Al/ml/hr, n=13). Furosemide significantly stimulated renin release (8.5±1.0 ng Al/ml/hr, n=7), an effect abolished by L-PIA (2.9±0.7 ng Al/ml/hr, n=7).

Activation of adenosine A1 receptors is reported to inhibit basal renin release and the reduction of PRC induced by L-PIA in anaesthetised rats supports this hypothesis. The results obtained with L-PIA in conscious rats is not consistant with the above hypothesis and may indicate an interaction with the effect of the anaesthetic on PRC. However the attenuation, by L-PIA, of the stimulatory action of furosemide on renin release supports a modulatory role for A1 receptors. Thus we propose that when renin release is stimulated, either by adrenergic activation (as seen during anaesthesia) or by 'a signal from the macula densa' (activated by furosemide administration), then adenosine A1 receptors may modulate this release.

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A RADIOISOTOPIC MODEL OF PLATELET AGGREGATION IN THE RABBIT IN VIVO

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111-Indium oxine labelled platelets have been used in a variety of species to assess platelet behaviour in vivo. We have previously described a non-invasive technique for the automated, continuous, external imaging of 111-Indium labelled platelets in experimental animals (Page et al, 1982; Oyekan & Botting, 1986). However, in vitro studies have suggested that 111-Indium oxine is not a suitable label for rabbit platelets as the 111-Indium binds to dense bodies rather than cytosolic components and that this 111-Indium is released upon activation (Baker et al,1982). Since the rabbit is a useful model for investigating a number of platelet related disorders e.g. atherosclerosis (Jayakody et al, 1988), we have evaluated the usefulness of 111-Indium oxine as an in vivo label for platelets in this species.

Platelets were isolated from citrated blood collected from the marginal ear vein of donor rabbits (NZW 2-3kg), washed free of plasma by centrifugation (640 G) and labelled with 111-Indium oxine as previously described (Page et al,1982). Recipient rabbits were anaesthetized with Hypnorm (Janssen Pharmaceutica, 0.4ml/kg) and one inch scintillation detectors placed over the thoracic, abdominal and cranial regions. 111-Indium labelled platelets were infused slowly into the marginal ear vein and allowed to equilibrate for one hour. Counts were recorded continuously in the various anatomical regions using an automated isotope monitoring system (AIMS 8000, Mumed Ltd) as descibed elsewhere (Page et al, 1982).

I.v. collagen (10-180 ug/kg) elicited a dose related increase in thoracic counts which was maximal 3 minutes after the injection and which returned to preinjection levels within 30 minutes. A concomitant fall in both the cranial and abdominal regions was also observed. The percentage of free 111-Indium oxine in the plasma before and after collagen (100 ug/kg) was not significantly different (9.84 $\pm$ 6.5 and 10.49 $\pm$ 6.7 respectively, n=5). I.v. ADP (0.56-100 ug/kg) and thrombin (10-32 iu/kg) also induced increases in thoracic counts with concomitant decreases in counts in the cranial and abdominal regions. Intracarotid (i.a.) ADP (3.2-100 ug/kg), collagen (25-500 ug/kg) and thrombin (18-100 iu/kg) also induced dose related increases in thoracic counts and decreases in cranial and abdominal counts. The initial fall in cranial counts induced by thrombin was followed by an increase which was sustained for up to 3 hours.

The present results suggest that 111-Indium oxine is a suitable label for the investigation of rabbit platelets in vivo and the platelets will respond to various agonists. It is hoped that this model will allow further investigation into the mechanisms of platelet activation in the arterial and venous circulation and may provide a novel model for investigating thromboembolic disorders in the cerebral circulation.

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INHIBITORY EFFECTS OF α_1- AND α_2-ADRENOCEPTOR AGONISTS ON GASTRO-INTESTINAL MOTILITY IN THE RAT IN VIVO

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The non-selective α-adrenoceptor agonist clonidine (Medgett et al., 1978) has been reported to inhibit gastric emptying (Cooper and McRitchie, 1985) in the rat in vivo. Other reports have demonstrated an effect of clonidine on small intestinal transit in the rat without an effect on gastric emptying (Ruwart et al. 1980). These effects are thought to be due to stimulation of inhibitory prejunctional α_2-adrenoceptors located on intrinsic excitatory cholinergic neurones in the myenteric plexus resulting in a decrease in acetylcholine (ACh) release and an inhibition of motility. In the present study the effects of selective α_1- and α_2-adrenoceptor agonists and antagonists were examined on three parameters of gastrointestinal motility in conscious rats; gastric emptying, small intestinal transit and distal colonic transit.

Male Sprague-Dawley rats (150-300g) were starved overnight and deprived of access to water two hours before the beginning of the experiments. Gastric emptying was measured by the phenol red liquid meal technique of Scarpignato et al., (1980). Small intestinal transit was measured by the method of Boura and Fitzgerald (1966) and involved measuring the distance travelled by a charcoal meal bolus. Colonic transit was measured by a new non-invasive technique modified from a method first described by Schottek (1967) by measuring the time of expulsion of a coloured agar bolus injected 5cm from the anus into the distal colon.

The selective α_1-adrenoceptor agonist cirazoline (3 and 10mg/kg p.o.) significantly inhibited gastric emptying, small intestinal transit and colonic transit in the rat all at the same dose levels. This inhibition of gastro-intestinal motility was antagonised by prazosin (3mg/kg p.o.), but unaffected by idazoxan (3mg/kg p.o.). In contrast the selective α_2-adrenoceptor agonist UK-14,304 (0.1-3mg/kg p.o.) had no effect on gastric emptying, but did inhibit small intestinal and distal colonic transit at a dose level below 0.3mg/kg p.o.. The inhibitory effect of UK-14,304 on small intestinal transit was antagonised by idazoxan (3mg/kg p.o.), but unaffected by prazosin (3mg/kg p.o.). UK-14,304 (0.03-0.3mg/kg p.o.) inhibited distal colonic transit and this inhibition was antagonised by idazoxan (0.3mg/kg p.o.), but unaffected by prazosin (0.3mg/kg p.o.). Neither prazosin or idazoxan in dose levels up to 10mg/kg p.o. had any effect on any parameter of gastrointestinal motility studied.

In conclusion, the present study demonstrates that there is no separation between the α_1-adrenoceptor mediated inhibition of gastric emptying, small intestinal transit and colonic transit induced by cirazoline. Small bowel and distal colonic transit are much more susceptible than gastric emptying to inhibition by the selective α_2-agonist UK-14,304. Therefore, α_2-adrenoceptors may be involved in regulating intestinal motility by modulating the activity of intrinsic neurones controlling normal gastrointestinal reflexes such as peristalsis.

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EFFECT OF CHRONIC TREATMENT OF THE GUINEA-PIG WITH β-ADRENOCEPTOR AGONIST AND PROPRANOLOL ON THE Na^+, K^+-PUMP IN THE SKELETAL MUSCLE

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Recent evidence suggest a close relationship between the Na^+,K^+-pump and β-adrenoceptors. Thus β-adrenoceptors coupled to Na^+,K^+-pump through cyclic AMP-protein kinase mechanisms in the skeletal muscle has been reported to mediate the hypokalaemic response to adrenaline (Buur et al 1982). We have previously shown that chronic pretreatment of the guinea-pig with β-adrenoceptor agonists desensitised and reduced the density of β-adrenoceptors while pretreatment with propranolol increased the density of β-adrenoceptors in the skeletal muscle (Elfellah & Reid 1987). In the present study the effect of chronic pretreatment with these drugs on the Na^+,K^+-pump was further investigated.

Male guinea-pigs weighing 250-350 gm were divided into 4 groups, Each group received vehicle (0.9% saline containing 0.1% ascorbate), isoprenaline (50 $ug.kg^{-1}$) terbutaline (125 $ug.kg^{-1}$) or propranolol (20 $mg.kg^{-1}$) subcutaneously three times daily for one week. The animals were sacrificed and crude membrane preparations from the gastrocnemius muscle were prepared for binding studies with [^{3}H]-ouabain (Elfellah & Reid 1988). ^{86}Rb uptake in isolated intact soleus muscle was used to measure the function of the Na^+,K^+-pump (Hougen & Smith 1978). The difference between Rb uptake in the presence and absence of ouabain (1mM) was taken to present the activity of the Na^+,K^+-pump.

Chronic pretreatment of the guinea-pig with isoprenaline or terbutaline significantly reduced the density of [^{3}H]-ouabain binding sites in gastrocnemius muscle membranes without altering the K_D (Table 1). Pretreatment with propranolol did not significantly affect the B_{max} or the K_D. Ouabain-sensitive Rb-uptakes in soleus muscle were 10.8 ± 1.2 (8) and 9.0 ± 0.4 (10) $mmol.gm^{-1}$ wet tissue 20 min^{-1} in control and terbutaline pretreated guinea-pigs respectively ($p > 0.05$ results are mean ± s.e. with number of animals).

Table 1 Effect of chronic pretreatment of the guinea-pigs with β-adrenoceptor agent on binding of [^{3}H]-ouabain in gastrocnemius muscle (mean ± s.e., n = number of guinea-pigs) * $p < 0.05$ ** $p < 0.01$ ANOVA

Pretreatment (n)	Control (14)	Isoprenaline (7)	Terbutaline (7)	Propranolol (8)
B_{max} ($pmol.mg^{-1}$protein)	5.3 ± 0.6	3.1 ± 0.4*	2.0 ± 0.2**	3.9 ± 0.4
K_D (nM)	159 ± 24	176 ± 49	215 ± 50	146 ± 23

The results show that chronic pretreatment of the guinea-pig with β-adrenoceptor agonists reduced the density of the [^{3}H]-ouabain binding sites without altering the function of the Na^+,K^+-pump measured by Rb uptake. This suggest the presence of spare receptors or ouabain binding sites unrelated to ^{86}Rb uptake function of the pump.

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CHARACTERISATION OF [^{3}H]IDAZOXAN BINDING TO NON α_2-ADRENOCEPTOR SITES IN RABBIT FAT CELL MEMBRANES

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As human fat cells, rabbit fat cells are characterized by a weak responsiveness to physiological amines. This response could be explained by an increased α2-responsiveness (Lafontan, 1981). In recent years selective [3H]antagonists ([3H]yohimbine, [3H]rauwolscine, [3H]idazoxan) have been used as probes for α2-site identification in a large variety of tissues and species. Biological assays and binding analysis have revealed species differences in α2-adrenoceptor characteristics (Bylund, 1985). To characterize rabbit fat cells α2-adrenoceptors, we performed binding studies in crude plasma membranes from rabbit fat cells with [3H]yohimbine, [3H]idazoxan, the imidazoline antagonist and [3H]UK-14,304, the imidazolidine agonist.

Perirenal fat cell ghosts were prepared and binding studies were performed in a 50mM Tris-HCl buffer (pH=7.5) containing 0.5mM Mg2+ as previously described (Berlan & Lafontan, 1985).

In rabbit fat cell membranes, [3H]yohimbine was unable to bind to α2-sites (low affinity whatever the buffer used -Tris or glycylglycine-, no clear saturation, low percentage of specific binding) while [3H]UK-14,304, an α2-adrenergic agonist, clearly revealed the existence of typical α2-sites. Saturation analysis of specific binding of [3H]UK-14,304 (defined as binding inhibited by 100 µM epinephrine) gave the following binding parameters: KD=1.9±0.2 nM and Bmax=385±41 fmoles/mg protein with a nH coefficient of 1.05 (n=3). The existence of alpha2-adrenoceptors was confirmed in biological assays; UK-14,304 and epinephrine (associated with propranolol) exhibited antilipolytic effects in rabbit fat cells.

Binding assays performed with [3H]idazoxan revealed some striking differences. The binding of the imidazoline antagonist was not displaced by 200 µM epinephrine or norepinephrine. However, when non-specific binding was defined with 10 µM naphazoline or tramazoline, a specific binding of [3H]idazoxan to rabbit fat cell membranes was observed (KD=1.6±0.6 nM; Bmax=1370±91 fmoles/mg protein; n=3). The Scatchard plot was linear and Hill coefficients (nH=1.01) revealed the existence of a single-class of [3H]idazoxan binding sites in rabbit fat cell membranes.

In a second step, we investigated ligand specificity of [3H]idazoxan binding (naphazoline defined) to rabbit fat cell membranes. The naturally occurring phenylethanolamines (epinephrine and norepinephrine) as well as the α1-agonists (phenylephrine and amidephrine) failed to displace [3H]idazoxan binding sites. Moreover, yohimbine, prazosin, propranolol and histamine did not compete [3H]idazoxan binding sites. Imidazoline and imidazolidine compounds competed for these sites with an unclassical order of potency: idazoxan (Ki=2.1±0.3nM) > naphazoline (9.2±0.7nM) > tramazoline (27±7nM) > UK-14,304 (948±135nM) > clonidine (975±416nM) > RX821037 (5210±330nM) > oxymetazoline (>10000nM).

A striking observation concerns amiloride (inhibitor of the Na+/H+ exchanger) which strongly competed with [3H]idazoxan binding with a mean Ki value of 48±6 nM (n=4). Pseudo-Hill coefficient (nH) was not significantly different from 1, suggesting interaction at a single site with no cooperativity among the sites.

These data provide evidence of high affinity, large capacity [3H]idazoxan binding sites in rabbit fat cell membranes, which are not α2-adrenoceptors.

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ENDOGENOUS CATECHOLAMINES DIFFERENTIALLY REGULATE β_1 AND β_2 DOG FAT CELL ADRENOCEPTOR

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The effects of long term endogenous catecholamine exposure on the regulation of leukocyte and adipocyte β-adrenoceptor subtypes were studied in an experimental model of chronic neurogenic hypertension induced by sinoaortic denervation (SAD) in dogs (Valet et al, 1988).

Using highly selective β agonist (procaterol for β2-adrenoceptors) and antagonists (bisoprolol for β1-; ICI 118,551 for β2-adrenoceptors) in lipolysis and binding studies, we first demonstrate that the normal dog fat cell possesses both β1- and β2-adrenoceptors in relative proportions of 67% and 33% .

Chronic SAD is associated after 45 days with a significant increase ($p<0.05$) in plasma noradrenaline (330%) and adrenaline (250%) levels, a 52% reduction in total leukocyte β-adrenoceptor number (125I cyanopindolol binding sites) and a 61% decrease in total omental adipocyte β-adrenoceptor number (3H dihydroalprenolol binding sites).

The increase in catecholamine levels elicited by SAD also promoted a strong reduction in β2- (but not β1-) adrenoceptor number (table 1) leading to a new relative distribution in β-adrenoceptors in adipocyte membranes: 36% for β2- and 64% for β1-adrenoceptors.

Table 1 Plasma catecholamines and β-adrenoceptors in control and SAD dogs

	CATECHOLAMINES (pg/ml)		β-ADRENOCEPTORS (fmol/mg protein)			
	NA	ADR	β-LEU	β-ADI	β1-ADI	β2-ADI
CONTROLS	212±36	86±48	48±2	355±73	118±16	237±28
SAD DOGS	700±71*	214±19*	23±4*	146±19*	94±15	52±13*

Means are values±sem, *$p<0.05$ when significantly different from control values. NA: noradrenaline; ADR: adrenaline, β-LEU: total 125I cyanopindolol binding sites on leukocytes, β-ADI: total 3H dihydroalprenolol binding sites on adipocytes.

Such a differential regulation provokes major consequences in the lipolytic response of the adipocytes. Lipolysis induced by adrenaline or isoprenaline (two mixed agonists) was weakly but significantly ($p<0.05$) reduced whereas lipolysis induced by procaterol was strongly reduced (about 80%). These data fit with binding studies and demonstrate the loss in β2-adrenoceptor number and response in SAD dogs.

The present study demonstrates a differential regulation of β1- and β2-adrenoceptors: β2- but not β1-adrenoceptor number are decreased by long term exposure to high plasma levels of endogenous catecholamines in the dog.

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FURTHER CHARACTERISATION AND PURIFICATION ON GADDUM'S 'SUBSTANCE R'

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We recently confirmed that the biologically active principle released from perfused rat intestine, and dubbed 'substance R' by Gaddum (1953), is a protein with oxytocic properties (Douglas et al 1988). Furthermore, we observed that the spectrum of pharmacological actions of substance R were similar, but not identical, to those of a glandular kallikrein. We now report the results of experiments designed to purify and further characterise the nature of substance R.

Substance R was collected as described previously (Douglas et al, 1988). Briefly, intact mesenteric beds of rats were perfused with Tyrode's solution and after 30 min equilibration effluent was collected for 6 hours. The effluent from 3 experiments were combined and dialysed overnight against 15 volumes of 50 mM ammonium bicarbonate and then freeze dried. Three such preparations were re-dissolved in 6 ml of phosphate buffer (50 mM, pH 7.5) and filtered.

Two purification strategies were used. Firstly, the soluble extract was applied to a Sephacryl S 300 column (2.6 x 100 cm) equilibrated in PBS (flow rate 0.4 ml/min) and 5.0 ml fractions were collected. The biological activity in each fraction was tested on the superfused rat uterus as described (Douglas et al 1988) and the molecular weights of the biologically active substance calculated. Secondly, the crude dialysed substance R was applied to a DEAE Sepharose ion exchange column (2.6 x 40 cm) equilibrated in Tris buffer 50 mM pH 7.5 . Fractions of 5.0 ml were eluted (0.4 ml/min) by a linear salt gradient, and tested for oxytocic activity.

Chromatography on Sephacryl alone resolved a single peak with strong biological activity (apparent molecular mass of 19-25 KD). SDS-PAGE of these fractions revealed that they were still contaminated with many other proteins. When applied to the DEAE ion exchange column, substance 'R' could be eluted with approximately 0.2 M NaCl. When the two techniques were combined so that the active ion exchange fractions were applied to the Sephacryl column the molecular weight of the biological activity was estimated at approximately 40 KD. When examined by SDS-PAGE the fractions contained small quantities of proteins of approximately 90, 80 and 55 KD but in addition, fractions displaying peak biological activity also contained a protein of 40 KD. Urinary kallikrein when chromatographed in the same manner had an apparent molecular weight of 42 KD. It is possible that the discrepancy in molecular weights of substance R was caused by the removal, during ion exchange chromatography, of a proteolytic enzyme.

In collaboration with Mr Carlos Figueroa at the University of Bristol we have established that substance R shares immunological epitopes with tissue kallikrein as assessed in an Ouchterlony double diffusion assay system.

Our data suggests that substance R is a proteolytic enzyme with a molecular weight of approximately 40 KD which is immunologically and biochemically related to the kallikrein family of enzymes and yet is not identical to tissue or plasma kallikrein.

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ANTAGONISTS OF BRADYKININ-INDUCED BICARBONATE SECRETION BY GUINEA-PIG GALLBLADDER

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Modifying the amino acid sequence of bradykinin (BK) has led to the discovery of peptides which antagonise the actions of BK on rat uterus, guinea pig ileum, rat blood pressure, vascular permeability, PI turnover and PGE_2 release from cultured cells (Stewart & Vavrek, 1987) as well as BK stimulation of colonic chloride secretion (Baron et al., 1987). In this study we have investigated the effects of five BK analogues on a novel action of BK: stimulation of bicarbonate secretion by guinea pig gallbladder (Baird & Margolius, 1988).

Hemi-gallbladders from female guinea pigs were mounted in Ussing chambers (window area 0.6 cm^2), bathed on either side with identical Krebs-Henseleit solutions, gassed with 95%O_2, 5%CO_2 and maintained at 37°C. The electrical potential difference across the epithelium was maintained at zero by continuously applied short circuit current (SCC), a measure of net electrogenic ion transport. All drugs were added to the mucosal bathing solution. Cumulative dose response curves to BK (0.01-3.3 µM) were constructed using a two minute time cycle. In control experiments BK produced dose related increases in SCC, previously shown to represent electrogenic bicarbonate secretion (Baird & Margolius, 1988). The ED_{50} was 0.1 µM and the maximum change in SCC was 44.0 ± 4.1 µA/cm^2 (n=25). BK analogues (1,3,10 µM) were added one minute before starting a dose response study. The results are summarized in the table:

Table 1. Effects of BK analogues on BK-stimulated SCC

		n
[DPhe^7,Hyp^9]-BK	no effect	6
[Phe^2,DPhe^7]-BK	no effect	5
[DPhe^{2,7}]-BK	no effect	4
DArg-[Hyp^3,Thi^{5,8},DPhe^7]-BK	pA_2 = 6.4	5
Lys-Lys-[Thi^{5,8},DPhe^7]-BK	pA_2 = 6.2	4

Thi = β-(2 thienyl)-L-alanine, Hyp = L-4-hydroxyproline

None of the analogues had any effect on basal SCC even at 10 µM. Two of the peptides with basic amino acids added at the N-terminus and Thi substituted for Phe at positions 5 and 8 of the parent molecule acted as competitive antagonists. The BK dose response curves were shifted to the right in parallel permitting the estimation of pA_2 values by Schild analysis.

We have previously shown that kinin stimulation of gallbladder bicarbonate secretion is dependent upon eicosanoid synthesis. SCC stimulated by 0.1 µM PGE_2 (42.3 ± 4.3 µA/cm^2, n=4) was not altered by one minute pretreatment with 5 µM DArg-[Hyp^3,Thi^{5,8},DPhe^7]-BK (44.2 ± 5.3 µA/cm^2, n=4).

These results identify selective, potent and competitive antagonists of kinin stimulated gallbladder bicarbonate secretion.

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INHIBITION OF NEUTROPHIL ACCUMULATION BY DEXAMETHASONE IN VIVO: EVIDENCE FOR SITE OF ACTION

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Glucocorticosteroids inhibit neutrophil infiltration in inflammatory reactions. Possible sites of action are: (1) the generation of chemotactic mediator; (2) the microvascular endothelial cell; or (3) the neutrophil. Previously we showed that neutrophil accumulation in response to exogenous chemoattractants was inhibited by dexamethasone in rabbit skin, indicating a site of action on (2) and/or (3) (Yarwood et al., 1988). In these experiments ^{111}In-labelled neutrophils from donor rabbit blood were injected i.v. into recipient rabbits treated with i.v. steroid 4h before. The experiments described here were designed to investigate a possible effect of the steroid, or a steroid-induced product, on (3), the neutrophil itself.

The procedure was as previously described (Rampart & Williams , 1988) except that donor rabbits were injected i.v. with dexamethasone (3mg/kg), 4h before taking blood for the preparation of neutrophils and labelling with ^{111}In. Labelled cells were then injected i.v., together with an oedema marker ^{125}I-albumin, into untreated recipient rabbits. Inflammatory mediators, mixed with a potentiating dose of PGE_2 ($3x10^{-10}$moles) were then injected intradermally (i.d.) into the back skin. Rabbits were killed 30 min later and skin sites counted for labels. Results are shown in table 1. The data are subtracted for the low values obtained in saline injected sites. Results are means±s.e.mean for n rabbit pairs; p is by paired t-test.

Table 1

Pretreatment of donor rabbits with i.v. dexamethasone (Dex): effect on neutrophil accumulation in recipient rabbit skin.

i.d. mediator (moles/site)	^{111}In-neutrophils/site Saline	Dex	n	p
C5a $3x10^{-12}$	459±103	228±76	9	0.013
C5a $3x10^{-11}$	857±128	497±108	9	0.01
FMLP $5x10^{-12}$	990±320	444±119	9	0.052
FMLP $5x10^{-11}$	1718±457	749±180	9	0.015
LTB_4 $5x10^{-11}$	589±88	350±69	9	0.012
LTB_4 $5x10^{-10}$	1281±236	551±117	9	0.006

Donor pretreatment significantly suppressed neutrophil accumulation for all stimuli except the low dose f-met-leu-phe (FMLP). There was no significant difference between the percentage of circulating labelled cells or neutrophil-dependent oedema responses induced by mediators in the two groups of rabbits (donor cells account for only 3% of the circulating neutrophils in recipients).

Thus, dexamethasone or a factor induced by the steroid appears to exert at least part of its action on neutrophil accumulation in vivo by an effect on the neutrophil itself.

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ENLARGEMENT OF THYMUS IN AGEING MALE RATS TREATED WITH 4-HYDROXYANDROSTENEDIONE

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The thymus of ageing male rats can be regenerated by removal of the testes, and this effect is prevented by testosterone which may exert this effect through aromatisation to oestradiol (Greenstein et al, 1986; 1988). To explore this hypothesis further, 18-month-old male rats were divided into two groups; each rat in one group was given s.c. "Silastic" implant containing cholesterol under ether anaesthesia, while the other group received an implant containing 25 mg 4-hydroxyandrostenedione (4-OH), an inhibitor of the aromatase system used to treat breast and prostatic carcinoma (Henderson, 1987). After 14 days, the animals were anaesthetised with pentobarbital (40 mg/kg i.p.), blood taken by cardiac puncture and the animals killed by cervical dislocation. The thymus, testes and adrenals were taken for measurement of tissue weight. Thymus weights were: control, 76±6 mg (n=4; mean±SEM); 4-OH, 231±42 mg (n = 5). These means were statistically different ($P<0.001$; Mann-Whitney U-test). Testis and adrenal weights were not different, and neither were serum concentrations of testosterone or oestradiol. These results suggest the ageing thymus can be regenerated by 4-OH, and the consequences for the immune system should be explored.

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CARDIOVASCULAR EFFECTS OF DIETARY RESTRICTION AND SELECTED ANORECTIC DRUGS IN THE DIETARY OBESE RAT

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While weight loss in man, caused by caloric restriction alone, has been shown to lower blood pressure (MacMahon et al, 1985), the effect of drug-assisted weight reduction has not been fully studied. The present work investigates the effects of amphetamine-, fenfluramine- and mazindol-induced weight loss on cardiovascular function in the dietary obese rat.

Female Wistar rats (University of Bath strain) of initial weights 203.4 ± 3.7g (mean ± s.e.m), housed under conditions of 12:12 hours light:dark at 22°C, in cages of 6 rats (except for groups WD and CD, both n=8), were allocated to the following groups: lean control (L); dietary obese (D); diet withdrawn (WD); amphetamine-dosed (AMPH); fenfluramine-dosed (FEN); mazindol-dosed (MAZ) and dietary obese, control-dosed (CD). All groups received water and laboratory chow for the first 4 weeks of the study. In addition, groups D, WD, AMPH, FEN, MAZ and CD received an excess of the palatable diet (luncheon meat, digestive biscuits, Rice Krispies and chocolate). After 4 weeks the palatable diet was withdrawn from group WD, while groups D, AMPH, FEN, MAZ and CD continued to receive it. During the second 4 weeks of the study AMPH and FEN received 5 and 10mg kg^{-1} drug respectively daily in the drinking water. Due to the low solubility of mazindol in water, MAZ received 4mg kg^{-1} drug daily by oral dosing with a 10% alcoholic suspension, with CD receiving the vehicle alone. Doses were selected so that similar weight loss could be produced. Systolic blood pressure (BP) and heart rate (HR) were recorded weekly using the tail cuff method and a Narco Programmable Electrosphygmomanometer. Weight was also measured. At 8 weeks the rats were killed and their hearts studied in vitro, being perfused with Kreb's solution. Baseline values of beating rate and contractility against 2g tension were recorded.

	Changes occurring during the second 4 weeks of study			'In vitro' heart data Baseline values	
Group(n)	Weight(g)	BP(mmHg)	HR(beats/min.)	Beating rate (beats/min.)	Contractility (g)
L(12)	+21.2±1.3	+1.2±3.9	+7.4±7.1	279.4±8.1	11.8±1.0
D(12)	+36.5±4.2	-0.7±4.2	+17.3±7.9	275.5±13.3	7.9±0.6
CD(8)	+25.9±4.0	+2.6±4.2	-11.4±8.3	----------	--------
WD(8)	-19.0±2.9	-14.4±7.4	-19.5±8.5	256.7±9.3	10.1±1.2
AMPH(12)	-22.9±5.1	-4.6±4.4	-44.3±10.7	250.6±7.0	9.5±0.5
FEN(12)	-11.7±1.0	-27.3±3.7	-36.3±6.5	262.3±4.4	7.6±0.8
MAZ(6)	-11.5±6.1	+4.5±8.9	+74.3±13.1	259.0±15.1	8.1±0.9

All values expressed as mean±s.e.m.

Group D gained significantly more weight ($p<0.001$) during the second 4 week period than all groups except CD. BP of WD and FEN fell significantly more than D ($p<0.01$ and $p<0.001$), while the HR of WD, AMPH, and FEN fell consistently more than either D or L ($p<0.001$). However, the HR of MAZ increased significantly. Although there were no significant differences in the isolated beating rates all drug treatment groups and WD showed small reductions. The baseline contractility of L was significantly greater than that of AMPH ($p<0.01$) or D, FEN, and MAZ ($p<0.001$).

Thus there are clear differences between the effect of the 3 anorectic drugs on the cardiovascular system in the dietary obese rat.

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IN VITRO ASSESSMENT OF THE MOUSE AS AN EXPERIMENTAL MODEL FOR THE POLYMORPHIC OXIDATION OF METOPROLOL

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The oxidative metabolism of metoprolol exhibits genetic polymorphism in man (McGourty et al, 1985). The female Dark Agouti (DA) rat has been proposed as a model for the human poor metaboliser phenotype (Al-Dabbagh et al, 1981). However, in vitro kinetic analysis (Boobis et al, 1986) and studies at the protein and gene level (Gonzales et al, 1987) have subsequently indicated that this model does not entirely reflect the human polymorphism. Since the mouse has some advantages over the rat for biochemical genetic studies, we have investigated the oxidation of metoprolol by hepatic microsomes from 12 inbred strains of mouse.

Microsomes were prepared from the livers of 6 male mice (25-30g) from each of the following strains: A2G/Ola, BALB/c/Ola, BXSB/Mp/Ola, CBA/Ca/Ola, C3H/He/Ola, C57BL/6J/Ola, C57BL/10/ScSn/Ola, DBA/1/Ola, DBA/2/Ola, NIH/Ola, NZB/Ola and 129/Ola.

All strains metabolised metoprolol to its O-demethyl and α-hydroxy products and the kinetics of both reactions were monophasic over the concentration range studied (10-160 μM). Although metabolic activity varied considerably within each strain, there were no significant differences between strains in the apparent Michaelis-Menten constants for either reaction. Mean ($\pm$ s.d.) values describing O-demethylmetoprolol formation in each strain ranged from 26.0 $\pm$ 10.6 (n = 5 mice) to 50.4 $\pm$ 19.2 μM (n = 6) for Km and from 0.57 $\pm$ 0.1 (n = 6) to 0.92 $\pm$ 0.21 nmoles/mg protein/min (n = 6) for Vmax. Corresponding values for α-hydroxymetoprolol production were 42.1 $\pm$ 21.6 (n = 5) to 64.3 $\pm$ 32.9 μM (n = 6) and 0.15 $\pm$ 0.03 (n = 6) to 0.25 $\pm$ 0.25 (n = 5) nmoles/mg protein/min. There was a significant correlation between the Vmax values for O-demethylation and α-hydroxylation (r_s = 0.62, $p < 0.001$) but not between Km values (r_s = 0.12, $p > 0.1$) (n = 65).

The O-demethylation and α-hydroxylation of both enantiomers of metoprolol (30 μM) were only weakly inhibited by quinidine and quinine (IC_{50} range = 5 to >100 μM). These findings are in marked contrast to observations with human liver microsomes where metoprolol oxidation was found to be highly sensitive to inhibition by quinidine (IC_{50} = 40 nM) but not quinine (IC_{50} = 10 μM) (Otton et al, 1988). Similarly, the selective and potent inhibition of debrisoquine 4-hydroxylase and bufuralol 1'-hydroxylase by quinidine observed in human liver microsomes was not confirmed in the rat (Zhi-Guang et al, 1988).

In conclusion none of the mouse strains studied appear suitable for use as a model for the human poor metaboliser phenotype. Furthermore, our data indicate that the enzyme or enzymes catalysing metoprolol oxidation in the mouse have markedly different catalytic properties compared to their human orthologues.

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EFFECT OF EFAROXIN, AN α_2-ADRENOCEPTOR ANTAGONIST, ON INSULIN RELEASE IN CONSCIOUS RATS

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The effect of idazoxan, an α_2-adrenoceptor antagonist, on the slow and fast release phases of insulin secretion in the rat have been previously reported (Roach et al., 1984; Berridge et al., 1986). Efaroxan (2-ethyldihydrobenzofuran-2-imidazoline hydrochloride) is an α_2-adrenoceptor antagonist with 4.2 times the potency and 11 times the selectivity of idazoxan. In this study we looked at the effect of efaroxan on plasma glucose and insulin levels under a variety of test conditions.

Male Sprague-Dawley rats (275-325 g) had their descending aortas cannulated according to the method of Weeks et al. (1960) and allowed to recover for two days. Blood samples (0.6 ml) were taken before and at various times after treatment. The effect of efaroxan (1 and 5 mg/kg, p.o.) on basal levels of glucose and insulin were investigated in fed and fasted (food withdrawn 18 hours prior to the test) rats and on the hyperglycaemic response to UK-14,304 and glucose (1 g/kg, s.c. and 250 µg/kg, i.a.). The two glucose challenges were designed to stimulate the slow and fast release phases of insulin respectively.

UK-14,304, an α_2-adrenoceptor agonist (Cambridge, 1981) (150 µg/kg, s.c.), produces hyperglycaemia, but had little effect on plasma insulin levels in fasted rats. Efaroxan (1 and 5 mg/kg, p.o.), administered 15 minutes prior to UK-14,304, produced a dose-related hyperinsulinaemia and antagonism of the UK-14,304-induced hyperglycaemia, indicating antagonism of pancreatic α_2-adrenoceptors. When efaroxan was tested on the basal plasma glucose and insulin levels of fed and fasted rats, it produced a modest reduction in glucose, but a 4- to 5-fold increase in insulin levels in fasted rats and a 2-fold increase in fed rats. Efaroxan (5 mg/kg, p.o.) produced significant increases in the slow and fast release phases of insulin secretion in response to a subcutaneous (1 g/kg) and intra-arterial (250 mg/kg) glucose challenge respectively.

These data provide further evidence that α_2-adrenoceptors are involved in insulin release and that α_2-antagonists can enhance insulin release.

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THE EFFECT OF PHORBOL ESTERS ON STEROID METABOLISM IN ISOLATED RAT HEPATOCYTES

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Hormones can modulate hepatic steroid metabolism and many are known to act by altering the turnover of phosphatidylinositol. Breakdown of this intracellular phospholipid produces two intracellular second messengers, Inositol-1,4,5-trisphosphate which increases intracellular calcium levels and Diacylglycerol (DAG), which interacts with calcium and phosphatidylserine, to activate the phosphorylating enzyme protein kinase C (PKC) (Vaartjes et al., 1986). Tumor-promoting phorbol esters, such as 4β-Phorbol-12-Myristate-13-Acetate (PMA), are known to substitute for endogenous DAG and directly activate PKC, without requiring calcium or phospholipid, both in vivo and in vitro. It has been reported that PMA (50nM) can affect the metabolism of the steroid substrate Androst-4-ene-3,17-Dione in isolated rat hepatocytes and this effect can be mimicked by adding exogenously, the synthetic diacylglycerol, 1,2-Dioctanoylglycerol (Allan & Skett, 1988). This suggested an involvement of PKC in PMAs action on steroid metabolism and the hypothesis was investigated using a series of protein kinase inhibitors. Several specific and non-specific kinase inhibitors derived from bacteria have been described (Kase et al., 1987) and we utilized the general, non-specific kinase inhibitor K252a (20nM) and the specific protein kinase C inhibitor K252b (20nM). Pretreatment with the inhibitors revealed that the non-specific K252a had no effect upon PMA action, whereas K252b caused a blockade of PMA action upon steroid metabolism. The results showed that PMA was capable of activating PKC, a result further confirmed employing a Histone H1 phosphorylation assay and the technique of Pelech et al. (1986). Thus, activation of PKC by PMA was found to affect steroid metabolism, specifically causing inhibition of the metabolism of the steroid Androst-4-ene-3,17-Dione. Activation of PKC may be one of the mechanisms by which regulatory hormones can affect steroid metabolism.

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CYCLOSPORIN IS A POTENT AND SELECTIVE INHIBITOR OF HUMAN LYMPHOCYTE MIGRATION IN VITRO

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Lymphocytic infiltrates are considered to play an important role in the pathogenesis of certain inflammatory diseases such as psoriasis and atopic dermatitis. In view of the therapeutic efficacy of Cyclosporin A (CyA) in these diseases we have studied the effect of CyA on in vitro lymphocyte migration and have compared its effects with those due to Dexamethasone (Dex).

The lymphocyte migratory responses to recombinant interleukin 1α (rIL 1α), rIL 2, leukotriene B_4 (LTB_4) and zymosan activated plasma (ZAP), were determined by using mixed human peripheral blood lymphocytes in a 48-well microchemotaxis assay as previously described (Bacon et al, 1988). Results are expressed as a migration index, calculated by dividing the area of the chemotaxis filter occupied by cells stimulated with chemoattractant (mm^2) by the area of filter occupied by randomly migrating cells (mm^2). In further experiments lymphocytes were pre-incubated for 30 minutes at 37°C with a range of concentrations of CyA or Dex. The migratory responses of these pre-incubated cells to fixed concentrations of agonists were then determined, the concentrations of each agonist being that which gave a migration index of between 50-70% of the maximal.

In the absence of either drug, bell-shaped dose response curves were seen with 0.1-10% ZAP (maximal migration index [mMI] at 5% = 2.8 ± 0.2, mean ± s.e.mean, n=12), 5 x 10^{-9} to 5 x 10^{-7}M LTB_4 (mMI = 2.6 ± 0.3 at 1 x 10^{-7}M, n=4), 1.5 x 10^{-11} to 2.9 x 10^{-10}M rIL 1α (mMI = 2.3 ± 0.4 at 1.5 x 10^{-10}M, n=6) and 1.0 x 10^{-11} to 1.0 x 10^{-9}M rIL 2 (mMI = 3.0 ± 0.3 at 2.2 x 10^{-10}M, n=4). CyA (4.0 x 10^{-13} to 1.6 x 10^{-7}M) caused concentration dependent inhibition of lymphocyte migration in response to 6.0 x 10^{-11}M rIL 1α (n=4) and 1.0 x 10^{-10}M rIL 2 (n=4). The IC_{50} value for CyA in both cases was approximately 1.0 x 10^{-11}M. Similar concentration-related inhibition of lymphocyte migration in response to rIL 1α (n=4) and rIL 2 (n=4) was seen using lymphocytes pre-incubated with CyA for 30 minutes, washed and then tested for locomotor activity in the microchemotaxis assay without the addition of further CyA. In contrast the same concentration of CyA did not significantly inhibit the lymphocyte migratory responses towards 0.5% ZAP (n=4) or 2.5 x 10^{-8}M LTB_4 (n=4). Pre-incubation with Dex (4.0 x 10^{-13} to 4.0 x 10^{-6}M) caused concentration-dependent inhibition of rIL 1α and LTB_4-induced lymphocyte migration (IC_{50} = approximately 1.5 x 10^{-8}M for rIL 1α, n=4 and 1.5 x 10^{-7}M for LTB_4, n=4), but had no effect on the responses to rIL 2 (n=6) or ZAP (n=4).

CyA is therefore a potent and selective inhibitor of lymphocyte migratory responses to IL 1α and IL 2, an action which may contribute to its therapeutic efficacy in inflammatory diseases. Dex also inhibited IL 1α-induced migration but was less potent than CyA and expressed a different selectivity towards the other agonists tested.

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THE EFFECT OF GLYCEROL-INDUCED ACUTE RENAL FAILURE ON WARFARIN PHARMACOKINETICS IN THE RAT

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Renal disease is known to affect the metabolic clearance of many drugs (Terner et al., 1978). A reduced content of cytochrome P450 and a significant decrease in aminopyrine clearance has been found in rats with experimental renal failure (van Peer and Belpaire, 1977; Patterson and Cohn, 1984). However, few studies have involved drugs widely used in clinical practice. The present study was designed to investigate the pharmacokinetics of warfarin in rats with glycerol-induced acute renal failure (ARF).

Male Sprague-Dawley rats (200-340 gm) were randomly assigned to ARF or control groups. ARF was induced by i.m. injection of glycerol in physiological saline (50% v/v). The control group were injected with only physiological saline and all animals were used 48 hours after treatment. Under urethane anaesthesia (15% w/v, 10 ml/kg, i.p.), the jugular vein and the contralateral carotid artery were cannulated for drug administration and sample collection respectively. Warfarin (1 mg/kg) was rapidly injected and 0.5 ml blood samples collected between 0.5 and 60 minutes. Warfarin was extracted from plasma samples and determined by HPLC using a modification of the method of Breckenridge et al. (1985).

A summary of the pharmacokinetic parameters is shown in table 1 below.

Table 1. Pharmacokinetic parameters for Warfarin in controls and ARF rats

Parameter	Controls (n = 7)	ARF (n = 7)	Statistical significance
Cl (ml/min/kg)	1.20 ± 0.15	1.33 ± 0.34	N.S.
Vd (1/kg)	0.110 ± 0.025	0.153 ± 0.014	$p < 0.05$
Vc (1/kg)	0.053 ± 0.013	0.073 ± 0.006	$p < 0.05$
$t_{\frac{1}{2}}$ (β) (min)	69.0 ± 19.6	86.7 ± 28.7	N.S.
Dose/AUC (0-40 min) (ml/min/kg)	3.48 ± 0.50	4.49 ± 0.65	$p < 0.05$

Results are given as mean ± standard deviation.
Legend: Cl = plasma clearance, Vd = volume of distribution
Vc = volume of central compartment, $t_{\frac{1}{2}}$ (β) = biological half-life
AUC = area under plasma concentration time curve

There was no significant difference in clearance of Warfarin between controls and ARF rats. However the plasma concentration versus time plot and the parameter Dose/AUC (0-40) which measures warfarin clearance during the period of the experiment, suggested that warfarin was disappearing faster from plasma of ARF rats. This apparent contradiction may reflect a more rapid uptake of warfarin, counterbalanced by a slower rate of biotransformation, in the liver of ARF rats. The significant increase in Vd and Vc in rats with ARF may be due to plasma expansion in end-stage renal failure or possibly a decrease in protein binding in the uraemic state.

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IN VIVO COMPARISON OF AMINOGLUTETHIMIDE AND A NOVEL PYRROLIDINE-DIONE ON OESTROGEN PRODUCTION IN THE RAT

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Blockade of oestrogen synthesis by inhibition of aromatase is considered to be an important treatment modality for hormone-dependent post-menopausal breast cancer (Santen 1986). Aminoglutethimide (AG), which is currently in use for this purpose, has several notable side-effects e.g. inhibition of corticosteroid synthesis and CNS depression. However, a novel analogue 3-(4'-aminophenyl)-3-ethyl pyrrolidine-2,5-dione (WSP-1) is equipotent with AG as an aromatase inhibitor both *in vitro* and after acute doses *in vivo* in rats (Pourgholami *et al.* 1987) but with fewer side effects (Ahmad *et al.* 1987). The present study examines the oestradiol-lowering effect of WSP-1 in rats receiving multiple doses of the compound in comparison with AG.

Female Wistar rats (200g) received pregnant mares' serum gonadotrophin (PMSG; 100 IU in 0.1ml saline, s.c.) at 2 day intervals on 5 occasions. Commencing on the fifth day of this regimen, the animals also received twice daily i.p. doses of either AG (50mg/kg), WSP-1 (50mg/kg) or vehicle (1% w/v carboxymethylcellulose; control) for 5 days. Blood was collected by cardiac puncture under terminal ether anaesthesia 3h after the last dose. In addition, the ovaries were excised. Plasma oestradiol was measured by radio-immunoassay and ovarian aromatase activity was assayed by the method of Graves and Salhanick (1979).

Both AG and WSP-1 significantly ($P<0.001$) reduced plasma oestradiol levels in the treated rats (1440±129 pM in controls, n=24; 63±9 pM in AG group, n=8; 99±16 pM in WSP-1 group, n=8). The ovarian aromatase was also found to be significantly ($P<0.05$) inhibited by this multiple dose regimen of the aromatase inhibitors (71 and 54% inhibition by AG- and WSP-1-treatment respectively). In a situation where PMSG stimulation of ovarian aromatase had declined (PMSG regimen as above followed by aromatase inhibitors, 50mg/kg i.p. daily for 5 days, blood being collected 3h after last dose), the aromatase inhibitors were also effective (P 0.05) in lowering plasma levels of oestradiol (37±7 pM, control; 20±2 pM, AG group; 25±1 pM, WSP-1 group; n=8 in each case).

The results indicate that WSP-1 has a similar order of activity to AG in lowering plasma levels of oestradiol and in inhibiting ovarian aromatase in the rat. WSP-1 thus appears to be a compound worthy of further evaluation.

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ELECTRODE SEPARATION AND TISSUE BATH RESISTANCE: AN UNEXPECTED FINDING

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Tissue bath electrical resistance (R) might reasonably be expected to increase as electrode separation increases (Hardebo et al., 1986). In cylinders (e.g. wire), for example, R is proportional to length. We wished to quantify such changes in R to determine whether these might: i) influence tissue responses and ii) exceed the effective working range of a recently-developed constant current unit (CCU) (Jamieson & Selbie, 1988).

0.5mm diameter steel electrodes were suspended in a 25ml perspex bath of Krebs' solution (KPSS) and separated in parallel by micrometer. Conductance (G) was measured by Wayne Kerr Universal Bridge at 1.589kHz. R was calculated as 1/G. Preliminary experiments indicated (predictable) variation in R with length of exposed/immersed electrode and tendency of R to increase with repetition (probably reflecting some electrolysis despite the low a.c. bridge current). Measurements were made, in random order of separation, using i) uninsulated electrodes (~ 10mm immersed), from 0.25-10mm apart (Figure 1A) ii) insulated electrodes with exposed ends of 22, 10 and 1mm, from 1.2-10mm apart (Figure 1B). KPSS was renewed between observations in ii) only.

Contrary to our expectation R fell with increasing separation. We did not examine the effect of tissue interposed between the electrodes but would not expect electrode separation to influence tissue responses measurably. The range of resistances is within the constant current range of the CCU.

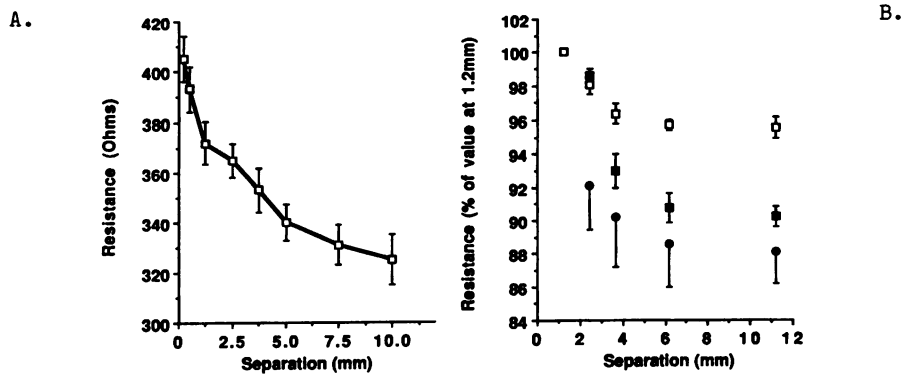


Figure 1 Resistance of KPSS vs electrode separation. A: uninsulated. Means (SEM) of 4 observations. B: insulated; exposed lengths □ 22, ●10, ■1 mm. Means (SEM) of 3 observations. R expressed as % of initial value (at 1.2mm): initial values 186, 454, 3577 Ohms respectively.

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CHANGES IN POLYAMINE CONTENT IN HUMAN CANCER CELLS IN RESPONSE TO TREATMENT WITH METHOTREXATE

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The polyamines, putrescine, spermidine and spermine, are essential polycations necessary for the regulation of cell growth in mammalian cells. Increased intracellular concentrations of all three polyamines are required for increased rates of cell growth and similarly, decreased concentrations are associated with low growth rates (Wallace & Keir, 1981). Methotrexate is a commonly used drug in the treatment of a variety of malignant diseases. It is a folate antagonist which inhibits dihydrofolate reductase and prevents purine nucleotide synthesis and consequently inhibits DNA synthesis. It is a cytotoxic drug which has a marked effect on cell growth. Therefore, if polyamines are essential for the regulation of cell growth, treatment of cells with methotrexate might be expected to influence polyamine content.

The aim of this study was to investigate the effect of methotrexate on polyamine metabolism in order to determine whether there is a link between inhibition of cell growth rate and polyamine content.

Human colonic cancer cells (HT115) were grown routinely at 37°C in monolayer culture in Dulbecco's medium supplemented with 10% (v/v) horse serum. Methotrexate, at the required concentration, was added to the cultures at 4h after cell attachment had taken place. Cell growth and polyamine content were measured as described previously (Lowry et al., 1951; Wallace et al., 1988).

Table 1

Addition	Protein Content	Polyamine Content		
		Put	Spd	Spm
	(mg/plate)	(nmoles/mg protein)		
None	0.71 ± 0.05	0.80 ± 0.12	4.23 ± 0.76	7.82 ± 0.35
50nM-Methotrexate	0.43 ± 0.03*	n.d.	0.51 ± 0.14*	2.49 ± 0.82*
1μM - Methotrexate	0.36 ± 0.02*	n.d.	0.43 ± 0.02*	2.63 ± 0.36*

Results are mean ± SD (n = 3). Statistical analyses by ANOVA and Dunnetts test
* $p<0.01$ n.d. not detected.

Both concentrations of methotorexate produced a significant inhibition of cell growth after 48h treatment. This was accompanied by decreases in the intracellular content of all three polyamines; putrescine was below the limit of detection while both spermidine and spermine decreased by approximately 90% and 70% respectively. Analysis of the extracellular medium has revealed the presence of elevated concentrations of polyamines in drug-treated cultures. Thus it may be that methotrexate stimulates the catabolism and excretion of polyamines either as a part of, or, as a consequence of its growth inhibitory effect.

We thank the Carnegie Trust for financial support.

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QUANTIFICATION BY H.P.L.C. OF RP 49356 IN LIVER PERFUSATE FOLLOWING PERFUSION OF AN ISOLATED RAT LIVER

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The extent of hepatic metabolism of RP 49356 [N-methyl-2-(3-pyridyl)-tetrahydropyrane-2-carbothioamide-1-oxide], a novel antihypertensive agent, has been studied in the perfused isolated rat liver.

The experiment was performed using 3 mg of RP 49356 which had been dissolved in 0.3 ml of methanol before being dispersed in the total volume of perfusion medium (Brauer's, 120 ml). Sampling occurred simultaneously, both before and after passage through the liver over the period 0-120 min after drug addition. Samples were subsequently centrifuged and the resulting supernatant stored at -20°C until analysed.

Samples were assayed by h.p.l.c. with internal standardisation following extraction of RP 49356 into dichloromethane containing 1% iso-propyl alcohol at neutral pH. After vortex mixing and centrifugation, the lower organic layer was decanted and evaporated to dryness under a constant stream of oxygen free nitrogen at 50°C. Each residue was reconstituted in 100 ul of the mobile phase before analysis.

The mobile phase consisted of methanol: water (distilled) 1:1 v/v. The analytical column was a 250 x 4.6 mm LiChrosorb 'Select B' cartridge (5um, C8) protected by a 4 x 4 mm C18 (7 um) guard cartridge positioned between the injector and the analytical column. Chromatography was performed at ambient temperature at a flow-rate of 1.0 ml/min resulting in a back pressure of 2500 p.s.i. RP 49356 and the internal standard were detected by their U.V. absorption at 275 nm.

Levels of RP 49356 decrease steadily over the period of the perfusion, with a half-life, calculated after passage through the liver, of about 90 minutes. The only deviation from this trend is the secondary peak before passage through the liver which is very possibly due to incomplete mixing of the parent drug in the perfusate reservoir at the start of the experiment.

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THE EFFECT OF MALARIA INFECTION ON QUININE DISPOSITION IN THE ISOLATED PERFUSED RAT LIVER PREPARATION

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There is clinical and pathological evidence of liver damage associated with malaria infection (Aikawa, et al, 1980; Hollingdale, 1985) and the possibility of impaired drug metabolism (Trenholme, et al, 1976). This is of particular importance for quinine, a drug with a narrow therapeutic index, the disposition of which is reported to change during malaria infection in man (White, et al, 1982).

We have studied the effect of malaria on the disposition of quinine in the isolated perfused liver (N=5) from rats infected with merozoites of P. berghei, a rodent malaria model, in comparison with non-infected controls (N=5). The mean parasitaemia produced in the infected rats was 38 ± 8%. Following bolus administration of quinine (1mg) to the 100ml recycling perfusion circuit, perfusate plasma was sampled at intervals to 4hr and assayed by HPLC (Mihaly, et al, 1987). Statistical comparisons were by Students 't' test accepting significance when $P < 0.05$.
Resultant pharmacokinetic parameters are tabulated below:

Parameter	Control	Infected	Significance
AUC (ng.h/ml)	3822 ± 347	6470 ± 1101	$P < 0.001$
Cl (ml/min/g.liver)	0.64 ± 0.09	0.33 ± 0.08	$P < 0.001$
Vd (ml/g.liver)	81.2 ± 23.7	53.0 ± 13.3	$P < 0.05$
$T_{\frac{1}{2}}$ (h)	1.37 ± 0.25	1.93 ± 0.60	$P > 0.05$

Following quinine dosage to both control and infected livers, drug concentrations fell biexponentially. Higher quinine concentrations were observed during malaria as indicated by the increase in AUC (69%). Malaria infection resulted in a significant decrease in quinine clearance (48%) and volume of distribution (35%). The combined effects of these changes was a trend towards a longer elimination half life in infected livers although this did not reach significance ($P < 0.1 > 0.05$). In addition, the extent to which quinine clearance was impaired was related linearly to the severity of malaria infection ($r = 0.898$, $P < 0.02$) although the volume of distribution was not. This study indicates that both the hepatic metabolism and the distributional characteristics of quinine are altered during malaria infection in the rat and emphasises the need for a proper understanding of the pharmacokinetics of drugs in disease states before therapeutic regimens are devised.

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SEROTONIN EXERTS A BIPHASIC EFFECT ON α-MELANOCYTE-STIMULATING HORMONE RELEASE FROM SUPERFUSED SLICES OF RAT HYPOTHALAMUS

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Previous work has shown that an endogenous dopaminergic system exerts a tonic inhibitory control over the release of α-melanocyte-stimulating hormone (α-MSH) from rat hypothalamic slices (Tiligada & Wilson, 1987; Tiligada & Wilson, 1988). This study has investigated the influence of exogenous serotonin (5-HT) on α-MSH release from this *in vitro* system.

Release of α-MSH from 250μm frontal slices of rat hypothalamus superfused at 37°C and 0.5ml/min with oxygenated artificial cerebro-spinal fluid (ACSF) (Barnes *et al.*, 1988) was quantified in freeze-dried 2min ACSF samples by radioimmunoassay (Wilson & Morgan, 1979). Four minute pulses of ACSF containing 50mM KCl or varying concentrations of 5-HT were introduced both in the absence and presence of the 5-HT antagonist cinanserin (10^{-6}M).

Control depolarisations with 50mM KCl-containing ACSF produced significant ($P<0.01$) increases in α-MSH release above baseline. Superfusion of the tissue with varying concentrations of 5-HT (10^{-7}M - 10^{-4}M) resulted in a U-shaped dose-response curve. Maximum stimulation of α-MSH release was obtained with 10^{-6}M 5-HT, whilst no significant stimulation of release occurred when 5-HT concentrations were further increased to 10^{-4}M (Figure 1). Cinanserin caused an inhibition of the stimulatory effect of 10^{-6}M 5-HT on α-MSH release, whilst peptide release in the presence of 10^{-4}M 5-HT and cinanserin was stimulated. Thus the 5-HT dose-response curve was shifted to the right in the presence of cinanserin (Figure 1). In addition, 10^{-6}M cinanserin significantly reduced 50mM KCl-induced α-MSH release from 329±44% to 174±27% (n=5, ±SEM) of basal release ($P<0.001$) without altering baseline peptide release.

These data demonstrate that 5-HT exerts a biphasic regulatory effect on α-MSH release from superfused rat hypothalamic slices and that this action can be antagonised by cinanserin. Endogenous 5-HT may play a role in K^+-stimulated peptide release.

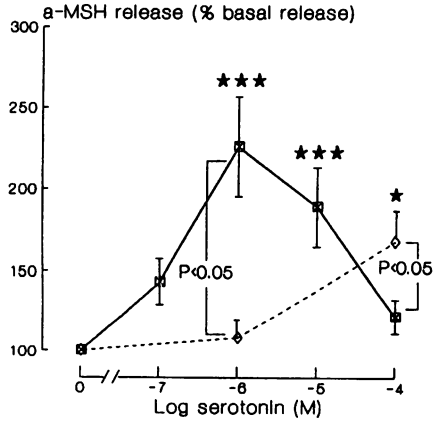


Figure 1 Increases ±SEM in release of α-MSH above baseline from superfused slices of rat hypothalamus caused by various concentrations of 5-HT in the absence (——) and presence (- - -) of 10^{-6}M cinanserin. * $P<0.05$ and *** $P<0.001$ versus basal release (ANOVA).

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THE DEPRESSION BY FLURAZEPAM OF 5-HT RELEASE IN RAT HIPPOCAMPUS IS MEDIATED BY GABA RECEPTORS

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Serotonergic pathways in the brain are thought to be involved in the anxiolytic actions of benzodiazepines which decrease 5-HT turnover in cerebral cortex (Collinge et al., 1983) and release in striatum (Soubrie et al., 1983). The activity of serotonergic neurones projecting to the hippocampus is tonically depressed by the action of GABA in the median raphe nucleus (Forchetti & Meek, 1981). In the present studies, we have investigated the effect of flurazepam (FLU) and of the $GABA_A$ antagonist picrotoxin (PTX) on 5-HT release in rat hippocampus using microdialysis.

Male Sprague-Dawley rats (200-300g) were stereotaxically implanted with microdialysis probes in hippocampus during chloral hydrate anaesthesia (400 mg/kg,i.p.). Following a 24 h recovery period, rats were connected to a perfusion system which allows them to move around freely. Due to the low basal level of 5-HT in hippocampal dialysates, a 5-HT uptake blocker citalopram (1 μM) was added to the perfusion medium (Ringer solution). Dialysates were collected every 20 min and assayed by HPLC with electrochemical detection for 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA).

FLU (10 mg/kg, i.p.) caused a decrease in 5-HT concentration in hippocampal dialysates which reached 50±5% of control 1 h after drug injection and the effect was still present 2 h later; there was no change in 5-HIAA levels. Local application of FLU (10 μM) during a 20 min period produced a similar reduction (-37±10%) in 5-HT concentration with no change in 5-HIAA. The anxiogenic partial inverse agonist FG 7142 (3 mg/kg, s.c.) had no effect on either 5-HT or 5-HIAA.

In order to see whether the effect of FLU is mediated by GABA receptors, the chloride channel blocker PTX was added to the perfusion fluid in a final concentration of 10 μM during 40 min of perfusion. This caused a sharp increase in 5-HT concentration in the dialysates to 385±39% above control by the end of a 40 min period. As soon as normal medium was again perfused, 5-HT concentration declined sharply and reached control level within 40 min. The perfusion of PTX caused a pronounced behavioural activation, which had a delayed onset and outlasted the perfusion with PTX.

When FLU was added to the perfusion fluid 20 min after PTX, its effect was completely suppressed.

These results show that both systemic and local administration of flurazepam decrease 5-HT release in hippocampus; the effect of picrotoxin suggests that GABA receptors exert a tonic inhibition at the nerve terminals on the release of 5-HT in the hippocampus and that the depression of 5-HT by FLU is mediated by $GABA_A$ receptors.

We thank Action Research for the Crippled Child for financial support.

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DIFFERENTIAL RESPONSIVENESS OF DORSAL AND MEDIAN RAPHE SEROTONIN (5-HT) SYSTEMS TO 5-HT_1 AGONISTS AND p-CHLOROAMPHETAMINE

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It was recently demonstrated that dorsal and median raphe 5-HT neurones give rise to projections that are morphologically different and that 5-HT terminals originating from the dorsal raphe can be selectively destroyed by chlorosubstituted amphetamines (Kosofsky & Molliver, 1987; Mullen et al., 1987). We undertook an in vivo voltammetric study of the dorsal raphe-frontal cortex and the median raphe-dentate gyrus 5-HT systems to assess whether differential functional properties could be demonstrated for each of these two subsets of 5-HT neurones.

Male Sprague-Dawley rats were tracheotomized under light ether anaesthesia and then paralyzed and artificially ventilated using a 0.5% fluothane in a 3:1 N_2O-O_2 mixture. Carbon fiber electrodes were prepared and electrochemically pretreated essentially as described by Cespuglio (1982) in order to detect extracellular 5-hydroxyindoleacetic acid (5-HIAA), assessed by its oxidation peak occuring at 250 mV. Differential pulse voltammograms (0 to 500 mV) were generated every 5 min at a rate of 10 mV/s.

The selective 5-HT1A agonist 8-OH-DPAT produced a gradual decrease in extracellular 5-HIAA levels in the dorsal raphe and in the frontal cortex reaching a maximum of 60% 3h after an i.v. injection of 30 µg/kg. However, the same dose of 8-OH-DPAT was ineffective in the median raphe and in the dentate gyrus which receives its 5-HT innervation exclusively from the median raphe. A higher dose of 8-OH-DPAT (150 µg/kg iv) produced a 60% decrease in extracellular 5-HIAA concentrations in the median raphe whereas only a 20% decrease was obtained in the dentate gyrus. In contrast, the non-selective 5-HT1A/B agonist RU 24969 (10 mg/kg ip), caused a 70% reduction in extracellular 5-HIAA levels in both the dorsal and median raphe and a 50% decrease in both the frontal cortex and dentate gyrus. Three h after the administration of p-chloroamphetamine (5 mg/kg ip), extracellular 5-HIAA levels were increased by 60% in the frontal cortex but remained unaffected in the dentate gyrus.

Several conclusions may be drawn from the present investigation: 1) The dorsal raphe-frontal cortex 5-HT system shows a greater responsiveness (decreased extracellular 5-HIAA levels) to a 5-HT1A agonist (8-OH-DPAT) than does the median raphe-dentate gyrus 5-HT system; 2) In the latter 5-HT system, 5-HT1A receptor stimulation caused a greater reduction of extracellular 5-HIAA in cell bodies than in the corresponding projection fields (dentate gyrus); 3) The terminal 5-HT1B autoreceptor seems to play a more important role in the control of 5-HT release in the dentate gyrus than in the frontal cortex; 4) 5-HT terminals in the dentate gyrus appear to be insensitive to the 5-HT releasing effect of p-chloroamphetamine which is consistent with the inability of this drug to destroy 5-HT terminals in that region of the brain.

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300P

AN ELECTRONMICROSCOPIC STUDY OF THE NEW VOLTAMMETRIC CARBON FIBRE BIOSENSOR DETECTING BASAL 5-HT IN VIVO

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It is established that the 8 or 12 μm in diameter carbon fibre electrodes (CFEs) associated with differential pulse voltammetry (DPV) do not monitor extracellular dopamine (DA) or 5-hydroxytryptamine (5HT) but their deaminated metabolites in vivo (Crespi et al., 1984). To selectively detect these amines we have recently developed a new 30 μm diam CFE chemically (chromic acid) and electrically pretreated and then electrically coated with nafion: a sulphonated polymer selectively permeable to cations (Crespi et al., 1988). In this study we have electronmicroscopically investigated the effects of pretreatments allowing the selective detection of DA and 5HT oxidation (Crespi et al., 1988).

The untreated 30 μm CFEs present a uniform surface when observed with the scanning electron microscopy (SEM). In a phosphate buffered solution (PBS) containing ascorbic acid (AA 500 μM), 3,4 dihydroxyphenylacetic acid (DOPAC 50 μM) and 5-hydroxyindoleacetic acid (5HIAA 20 μM) only a wide voltammetric oxidation wave was obtained with the electrodes. Once dipped (20 sec) in chromic acid the surface of the 30 μm CFEs appeared clearer and smoother. Chemical treatment enhanced the voltammetric sensitivity but not the selectivity of the electrodes towards the electroactive compounds in PBS. The combination of chromic and electrical pretreatment (Crespi et al., 1988) profoundly modified the surface of the electrodes. Groups of crystals were regularly spaced along the carbon fibre with depressions or craters, and a consistent distribution of holes (100-150 nm diam) also present. The DPV detection of Peak 3 at 300 mV (5HIAA oxidation) was then clearly possible, while AA and DOPAC oxidation (Peak 1 and Peak 2 respectively (Crespi et al., 1988) were merged in a single wave at 50 mV. Washing these treated CFEs with distilled water removed the crystals leaving a smoother surface with clearly observable holes and narrow protuberances but there were no alterations in the voltammogram described above. The treated CFE were very fragile and shattered during cutting with an ultramicrotome for subsequent transmission electron microscopy observation (TEM). The untreated fibres were less fragile, and the TEM study showed on their sections numerous small circular discs (2-3 μm in diameter), uniform in shape and very dense which may correspond to the core of the fibres (Bennett & Johnson, 1978). These discs were not detectable following pretreatment which probably disrupts them. Nafion coating of the electrically and chemically pretreated CFE resulted in a smooth surface with neither detectable holes nor protuberances indicating successful filming of the carbon surface. These nafion electrodes were insensitive to anions (AA, DOPAC and 5HIAA) but showed selective voltammetric sensitivity to 5HT and DA.

Thus, pretreatment alters the structure of the 30 μm carbon fibre by pitting, roughening and fracturing the carbon as observed with the 12 μm CFE (Self et al., 1988). This probably allows easier penetration into the CFE of electroactive compounds which may explain the high sensitivity of the treated CFEs.

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EFFECT OF CHRONIC RITANSERIN AND CLORGYLINE REGIMENS ON 5-HT_2 RECEPTORS IN RAT CORTEX AND RAT PLATELETS

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$5HT_2$ receptors are concentrated in the frontal cortex but are also present in the striatum, mesolimbic areas (Leysen et al, 1982) and on blood platelets (Leysen et al, 1983). Receptor "down regulation" has been observed following antidepressant drugs (Peroutka and Snyder, 1980). However, antagonists eg ritanserin, have also been reported to decrease $5HT_2$ receptor numbers (Leysen et al, 1986). We have compared the effect of clorgyline (MAO type A inhibitor) and ritanserin on receptors in platelets and cortex to determine if they have comparable effects in the two tissues.

Male Wistar rats (400g) received ritanserin (10mg/kg/day), clorgyline (2mg/kg/day) or tartaric acid (1mM, pH5, vehicle) for 28 days: over this period all 3 groups of animals gained weight. After a 3 day drug free period, the animals were anaesthetised and exsanguinated prior to decapitation. Frontal cortices were rapidly removed and platelets were isolated. Samples were stored at -70°C. [^{3}H]ketanserin (0.06-4nM) was used for the cortical receptor assay. Non-specific binding (approx 30%) was assessed using methysergide (1μM) in the assay. Approximately 2.5mg of tissue (wet weight) was used per assay tube and the samples were incubated for 15 mins at 37°C. [^{125}I]iodo LSD (0.06-4nM) was used in the platelet binding assay and ketanserin (1μM) was included to assess non-specific binding (approx 40%). 2.0 x 10^8 platelets were used per assay tube and the assay was incubated for 30 min at 37°C. Incubations were terminated by vacuum filtration using Whatman GF/C filters and 3 x 4ml washes with cold buffer. Direct Linear Plot analysis was used to obtain a measure of Bmax and Kd.

	CORTEX (n = 7)		PLATELETS (n = 6)	
	Bmax (fmol bound/ mg protein)	Kd (nM)	Bmax (fmol bound/ mg protein)	Kd (nM)
Control	315.23 ± 10.72	1.51 ± 0.20	26.18 ± 3.83	0.80 ± 0.18
Clorgyline	249.63 ± 13.99*	1.54 ± 0.23	21.32 ± 4.78	0.41 ± 0.08
Ritanserin	222.55 ± 17.17**	1.66 ± 0.16	50.94 ± 7.96*	1.28 ± 0.41

Values expressed as mean ± SEM: *$p < 0.05$, ** $p < 0.001$ compared to control tissue.

Both clorgyline and ritanserin cause a decrease in $5HT_2$ receptor number in frontal cortex. However, in platelets, ritanserin causes an increase in receptor number and clorgyline has no effect. The effect of ritanserin on platelet $5HT_2$ receptors is what one would predict, ie antagonist induced supersensitivity. The ritanserin effect on cortex confirms previous reports and is presently inexplicable. The effect may be indirect and due to the drug affecting other neuronal systems. The data also shows that platelet and brain changes are not necessarily comparable.

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5-HT_1 BINDING SITES IN POST-MORTEM HUMAN BRAIN

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In recent years a number of 5-HT receptors have been identified and characterised. Functional 5-HT receptors have been divided into three subtypes : 5-HT_1-like, 5-HT_2 and 5-HT_3 (Bradley et al., 1986). 5-HT_1-like sites have been further subdivided on the basis of radioligand binding into 1A, 1B, 1C and 1D sites.

We have used [^{3}H]5-HT (3nM, non-specific binding defined by unlabelled 10μM 5-HT) to label 5-HT binding sites in post-mortem human brain (frontal cortex, hippocampus and amygdala) and have studied displacement with subtype selective drugs (5×10^{-10} - 10^{-3}M). Binding parameters were estimated using non-linear regression analysis fitting a one component model in self-displacement of [^{3}H]5-HT or two component model in displacement of [^{3}H]5-HT by various drugs. The computer program was an adaptation of the method of De Lean et al., (1982).

In all three brain regions, [^{3}H]5-HT binding consistently exhibited a single binding component in self-displacement experiments. However, some drugs were able to distinguish high and low affinity binding components. In the frontal cortex, the 5-HT_{1A} selective drugs, 8-OH-DPAT, buspirone, ipsapirone, spiperone and 5-CT displaced from a similar proportion of sites (35-53%) with high affinity (K_D 7-200nM) and the remaining sites with low affinity (4-69μM). TFMPP (5-HT_{1B} selective) was only a weak displacer (32% inhibition of specific binding at 1μM). Ketanserin, a 5-HT_2 selective drug, displaced only a proportion of [^{3}H]5-HT binding (78% inhibition of specific binding at 10μM) with low affinity (K_D 1.6μM). The 5-HT_3 selective compounds, GR38032F and MDL 72222 displaced from 31% and 15% of sites, respectively, at 10^{-4}M. 8-OH-DPAT displaced with high affinity from a similar proportion of sites in the amygdala (43%) as in the frontal cortex, but a higher proportion (63%) in the hippocampus. RU 24969 (slightly greater affinity for 5-HT_{1B} than 5-HT_{1A} sites) displaced from a higher proportion of sites than 8-OH-DPAT in the frontal cortex (61% vs 42%), amygdala (70% vs 43%) and hippocampus (90% vs 63%).

The results demonstrate the selective labelling of 5-HT_1-like sites by [^{3}H]5-HT in the human brain with little or no labelling of 5-HT_2 or 5-HT_3 sites. Self displacement of [^{3}H]5-HT binding is consistent with the presence of a single population of sites, indicating that 5-HT, is non-selective for the 5-HT_1 subtypes. Around 40% of the 5-HT_1 sites in the frontal cortex and amygdala would appear to be of the 5-HT_{1A} subtype in contrast to 60% in the hippocampus. Despite the reported absence of 5-HT_{1B} sites in the human brain (Hoyer et al, 1986) RU 24969 consistently displaced with high affinity a greater proportion of sites than 8-OH-DPAT. It is possible that the additional sites displaced by RU 24969 are 5-HT_{1D} sites (Heuring & Peroutka, 1987). However 5-CT, which has similar affinity for 5-HT_{1A} and 5-HT_{1D} sites in human caudate (Waeber et al., 1988) displaced from the same proportion of sites with high affinity as 8-OH-DPAT in human cortex.

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ICI 169,369 - A NOVEL 5-HT_2 ANTAGONIST INHIBITS 5-HT-INDUCED WET DOG SHAKE BEHAVIOUR IN THE RAT

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Increased central serotonergic activity in the rat results in a number of specific behavioural responses which include head weaving, reciprocal forepaw treading, Straub tail, "wet dog" shakes, tremor and hind limb abduction (Grahame-Smith, 1971; Jacobs, 1976; Sloviter, Drust and Connor, 1978 and Bedard and Pycock, 1977). The "wet dog" shake (WDS) component of this complex repertoire of behaviours has been proposed for the in vivo study of drugs acting on central 5-HT receptors (Bedard and Pycock, 1977; Sloviter, Drust and Connor, 1978; Matthews and Smith, 1980). Recent evidence (Yap & Taylor 1983) indicates that 5-HT agonist-induced WDS may be, at least in part, mediated by activation of central 5-HT_2 receptors. The aim of this study was to examine the effect of several standard 5-HT antagonists and the novel 5-HT_2 antagonist ICI 169,369 (2-[2-dimethylaminoethylthio]-3-phenylquinoline hydrochloride) (Blackburn et al., 1987) in this model following central administration.

Antagonism of 5-HT- induced rat body shake was assessed in adult male rats of the Alderley Park SPF strain and weighing 180-220g, on automated microcomputer controlled activity monitors. The rats were pretreated with desmethylimipramine (25mg/kg i.p.) before an intracerebroventricular (icv) injection of 5,7-dihydroxytryptamine (5,7-DHT) dissolved in 20µl of artificial cerebrospinal fluid (CSF) 160µg/20µl. The neurotoxin was injected over 5 minutes through a previously implanted icv guide cannula under sodium pentobarbitone (40mg/kg i.p.) anaesthesia. After a period of seven days, lesioned rats received an approximate ED_{50} dose of 5-HT (45µg/10µl) and only those rats which responded in the expected manner i.e. rapid rotational movements of head, neck and trunk (>200/75min observation period) were used in the 5-HT antagonist studies. Following a 4 day recovery period, 5-HT antagonists were adminstered icv in 10µl CSF 15min prior to the standard dose of 5-HT.

Standard 5-HT antagonists effectively reduced the body shake response with a relative order of potency:- methiothepin > methergoline > cyproheptadine > cinanserin > ICI 169,369, > methysergide and (-)-propranolol, based on ID_{50} estimations obtained by regression analysis of the log dose antagonist v 5-HT response plot. In this study the novel 5-HT_2 antagonist ICI 169,369 inhibited 5-HT-induced WDS behaviour with a similar potency to methysergide and cinanserin. The clinical significance of antagonism of central 5-HT_2 receptor is currently under investigation with ICI 169,369.

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INDUCTION OF THE 5-HT SYNDROME IN THE RAT FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 5-HT_{1A} LIGANDS

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Reports that the selective 5-HT_{1A} binding ligand 8-hydroxy-N,N-dipropylamino-tetralin (8-OHDPAT) induces a '5-HT syndrome' in the rat (eg: Hjorth et al., 1982) suggest that central 5-HT_{1A} receptors are involved in the initiation of this behaviour. However, other selective 5-HT_{1A} agonists/partial agonists, eg: buspirone (Skolnick et al., 1985) and LY165,163 (Donohoe et al., 1987), have been reported not to induce a 5-HT syndrome. Here we describe the overt behavioural effects of several 5-HT_{1A} binding ligands following intravenous administration to rats.

Drugs were administered to male Sprague-Dawley rats (320-400g) via a lateral tail vein. Maximal behavioural responses were attained within 1-5 min. following drug administration. Therefore, assessment of the syndrome response was made during this period. The most clearly defined features of the syndrome following administration of 8-OHDPAT were fore-paw treading and flat posture. These criteria were employed to score the syndrome quantally (unequivocal response or non-response) by an observer 'blind' to drug pretreatments. ED_{50} values (with 95% confidence limits) were determined by the sequential up/down method ($n \geq 10$ rats per compound) and calculated using a modified probit analysis (Kimball et al., 1957).

Table 1 Relative potencies of 5-HT_{1A} ligands for induction of syndrome in the rat

8-OHDPAT	0.04 (0.03-0.06)	Gepirone	0.50 (0.36-0.68)
5MeODMT	0.06 (0.05-0.09)	Ru24969	0.83 (0.62-1.13)
Buspirone	0.34 (0.27-0.42)	Ipsapirone	2.12 (1.52-2.96)
LY165,163	0.39 (0.34-0.45)	Methysergide	11.35 (9.70-13.30)

Potency is expressed as an ED_{50} value (with 95% confidence limits) in mg base/kg i.v. 5MeODMT = 5-methoxy-N,N-dimethyltryptamine.

All compounds listed in Table 1 unequivocally induced a syndrome similar to that evoked by 8-OHDPAT. The effect of methysergide in this model is consistent with its reported partial agonist activity at peripheral "5-HT_1-like" receptors (Bradley et al., 1986). In addition to the syndrome, Ru24969 also induced brief episodes of hyperactivity. Although buspirone, gepirone and ipsapirone rapidly induced a syndrome, marked sedation was apparent within 5-10 min. of drug administration. The putative 5-HT_{1A} antagonist BMY7378 (Yocca et al., 1987) failed to induce any components of the syndrome (up to a dose of 10mg/kg i.v.). However, the ED_{50} for 8-OHDPAT to induce syndrome was increased significantly in animals pre-dosed (s.c.;30 min.) with BMY7378 (controls 0.04(0.03-0.05); BMY7378(0.1 mg/kg s.c.) 0.11(0.07-0.18); $p<0.05$; values are ED_{50}'s(95% conf. limits) mg/kg 8-OHDPAT)

These data indicate that several compounds possessing appreciable affinity for the 5-HT_{1A} binding site exhibit central 5-HT_{1A} agonist activity in vivo. The transient behavioural effect of buspirone may be due to rapid metabolism since a recent report (Sethy and Francis, 1988) is consistent with this suggestion.

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BEHAVIOURAL EFFECTS OF GR 43175 AND OTHER 5-HT_1 AGONISTS FOLLOWING CENTRAL ADMINISTRATION IN THE MOUSE

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In vitro studies have identified GR 43175 (Humphrey et al., 1988) as a highly selective agonist for 5-HT_1-like receptors in the dog saphenous vein. To date behavioural actions of the compound have not been described. We now report the effects of GR 43175 on mouse behaviour following peripheral and central administration, and compare it with a number of other standard 5-HT_1 agonists.

In initial experiments, GR 43175 administered s.c. and i.v. to female Tuck mice (20-24g) and male Sprague-Dawley rats (250-300g) up to doses of 30 mg kg^{-1} was devoid of overt behavioural or autonomic effects. This lack of activity 'in vivo' after peripheral administration prompted us to examine the effects of GR 43175 and other 5-HT_1 agonists following direct central administration to mice. Drugs were dissolved in sterile isotonic saline and administered intracerebroventricularly (i.c.v.) to female mice (18-22g; n = 6) in a dose volume of 5µl/mouse using the method of Brittain (1966). Rectal body temperatures were recorded prior to dosing and at various times up to 120 min after drug injection. The locomotor activity of individual animals was measured at 15 and 90 min after dosing in activity monitors. Additionally, any overt behavioural effects occurring over the 2h period post drug administration were noted.

The i.c.v. administration of vehicle induced a slight (1-1.5°C) transient hypothermia, but did not modify overt behaviour. GR 43175 (0.3-100 µg i.c.v. per mouse) evoked an initial dose-related hypothermia (2-5.5°C), which was maximal at 15-30 min and was accompanied by hypolocomotion at 15-19 min post dosing. At doses of 10-100 µg these initial effects were superseded by hyperlocomotion, which was apparent at 30-60 min and marked in intensity (approx. 200% increase in locomotor activity counts cf. saline-treated controls) at 90-100 min. These doses of GR 43175 also evoked a pronounced retraction of the lower lip. Higher doses of the agonist (100-300 µg) initially induced some components of the 5-HT syndrome (Jacobs, 1976), namely forepaw treading, head weaving and tremor, but this was disrupted by the onset of hyperactivity. The 5-HT_1 agonist, RU 24969 (Gardner and Guy, 1983) at doses of 1-30 µg i.c.v. elicited hypothermia (4-6.5°C) and marked hyperlocomotion. This latter response was evident at 15 min and was pronounced at 90-100 min. Higher doses (60-100 µg) induced a clear 5-HT syndrome. The 5-HT_1 agonist, 5-carboxamidotryptamine (5-CT) (10-100 µg i.c.v.) evoked a marked 5-HT syndrome and head twitches within 5 min after injection. 5-CT also induced hypothermia (maximum 6°C), which was maximal at doses of 1-3 µg. None of the doses tested produced hyperlocomotion. The 5-HT_{1A} agonist, 8-hydroxy-dipropylamino-tetralin (8-OH-DPAT) evoked hypothermia (2.5-5°C) and hypolocomotion at doses of 0.3-30 µg i.c.v., but failed to induce any signs of the 5-HT syndrome. Apart from GR 43175, none of the other agonists elicited lower lip retraction.

These data indicate that several 5-HT_1-like receptor agonists induce characteristic behavioural/physiological changes (hyperactivity, hypothermia and 5-HT syndrome) following central administration in the mouse. GR 43175 additionally induced retraction of the lower lip. The effects of GR 43175 were seen following i.c.v., but not peripheral administration. Of the other agonists examined, the profile of activity of RU 24969 most closely resembled that of GR 43175, except that RU 24969 did not induce lower lip retraction.

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THE INTERACTION OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)TETRALIN (8-OH-DPAT) WITH α_2-ADRENOCEPTOR MEDIATED BEHAVIOURAL RESPONSES

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The 5-HT_{1A} agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8OH-DPAT), has been shown to have α_2-adrenoceptor antagonist activity in electrophysiological and behavioural studies (Crist and Surprenant, 1987; Winter, 1988). The hypoactivity (sedation) and mydriasis (pupil dilatation) induced in mice by clonidine respectively result from activation of pre- and postsynaptic CNS α_2-adrenoceptors (Heal et al 1988a). We have now used the hypoactivity and mydriasis models to determine whether the behavioural responses of mice to 8OH-DPAT involve actions on central α_2-adrenoceptors. In addition, the effects of 8OH-DPAT on 3-methoxy-4-hydroxyphenylglycol (MHPG) formation in mouse brain were determined, because this is a good index of noradrenaline turnover (Heal et al, 1988b).

Adult male C57/Bl/6Ola mice (Olac, Bicester) weighing 20-30g were used. Hypoactivity was scored 0-3 on 5 behavioural parameters at 10 min intervals for 60 min after injection as described by Heal et al (1981). Pupil diameter was measured in conscious mice 10 min after drug injection using the method of Heal et al (1988c) and MHPG was measured in whole brain minus cerebellum by HPLC with electrochemical detection as previously described (Heal et al, 1988b). Drugs were injected i.p. dissolved in saline. The following antagonists were studied:- prazosin (1 mg/kg), idazoxan (1 mg/kg), yohimbine (1 mg/kg), pindolol (1 or 10 mg/kg), spiroxatrine (1 or 5 mg/kg) and quipazine (2 mg/kg). Results are given as mean ± s.e. 8OH-DPAT (0.5 - 5 mg/kg) dose-dependently induced hypoactivity. The sedation produced by 8OH-DPAT (2 mg/kg) was only reduced by pretreatment with quipazine (48%, $P<0.01$) and not other antagonists, including idazoxan and yohimbine, suggesting this response was not α_2-adrenoceptor mediated. This was also indicated from an analysis of the individual behavioural components. Although 8OH-DPAT (2 mg/kg) and clonidine (75 µg/kg) produce similar total hypoactivity scores of 22.5 ± 0.7 and 25.1 ± 1.4, respectively, 8OH-DPAT, unlike clonidine, did not induce passivity or impair posture. Conversely, 8OH-DPAT suppressed tactile responsiveness to a greater extent ($P<0.01$). 8OH-DPAT (1 - 50 mg/kg) also dose-dependently increased pupil diameter. Mydriasis induced by 8OH-DPAT (2 mg/kg) was inhibited by idazoxan (50%, $P<0.001$), yohimbine (36%, $P<0.01$) and also spiroxatrine (5 mg/kg, 20%, $P<0.05$), pindolol (10 mg/kg; 67%, $P<0.001$) and quipazine (54%, $P<0.01$). Since 8OH-DPAT mydriasis was inhibited by both 5-HT_1 and α_2-adrenoceptor antagonists, this suggested the response may be indirectly mediated via noradrenaline release. The findings that 8OH-DPAT 0.5 and 5 mg/kg increased MHPG levels by 21% ($P<0.01$) and 37% ($P<0.001$) respectively support this hypothesis.

In conclusion, 8OH-DPAT caused hypoactivity and mydriasis in mice. The receptor mediating the former has not been clearly identified, but is almost certainly not a presynaptic α_2-adrenoceptor. The latter response is probably initiated by 5-HT_{1A} receptor activation leading to increased noradrenaline release and subsequent stimulation of postsynaptic α_2-adrenoceptors.

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ATTENUATION OF THE HYPOTHERMIA BUT NOT THE ANTIDEPRESSANT-LIKE EFFECT OF 8-OH-DPAT FOLLOWING ITS REPEATED ADMINISTRATION TO MICE

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The 5HT-1A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) has an antidepressant-like effect in a mouse model capable of detecting potential antidepressants, the response apparently involving 5HT-1A receptors (Luscombe et al, 1988). 8-OHDPAT also induces hypothermia in mice, a response probably involving presynaptic 5HT-1A receptors which is attenuated after repeated 8-OHDPAT treatment (De Souza et al, 1986). We have studied the effect of repeated 8-OHDPAT treatment on the antidepressant-like response and hypothermia evoked by 8-OHDPAT and report that only hypothermia is attenuated.

Male CD1 mice (Charles River; 22-24g at initiation of treatment) were injected twice daily for 10 days with either 8-OHDPAT (3 mg/kg s.c.) or saline (s.c.). Twenty three hours after the final dose the test treatment of either 8-OHDPAT (3 mg/kg) or saline was administered subcutaneously. Body temperature was measured 0.5h before and 0.5h after this treatment whilst the mobility of these mice in the Porsolt test was measured by a Doppler recording system (Buckett et al, 1982) during a 4 min period 1h after treatment.

8-OHDPAT (3 mg/kg s.c.) did not induce hypothermia in mice pretreated for 10 days with 8-OHDPAT whereas hypothermia was evoked by 8-OHDPAT in saline pretreated mice (Table 1). In contrast, the antidepressant-like effect of 8-OHDPAT (3 mg/kg) was observed in both 8-OHDPAT and saline pretreated mice (Table 1) despite chronic 8-OHDPAT administration decreasing base-line mobility in this test (see 8OH + SAL column in Table 1).

Table 1 Effect of repeated 8-OHDPAT or saline treatment on 8-OHDPAT-induced hypothermia and the 8-OHDPAT-induced increase in mobility in the Porsolt test

Test time‡	10 day treatment + acute treatment (all s.c.) SAL + SAL	8OH + SAL	SAL + 8OH	8OH + 8OH
	Rectal temperature (mean ± s.e.mean; °C)			
- 0.5h	38.47 ± 0.10	38.16 ± 0.13	38.21 ± 0.15	38.14 ± 0.13
+ 0.5h	38.05 ± 0.12	38.17 ± 0.15	36.79 ± 0.22***	37.98 ± 0.12
	Mobility (mean ± s.e.mean; arbitrary units)			
+ 1.0h	338 ± 35	250 ± 25*	464 ± 31**	448 ± 39*

‡ Test times are relative to acute treatment of saline (SAL) or 8-OHDPAT (8OH). ***P<0.001, **P<0.01, *P<0.05 vs SAL + SAL treatment group at that test time (Student's t-test, 2-tailed). n = 16-20 mice/treatment.

The failure of repeated 8-OHDPAT administration to attenuate the 8-OHDPAT-induced antidepressant-like effect compared to its marked reversal of 8-OHDPAT-induced hypothermia suggests that the two responses may be mediated by distinct groups of receptors. Since 8-OHDPAT-induced hypothermia in mice involves presynaptic 5HT-1A receptors (De Souza et al, 1986), these sites would not appear to be involved in the antidepressant-like activity of 8-OHDPAT.

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COMPARATIVE EFFECTS ON BODY WEIGHT, FOOD INTAKE AND TEMPERATURE OF WITHDRAWAL FROM IPSAPIRONE AND CHLORDIAZEPOXIDE IN RATS

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To investigate possible effects of withdrawal from the putative 5-HT_{1A} agonist anxiolytic ipsapirone, and compare it with withdrawal from chlordiazepoxide, female rats were allocated to groups of 10 subjects each.

Different experimental groups received i.p. injections twice daily (1000 and 1600 hours) of either saline (S), ipsapirone (I) or chlordiazepoxide (C). Doses administered (twice daily) increased daily by 2 mg/kg from 10 (day 1) to 30 mg/kg (day 11). Subsequently, animals received 30 mg/kg of the relevant drug twice daily for 10 further days - i.e. 21 days treatment in toto. After such chronic treatment, over the next 5 days three groups of subjects (S-S, I-I and C-C), continued to receive the same treatment they had received for the first 21 days of the study (i.e. up to day 26 of the study). Two groups (C-S and I-S) received twice daily saline injections over days 22 to 26 inclusive, instead of their previous drug injections. These two groups of animals therefore experienced withdrawal from the relevant drug treatments.

Withdrawal was assessed in Groups C-S and I-S 18 hours after the final drug injection on day 21, and at 24 hour intervals thereafter, up to experimental day 27. The main indices of withdrawal recorded were:- body weight, food intake and core body temperature. Significant (α =0.05) withdrawal effects were seen in group C-S as determined by food intake and body weight measures, which fell significantly and then recovered over the 6 days during which withdrawal was assessed. During withdrawal, the mean body weight of group C-S fell from 215 to 206 gms, a fall of some 4% of total body weight. Withdrawal effects peaked two days after the cessation of chronic chlordiazepoxide treatment (Day 23).

No effects of withdrawal on body weight and food intake were seen in subjects withdrawn from ipsapirone (Group I-S); nor were any such withdrawal signs seen in ipsapirone treated animals (Group I-I) which were subsequently (days 27 and 28) given the benzodiazepine antagonist flumazenil (Ro 15 - 1788, 10 mg/kg, i.p.). This antagonist also failed to induce any overt behavioural signs of "precipitated" withdrawal in ipsapirone treated animals. Ro 15-1788 failed to precipitate withdrawal even though there is evidence that buspirone, a 5-HT_{1A} anxiolytic, can potentiate benzodiazepine binding in vivo (Goeders et al. 1988).

Body temperature measurements during withdrawal indicated that animals withdrawn from chlordiazepoxide (group C-S) showed evidence of withdrawal-induced hyperthermia. Such hyperthermia was not seen in animals maintained on chlordiazepoxide (group C-C). However, "withdrawal" hyperthermia was also seen to a significant extent in control animals that received saline throughout the study (Group S-S). It was therefore difficult to attribute this effect unequivocally to withdrawal from chlordiazepoxide. Nevertheless, there was no sign that animals withdrawn from ipsapirone showed hypo- or hyperthermia during withdrawal.

These data suggest that ipsapirone can be given at high doses to drug-naive subjects for long periods of time without inducing the type of withdrawal effects seen with benzodiazepines.

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DORSAL RAPHE LESIONS ABOLISH EFFECTS OF 8-OH-DPAT AND IPSAPIRONE IN X-MAZE

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8-hydroxy-2(di-n-propylamino)tetralin (8-OHDPAT) and ipsapirone bind to 5-HT1A receptors. Ipsapirone is anxiolytic in several tests but the effect of 8-OHDPAT depends on the model used. Both its anxiogenic effect in X-maze and its anxiolytic action in punished drinking appear to be presynaptic since they are abolished by 5-HT depletion with p-chlorophenylalanine (Critchley & Handley, 1987; Engel et al., 1984). It may therefore have more than one site of action. We have here examined whether dorsal raphe is involved in the effects of these agents in the X-maze.

12 male PVG rats (240-260g) received 5,7-dihydroxytryptamine (4ug in 0.4ul of 0.2% ascorbic acid saline) into dorsal raphe while 12 sham operated rats received vehicle. At 21 days, rats received 8-OHDPAT (100 ug/kg ip), at 28 days, ipsapirone (1 mg/kg) and at 35 days, yohimbine (0.5 mg/kg) each with vehicle control. Subsequent assay showed 11 successful dorsal raphe lesions which spared median raphe (mean 9.25±7% depletion of 5-HT in hippocampus and 67±5% depletion in striatum). Results are given for these animals.

Dorsal raphe lesions (DRX) did not affect open/total entry ratio in X-maze (saline in shams 0.32 ±0.005; in DRX 0.31±0.02), DRX did reverse the anxiogenic (0.14±0.01, $p<0.01$) effect of 8-OHDPAT to anxiolytic (0.46±0.05, $p<0.01$ cf saline/DRX) and the anxiolytic action of ipsapirone (0.40±0.01) was abolished (0.30±0.02). The anxiogenic action of yohimbine (0.20±0.01, $p<0.01$) was unaffected by DRX (0.22±0.01). Total entries were unchanged throughout.

Thus, in the X-maze, both the anxiogenic effect of 8-OHDPAT and the anxiolytic effect of ipsapirone depend on dorsal raphe. It appears likely that 8-OHDPAT is acting as an agonist and ipsapirone as an antagonist at this site. This is concordant with the ability of ipsapirone to prevent the anxiogenic effect of 8-OHDPAT in this model (Critchley & Handley, 1987). An anxiogenic effect of 5-HT has also been observed after injection directly into dorsal raphe in mouse light aversion, whereas buspirone was anxiolytic (Costall et al., 1988). The anxiogenic effect of yohimbine appears to be independent of dorsal raphe.

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PROLONGED REVERSAL OF CHLORDIAZEPOXIDE AND ETHANOL WITHDRAWAL ANXIETY IN THE RAT BY A SINGLE DOSE OF FLUMAZENIL

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We recently demonstrated that the benzodiazepine (BDZ) antagonist, flumazenil (RO 15-1788) reversed the anxiogenic response detected by the elevated plus-maze test following chlordiazepoxide (CDP) withdrawal (Baldwin & File 1988). Flumazenil has also been shown to attenuate development of BDZ withdrawal signs in primates (Gallager et al 1986; Lamb & Griffiths 1985). Here we have investigated the effect of flumazenil on the development of the anxiogenic response displayed by rats withdrawn from chronic CDP, using the social interaction test of anxiety (File 1980). In addition, we examined the effect of flumazenil on withdrawal from chronic ethanol.

Male hooded Lister rats were used in 2 separate experiments. In experiment 1, rats were tested in the social interaction test (for details see File 1980) 24h after withdrawal from CDP (10mg/kg/day for 27 days). One CDP-treated group received flumazenil (4mg/kg) once during chronic treatment, 6 days before testing. A different CDP-treated group received flumazenil (4mg/kg), 24h after CDP-withdrawal, 20 mins before testing. Control group animals received vehicle injections throughout. In experiment 2, rats were fed a liquid diet containing 10% ethanol or control for 5 weeks (mean ethanol intake 12g/kg/day). Rats were tested 8h after ethanol was withdrawn. One group received flumazenil (4mg/kg) 20 mins prior to testing. Rats were then returned to their respective diets for 3 days and retested, undrugged, 8 hours after ethanol was withdrawn.

In experiment 1, CDP withdrawal significantly reduced time spent in social interaction compared with controls, indicating an anxiogenic response. Flumazenil (4mg/kg) given 20 mins before testing or 6 days before withdrawal completely reversed the reduction in social interaction during CDP withdrawal. In experiment 2, ethanol withdrawal significantly reduced social interaction scores compared with controls indicating an anxiogenic response. Flumazenil (4mg/kg) given 20 minutes before testing significantly reversed this effect. Three days later, withdrawal from ethanol again produced a significant decrease in time spent in social interaction. Rats that received flumazenil (4mg/kg) three days earlier, again spent significantly more time in social interaction than withdrawn rats.

In agreement with previous results in the plus-maze (Baldwin & File 1988), flumazenil reversed the anxiogenic CDP-withdrawal response in the social interaction test; a single injection of flumazenil, given during CDP-treatment also prevented the development of this anxiogenic response. Interestingly, flumazenil also produced a long-lasting reversal of the anxiogenic withdrawal response seen after ethanol-withdrawal.

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A SINGLE DOSE, BUT NOT CHRONIC INJECTIONS, OF FG 7142 UPREGULATES ß-ADRENOCEPTORS IN RAT CEREBRAL CORTEX

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Repeated injections of the benzodiazepine-receptor partial inverse agonist, FG 7142 produced an upregulation of α_2- and ß-adrenoceptors in mouse cerebral cortex (Stanford et al 1986a); a single injection caused a larger increase in ß-adrenoceptor number (Stanford et al 1986b). These changes were seen 7 days after the last injection. The aims of this study were to determine whether similar changes are found in rats and whether there are associated changes in behaviour and behavioural responses to noradrenergic receptor ligands. The effects of yohimbine and clenbuterol were examined in the elevated plus-maze test of anxiety (Pellow et al, 1985) and the holeboard test of exploration (File & Wardill, 1975), respectively.

Male hooded Lister rats were randomly allocated to the treatment groups: (a) 12 days FG 7142 (20mg/kg/day); (b) 11 days vehicle (water + tween) then 1 injection of FG 7142 (20mg/kg); (c) 12 days vehicle. All rats were then left in their home cages for 7 days. On the following 2 days rats were tested in the elevated plus-maze (for details see Pellow et al 1985) 30 min after yohimbine (1.25 or 2.5 mg/kg) or vehicle (water) and in the holeboard (for details see File & Wardill 1975) 30 min after clenbuterol (0.02 or 0.2 mg/kg) or vehicle (water). Rats that had received only vehicle on the previous 2 days were killed at least 3h after testing; cerebral cortices were taken for radioligand binding to ß- and α_2-adrenoceptor binding sites and measurement of noradrenaline content by HPLC-ECD.

TABLE 1 Effects of FG 7142 on ß-receptor binding in rat cortex

means ± SEM	ß-Bmax	n	kd	n
Vehicle control	151 ± 15	(9)	1.6 ± 0.3	(9)
acute FG 7142	184 ± 19	(10)*	1.93 ± 0.17	(10)
chronic FG 7142	154 ± 17	(9)	1.4 ± 0.17	(9)

* $p<0.05$ (sig.diff. from chronic FG 7142; Wilcoxon matched pair signed ranks test).

Acute FG 7142 significantly increased the number of ß-adrenoceptors compared with chronic FG 7142. There was no difference between the ß-Bmax for chronic FG 7142 and controls. There were no effects on α_2-receptor binding or noradrenaline levels, baseline levels of anxiety or exploration, or on the behavioural effects of yohimbine or clenbuterol.

Thus, in rats, a single but not chronic injection of FG 7142 caused ß-adrenoceptor upregulation; this is not associated with any behavioural changes and may be an adaptive response to the acute effects of FG 7142.

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USE OF THE ELEVATED PLUS MAZE TO ASSESS ANXIOLYTIC POTENTIAL IN THE RAT

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Many laboratories now utilise the elevated plus maze as an animal model for the detection of potential anxiolytic and anxiogenic agents. The behavioural measure in this model reported to be most sensitive to these types of compounds is the time spent on the open arms. A second measure also shown to be altered by these compounds is the number of open arm entries though it has been suggested that this is a less sensitive measure. In our initial studies using an elevated plus maze based on that described by Handley and Mithani (1984) it was observed that when a control rat entered a closed arm it tended to explore its full length yet rarely on entry to an open arm did it venture to the end. As a result it is suggested that it may be more appropriate to divide each arm into sections and to record the extent to which the animals explore each arm type. In this study the behavioural effects of chlordiazepoxide (CDP) on in-house bred male Hooded Lister and Sprague-Dawley strains of rat were investigated using the altered methodology outlined above in order to give some indication as to its sensitivity.

Animals (250-350g) were transferred to the experimental room at least 1h prior to testing. The apparatus consisted of an X-shaped maze constructed of perspex, elevated 70cm from the floor and comprising of two (opposite) closed arms and two open arms. These arms were 45cm long and 10cm wide. The closed arms had sides 10cm high while the open arms had no sides. The floor was covered with rubber matting and lined so that each arm was divided into 15cm sections. The 5 min test period commenced by placing a rat on the centre square (all facing the same open arm) and time spent and the number of arm sections crossed on both the open and closed arms was recorded. Time spent in the open arms and the sections traversed are shown in the table below.

Strain	CDP mg/kg i.p.	Time in open arm(s)	Open arms sections traversed 1st	2nd	3rd
Sprague-Dawley	veh	40.6± 6.5	3.8±0.4	2.2±0.3	1.0±0.4
	0.3	29.8±10.7	2.8±1.0	1.6±0.5	1.4±0.5
	1.0	70.0± 9.8*	5.2±0.4	4.0±0.9	3.6±0.7*
	3.0	89.6±19.0*	6.8±1.2	5.8±1.0*	5.6±1.2**
Hooded Lister	veh	73.2± 8.8	4.5±0.6	3.7±0.5	3.3±0.5
	0.3	76.6±19.1	4.0±0.6	3.6±0.7	3.0±0.5
	1.0	120.7±17.7*	9.0±1.2**	8.2±1.5**	8.3±1.6***
	3.0	105.4±21.9	6.6±1.7	5.0±1.3	4.2±1.2

n = 6-10. *P<0.05, **P<0.01, ***P<0.001. Total arm entries were not generally influenced by CDP.

Data indicates that entries into the furthermost section of the open arms is markedly increased by the known anxiolytic agent, chlordiazepoxide, in both strains of rat and it is therefore suggested that the assessment of open arm sections traversed, rather than simple measures of entries into the open arms, may provide a more sensitive measure of anxiolytic drug potential in the rat using the elevated plus maze.

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LIMITATIONS OF THE ELEVATED PLUS-MAZE TEST OF ANXIETY FOR ASSESSING THE EFFECTS OF CHRONIC BENZODIAZEPINE ADMINISTRATION

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Tolerance to the anxiolytic effect of diazepam (DZP) has been shown in the elevated plus-maze test (Fritz et al, 1986). However, rats were tested repeatedly, which may have influenced the results. The present experiments were undertaken to validate this test under our conditions and to investigate the time-course of tolerance to the anxiolytic effects of DZP. The apparatus consists of 2 open and 2 enclosed arms of a plus-shaped maze elevated to a height of 1m. Anxiolytic drugs increase the proportion of entries into and time spent on the open arms (Pellow et al, 1985).

For the validation experiment, male PVG rats (Bantin & Kingman) were pre-treated for 3 days with vehicle (1% Tween 20 in saline i.p.; control and acute groups) or DZP (1.25 mg/kg i.p.; subacute group). On day 4 animals were given vehicle (controls) or DZP (acute and subacute groups) i.p. 30 min before a 10-min test in the maze. The open/total ratios both for entries and for time were significantly higher ($p<0.05$) in the DZP groups (entries: acute 0.57±0.04; subacute 0.60±0.04) than control (0.38±0.02), demonstrating an anxiolytic effect of DZP. Mean total entries were significantly raised ($p<0.05$) in the acute group only.

For the chronic experiment, rats were pre-treated with 27 days vehicle (control and acute groups), 21 days vehicle + 6 days DZP, 7 days vehicle + 20 days DZP or 27 days DZP (DZP 2.5 mg/kg i.p.). A further group was weighed and then left unhandled for 27 days (unhandled control group). On day 28 vehicle (control and unhandled control groups) or DZP (1.25 mg/kg i.p.) was administered 30 min prior to testing. The test period was 15 min as previous experiments had shown that long-term handling tended to reduce the total number of entries. Although there was a trend for ratios to be higher in the treated groups, there were no significant differences between groups, and no clear pattern of tolerance. Most strikingly, there was no significant anxiolytic effect of DZP in the acutely treated group (Table 1).

Table 1 Open/total ratios ± s.e.mean for 15 min test in +-maze

	unhandled control	control	acute	6 days pre-treatment DZP	20 days pre-treatment DZP	27 days pre-treatment DZP
	n=6	n=6	n=5	n=5	n=7	n=7
Entries	0.54±0.04	0.45±0.05	0.60±0.06	0.53±0.06	0.65±0.04	0.58±0.07
Time	0.48±0.11	0.48±0.12	0.61±0.14	0.67±0.08	0.67±0.08	0.64±0.13

We have observed similar results in two other strains of rat. It is possible that after chronic handling the elevated plus-maze does not present a sufficiently anxiogenic stimulus for the DZP effect to be measured. However, the ratios in unhandled control rats were not significantly different from handled control rats. This phenomenon requires further investigation, but suggests that this test may not always be valid for the investigation of the effects of chronic benzodiazepine treatment.

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THE EFFECT OF MORPHINE TOLERANCE ON BRAIN AND SPINAL CORD OPIOID AND α_2-ADRENERGIC RECEPTORS IN THE RAT

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There have been conflicting reports on the effect of morphine tolerance on central α_2-adrenergic receptors. Hamburg & Tallman (1981) demonstrated an increase in the number of α_2-adrenergic receptors in morphine tolerant rat cortex and brain stem, whereas later workers were unable to show any changes (Sethy & Harris, 1982). In this study we have examined the effect of chronic morphine administration on [^{3}H] clonidine and [^{3}H] dihydromorphine binding to adult (250-300g) female rat (Porton, Wistar derived) brain and spinal cord homogenates. Rats were made morphine tolerant by injecting them daily for 10 days with morphine sulphate (7.5 mg/kg s.c.). Their tolerance was confirmed using the tail flick test. The rats were stunned and killed by decapitation and the spinal cord and brain (minus medulla) rapidly dissected out. The tissues ($\cong$ 0.5g) were homogenised in ice-cold Tris-HCl buffer (50 mM, pH 7.5), then centrifuged (50,000g for 10 minutes). The supernatant was discarded and the membrane pellet resuspended in Tris buffer. After a preincubation period of 30 minutes the membranes were recentrifuged and resuspended for use in the radioligand saturation binding studies. [^{3}H] clonidine (Amersham Int.) and [^{3}H] dihydromorphine (NEN) were used to study α_2-adrenergic and opiate binding respectively (concentration range 0.1 to 10 nM). Non-specific binding was defined using the competing ligands idazoxan (100 µM) and naloxone (10 µM) for α_2-adrenergic and opiate binding. Specific binding was taken to be the difference between the total and non-specific binding. The results produced are summarised in the following table.

[^{3}H] clonidine and [^{3}H] dihydromorphine (DHM) binding. mean ± S.E.M. (n = 5-6)

Tissue		K_D (nM)		B_{max} (fmol/mg protein)	
		[^{3}H] clonidine	[^{3}H] DHM	[^{3}H] clonidine	[^{3}H] DHM
Brain:	control	2.36±0.19	2.78±0.41	383.0±34.3	307.8±6.1
		N.S.	N.S.	N.S.	P<0.001
	tolerant	2.70±0.23	2.93±0.38	413.7±23.8	507.7±21.7
Cord:	control	3.51±0.64	2.38±0.52	207.8±18.8	163.3±19.1
		N.S.	N.S.	N.S.	N.S.
	tolerant	2.96±0.38	2.98±0.52	179.9±18.2	166.6±16.4

Statistics performed using the Mann-Whitney U-test. (N.S. = not significant)

From Hill plots there appears to be only one binding site for both ligands and tissues. There was no change in the number of binding sites (B_{max}) or affinity (K_D) of [^{3}H] clonidine in either the brain or spinal cord following induction of morphine tolerance. The affinity of [^{3}H] dihydromorphine also remained unchanged in morphine tolerant rat brain and spinal cord. However the number of brain binding sites increased significantly ($p<0.001$) in morphine treated animals, although in the spinal cord binding remained unaltered. These data would support the findings of Sethy & Harris (1982) that morphine tolerance does not modify α_2-adrenergic receptor binding in the CNS.

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ADENOSINE INHIBITS CALCIUM UPTAKE INTO RAT BRAIN SYNAPTOSOMES

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The neuromodulatory properties of adenosine may involve regulation of calcium uptake into presynaptic terminals. The use of synaptosomes to study this possibility has led to conflicting results, (Barr et al., 1985; Wu et al., 1982; Ribeiro et al 1979). We have reinvestigated this problem using purified synaptosomes and different incubation times and conditions.

Synaptosomes were prepared as described by Gray and Whittaker (1962) with the crude synaptosomal (P_2) fraction being purified on a discontinuous sucrose density gradient. The synaptosomes were suspended in a Tris buffered ACSF (composition mM): Tris HCl 20, NaCl 135, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 0.6, glucose 10, pH 7.7, 37°C). Experimental solutions contained 50µl 45Ca (1µCi), 50µl ACSF, and 50µl synaptosomes to initiate the incubation period. The tubes were placed in a water bath at 37°C for at least 30 mins before experimentation. Uptake was terminated by adding lanthanum stopping buffer (1mM). Solutions were then filtered and washed, and the amount of radioactive calcium measured by scintillation spectrometry.

The effect of adenosine and a series of analogues on calcium influx was tested by adding cold synaptosomes directly to the test solution and incubating at 37°C for 2 mins. Alternatively, the compounds were preincubated with the synaptosomes for 10 mins at 5°C or 37°C and uptake measured over 1 or 20 secs. Inhibition of calcium uptake was observed only with preincubation and prewarming.

		nmol $^{45}Ca^{2+}$/mg protein, mean ± s.e.m.		
Compound (µM)	n	Control	Test	Significance
Adenosine (10)	3	26.7±4.4	25.2±0.8	N.S.
CPA (1)	5	27.9±2.6	20.0±2.3	$P<0.01$
CHA (1)	6	29.4±2.0	25.8±1.4	N.S.
R-PIA (5)	6	24.1±2.1	21.4±1.6	N.S.
S-PIA (5)	6	22.3±1.5	22.5±2.3	N.S.
CADO (1)	3	31.2±0.7	26.5±0.8	$P<0.01$
NECA (10)	6	11.9±0.5	7.4±1.4	$P<0.05$

All samples preincubated at 37°C, 20 sec.uptake N.S:$P>0.05$ (PTT).

A small but significant inhibition of depolarisation induced calcium uptake was shown by N6-cyclopentyladenosine (CPA), 2-chloroadenosine (CADO) and N-ethylcarboxamide adenosine (NECA). Since N6-cyclohexyladenosine (CHA) and N6-phenylisopropyladenosine were inactive, an A2 class of receptor may be involved.

J.T.B. is a S.E.R.C. C.A.S.E. research student with Pfizer (UK) Ltd.

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316P

KAINIC ACID-PREFERRING RECEPTORS STIMULATE INOSITOL PHOSPHATE FORMATION IN CULTURED CEREBELLAR GRANULE CELLS

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Electrophysiological experiments have identified three subtypes of excitatory amino acid (EAA) receptor for which NMDA, quisqualic acid and kainic acid are selective agonists. In addition, studies of second messenger formation in response to EAAs suggest the existence of further receptor subtypes. In cerebellar granule cells, EAAs stimulate the formation of inositol phosphate (IP): this stimulation has two components, one of which is sensitive to 2APV (Nicoletti et al., 1984). Wroblewski et al. (1987) have classified these receptors as GP1 (2APV-sensitive) and GP2 (2APV-insensitive). The GP1 receptor appears to correspond to the NMDA-preferring receptor defined electrophysiologically. We now present pharmacological evidence suggesting that the GP2 receptor corresponds to the kainate-preferring receptor.

Granule cell cultures were prepared from neonatal rat cerebellum as described by Gallo et al. (1984). After 24 h, the cells had formed into clusters and extended neurites. The cultures were essentially devoid of other cell types. The cells were prelabelled with [3H]myo-inositol (0.3 mM) for 24 h before use. The culture medium was then replaced with Krebs-HCO_3 buffer containing LiCl (10 mM) and the antagonist if tested. The EAA was then added after 15 min for a further 30 min. IP formation was measured using the method of Schmidt et al. (1987).

In freshly-prepared cultures, IP formation could be stimulated by glutamic acid. This effect was sensitive to the NMDA antagonist MK 801. These cultures were insensitive to kainic acid. However, over a period of 7 days, the cells gradually became sensitive to kainic acid. The glutamate response in the 7 day-old cultures was not sensitive to MK 801. For all subsequent studies, 7 day-old cultures were used.

Dose-response curves were obtained for the different EAAs, and the following rank order of potency observed: domoate (EC50 = 5 x 10^{-6} M) > kainate (EC50 = 5 x 10^{-5} M) > glutamate (EC50 = 10^{-4} M) > quisqualate > ibotenate. The maximal effects of the latter two were considerably smaller than those of the other EAAs. NMDA was inactive. A variety of antagonists were tested using kainic acid (10^{-4} M) as the agonist. The most potent inhibitor identified was CNQX (IC50 = 2 x 10^{-5} M). The response was also inhibited by kynurenic acid (IC50 = 5 x 10^{-4} M). No inhibition could be observed with MK 801 (10^{-6} M), riluzole (10^{-4} M), 2APV, 2APB, L-serine-O-phosphate, α-aminoadipate, GDEE or GAMS (all at 10^{-3} M).

When the magnesium was removed from the incubation medium, IP formation could be stimulated by NMDA (50% stimulation at 10^{-4} M). The effect of ibotenic acid was also increased, whilst the responses to glutamic and kainic acids were unchanged.

These results demonstrate that the pharmacological specificity of the receptor mediating EAA-stimulated IP formation in cerebellar granule cells is consistent with that of the kainic-acid preferring receptor observed in electrophysiological models. Preliminary evidence suggest that, under certain conditions (low magnesium, immature cell cultures) NMDA-preferring receptors may also be coupled to IP formation.

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THE USE OF [^{125}I]7-AZIDOIODOKETANSERIN AS A PHOTOAFFINITY LABEL FOR HISTAMINE H_1 RECEPTORS IN GUINEA-PIG CEREBELLUM

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In the central nervous system, histamine H_1 receptors are thought to be mediate the sedative effects of antihistamine drugs. A particularly rich source of these receptors is the guinea-pig cerebellum, where they are coupled to phospholipase C (Daum et al., 1984). Recently, it has been demonstated (Wouters et al., 1985) that azido derivatives of ketanserin will bind covalently to histamine H_1 and serotonin $5HT_2$ receptors on exposure to ultraviolet radiation. It has since been shown (Wouters et al., 1987) that one of these compounds, 7-azido-8-[^{125}I]iodoketanserin ([^{125}I]AZIK), can be used as an irreversible radioligand to identify the $5HT_2$ receptor. We now report on the use of this ligand to photolabel the H_1 receptor.

A crude particulate fraction of guinea-pig cerebellum was used. Tissue was homogenised in sodium phosphate buffer (50 mM; pH 7.4) and centrifuged at 50,000 x G for 10 min. The pellet was recovered and resuspended in the same buffer (10 mg prot/ml). Binding assays were performed by filtration. Soluble receptor preparations were obtained by incubating the membrane preparations with digitonin (1%) for 1 h and recovering the soluble protein by ultracentrifugation (150,000 x G for 1 h).

When guinea-pig cerebellar membranes were irradiated for one minute with [^{125}I]AZIK (0.2 nM) and the proteins contained therein subsequently subjected to polyacrylamide gel electrophoresis, two labelled peptides were observed with molecular weights of 58,000 and 63,000 Daltons. The labelling of both these peptides was completely supressed when the photolabelling was performed in the presence of mepyramine (10^{-6} M). Minor incorporation was sometimes observed in other molecular weight ranges, but this was not mepyramine-sensitive. The incorporation was abolished in a concentration-dependant manner by mepyramine and d-chlorphenyramine, but not by pipamperone, prazosin or tetrabenazine (10^{-6} M). By contrast, when membranes from rat frontal cortex were irradiated in the presence of [^{125}I]AZIK, the ligand labelled a 66,000 Da peptide: this labelling was sensitive to pipamperone but not mepyramine. The presence of protease inhibitors in the incubation media did not change the labelling pattern, whilst, without 2-mercaptoethanol in the electrophoresis buffer, the molecular weights of the labelled peptides were increased slightly.

Solubilised [^{125}I]AZIK binding sites from guinea-pig cerebellar membranes co-migrated with H_1 receptors identified by reversible [^{3}H]mepyramine binding upon chromatography on both gel filtration and chromatofocussing columns. The molecular weight of the solubilised receptor-detergent complex was estimated to be 450,000 Da, and its isoelectric point to be 4.2.

These results demonstrate the feasibility of using [^{125}I]AZIK to photolabel the histamine H_1 receptor. Although this ligand also binds to the serotonin $5HT_2$ receptor and the monoamine transporter, specificity of labelling can be acheived by the use of appropriate tissue sources and compounds to define the non-specific binding. The molecular weights and isoelectric points of the identified receptor proteins were comparable to those of previously characterised monoamine receptor proteins.

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4-β-PHORBOL-12,13-DIACETATE POTENTIATES MONOSYNAPTICALLY-EVOKED EXCITATIONS IN THE OLFACTORY CORTEX

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Protein kinase C (PKC) is an enzyme which requires Ca^{2+}, phospholipids and diacylglycerol for full activity. Phorbol esters which mimic diacylglycerol, selectively activate the enzyme (Baraban, 1987) and have proved useful in elucidating the possible roles of PKC. The aim of the present study was to determine whether PKC might regulate transmission at the lateral olfactory tract-pyramidal cell (LOT-PC) synapse of the olfactory cortex, a site where glutamate and/or aspartate are likely transmitters (Collins, 1986).

Rat olfactory cortex slices (500μm thick) were preincubated and perfused at room temperature (Pickles & Simmonds, 1976). Transmission at the LOT-PC synapse was monitored by recording the surface field potential known as the N-wave evoked on stimulation of the LOT. Dropwise application of 4-β-phorbol-12,13-diacetate (PDA_c; 0.33 to 20 μM) had no effect on the configuration of the tract action potential but caused a concentration-dependent increase in the area and peak amplitude of the N-wave together with the appearance of multiple population spikes. The maximum effect of PDA_c was at a concentration of 10 to 20 μM when the area and peak amplitude of the N-wave was 271±18 and 197±13 percent of control respectively (n=12). Each dose of PDA_c required 18 min contact time to evoke a peak response and the effects were essentially irreversible (4 slices). 4-β-Phorbol (20μM), which does not stimulate PKC activity, had no effect on the N-wave (4 slices).

In order to identify the site of action of PDA_c, the following experiments were carried out using slices perfused with solution containing 25μM picrotoxin to block γ-aminobutyric acid receptors and 100μM 2-amino-5-phosphonopentanoic acid to block polysynaptic excitations (Collins & Howlett, 1988). 1 Synaptic facilitation of the N-wave evoked by stimulation of the LOT twice in rapid succession (Anson & Collins, 1987) was abolished by 5μM PDA_c (4 slices). 2 In a series of 4 input-output studies, PDA_c (5μM) had no effect on the relationships between stimulus voltage and action potential amplitude or N-wave amplitude and population spike latency; however, for a given action potential, the amplitude of the N-wave was markedly increased. 3 Depolarizations evoked by single submaximal concentrations of L-glutamate or L-aspartate (Surtees & Collins, 1985) were unaffected by 5μM PDA_c (n=3). These results strongly suggest that the potentiation of transmission at the LOT-PC synapse produced by PDA_c is caused by an increase in transmitter release although an increase in PC excitability cannot be entirely excluded. Thus PKC might have a role in regulating monosynaptic excitatory transmission in the olfactory cortex.

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THE INFLUENCE OF CENTRAL HISTAMINERGIC MODIFICATION ON THE SUSCEPTIBILITY OF MICE TO CHEMICALLY-INDUCED SEIZURES

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High doses of antihistamines (H1 antagonists) have been reported to produce convulsions, particularly in children, and studies on the use of these drugs in epileptic patients have shown that there may be an increase in seizure frequency (Churchill & Gammon, 1949). In 1986 Tuomisto & Tacke suggested that histamine may be acting as an anticonvulsant agent in the brain. In this study, the effect of pretreating mice with differing doses of several antihistamines (H1 antagonists) on the incidence and frequency of clonic convulsions induced by either pentylenetetrazol (leptazol) or picrotoxin was investigated. The results obtained were analysed using non-parametric statistics (Mann-Whitney U Test).

It was found that both diphenhydramine (20-30mg kg^{-1}) and mepyramine (10-20mg kg^{-1}) produced dose-related potentiation of leptazol- or picrotoxin-induced clonic convulsions in the mice, whilst astemizole (10-20mg kg^{-1}), a non-CNS penetrating H1 antagonist, (Van Wauwe, Awouters et al, 1981) had no effect on the pattern of the chemically-induced seizures (Table 1). These results suggest that a reduction in central histaminergic (H1) function increases the risk of seizures in mice and supports the suggestion that there may be involvement of the central histaminergic system in convulsive disorders in man. The results of this study are in agreement with Gerald & Richter (1976) who showed that a reduction in histaminergic function modified the risk of seizures in mice while an enhancement was without effect.

Table 1 The relative frequency of chemically-induced seizures in mice after antihistamine pretreatment. Controls = 1.0

Drug	mg kg^{-1}	Leptazol Relative seizure frequency	Group size.	Picrotoxin Relative seizure frequency	Group size.
Diphenhydramine	30	2.8 ***	9	4.5 ***	10
	20	1.5 **	28	2.1 *	18
Mepyramine	20	4.5 **	40	4.5 **	20
	10	3.8 **	20	1.2	20
Astemizole	20	1.5	20	1.5	20
	10	1.0	10	1.0	10

***=$p<0.001$, **= $p<0.025$, *= $p<0.01$ compared to vehicle-injected control mice.

Further work on modifying brain histamine levels and the effects of other histaminergic agonists/antagonists on animal models of epilepsy is being undertaken.

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U50488H DECREASES KAINIC ACID-INDUCED NEUROTOXICITY IN THE HIPPOCAMPAL CA3/CA4 REGION OF THE RAT

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Systemic administration of the neurotoxin, kainic acid (KA), to rats elicits seizures which are accompanied by a loss of neurones from selective brain regions, including the hippocampus (Lothman and Collins, 1981). U50488H has been reported to protect hippocampal CA1 neurones against KA neurotoxicity (Lason, Simpson, and McGinty, 1987). In the present study the effect of U50488H on KA-induced neuronal loss has been quantified.

Male Sprague-Dawley rats (200-450g) were given KA (10mg/kg s.c.). The animals were then observed from 30min after the injection for periods up to 3h. U50488H (1-30mg/kg s.c.) was administered 30min prior to KA and again 3h later. Animals were killed after 4 days by cervical dislocation and the brains removed and frozen rapidly. Coronal sections (20μm) containing the dorsal hippocampus were cut on a cryostat and stained with haemotoxylin. The area of staining in the hippocampal regions CA1/CA2 and CA3/CA4 were then measured using a Cambridge Instruments Image Analyser (Quantimet 970).

In rats which had received KA, wet dog shakes (WDS) commenced 53±3min after injection (n=16) and seizures at 81+4min (n=13). U50488H (1-30mg/kg) caused a dose-dependent increase in the latency to onset of WDS and seizures. The highest dose of U50488H (30mg/kg) prevented WDS in 13 out of 22 animals and seizures in 10 out of 22 animals and in the remainder increased the latency to onset. In rats that had experienced seizures the area of neurones stained in the CA1/CA2 and CA3/CA4 regions of the hippocampus was significantly less than in saline-treated rats (t-test, * $p<0.05$; Table 1). However, in rats that had received kainic acid but had not experienced seizures, no loss of staining was observed. Thus, neurotoxicity was associated with kainic-acid induced seizures.

Table 1

Hippocampal region	Area of staining ($\mu m^2 \times 10^3$) Control	KA	KA+U50488H (30mg/kg)
CA1/CA2	344±20 (13)	250±25 (11)*	253±28 (12)*
CA3/CA4	164±11 (13)	85±15 (11)*	141±15 (12)*

Only data from rats that convulsed is included in the table

U50488H (1-30mg/kg) protected neurones in the CA3/CA4 region of the hippocampus from KA neurotoxicity but not those in the CA1/CA2 region. The protection did not appear to be dose-dependent, and the effect of U50488H (3mg/kg) was apparently not reversed by co-administration of the opioid antagonist, naltrexone (3mg/kg; n=8). Naltrexone alone had no effect. A loss of neurones from the CA3/CA4 region of the human hippocampus is observed in post-mortem brains of patients that have suffered repetitive epileptic attacks (Nadler, 1981). U50488H appears to protect the corresponding neurones in rats from kainic acid-induced neurotoxicity.

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THE EFFECT OF CROMAKALIM ON [^{86}]Rb EFFLUX FROM RAT BRAIN SYNAPTOSOMAL AND SLICE PREPARATIONS

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The novel antihypertensive agent, cromakalim (BRL 34915), relaxes vascular smooth muscle, an effect that may be due to the opening of K^+ channels and enhancement of K^+ efflux, resulting in a hyperpolarisation of the cell membrane (Buckingham et al. 1986; Coldwell and Howlett, 1987; Hamilton et al. 1986). Bartschat and Blaustein (1985), using [^{86}]Rb as a tracer for K^+ ions in rat brain non-superfused synaptosomes, demonstrated that Ca^{++}-sensitive K^+ efflux was sensitive to tetraethylammonium (TEA) and 3,4-diaminopyridine (DAP), known K^+ channel blockers. The objective of this present study was to compare the effects of cromakalim with other K^+ channel activators, nicorandil and pinacidil, on K^+ channel function as measured by [^{86}]Rb efflux in rat brain superfused synaptosomal and slice preparations.

Rat whole brain, cortical and hippocampal synaptosomes or cortical and hippocampal slices (200 x 200μ) were incubated in oxygenated HEPES physiological saline (PSS) and loaded with [^{86}]RbCl (10μCi/ml) for 30 minutes at 30°C. Aliquots of preloaded synaptosomes (~20mg tissue) or slices (~12mg tissue) were filtered onto GF/B filters and rinsed with 4 x 5ml of ice-cold PSS. The rinsed filters were placed individually in wells of a 6 chamber release apparatus (Raiteri et al. 1974) and superfused with PSS (30°C) at a rate of 0.5ml/min. After a 20 minute initial superfusion period (to achieve steady efflux rates), 36 successive 2 minute fractions were collected and at various times pulses of drug introduced (S1: 10-12; S2: 24-26 fractions). At the end of the experiment the tissue on the filters was solubilised with Soluene and the radioactivity in the fractions and extracts was determined. Efflux was expressed as fractional rates.

Initial experiments with whole brain synaptosomes demonstrated that raising extracellular [K^+] (15-60mM) stimulated [^{86}]Rb efflux in a concentration related manner, an effect shown to be Ca^{++}-dependent. TEA (IC_{50} 8mM) and DAP (IC_{50} 1mM) both inhibited K^+(20mM)-evoked [^{86}]Rb efflux of the S2 peak, when added between the S1 and S2 stimulation, confirming that K^+ channels present in nerve terminals are amenable to drug manipulation. Cromakalim (1-10μM), concentrations known to stimulate [^{86}]Rb efflux in vascular tissue (Coldwell and Howlett, 1987), did not affect basal [^{86}]Rb efflux from whole brain synaptosomes whilst higher concentrations (50-100μM) failed to stimulate [^{86}]Rb efflux above that of the ethanol-control values. Nicorandil and pinacidil (up to 100μM) were similarly without effect. In addition, cromakalim (1-10μM) did not alter K^+-stimulated [^{86}]Rb efflux from whole brain synaptosomes.

Regional studies demonstrated that cromakalim (up to 100μM) did not increase significantly basal [^{86}]Rb efflux above the ethanol-control values in cortical and hippocampal synaptosomes. Similarly, studies with preloaded hippocampal or cortical slices demonstrated that cromakalim (10-30μM), nicorandil (30-100μM) and pinacidil (30-100μM) did not stimulate [^{86}]Rb basal efflux.

In summary, rat brain superfused synaptosomal or slice preparations possess Ca^{++}-dependent K^+ channels that can be blocked by TEA and DAP but which are cromakalim, nicorandil and pinacidil insensitive.

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EFFECTS OF CROMAKALIM ON THE RELEASE OF ENDOGENOUS GLUTAMATE FROM CEREBELLAR SLICES

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In a previous communication to the Society we have shown that the potassium channel blocking drug tetrapentylammonium (TPeA) stimulates the release of endogenous glutamate from cerebellar slices, and that this release is not calcium-dependent (Barnes et al., 1987). Cromakalim, a potassium channel activating compound, has been shown to open channels in smooth muscle preparations (Weir & Weston, 1986) and also to reduce the excitability of pyramidal neurones in the hippocampal slice preparation (Alzheimer & ten Bruggencate, 1988). In this present study we have investigated the effects of cromakalim on both TPeA and potassium-induced release of glutamate.

Parasaggital slices (250 um) were superfused (0.5 ml/min) with artificial cerebrospinal fluid (aCSF) gassed with 95% O_2/5% CO_2 at 37°C. Two minute aliquots of aCSF were collected and assayed for glutamate by HPLC with fluorescence detection.

Two-minute pulses of TPeA produced a concentration-dependent ($1x10^{-5}$ to $1x10^{-3}$M) increase in glutamate release under calcium-free conditions. At $1x10^{-3}$M the release in calcium-free aCSF was significantly greater than in normal aCSF ($p<0.05$). Further experiments, using TPeA in normal aCSF, showed that three, two-minute pulses of the drug exhibited no decay over the three pulses (100%, 105% and 104% respectively). In addition, the elevated transmitter release in calcium-free aCSF also persisted, exhibiting no decay over a 30 minute perfusion period. The glutamate releasing activity of TPeA in both normal and calcium-free aCSF was significantly ($p<0.01$ in both cases) decreased by cromakalim ($1x10^{-3}$M) although the difference between normal and calcium-free aCSF was maintained. In addition, cromakalim ($1x10^{-3}$M) also significantly decreased K^+-induced (60mM) glutamate release ($p<0.001$).

The effect of TPeA on glutamate release in normal aCSF is consistent with an inhibition of the repolarising phase of the action potential and thus a prolongation of spike duration. This effect was antagonised by cromakalim, presumably by its ability to open potassium channels. That the release continues in the absence of external calcium might be explained by an increased influx of sodium, due to the prolongation of the action potential, releasing calcium from internal stores (Sandoval, 1980). This inhibition of glutamate release with cromakalim correlates well with the findings of Alzheimer and ten Bruggencate (1988) where they showed that epileptiform activity in hippocampal slices induced by high potassium concentrations or the omission of calcium or magnesium ions from the perfusion medium was reduced by cromakalim. These results suggest that cromakalim and other potassium channel activators might be useful as anticonvulsants.

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SEIZURE KINDLING BY REPEATED FOCAL INJECTIONS OF GLUTAMATE, ASPARTATE OR A GLUTAMATE/ASPARTATE MIXTURE

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It has recently been demonstrated that full limbic seizures can be kindled by repeated amygdaloid injections of a particular mixture of glutamate and aspartate (1:3) at high doses (1.5 μmol in 0.5 μl) (Mori & Wada, 1987). We confirm this observation here and further report that repeated focal injections of glutamate or aspartate given alone, at similar high doses, are equally effective at causing seizure kindling.

Male Sprague-Dawley rats (260-340g) were implanted with a combined guide cannula and stainless steel bipolar electrode unit into the amygdala (coordinates of tip of inserted injection cannula: 2.0mm posterior and 4.5mm lateral to bregma, 9.0mm below skull surface; Paxinos & Watson, 1982). One week after surgery focal amygdaloid injections were delivered every second day (in 0.5 μl phosphate buffer; 2 min) and electroencephalographic and behavioural responses were recorded, as previously described (Croucher et al, 1988).

Initial administration of glutamate/aspartate mixture (1:3 ratio) (1.5 μmol) caused little response, but repeated administration resulted in the development of fully kindled (stage 5) limbic seizures after 5.7 ± 0.9 injections (mean ± s.e.m; n=3). In a second group, given half this dose, stage 5 seizures developed after 8.6 ± 1.0 injections (n=5). The seizures were accompanied by fully developed after-discharges of similar amplitude and frequency to those seen during electrically kindled seizures (Peterson et al, 1983). Moreover, fully kindled seizures could be inhibited by co-injection of 'NMDA receptor' antagonists (AP7 or CPP; 70nmols), thus demonstrating the receptor mediation of the effects. In fully kindled animals single injections (1.5 μmol) of glutamate, aspartate, glutamate/aspartate (1:1) or glutamate/aspartate (3:1), but not sodium chloride, evoked maximal stage 5 seizures. In 2 further groups of animals, administration of glutamate (1.5 μmol) or aspartate (1.5 μmol) alone was also shown to kindle limbic seizures, after 10.5 ± 0.9 (n=6) and 7.7 ± 0.9 (n=6) injections, respectively. Eight weeks after kindling animals given a single injection of glutamate or aspartate showed maximal seizure responses.

Thus, repeated injection of high doses of excitatory amino acids (EAA) into the amygdala results in a gradually increasing excitatory response to the same dose, which is eventually manifested as full stage 5 kindled seizures. This kindling effect is stable for periods of at least 2 months after development and shows many features in common with electrical kindling. Mori & Wada (1987) have shown transference between electrical and EAA kindling, suggesting that they share mechanisms in common. This highlights once again the important role of EAA in basic mechanisms of epilepsy (Bradford & Peterson 1987, Croucher et al, 1988).

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ALTERATIONS IN THE NMDA-RECEPTOR COMPLEX FOLLOWING FOCAL CEREBRAL ISCHAEMIA IN THE RAT

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Glutamate receptor activation plays a key role in the development of ischaemic damage in cerebral tissue. Systemic administration of NMDA receptor antagonists, such as MK-801, attenuates ischaemic brain damage (Gill et al. 1987; Ozyurt et al. 1988). We now report persistence of the glutamate-induced enhancement of (^{3}H)-MK-801 binding 12 hours following focal ischaemia in the rat.

Permanent occlusion of the right middle cerebral artery (MCAO) was performed on six rats (Sprague-Dawley, 300-350g) anaesthetised with 2% halothane as described by Tamura et al. (1981). In six sham-operated rats the middle cerebral artery was exposed but not occluded. Twelve hours after surgery the rats were sacrificed and the following two brain regions dissected from both hemispheres: an area of largely ischaemic cortex termed lateroventral cortex and an area of largely non-ischaemic cortex termed dorsomedial cortex. (^{3}H)-MK-801 (2nM) binding assays were carried out using extensively washed synaptic membranes either in the presence or absence of L-glutamate (10μM). A further four rats received MCAO as described above, and after 12 hours the brains were prepared for histology.

Histological examination of brain sections from these four rats revealed that in the ipsilateral lateroventral cortex, almost every neurone displayed signs of ischaemic cell change, whilst cellular morphology appeared normal in the ipsilateral dorsomedial cortex and in both areas of the contralateral hemisphere. In the ischaemic lateroventral cortex (^{3}H)-MK-801 binding in the absence of glutamate was somewhat reduced relative to sham controls (Table 1). In the presence of glutamate (10μM), (^{3}H)-MK-801 binding in lateroventral cortex was markedly enhanced both in sham-operated and MCAO animals (Table 1). In all other areas similar levels of (^{3}H)-MK-801 binding were observed in sham-operated and MCAO animals in both the absence and presence of L-glutamate.

Table 1

	(^{3}H)-MK-801 Bound (fmol/mg protein)	
	Sham	MCAO
Lateroventral Cortex		
No Glutamate	163 ± 35	94 ± 16
Glutamate (10μM)	697 ± 85	392 ± 80 *
	(480%)	(430%)
Dorsomedial Cortex		
No Glutamate	104 ± 21	121 ± 23
Glutamate (10μM)	557 ± 71	467 ± 67
	(660%)	(410%)

Values are mean ± SEM (n=6 except contralateral lateroventral cortex in MCAO group n=5). Numbers in parenthesis are the mean percentage increase in binding induced by glutamate. *$P < 0.05$ compared to sham group.

Thus while gross, irreversible neuronal damage has occurred in cerebral cortex 12 hours following MCAO, this was not associated with a complete loss of (^{3}H)-MK-801 binding sites. Moreover, the ability of glutamate to enhance (^{3}H)-MK-801 binding is maintained even in ischaemically damaged tissue.

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THE α_2-ADRENOCEPTOR ANTAGONISTS ATIPAMEZOLE AND IDAZOXAN REVERSE THE HYPOTHERMIC EFFECTS OF ETHANOL

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Previous studies have found that the selective alpha$_2$-adrenoceptor antagonist atipamezole can reverse some of the behavioural effects of ethanol, such as the ethanol-induced changes in exploratory head-dipping in a holeboard test (Durcan et al, 1989a,b) and its ataxic effects (Lister et al, 1988), whilst leaving others, such as locomotor stimulant actions, anti-anxiety and anti-convulsive effects, unaffected (Durcan et al, 1989a,b; Lister et al, 1988). In this experiment the effects of the selective alpha$_2$-adrenoceptor antagonists atipamezole and idazoxan on ethanol-induced hypothermia were investigated in mice. The doses of the antagonists were chosen on the basis of their previous effectiveness against some of ethanol-induced behaviour changes.

NIH Swiss mice were administered either 2g/kg ethanol or distilled water vehicle (i.p.) simultaneously with a contralateral injection (i.p.) of atipamezole (1 or 3 mg/kg), idazoxan (1 or 3 mg/kg) or distilled water vehicle. Core body temperatures were measured using a rectal probe and a digital thermometer (Sensortek Inc.). A baseline temperature was taken 20 minutes prior to drug administration. Temperature readings were also taken 20 and 40 minutes after drug administration.

Ethanol significantly reduced ($p<.001$) core temperature whilst both alpha$_2$-adrenoceptor antagonists were without effect when administered alone. However, both the 1 and 3 mg/kg doses of atipamezole significantly ($p<.05$) reversed the ethanol-induced reduction in body temperature 20 and 40 minute after administration. The 3 mg/kg dose of idazoxan (but not the 1 mg/kg dose) also significantly ($p<.05$) reversed ethanol's hypothermic effects 20 minutes after administration but this effect was not statistically significant at 40 minutes. These results suggest that alpha$_2$-adrenoceptor can, at least partially, modulate the hypothermic effects of ethanol. The efficacy of the alpha$_2$-adrenceptor antagonists in reducing ethanol-induced hypothermia contrasts with the reported ineffectiveness of another putative 'alcohol antagonist', Ro 15-4513, in reducing the hypothermic effects of ethanol (Hoffman et al, 1987)

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ELECTROPHYSIOLOGICAL CHANGES IN VITRO FOLLOWING CHRONIC TREATMENT WITH ETHANOL IN VIVO

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Long term intake of ethanol is known to cause a withdrawal syndrome on cessation of intake. Clinically this takes the form of tremors, hyperreflexia, hallucinations and potentially fatal convulsions. The neurophysiological basis is uncertain. Effects of ethanol demonstrated using electrophysiological techniques include increases in calcium-mediated potassium conductance (Carlen et al, 1982) and potentiation of GABA transmission (Nestoros, 1980), but there has been little such investigation of the changes which take place on withdrawal from chronic treatment. Durand & Carlen (1984) found decreases in calcium-mediated potassium conductance three weeks after cessation of chronic ethanol administration; no withdrawal signs were evident at this time. We have measured field potentials in hippocampi removed from mice immediately after chronic ethanol treatment, during the period when a clear withdrawal syndrome could be seen *in vivo* (Whittington & Little, 1988).

Male mice, C57 strain, 25-30g, were given ethanol, 24% v/v, as sole fluid, for twelve weeks; ethanol intake was 10-14 g/kg/day. Controls drank water. Hippocampal slices were made between 9 and 10am, without withdrawing the animals from the ethanol, and perfused with standard Ringer solution. Extracellular recordings from CA1 area were started 30 min later, using orthodromic stimulation of Schaffer collateral fibres. Thresholds for production of first and multiple population spikes, paired pulse potentiation (70 msec interval at 1.25 x threshold stimulation) and slope of the EPSP component of first reponses were measured every 15 min. Input/output curves were determined at 1, 3, 5, and 7h from start of recording. Results were recorded on videtape and the areas of the population spikes measured by computer analysis. Results, expressed as mean ± s.e.m., from slices from ethanol treated and control animals were compared by analysis of variance (n=5 each group).

At the beginning of recording the thresholds for elicitation of single population spikes were higher in slices from ethanol treated animals than from controls (possibly due to residual ethanol). After 2-3h they were consistently lower than in controls and remained significantly so for the remainder of recording ($P<0.01$). At 7h these thresholds were: controls 23.3 ± 4.9 μAmp, ethanol 14.4 ± 1.4 μAmp. Thresholds for multiple spikes, initially similar in both sets of preparations, were lower in slices from ethanol treated animals from 3h to 7h ($P<0.01$). At 7h these thresholds were: controls 223 ± 3 μAmp, ethanol 182 ± 4 μAmp. In slices from ethanol treated animals paired pulse potentiation increased, compared with controls, from the beginning of recording, reached a peak at 2h 15 min and returned to controls values by 4h. At the peak the increase was 78% of control values (second response/first response, %, controls: 204 ± 8, ethanol 363 ± 48). The input/output curves for population spike area and EPSP slope showed a shift to the left for the slices from ethanol treated animals, but no change in the gradient or maximal response, indicating a greater response of the pyramidal cells to a given stimulus.

These results show that the mechanisms controlling excitation of CA1 pyramidal cells are modified during withdrawal from chronic ethanol treatment. The changes in paired pulse potentiation suggest that the hyperexcitability may originate at the synapse. Changes in multiple spike threshold may indicate alterations at postsynaptic sites. These studies show that *in vitro* recording can provide a detailed analysis of the changes which occur during withdrawal from chronic drug treatment.

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BENZODIAZEPINE-INDUCED SHIFTS OF ENTRAINED CIRCADIAN LOCOMOTOR BEHAVIOUR IN THE SYRIAN HAMSTER

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Administration of short-acting (e.g. triazolam) or long-acting (e.g. diazepam) benzodiazepines induces phase shifts in circadian locomotor activity (Turek & Losee-Olson, 1987; Ralph & Menaker, 1986). The present study describes the effects of single injections of benzodiazepines on entrained circadian locomotor behaviour and the blockade of these responses by pretreatment with benzodiazepine (Ro 15-1788) and GABA (bicuculline) antagonists.

Male Syrian hamsters (Mesocricetus auratus) were housed individually in cages containing running wheels. Animals were kept on a 6h light:18h dark lighting regimen to avoid the masking of drug-induced effects by light, which occurs in nocturnally active species. Entrained circadian locomotor (wheel running) activity was monitored continuously by a microcomputer system and displayed as actograms. Hamsters were injected (1-10mg/kg, i.p) with chlordiazepoxide, flurazepam, triazolam or vehicle (0.9% NaCl or DMSO) 4-6 hours prior to the onset of locomotor activity in order to elicit phase advancements.

Pronounced phase advancements in the onset of locomotor activity (i.e. a shift to an earlier time of day), which persisted for 3-14 days (Fig 1), were produced following a single injection of triazolam (75 ± 15 min; n=7/8; mean shift ± s.e.m.; n animals), chlordiazepoxide (60 ± 15 min; n=7/8) or flurazepam (60 ± 15 min; n=6/8).

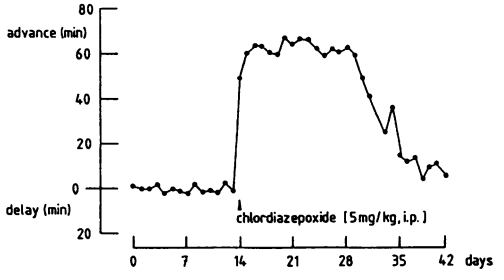


Figure 1 Chlordiazepoxide-induced advance in the onset of entrained wheel-running activity of a single hamster.

Benzodiazepine-induced phase advances were blocked by pretreatment with Ro 15-1788 (50 mg/kg in DMSO, i.p.) or bicuculline (10 mg/kg, i.p.). These data suggest that the effects of benzodiazepines on the circadian system are mediated via the benzodiazepine-$GABA_A$ receptor complex, presumably associated with the suprachiasmatic circadian pacemaker (Groos et al, 1983). These observations may have significance for the mangement (i.e. re-entrainment) of disturbed circadian behaviour found, for example in some affective disorders, insomnia and 'jet-lag'.

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THE VERTEBRATE NEURONAL $GABA_A$ RECEPTOR COMPLEX IS MODULATED BY GROUP IIB DIVALENT CATIONS

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The $GABA_A$ receptor complex possesses multiple binding sites for agents which can either enhance or antagonise the action of GABA in opening chloride ion channels (Schofield et al. 1987). Previously we observed that zinc enhanced the GABA response recorded from olfactory cortex brain slices in vitro (Smart & Constanti, 1983). However, zinc was recently reported to have either no effect on the GABA response recorded in rat pyriform cortical slices (Hori et al, 1987) or to inhibit the GABA response observed in dissociated hippocampal tissue cultures (Westbrook & Mayer, 1987). We have now reinvestigated the action of zinc and some other divalent cations on the $GABA_A$ receptor complex in vertebrate neurones.

GABA responses were recorded using either the whole-cell recording configuration from dissociated rat sympathetic ganglia in culture or by intracellular recording from guinea-pig cortical brain slices. On cultured rat superior cervical ganglion neurones 10-100μM zinc resulted in a reversible inhibition of the GABA-induced response. This inhibition appeared to be dependent on the stage of development of the neurones, since with older cultures or cultures prepared from adult rather than foetal animals, the inhibition produced by zinc was reduced and virtually absent when applied to the intact rat sympathetic ganglion in vitro. The antagonistic action of zinc was partly dependent on the agonist concentration, increasing with the dose of GABA. The inhibition of GABA responses was not solely limited to zinc; cadmium, another group IIB divalent cation also antagonised GABA-evoked responses, but the group IIA divalent cation, barium, was without effect.

In contrast, the superfusion of 100-500μM zinc over a guinea-pig cortical or hippocampal brain slice resulted in a time dependent increase in the GABA response which was maintained as long as zinc remained in the superfusate. This effect was not dependent on a reduction in GABA uptake since it also occurred in the presence of 1mM nipecotic acid. In addition, both barium and to a lesser extent cadmium induced enhancements in the GABA-evoked response concurrent with a reduction in the neuronal input conductance. These effects of the divalent cations were slowly reversible.

Our results suggest that the $GABA_A$ receptor at different stages of development is differentially sensitive to divalent cations, particularly zinc and cadmium. The GABA response of foetal neuronal cultures is most sensitive to block by zinc, whereas more mature, intact preparations are more resistant to the action of zinc.

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THE RESPONSIVENESS OF THE FERRET TO APOMORPHINE INDUCED EMESIS

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Apomorphine is believed to act as a directly acting dopamine receptor agonist to induce a reliable vomiting response in the cat, dog and primate (Wang, 1965). However, in the ferret, apomorphine is reported to cause an inconsistent emesis (Andrews et al. 1986) and in the present study we further investigate the ability of apomorphine to induce an emetic response in this species.

The effects of apomorphine injected subcutaneously into the neck were determined in male albino and Fitch ferrets (1.2-1.5kg) 2 h after feeding. Emesis was characterised by the expulsion of solid or liquid material, i.e. vomiting, or retching movements not associated with the passage of material from the gut.

Apomorphine induced an emetic response which achieved maximum intensity at 0.25mg/kg; higher doses of 1 and 2mg/kg were less effective (Table 1). A detailed analysis of the results from 12 ferrets receiving 0.25mg/kg indicated a marked variability in response, with 4 animals giving a mean number of 7 vomits/retches as compared to a mean of 37 in the 8 remaining animals. After 5 days animals were rechallenged with apomorphine and animals previously giving an intense emetic response again responded with a mean of 36 vomits/retches, with a mean value of 13 vomits/retches in the remaining 4 animals. In subsequent experiments animals were preselected as those giving the more intense response to apomorphine. In these animals a neuroleptic pretreatment prevented the emetic response to apomorphine. The 5-HT_3 receptor antagonist ICS 205-930 antagonised the response in 3 out of 12 animals at the high dose of 1mg/kg (Table 1).

Table 1: Apomorphine (Apo) induced emesis and its antagonism in the ferret

Treatment	(mg/kg)	n	No. of animals vomiting	Latency to first vomit (min)	Number of vomits ±SEM	Number of retches ±SEM
Apo.	0.062		0	>30	0	0
	0.125	8	7	9±3	2±1	12±3
	0.25	8	8	3±1.5	4±1	28±10
	1.0	8	5	15±5	1±1	15±5
	2.0	4	1	22±18	2±2	9±5
Apo.	0.25	8	8	5±1	3±1	17±4
+ Fluphenazine	0.1	4	0	>30*	0*	0*
+ Sulpiride	0.01	4	2	26±4*	1±1	1±1*
	0.1	4	0	>30*	0*	0*
+ Metoclopramide	0.1	4	4	12±1*	3±1 NS	10±1 NS
	0.5	4	1	24±6*	1±1	1±1*
	1.0	4	0	>30*	0*	0*
+ ICS 205-930	0.1	8	7	8±3 NS	5±2 NS	20±8 NS
	1.0	12	9	14±3*	2±1 NS	12±5

Significant difference compared to apomorphine 0.25mg/kg *P<0.05 (Mann Whitney).

The data indicate a considerable inter-animal variability in the emetic response to apomorphine and, whereas neuroleptic agents are effective to prevent emesis in all ferrets, the 5-HT_3 receptor antagonist ICS 205-930 prevented emesis in only a few animals. A study of such differences, and an apparent loss of the effect of apomorphine at high doses, may help to elucidate the dopamine-serotonergic interaction that is involved in the mediation of emesis in the ferret.

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330P

THE ANTI EMETIC EFFECTS OF ATROPINE SULPHATE IN THE FERRET

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The potent effects of $5HT_3$ receptor antagonists in ameliorating the emetic effects of cytotoxic drugs and radiation in experimental animals and patients has tended to distract attention from the possible involvement of other receptor types in the emetic pathway. Anti cholinergic agents are often considered to be useful only in motion sickness but a review of the literature reveals a number of examples of antagonism of emesis by cholinergic receptor antagonists (eg apomorphine, Isaacs 1956, cis platinum, Harris and Cantwell 1986, emetine, Cheymol and Quinquand 1950). In view of these reports we decided to investigate the anti emetic potential of atropine sulphate (AS) in the ferret.

Ferrets (400g-1200g) were deprived of food overnight but allowed access to milk prior to one of the following emetic stimuli : whole body X-radiation 800cGy (250Kv) emetine hydrochloride 20mg/Kg I.P., cyclophosphamide 200mg/Kg I.P.. Copper sulphate (30ml 100mg%) was given by oro-gastric tube to animals which had not received milk. AS 1mg/Kg S.C. was given at least 30 min prior to emetic challenge. Emetine: AS significantly reduced the total number of retches (R) and vomits (V) (4hrs observation control R 243±37R vs 70±8 $P<0.005$, control V 35±6 vs 13±1 $P<0.05$ $n=5$) without significantly affecting the latency. A significant reduction in R and V was also produced by 0.5mg/Kg AS. Cyclophosphamide: AS was without significant effect on the total R or V (4hrs observation control R 52±14 vs 59±31, control V 12±2 vs 9±4 $n=5$) although the pattern of the response was modified. Copper sulphate: AS was without significant effect on the response although there was a tendency for the total R and V to be reduced (30min observation control R 44±8 vs 31±9, control V 7±1 vs 5±1 $n=5$). X-radiation: AS was without significant effect on the latency of the response but significantly ($P<0.05$) reduced the total number of retches and vomits (2hrs observation control R 88±10 vs 46±11, control V 17±2 vs 7±1). AS was not noticeably sedative at a dose of 1mg/Kg nor did it evoke emesis.

These results illustrate a number of points. 1) AS posesses limited anti-emetic properties and its effects on one emetic agent do not predict those of another 2) the anti emetic effects of AS may depend upon the site of action of the emetic stimulus 3) these studies provide support for suggestions (Beleslin & Nedelkovski 1988) of a central cholinergic involvement in the emetic pathway. The cholinergic link is not common to all pathways evoked by different emetic agents.

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$5\text{-}HT_3$-SEROTONERGIC MECHANISMS AND STIMULATION OF GASTROINTESTINAL (GI) MOTILITY BY CISAPRIDE

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Cisapride enhances GI motility. It also antagonizes the effects of serotonin on $5\text{-}HT_3$ receptors. Both ICS-205-930, a selective $5\text{-}HT_3$ antagonist, and cisapride accelerate gastric emptying. Our aim was to determine whether the motor stimulating properties of cisapride are mediated via $5\text{-}HT_3$-serotonergic receptors. In vitro, both ICS-205-930 and cisapride antagonized serotonin (3.6×10^{-6} M)-induced contractions of the guinea-pig ileum (IC_{50}-values resp. 1.3×10^{-7} M, 1.1×10^{-7} M). Contractions of the ileum evoked by electrical stimulation were amplified to a similar extent by cisapride (3.4×10^{-7} M) alone (20 $\pm$ 4 %), or by cisapride in the presence of ICS-205-930 (1.4×10^{-7} M) 24 $\pm$ 5 %. ICS-205-930 did not affect these twitch responses. Similarly on the guinea-pig colon, cisapride induced a contraction (8.6 $\pm$ 1.6 mm) not affected by the presence of ICS-205-930 (5.6×10^{-7} M) that again by itself did not induce a contraction. In vivo, in dogs implanted with force-transducers cisapride (1.25 mg/kg p.o.) enhanced antroduodenal motility, e.g. it doubled the amplitude of antral contractions, whereas ICS-205-930 (0.63 mg/kg s.c.) slightly reduced it. ICS-205-930 did not affect the responses to cisapride. In conclusion: The effects of cisapride were not mimicked nor affected by selective $5\text{-}HT_3$ antagonists, indicating that in these tests $5\text{-}HT_3$-serotonergic receptors are not involved in the action of cisapride on GI motility.

THE TOXICITY OF MPTP AND MPP^+ IN RAT NEURONAL AND GLIAL CELL CULTURES

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The neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is thought to depend on the biotransformation to 1-methyl-4-phenyl-pyridinium ion (MPP^+), a reaction catalyzed by monoamine oxidase B which is present in glial cells. It is hypothesised that MPP^+ is released from glial cells and subsequently taken up and accumulated in dopamine containing neurons to initiate neuronal degeneration (Heikkila et al. 1985; Javitch et al. 1985). The present studies use a cell culture technique to investigate the sensitivity of neuronal and glial cells to the toxic effects of MPTP and MPP^+.

Rat foetuses were taken at 15 days gestation and the mesencephalon containing the substantia nigra was dissected out. For neuronal cultures, 0.15ml dissociated cell suspension in serum supplement medium (SSM) was plated in each cell well and incubated at 37°C, 100% humidity, 5% CO_2 for 18-20h. The medium was then replaced with serum free medium containing insulin 5μg/ml, progesterone 20nM, putrescine 100μM, selenium 30nM, transferrin 100μg/ml, L-glutamine 2mM, penicillin 100units/ml and streptomycin 100μg/ml in equal parts of Ham F12 solution and Dulbecco's Modification of Eagle medium and incubated for 48h before the replacement of fresh medium with or without MPTP and MPP^+ for 24h. For glial cultures, a cell suspension was plated and incubated in a 25cm^2 tissue flask for 48h before subculture in SSM.0.15ml of the cell suspension was plated in each cell well and incubated for 24h. MPTP or MPP^+ were added to the feeding medium and incubated for a further 24h. The live and dead cells were identified using ethidium bromide/acridine orange staining and fluorescent microscopy.

Table 1: MPTP and MPP+ toxicity on rat neuronal and glial cell cultures

	% live cells			
	MPTP		MPP^+	
Treatment	Neuronal	Glial	Neuronal	Glial
Control	80.7±2.5	90.5±0.4	72.6±0.9	83.1±2.4
10^{-8}	82.3±5.6	90.2±0.7	75.5±1.0	82.9±3.0
10^{-7}	77.3±4.7	91.3±0.5	68.7±1.1*	83.2±1.7
10^{-6}	65.7±4.0*	91.7±1.6	62.3±2.0**	81.8±2.1
10^{-5}	63.6±4.0*	91.9±0.5	46.2±4.6**	78.6±1.5
10^{-4}	57.1±5.4**	89.3±0.8	32.9±5.3***	69.2±4.2*
10^{-3}	27.1±3.6***	72.4±2.8**	7.7±1.6***	49.5±4.2**

Each value is the mean ±S.E.M., n = 3-5. Significant reductions compared to control *P<0.05, **P<0.01, ***P<0.001 (Student's t-test).

The results indicate that neuronal cells are approximately 100-1,000 times more sensitive to the toxic effects of MPTP and MPP^+ than glial cells and that MPP^+ is approximately 10 times more potent than MPTP. Therefore, it remains possible that the uptake of MPTP into glial cells and release of MPP^+ is a process that could occur without glial cell death, to the continuing detriment of neuronal cells. However, high concentrations of MPTP and micromolar concentrations of MPP^+ can also cause neuronal cell death in the absence of glial cells. It remains to be determined whether such toxic effects of MPTP and MPP^+ on neuronal cells in an 'in vitro' system can also occur 'in vivo'.

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THE SELECTIVE TOXICITY OF MPP^+ ON RAT NEURONAL CELL CULTURES

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The selective toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) for the dopamine neurone is considered to involve (a) its oxidation to the 1-methyl-4-phenylpyridinium ion (MPP^+) in glial cells followed by (b) the release of MPP^+ and uptake into the dopamine neurone via the high affinity dopamine reuptake process (Javitch et al, 1985). In the present study, we use neuronal and glial cell cultures to investigate the sensitivity of cells obtained from four different areas of the rat brain to the toxic effects of MPP^+.

Rat foetuses were taken at 15 days gestation and tissues dissected from the striatum, frontal cortex and mesencephalon to include the "substantia nigra" and "raphe nucleus". Neuronal and glial dissociated cell cultures were prepared from each area according to the technique of Barnes et al (1988). The medium was replaced after 72 h by feeding medium with or without MPP^+ and incubated for 24 h before identifying the live and dead cells using ethidium bromide/acridine orange staining and fluorescent microscopy.

Table 1: The toxic effects of MPP^+ on rat neuronal and glial cell cultures

Treatment	% live cells "Substantia nigra"	Frontal cortex	Striatum	"Raphe nucleus"
		neuronal cells		
Control	75.1±1.0	86.7±3.0	79.1±4.9	90.3±1.4
10^{-8}	77.4±0.7	72.3±2.6*	76.8±6.2	91.0±1.9
10^{-7}	70.1±1.2*	59.4±8.4*	72.6±2.1	90.8±1.3
10^{-6}	65.2±2.3*	55.8±8.4*	73.0±2.7	85.1±0.9*
10^{-5}	47.8±5.6**	55.1±6.4**	71.5±5.9	75.1±4.5*
10^{-4}	34.3±5.2***	53.1±6.4***	62.4±2.5*	67.5±3.5**
10^{-3}	12.5±2.7***	22.3±4.7***	42.0±5.2**	24.5±2.7***
		glial cells		
Control	93.8±2.0	91.6±1.4	94.5±1.5	90.8±1.9
10^{-6}	92.8±1.9	91.7±1.2	94.7±0.4	91.0±1.5
10^{-5}	87.2±2.4	85.9±1.1*	83.9±2.2*	91.4±1.5
10^{-4}	80.9±0.6*	82.8±0.9**	66.3±3.9**	72.5±2.8*
10^{-3}	60.2±4.5**	50.6±3.6***	40.1±1.3***	59.5±2.8***

Each value is the mean ±S.E.M. n = 3-6. Significant reductions compared to control *P<0.05, **P<0.01, ***P<0.001 (Student's t-test).

The results indicate a relative selectivity of action in the 'in vitro' toxicity of MPP^+ on the mesencephalic neuronal culture containing substantia nigra cells and cells from the frontal cortex , which were 10-1000 times more sensitive than striatal or raphe neuronal cells. With the exception of the striatal cultures, glial cells were 100-1000 times less sensitive than neuronal cells. It is concluded that the lower concentrations of MPP^+ cause a concentration related toxicity only in the "substantia nigra" neuronal culture. The significance of the toxicity in other cells remains to be determined.

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REVERSAL OF NEUROLEPTIC-INDUCED CATALEPSY IN RATS BY DOPAMINERGIC AGENTS

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Extrapyramidal side-effects are a major drawback for managing schizophrenic patients with neuroleptics. These drug-induced side-effects manifest themselves as Parkinsonian-like akinesia in man (Hornykiewicz, 1973) and catalepsy in rodents (Stanley & Glick, 1976). In the present study the reversal of neuroleptic-induced catalepsy in the rat was investigated by administration of dopaminergic agents. The aim of these experiments was to produce an animal model in which potential anti-parkinsonian compounds could be screened.

Male hooded rats (140-170g; Glaxo, Herts.) were originally injected subcutaneously (s.c.) or intrastriatally (i.s.) with either sulpiride or fluphenazine to elicit catalepsy. The onset and time for the catalepsy to plateau were determined using a variety of doses of each dopamine antagonist. Reversal of the neuroleptic-induced catalepsy was subsequently undertaken at a predetermined time point.

Results show that catalepsy can be elicited by fluphenazine, 0.03-0.3mg/kg s.c. and 0.1-3µg/rat i.s., and that this effect reaches the set cut-off point (180s) between 4 and 6h. The catalepsy observed after i.s. sulpiride, 1.25µg/rat, reaches the same level between 7 and 8h. Furthermore, it was found that the dose response curve for each neuroleptic, after either route of administration, varied slightly on different test days. Taking this possible shift into consideration, a number of doses of each dopamine antagonist were used to induce catalepsy when investigating the anticataleptic action of the agents in question.

The classical antiparkinsonian drug L-DOPA (100mg/kg i.p.) was found to reverse the catalepsy-induced by either neuroleptic, in the presence of the DOPA decarboxylase inhibitor, benserazide (10mg/kg i.p.). Furthermore, neuroleptic-induced catalepsy was reversed by the indirect sympathomimetic, methamphetamine (1mg/kg s.c.) and the dopamine agonist quinpirole (3mg/kg s.c.). The purported D1 agonist, SKF38393 (3 & 10mg/kg s.c.) did not reverse the drug-induced catalepsy.

The data presented here illustrates that neuroleptic-induced catalepsy, in the rat, can be used as a screen for potential anti-parkinsonian compounds. This was shown by the reversal of this behaviour with the commonly used anti-parkinsonian agent, L-DOPA.

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BIOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF THE DOPAMINE TRANSPORT COMPLEX IN RODENT AND DOG STRIATUM

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Dopamine (DA) is a major biogenic amine neurotransmitter in the mammalian central nervous system and has been implicated in the etiology of Parkinson's disease and schizophrenia (Niznik, 1987). Although much is known about the postsynaptic DA receptors there is a relative lack of information about the pre-synaptic DA-transport-complex (DTC). In the present study we have characterised the binding of [^{3}H][1-[2-(diphenyl methoxy)ethyl-4-(3-phenyl propyl)-piperazine ([^{3}H]GBR12935) to rat, mouse, guinea pig and dog striatal membranes in order to ascertain the properties of, and possible species differences in the DTCs.

Aliquots (0.4 ml) of striatal membranes (1.25 mg/ml;in 50 mM Tris.HCl + 120 mM NaCl + 0.01% BSA) were incubated with [^{3}H]GBR12935 (47 Ci/mmol;0.1-10 nM) in the presence or absence of test compounds in a total vol of 0.5 ml for 45 min at 22°C. Non-specific binding was defined by using 1 µM GBR12909. [^{3}H]GBR12935 binding was saturable and reversible and involved a single class of sites in rodent and dog striatum. Interestingly, the dog striatal DTC exhibited a significantly higher affinity (K_d = 1.8 ± 0.6 nM; P < 0.02-0.01) than the G.pig and a lower binding capacity (B_{max} = 0.6 ± 0.1 pmol/ mg protein; P < 0.05-0.01) than the rat (K_d = 2.3 ± 0.2 nM; B_{max} = 2.1 ± 0.17 pmol/mg), mouse (K_d = 2.4 ± 0.4 nM; B_{max} = 1.5 ± 0.36 pmol/mg) and G.pig (K_d = 4.9 ± 0.49 nM; B_{max} = 1.9 ± 0.35 pmol/mg) (n = 3-4) striatum. [^{3}H]GBR12935 binding to the striatal DTC in the rodents and dog was competitively inhibited by unlabelled GBR12909 (K_i = 6-12 nM), mazindol (K_i = 106-216 nM), benzotropin (K_i = 113-335 nM), nomifensine (K_i = 0.7-2.6 uM), cocaine (K_i = 1-2.3 µM) and phencyclidine (K_i = 1.6-2.6 µM). Compounds with K_is > 3-10 µM included amfonelic acid, dopamine (uptake substrate), amphetamine (DA releaser) and spiperone and haloperidol (DA receptor ligands). However, unlike Andersen (1987) we found amfonelic acid to be one of the least active competitors of [^{3}H]GBR12935 binding in all the species examined and this aspect requires further investigation. Again, the dog striatal DTC exhibited significantly different sensitivity to the rodent DTC since the majority of the competing agents were more active in the dog than in the rodents (P < 0.05-0.01). This could be explained, perhaps, by the fact that rodent striatal homogenates were prepared from fresh tissues while the dog preparations were made from previously frozen tissues, and thus the apparent differences between these species may be a reflection of methodological differences. Interestingly, the biochemical and pharmacological profile of binding of [^{3}H]GBR12935 in the above species compares well with that found in the human caudate nucleus (Janowsky et al.1987) and correlates well with the rank order of potency of compounds for inhibiting DA uptake but not well with that of DTC-substrates or with that of releasers of DA. Therefore, these results have reconfirmed the validity of using this radioligand for labelling the dopamine transport complex.

In conclusion, we have shown that the overall properties of the striatal DTC in different species appear to be similar and that [^{3}H]GBR12935 is a useful radioligand for future investigations of pathological changes in, and the plasticity of, the DA transport complex in the mammalian brain.

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DOPAMINE AND STRESS-INDUCED ANTINOCICEPTION IN THE MOUSE

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Reaction times of mice on a hot-plate are increased by a 3min swim at 20°C and in mice swum for 30s at 30°C the number of abdominal constrictions induced by acetic acid is reduced. The first model of stress-induced antinociception (SIA) is sensitive to mu- and delta- opioid receptor antagonists whilst the second does not appear to involve opioid pathways (Hart et al 1983). We describe the investigation of the involvement of dopamine (DA) in these two models of SIA and in a third model in which the stress is a 3min swim at 2°C.

Male LACA mice (25-35g) were allowed to acclimatise for 2h prior to use. Antinociception was assessed by measurement of the reaction time of the mouse on a hot-plate (56°C) with a maximum reaction time of 45s. On removal from the water, each mouse was warmed by lamps for 2min: unstressed mice were treated in a similar manner. Reaction times for each mouse were determined three times; 30min prior to the i.p. injection of vehicle or drug, 15min (agonist) or 30min (antagonist) after injection (immediately pre-swim) and 20min or 35min after injection (post-swim). Differences between the first and third measurements indicated changes due to drug administration and SIA was expressed as the difference in pre-swim and post-swim reaction times. Each treatment included at least 6 mice and reaction time values were analysed by the Mann-Whitney U-test.

Apomorphine (0.05mg/Kg) had no effect itself but in mice swum for 3min at 20°C SIA was 16.25s compared with 12.75s in vehicle treated mice ($P<0.002$). At a dose of 0.5mg/Kg apomorphine had no effect on either unstressed or stressed mice but at 5mg/Kg unstressed mice had a shorter reaction time on the hot-plate and, in stressed mice, SIA was reduced significantly. L-Dopa (200mg/Kg) behaved similarly to the high dose of apomorphine decreasing the reaction times in unstressed mice and reducing SIA. Neither haloperidol nor pimozide (both at 0.1 and 1mg/Kg) altered reaction times in unstressed mice but both produced significant increases in SIA (13s with vehicle increased to 17.5s with haloperidol and to 18.5s with pimozide, both 0.1mg/Kg). This augmentation of SIA was more marked when haloperidol and pimozide were given by the icv route. SIA in vehicle treated mice was 11.5s whilst in the presence of 5µg haloperidol SIA was 39s ($P<0.001$) and with pimozide (5µg) it was 28.75s ($P<0.01$). DA, by the icv route, produced similar responses to apomorphine with 1µg increasing SIA from 13s to 17.5s and 10µg causing a significant reduction to 9s ($P<0.001$). A similar pattern of activity was seen with the DA agonists and antagonists when the intensity of the stress was increased to 3min in water at 2°C. The low dose of apomorphine (0.05mg/Kg) enhanced SIA with the high dose of 5mg/Kg reducing SIA. Haloperidol and pimozide (both at 1mg/Kg) increased SIA significantly. Apomorphine, l-dopa, haloperidol and pimozide all reduced acetic-acid induced constrictions in unstressed mice making it impossible to assess the role of DA in the non-opioid model of SIA. Our results suggest an inverse relation between DA and SIA in the mouse similar to that seen with footshock SIA in the rat (Tricklebank et al 1984).

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IN VIVO MODULATION OF DOPAMINE RELEASE IN THE RAT NUCLEUS ACCUMBENS BY THE MUSCARINIC AGONIST OXOTREMORINE

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The muscarinic agonists, such as oxotremorine, are known to modulate in vitro the release of dopamine (DA), especially in the corpus striatum (Raiteri et al., 1982) and in the nucleus accumbens (De Belleroche et al., 1985). However recent experiments on the nucleus accumbens have led to conflicting results (Gongwer et al., 1987). The present experiments investigated in vivo whether oxotremorine could increase DA release in the nucleus accumbens and also attempted to locate more precisely the muscarinic receptors involved.

Differential normal pulse voltammetry associated with pretreated carbon fiber microelectrode was used to measure DA release in the nucleus accumbens (A 10.6, L 1.4, V 6.4 according to Paxinos and Watson atlas) in chloralhydrate anaesthetized rats (male Wistar, 300-350 g), pretreated with pargyline (75 mg/kg i.p., 2 h) (Gonon et al., 1984).

Extracellular DA levels increased to 214 $\pm$ 7 % of controls within two minutes after injection of oxotremorine (0.5 mg/kg s.c.) and this level was maintained for at least one hour. This effect was significantly reduced by prior administration of scopolamine (10 μg i.c.v., 30 min) or pirenzepine (40 μg i.c.v., 30 min) (-54 $\pm$ 14% and -67 $\pm$ 6%, respectively).

Pneumatic microinjections (50 nl, 2 min) of oxotremorine (2.10^{-3} M) close to the electrode tip did not produce a specific increase in DA extracellular levels, nor did they affect the DA release evoked by electrical stimulation (pulse : 0.3 ms, 250 μA, 25 Hz, burst duration 1s, period 2s, 20 bursts) of the medial forebrain bundle (A 4.4, L 1.3, V 9.0). Local administration of oxotremorine (50 nl, 2.10^{-3}M) in the ventral tegmental area (A 3.7, L 0.8, V 8.0) was unable to modify DA release in the nucleus accumbens.

In the same experimental conditions, pretreatment with clonidine (100 μg/kg i.p., 60 min) significantly reduced the oxotremorine-evoked DA release in the nucleus accumbens (-90 $\pm$ 9%). The latter result is in agreement with previous studies which suggest that DA release induced by oxotremorine may be produced by activation of noradrenergic pathways (Weinstock et al., 1980).

These experiments confirm that the stimulation of muscarinic receptors increases DA release in the nucleus accumbens, but they suggest that, in our experimental conditions, the receptors involved are located neither in the nucleus accumbens nor in the ventral tegmental area. Further experiments are in progress to clarify the contribution of noradrenergic processes to the increase in DA release induced by oxotremorine

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THE EFFECT OF REPEATED ISCHAEMIC EPISODES ON DOPAMINE RELEASE IN THE RAT FOREBRAIN AS MEASURED BY IN VIVO VOLTAMMETRY

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Cerebral ischemia causes a massive release of striatal dopamine (DA) in gerbils with unilateral strokes (Brannan et al., 1987), and in spontaneously hypertensive rats with bilateral carotid artery ligation (Yao et al., 1988). This has led to involve DA in the striatal tissue damage which occurs after cerebral ischemia (Globus et al., 1987).

Using differential normal pulse voltammetry associated with pretreated carbon fiber microelectrodes (Gonon et al., 1980), the present study aimed to investigate the effect of repeated global forebrain ischemia on the catechol peak in the rat striatum (ST) (A 10, L 2.2, V 4.5 according to the Paxinos and Watson atlas), and substantia nigra (SN) (A 3.8, L 2.0, V 8.4).

Freely moving rats (male Wistar 300-350 g, n = 4), were subjected to four successive 5-min-ischemic periods according to the four vessels occlusion model described by Pulsinelli and Brierley (1979). During each 5-min ischemia, a marked increase of the extracellular catechol peak was observed in the ST, occurring less than one minute after the loss of the righting reflex. The catechol signal increased to 1077 $\pm$ 54% (mean $\pm$ SEM of the four successive peaks, n = 4) of the basal value and returned rapidly to baseline after recirculation. There was no modification of this response over the four successive 5-min ischemic periods. The striatal catechol peak was significantly reduced 3 hours after administration of αMT (300 mg/kg i.p., -79 $\pm$ 6%, n = 4) or 2 hours after injection of reserpine (5 mg/kg i.p., -79 $\pm$ 21%, n = 3). GHB (1 g/kg i.p., n = 3) was unable to modify the successive ischemia-induced increases of the striatal catechol peak.

In anaesthetized rats (chloralhydrate 400 mg/kg i.p., n = 2) the catechol peaks were recorded simultaneously in the ST and in the SN. The catechol peaks were increased to 904 $\pm$ 106% of baseline in the ST, whereas in the SN, they were reduced (below detectable levels) during each ischemic period.

These findings show that increases in catechol signal, probably reflecting increase in DA release (Yao et al., 1988), occur during each ischemia together with a reduction of the nigral catechol peak. This latter result may suggest that the autoreceptors-mediated negative feed back in the substantia nigra is reduced during ischemia, thus leading to an increased firing of the nigrostriatal neurons resulting in a massive release of DA in the striatum. However, the unability of GHB to modify the ischemia-induced striatal DA release, does not support such hypothesis. As NMDA receptor activation affects DA release in the striatum (Scatton et al., 1988), experiments are in progress to investigate the effect of excitatory aminoacid antagonists on ischemia-induced striatal DA release.

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NICOTINE STIMULATES DOPAMINE SYNTHESIS RELEASE AND METABOLISM IN VIVO

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Studies have shown that nicotine stimulates *in vitro* DA release and metabolism (Balfour, 1982). Here we report the *in vivo* effect of nicotine on DA synthesis, release and metabolism in the caudate-putamen and nucleus accumbens using three techniques. As an index of DA synthesis, DOPA accumulation was measured after pretreatment with NSD-1015 (a decarboxylase inhibitor) (Mitchell et al., 1987). As indices of DA release and metabolism extracellular levels of DA and DOPAC were measured with intracerebral dialysis. To obtain better temporal resolution of DA metabolism, voltammetry with electrically pretreated carbon fibre electrodes was also used.

Male Sprague Dawley rats (250-320 g, Harlan Olac LTD) were used. In the DOPA accumulation study animals received nicotine (0.8 mg/kg free base, s.c.) or saline (1 ml/kg, s.c.) followed 15 min. later by NSD-1015 (100 mg/kg i.p.). Animals were killed 30 min. later, their brains removed and dissected into a number of regions including the caudate-putamen and nucleus accumbens. Tissue was prepared for assay by HPLC-ED. In the second part of the study either a dialysis loop or a pretreated carbon fibre electrode were stereotaxically implanted into the right nucleus accumbens whilst another loop or electrode was positioned in the contralateral caudate-putamen. Animals were anaesthetised throughout the experiment (chloral hydrate 600 mg/kg, i.p.). During dialysis, artificial CSF was perfused (1 µl/min.) through the loop, samples were collected every 20 min. and analysed using HPLC-ED. For voltammetry, a differential pulse voltammetric scan was performed on each electrode every 6 min. After a stabilization period of 90-120 min., either nicotine (0.8 mg/kg free base, i.v.) or saline (0.2 ml i.v.) were administered via the femoral vein and the effect monitored for a further 180 min.

In the nucleus accumbens DA synthesis increased significantly 45 min. after nicotine (+63%, $P < 0.01$ Student's unpaired t-test, nicotine vs saline n=6). In the dialysis sample collected 40-60 min. after nicotine, the levels of DA and DOPAC were significantly increased (+117%, $P < 0.001$, and +21.5%, $P < 0.02$ respectively; n=6). DOPAC measured voltammetricaly increased rapidly within 12 min. of nicotine injection, reaching a maximum of +36% within 42 min. ($P< 0.02$, n=6). In the caudate-putamen there was a +14% (n=6) change in DA synthesis. Extracellular DA and DOPAC, by dialysis, increased by +10% (n=6) and +17% (n=6) respectively, while extracellular DOPAC via voltammetry increased by +14% (n=6). In all cases these responses were not significant. The three techniques also showed that nicotine had no significant effect on 5-HT synthesis and metabolism.

These findings provide evidence that nicotine does stimulate DA release, metabolism and synthesis in the nucleus accumbens but not the caudate-putamen. Our dialysis results confirm those of Imperato et al. (1986), whilst the voltammetric data demonstrates the good temporal resolution of the technique.

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INHIBITION BY GR38032F OF BEHAVIOURAL EFFECTS IN THE RAT WHICH FOLLOW WITHDRAWAL FROM TREATMENT WITH DRUGS OF ABUSE

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It has been shown that subchronic treatment with nicotine, cocaine or alcohol reduces mouse aversion to a brightly lit environment and reduces the responsiveness of marmosets to a human threat. However, on withdrawal from these subchronic treatments mice become significantly more averse to a bright environment and marmosets exhibit significantly enhanced posturing and retreat from a human threat. These behavioural consequences of withdrawing from treatment with drugs of abuse are prevented by the selective $5HT_3$ receptor antagonist, GR38032F (Oakley et al., 1988; Costall et al., 1988). Here we extend these observations to the rat, using a social interaction test, and present evidence that the amygdala may provide a site for the described action of GR38032F. Unfamiliar male Sprague-Dawley rats (225-275g) were placed in an unfamiliar open-topped arena (52.5 x 52.2cm and 30cm high) with 17.5 x 17.5cm areas marked on the floor (to measure locomotor activity). From remote video recordings (10 min) social interaction was timed (s) to include sniffing of partner, crawling under or climbing over partner, following of partner, genital investigation of partner. Treatment groups were 6 pairs of rats.

Vehicle treated rats spent 76.5±8.3s in social interaction. Those receiving nicotine (0.1mg/kg i.p. b.d. 7 days) spent 131.0±14.6s in social interaction, whilst alcohol treated rats (8% w/v 14 days) spent 123±13.0s in social interaction ($P<0.001$). 24-48h after withdrawing treatment, these animals spent only 10.1±1.2s and 19.2±2.1s respectively in social interaction ($P<0.001$ compared both to vehicle responses and those recorded during treatment). If GR38032F (100ng-10μg/kg was given at the time of ceasing treatment with nicotine or alcohol, and then b.d. for the duration of the period during which the changed behaviour of withdrawal could be detected [up to 96h]), then the decrease in social interaction associated with the cessation of treatment with nicotine or alcohol could be prevented (animals withdrawn from nicotine and given 100ng/kg i.p. b.d. GR38032F spent 83.6±8.9s in social interaction, whilst those withdrawn from alcohol and similarly treated spent 90.4±10.0s in social interaction, $P<0.01$). There were no changes in locomotor activity from control values in any treatment group (64.7±7.1 to 70.6±6.9 crossings/10 min). Subsequent studies using cocaine (1.0mg/kg i.p. b.d. 14 days) or diazepam (10mg/kg i.p. b.d. 7 days) also showed suppression of withdrawal effects by GR38032F. Further, animals which have been prepared for intracerebral injection into the central area of the amygdala (Ant. 5.8, Vert. -1.8, Lat. ±4.5, atlas of De Groot) have been subject to subchronic treatments with nicotine or diazepam, and GR38032F (0.01μg, 0.25μl) has been injected into the amygdala at times when the disturbances in behaviour associated with withdrawal were maximum. In all situations the behaviour of rats returned to values close to that of control rats (vehicle or non-treated). Injections of GR38032F (0.01-10μg, 1μl) into the striatum (Ant. 7.8, Vert. +1.0, Lat. ±3.0) during withdrawal provided no protection.

These data support the suggestion that a selective $5HT_3$ receptor antagonist may be used to prevent the consequences of ceasing continued treatment with drugs of abuse, and indicate that one locus of action may be in the amygdala.

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INFLUENCE OF CAFFEINE ON THE BEHAVIOURAL PROFILE OF THE COMMON MARMOSET (CALLITHRIX JACCHUS)

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At high doses, caffeine (1,3,7 trimethylxanthine) has been shown to produce symptoms of anxiety and nervousness in healthy subjects and psychiatric patients (Charney et al, 1984) and to possess an anxiogenic-like profile in rodent models of anxiety (Pellow et al, 1985). The aim of this study therefore was to assess the behavioural profile of caffeine in the common marmoset (Callithrix jacchus). This species has recently been reported to be sensitive to the action of anxiolytic compounds, assessed in the human threat test (Costall et al, 1988a), and the action of the anxiogenic agent FG7142 (assessed in a situation of "low anxiety", that is in the absence of a human threat) (Costall et al, 1988b).

The behaviour of adult marmosets (n = 6-10), housed in pairs, was assessed over two 15 min periods in the animals' home cages adapted for video recording in a "low anxiety" situation (in the absence of a human threat). The initial assessment followed a period of habituation to the camera equipment and was, for the purposes of this study, termed "normal" behaviour. 40 min subsequent to subcutaneous dosing with drug/vehicle or following handling alone (control), a second behavioural assessment was made.

A number of behavioural parameters were measured: i) time spent on or in front of the front perch, ii) time spent in the nestbox, iii) the number of "postures", iv) the number of rears, that is leaning forward against the perspex front whilst standing on the front perch, v) the number of jumps and line crossings (arbitrary, central line on video screen dividing the cage into two sections).

Administration of caffeine to marmosets produced a profile of behaviour similar to that induced by FG7142 (Costall et al, 1988b) (behaviour compared to that of vehicle-treated animals which was indistinguishable from the behaviour of normal marmosets). Caffeine (10-50mg/kg s.c.) dose-dependently increased time spent in the nestbox, although this only achieved significance at 50mg/kg ($P<0.05$). Rearing behaviour was reduced (in the order of 40%) by caffeine treatment (20-50mg/kg s.c.). A similar profile was obtained for the number of jumps and line crossings; for example, caffeine (20mg/kg s.c.) reduced each parameter to approximately 60% of their pre-drug levels ($P<0.05$). Caffeine (5-50mg/kg s.c.) failed to significantly alter either time spent at the cage front or the number of postures. It should be noted that following treatment with 50mg/kg caffeine, animals exhibited behaviour consistent with a nausea response, for example, "nose rubbing" on cage surfaces, salivation and eye closure (Costall et al, 1986).

Data shows that caffeine alters marmoset behavioural patterns in a similar manner to an anxiogenic β-carboline. This further supports the proposal that the profile of action of anxiogenic agents may be assessed in the common marmoset in a situation of "low anxiety" provided by remote camera recording without a human threat.

D.N.C. Jones is a SERC/Glaxo CASE award student.

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INHIBITION OF DOPAMINE AND 5-HT UPTAKE BY DOPAMINE RECEPTOR AGONISTS AND BY GBR 12909

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The 2-aminotetralins 5,6-ADTN and 6,7-ADTN are dopamine (DA) receptor agonists which enhance basal efflux of [^{3}H]-DA from rat striatal slices (Hoffman et al., 1980), inhibit active uptake of [^{3}H]-DA and are accumulated into DA neurones (Woodruff, 1982). We have investigated two chemically-related compounds, N,N-dipropyl-5,6-ADTN (dpADTN) and N-0437 (Van der Weide, 1987), and a partial ergoline, quinpirole, for effects on DA and 5HT uptake and release in substantia nigra. In addition we have tested the DA uptake inhibitor GBR 12909 (Van der Zee et al., 1980).

For transmitter release experiments slices of substantia nigra from male AH/A rats were prelabelled with [^{3}H]-DA or [^{3}H]-5HT as described by Herdon et al (1985), then superfused with Krebs buffer or test compound. For the uptake experiments, a crude synaptosomal preparation was incubated with [^{3}H]-DA in the presence or absence of test compound. The synaptosomes were filtered, washed, and the tritium content was measured. All drugs were tested in triplicate. Ouabain was used to define active uptake.

The basal release of [^{3}H]-DA was increased by dpADTN (1-100μM) to a maximum of 660% whereas that of [^{3}H]-5HT was not increased by 1 or 10μM dpADTN, but 100μM caused an increase of 135%. N-0437 (1-100μM) had no effect on the basal release of either transmitter, but it was more potent than dpADTN at inhibiting the active uptake of both [^{3}H]-DA and [^{3}H]-5HT. Quinpirole had no effect (Table 1).

Table 1: Inhibition of active uptake of [^{3}H]-DA and [^{3}H]-5HT

Drug	IC_{50} (nM) and 95% confidence limits	
	[^{3}H]-DA	[^{3}H]-5HT
N-0437	417 (91-1253)	921 (589-1432)
dpADTN	1850 (500-4460)	7410 (3960-13380)
GBR 12909	79 (31-186)	195 (40-959)
Quinpirole	>10000	about 10000

These results show that dpADTN increases basal efflux of both [^{3}H]-DA and [^{3}H]-5HT, and this may be due to the drug acting as a substrate for the DA and 5HT uptake systems. N-0437, however, inhibited DA and 5HT uptake without affecting their basal release. Finally, the selectivity of GBR 12909 for inhibiting [^{3}H]-DA compared to [^{3}H]-5HT uptake was found to be relatively small, compared to that originally reported (Van der Zee et al., 1980). Similar results were obtained for inhibition of [^{3}H]-DA uptake in corpus striatum and [^{3}H]-5HT uptake in the raphé nucleus. GBR 12909 thus appears to be markedly less selective for DA uptake than was originally supposed.

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COCAINE MICRODIALYSIS: STEREOSELECTIVE DOPAMINERGIC EFFECTS AND IN VIVO AUTORADIOGRAPHIC STUDIES

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Cocaine is a symapathomimetic which blocks dopamine (DA) neuronal reuptake. Dialysis studies have shown it to increase extracellular DA levels in the brain either after i.p. administration (Church et al 1987) or when given directly via a dialysis probe (Glue et al, 1988). However it is not clear whether this is a stereospecific response. Additionally the extent of the spread of cocaine from the probe is not known. The present study addresses these two questions.

To investigate pharmacological specificity, the (+) and (-) enantiomers of cocaine HCl were dissolved at a 1.0mM concentration in artificial CSF and infused in a random order through a dialysis probe placed in the striatum of anaesthetised rats. Dialysate samples were obtained every 15 minutes and injected into an HPLC with an ESA coulometric detector. Baseline DA levels were 36 fmole $\pm$ 6 (sem)/sample. After dialysis of (-) cocaine levels increased to a peak of 1746 fmole $\pm$ 136 (sem), and returned to normal within 60 mins. (+) cocaine produced a much smaller peak of 102 fmole $\pm$ 28 (sem), returning to baseline within 30 mins.

To look at spread of cocaine from the probe, autoradiography was used. ^{3}H-cocaine HCl was added to 1mM cocaine in artificial CSF to give an activity of $1x10^{6}$ counts/ul, and this was infused at a rate of 1.3 ul/min for 15 mins via a microdialysis probe inserted into the striatum. Rats were sacrificed immediately after this infusion, their brains were frozen and A-P sections cut, and these were exposed on LKB Ultrofilm for 3 weeks. The resulting images were analysed on a computer-assisited image analysis system. This demonstrated that the maximal spread of ^{3}H-cocaine was within a 2mm radius from the probe, with a concentric distribution.

The difference in magnitude of DA responses between the two cocaine enantiomers suggests a high degree of pharmacological specificity in this response. The small rise with the (+) cocaine may be due to non-specific effects in the dialysate, such as more acidic pH. Autoradiography findings suggest that neurotransmitter responses seen in microdialysis studies may reflect more widespread changes than those of neurons immediately adjacent to the probe.

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CENTRAL EFFECTS OF NEUROPEPTIDE Y ON LOCAL CEREBRAL GLUCOSE USE IN THE CONSCIOUS RAT

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Neuropeptide Y (NPY) has a widespread distribution throughout the CNS and has been implicated in central cardiovascular control. NPY-immunoreactive nerves and NPY binding sites have been localised in important cardiovascular sites in the medulla oblongata and central administration of the peptide results in changes in blood pressure and heart rate (Macrae & Reid, 1988). In this study, we have used [14C] 2-deoxyglucose autoradiography (Sokoloff et al, 1977) to reveal the functional events in the CNS associated with intracisternal (i.c.) administration of NPY. Experiments were carried out on conscious, male, Wistar-Kyoto rats (274-312g) previously implanted with i.c. guide cannulae. NPY (1.25 nmols in 5µl) was made up in a vehicle of sterile saline containing 0.25% BSA which was without cardiovascular effect. Intracisternal NPY (n=8) produced a significant increase in blood pressure from 129±2.8 to 138±2.7 mmHg 2 minutes post-injection, followed by a significant fall in blood pressure which reached a maximum at 25 minutes post-injection of 120±2.6 mmHg.(compared to vehicle, n=6) Table 1 summarises the effect of NPY on local cerebral glucose utilisation (LCGU) in selected areas of the brainstem and forebrain.

TABLE 1. Local cerebral glucose use following i.c. NPY (1.25nmols).

	CONTROL (n=5)	NPY (n=7-10)
BRAINSTEM		
AREA POSTREMA	94±2	67±5 **
CAUDAL NTS	82±1	62±4 **
CAUDAL VLM	66±3	56±5
ROSTRAL NTS	74±1	60±3 **
ROSTRAL VLM	66±2	60±4
KOLIKER-FUSE NUCLEUS	64±3	55±2 *
INFERIOR COLLICULUS	172±9	141±9 *
HYPOTHALAMUS	62±2	56±4
FOREBRAIN		
SUBFORNICAL ORGAN	62±3	50±3 *
GLOBUS PALLIDUS	62±3	60±3
CAUDATE NUCLEUS	109±6	96±4

Data are presented as mean ±SEM (µmol/100g/min) * P<0.05; ** P<0.01 vs control (Student's t-test). NTS, nucleus tractus solitarius; VLM, ventrolateral medulla;

A significant reduction in LCGU in the area postrema, caudal NTS, rostral NTS, Koliker-Fuse nucleus and subfornical organ provides direct evidence for their functional involvement in the central response to NPY.

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THE EFFECT OF NPY ON cAMP LEVELS IN SLICES OF RAT MEDULLA

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Centrally administered neuropeptide-Y (NPY) reduces mean arterial pressure and heart rate. Studies have also shown its colocalisation and possible functional interaction with catecholamines (Macrae et al 1988; Fuxe et al 1986). Despite increasing evidence of a physiological role of NPY, little information is available concerning its mechanism of action.In human and rat cortex NPY, under certain conditions can reduce cAMP accumulation and may inhibit the effects of other agents on cAMP levels (Westlind-Danielsson et al 1987). The present study examined the effects of NPY on cAMP levels in slices of rat medulla oblongata.

Slices were pre-incubated in a physiological buffer (PB),gassed with 95%O_2/5%CO_2 at 37°C for 30 mins. The PB was then replaced with buffer containing theophylline (1.08mg ml^{-1}) and bacitracin (0.1mg ml^{-1}) to prevent the breakdown of cAMP and NPY respectively. NPY or control vehicle was added to appropriate tubes and incubated for 5 mins. Where the effects of NPY on raised levels of cAMP were investigated, forskolin, isoprenaline or appropriate vehicle control was added and incubated for a further 10mins in a buffer containing ascorbic acid (0.2 mg ml^{-1}) where necessary, to prevent the oxidation of isoprenaline. The cAMP content was determined using a competitive protein binding assay.

TABLE:1

DRUG	cAMP LEVEL	DRUG	cAMP LEVEL	DRUG	cAMP LEVEL
Control	2.73±0.30	Control	3.67±0.20	Control	2.57±0.14
Forsk(10^{-5}M)	6.08*±0.63	Forsk(10^{-5}M)	5.07*±0.28	Isopren(10^{-6}M)	3.70*±0.17
NPY(10^{-6}M)	2.80±0.26	NPY(10^{-7}M)	3.14±0.20	NPY(10^{-6}M)	2.80±0.43
Forsk+NPY	4.35*±0.43	Forsk+NPY	4.53*±0.36	Isopren+NPY	4.05*±0.16

Statistical analysis was carried out on the mean levels of cAMP using ANOVA with a Bonferroni correction for multiple comparison and expressed as pmols cAMP mg^{-1} protein. * $p<0.05$ from control values or as indicated by the bar. Isoprenaline;Isopren,Forskolin;Forsk n = 6-9 in each group.

Forskolin(10^{-5}M) significantly increased cAMP levels in the medulla slices compared to control responses, due to its direct activation of adenylate cyclase. NPY(10^{-6}M and 10^{-7}M) alone did not have any effect on the basal level of cAMP. However, NPY(10^{-6}M) caused a significant attenuation of the increase in cAMP levels produced by forskolin. Isoprenaline(10^{-6}M) also raised the cAMP level compared to control values, although this increase was not significantly affected by NPY(10^{-6}M). The results demonstrate that in the rat medulla, NPY can reduce raised levels of cAMP but does not appear to interact directly with beta-adrenoceptor function.

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BINDING OF [^{125}I]ENDOTHELIN TO RAT BRAIN AND ITS INHIBITION BY PEPTIDE ANALOGUES

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Many peptides that are now known to have actions in the central nervous system were first described and characterized in peripheral tissues. Accordingly we have investigated the binding of the newly described 21 amino-acid peptide, endothelin (Yanagisawa *et al.* 1988), in the central nervous system of the rat, both by autoradiography and by ligand binding studies with homogenates of cerebellum and hindbrain.

For ligand binding studies to cerebral homogenates, the cerebellum and hind brain were removed from male Sprague Dawley rats (180-250 g; Charles Rivers, France), which were killed by decapitation, and homogenised in 10 vol. ice-cold 50 mM HEPES buffer (pH 7.4) containing 1 mM 1,10-phenanthroline (Sigma). The homogenates were centrifuged at 1000 g for 10 min at 4° C and the binding studies carried out on the resulting supernatant. Porcine/human endothelin (Peptide Institute, Osaka, Japan) was iodinated by the method of McKee *et al.* (1986) and the purity checked by HPLC. Incubations were carried out at 25° C in 0.5 ml in microcentrifuge tubes using 500-750 µg protein and the binding reaction was terminated by centrifugation for 2 min at 14000g; the pellets were washed 3 times by dipping the microcentrifuge tubes into ice-cold incubation medium (50 mM HEPES, pH 7.4; 0.1% bovine serum albumin; 1 mM 1,10-phenanthroline; 140 µg/ml bacitracin). Autoradiographic studies were performed using 10 µm frozen serial sections of rat brain incubated with 120-250 pM [^{125}I]endothelin in the presence and absence of 100 nM cold endothelin. The labelled sections were then applied to either Ultrafilm or nuclear emulsion-coated cover slips and quantified densitometrically.

Endothelin inhibited [^{125}I]endothelin (120 pM) in rat hindbrain/cerebellar membranes with an apparent affinity of $2.5 \pm 0.7 \times 10^9\ M^{-1}$ (n = 4). The endothelin-sensitive (400 nM) binding was saturable with an apparent affinity constant of $3 \times 10^9\ M^{-1}$ and a capacity of about 200 fmol/mg protein. The rate of association (K_{obs}) was $1.6 \pm 0.4 \times 10^{-2}\ min^{-1}$ (n = 4) and dissociation (investigated by dilution with incubation buffer and addition of an excess of unlabelled endothelin) was less than 10% over 48 h. [Ala^{3,11}]endothelin and [Ala^{1,15}]endothelin also inhibited [^{125}I]endothelin to the same extent as endothelin with respective apparent affinity constants of $4.1 \pm 1.8 \times 10^8\ M^{-1}$ (n = 3) and $9.4 \pm 4.1 \times 10^7\ M^{-1}$ (n = 3). The Hill coefficients for inhibition of [^{125}I]endothelin binding by endothelin, [Ala^{3,11}]endothelin and [Ala^{1,15}]endothelin were 1.6 ± 0.1 (n = 4), 1.9 ± 0.4 (n = 3) and 1.8 ± 0.5 (n = 3).

The greatest density of endothelin-sensitive [^{125}I]endothelin binding sites determined by autoradiography was found in the cerebellum. Moderate densities of binding occurred in the cerebral cortex, hippocampus, thalamus, mid and hindbrains in ascending order of density. Dense concentrations of binding sites were observed in the lateral ventricular and fourth ventricular choroid plexuses. The coverslip technique showed that the binding was associated with neuronal rather than vascular elements.

These results suggest that endothelin may have a role in the function of the central nervous system that is unrelated to its vasoconstrictor activity. The values of the Hill slope determined for its binding are consistent with positive cooperativity. Two analogues, each of them lacking one or other of the two disulphide bridges in the parent molecule, inhibited specific [^{125}I]endothelin binding. Since these two analogues also have biological activity in smooth muscle (Hiley *et al.*, 1989) and behaviourally (Moser & Pelton, 1989) and inhibit [^{125}I]endothelin binding to the same extent, it seems that the binding sites described here are related to a functional receptor.

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BEHAVIOURAL EFFECTS OF CENTRALLY ADMINISTERED ENDOTHELIN IN THE RAT

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Endothelin is a recently discovered 21 amino acid peptide that has potent vasoconstrictor activity (Yanagisawa *et al*, 1988). High affinity binding sites for endothelin have been demonstrated in rat CNS (Jones *et al*, 1988) and we describe here the gross behavioural changes observed in rats following its central administration.

Male Sprague Dawley rats (250 - 300g) were prepared with indwelling guide cannulae under sodium pentobarbitone anaesthesia (60 mg/kg i.p.) using standard stereotaxic procedures. This allowed the placement of an injection cannula into the right lateral ventricle in lightly restrained conscious rats. Rats were allowed 7 days recovery period before experiments began and each rat was used a maximum of 3 times, with at least 7 days between each use. Endothelin, its [$Ala^{3,11}$] analogue and somatostatin were dissolved in sterile saline and given i.c.v. in volumes of 0.5 - 2 µl at a rate of 1 µl/min.

Following the administration of endothelin (1.25 - 5 pmol/rat) the principal effects observed, which had an onset latency of 5 - 10 min, were a marked ataxia, flat body posture and tendency to direct movements to one side. This latter effect showed itself at lower doses by the rats leaning to one side and a tendency to turn in the same direction. At higher doses the leaning was more severe and the limbs under the rat were held tightly to the body, while those on the other side were extended. This would then develop into longitudinal rolling of the body, similar to that previously described for somatostatin and called barrel rotation (Cohn & Cohn, 1975). The incidence of barrel-rolling induced by endothelin was dose-related (number of rats showing barrel-rolling/rats tested: 1.25 pmol/rat 0/8; 2.5 pmol/rat 3/8; 5 pmol/rat 10/13). Endothelin was thus more than 1000 fold more potent than somatostatin (7/8 rats showing barrel-rolling after 20 nmol somatostatin i.c.v.). [$Ala^{3,11}$]endothelin induced similar behavioural changes to endothelin, but was approximately 10-fold less potent (number of rats barrel rolling/ number tested: 18.8 pmol/rat 1/6; 37.5 pmol/rat 3/6; 75 pmol/rat 3/5).

Barrel-rolling lasted between 5 and 60 min but the duration and severity of this effect were not clearly related to dose. This may be because the rolling behaviour was very sensitive to sudden sounds and the presence of cage walls. Two to three hours after endothelin the rats appeared to be normal. Unlike somatostatin, endothelin did not induce tight head-to-tail circling. Other behaviours observed were a sensitivity to touch (either crying on touch or exaggerated startle reflex), head-twitches, forepaw treading, biting of the cage walls, increased exploration and increased rearing, with the rat often resting in a vertical position against the cage wall. Doses of 10 and 20 pmol/rat were each tested in one rat but these died, after 17 and 10 min respectively, of respiratory failure after showing all the effects mentioned above. Haloperidol (0.2 mg/kg s.c.), diazepam (2 mg/kg s.c.) or trihexaphenidyl (1 mg/kg i.p.) pretreatment failed to alter the response to endothelin (5 pmol/rat). In contrast to the results of Cohn & Cohn (1975) trihexaphenidyl also failed to antagonise the rolling response to somatostatin (20 nmol/rat). Metergoline (5 mg/kg s.c.) appeared to reduce the incidence of barrel-rolling (2/5 rats tested rolled) but nifedipine (10 mg/kg i.p.) seemed to be ineffective.

These results show that endothelin can have centrally mediated effects although further work is clearly necessary to rule out effects resulting from CNS vascular changes. It is tempting to speculate that the effects on motor coordination that were seen following endothelin and [$Ala^{3,11}$]endothelin are related to the high concentration of [^{125}I]-endothelin binding sites present in the rat cerebellum (Jones *et al*, 1988). The similar potency ratio between their binding affinities and behavioural effects supports this speculation.

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IMMUNOHISTOCHEMICAL LOCALISATION OF β-NERVE GROWTH FACTOR RECEPTORS IN HUMAN BRAIN

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β-Nerve growth factor (NGF) is known to be associated with cholinergic projection neurones of the basal forebrain in both rat and human brain. NGF and the content of $mRNA^{NGF}$ have been measured in the CNS of the rat and found to correlate with distribution of cholinergic innervation (Korsching et al., 1985). Receptors for NGF (NGFr) in rat brain have been visualized immunohistochemically and the distribution described (Dawbarn et al., 1988). This distribution was found to coincide with choline acetyltransferase immunoreactive neurones (Dawbarn et al., in press). The data reported here demonstrates the direct visualization of NGFr's in neurones of the diagonal band of Broca (dbB) and the nucleus basalis of Meynert (nbM) in the human brain.

Using a monoclonal antiserum to human NGFr we have examined the distribution of NGFr-like immunoreactivity (NGFr-IR) in post-mortem brain tissue. Brains were fixed in 4% buffered paraformaldehyde for 48 hours. The area containing the basal forebrain was transferred to 30% buffered sucrose. Sections (30um thick) were cut throughout the basal forebrain. Every 20th section was processed for NGFr-IR using the peroxidase anti-peroxidase method (Sternberger, 1979).

Neurones were visualized by the deposition of a dark brown reaction product. Staining was punctate in appearance in both perikarya and dendrites of the dbB and nbM. Four main groups of NGFr-IR neurones were observed: the nucleus of dbB, the anterior, intermediate and posterior portions of the nbM. Neurones could also be seen in the medial septal nucleus, in the anterior perforated substance and in the medullary laminae of the globus pallidus. The high percentage of magnocellular NGFr-IR neurones compared with Nissl stained hyperchromic cells indicates that most of the hyperchromic neurones in the magnocellular forebrain system express receptors for NGF. NGFr-IR fibre processes could be observed, running along the innermost edge of the cingulum and external capsule to the dorso-lateral part of the putamen and were seen as a partial or complete tract throughout the nuclei of the dBB and nbM.

In the human brain, neurones in the dbB and nbM are known to degenerate in Alzheimer's disease. It is possible that a defect in either NGF or NGF receptor function is causally involved in this disease. We are currently examining the immunohistochemical distribution of these receptors in Alzheimer's disease, on the basis that NGF therapy can only be successful if these receptors are present.

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QUANTITATIVE AUTORADIOGRAPHIC MEASUREMENT OF [^{3}H]-FORSKOLIN BINDING SITES IN HUMAN BRAIN: DEFICITS IN ALZHEIMER'S DISEASE

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Forskolin is a potent activator of adenylate cyclase and the distribution of [^{3}H]-forskolin binding sites has been described in rat brain (Gehlert et al. 1985). We have used quantitative autoradiography to map the distribution of [^{3}H]-forskolin binding sites in human brain and to investigate alterations in their distribution in Alzheimer's Disease.

Brains from six persons dying with no history of dementia or neurological disease (mean age ± SEM, 79 ± 3 years; mean postmortem delay ± SEM, 19 ± 7 hours) and 7 with Alzheimer's Disease (mean age ± SEM, 73 ± 3 years; mean postmortem delay ± SEM, 8 ± 2 hours) were obtained postmortem. 20µm thick frozen cryostat sections were thawed then incubated at room temperature for 20 min. with 50mM Tris HCl (pH 7.5) containing 10mM $MgCl_2$ and 20nM [^{3}H]-forskolin. Adjacent sections were incubated in the presence of 20µM forskolin. Following 2 x 1 min. rinses, sections were dried and apposed to tritium-sensitive film for 4 weeks. Autoradiograms were quantified against a range of [^{3}H] microscales (Amersham) using a Quantimet 970 image analyser (Cambridge).

Saturation analysis of [^{3}H]-forskolin binding sites (2-150nM) in tissue sections from 3 control brains revealed apparent Kd values of 9.2 ± 0.7nM in frontal cortex and 9.0 ± 0.8nM in hippocampus. [^{3}H]-Forskolin (20nM) binding sites exhibited a heterogeneous distribution in cortical and hippocampal regions (Table 1). A marked reduction in [^{3}H]-forskolin binding was observed in middle frontal cortex in the Alzheimer group, while other areas were no different from controls (Table 1). There was no correlation between [^{3}H]-forskolin binding and postmortem delay in either control (r=0.253) or Alzheimer (r=0.100) groups in frontal cortex.

TABLE 1.

	[^{3}H]-Forskolin Bound (pmol/g tissue)	
	Control	Alzheimer
Frontal Cortex		
Layers I-III	45.2 ± 3.5	22.2 ± 3.3 *
Layers IV	37.5 ± 2.8	18.4 ± 2.9 *
Layers V-VI	40.7 ± 3.5	22.3 ± 2.3 *
Hippocampal Region		
Parahippocampal gyrus	29.0 ± 3.9	25.0 ± 3.5
Subiculum	16.2 ± 3.3	17.2 ± 3.3
CA3	16.0 ± 2.9	16.6 ± 1.9
Hilus (dentate gyrus)	25.1 ± 5.7	26.1 ± 2.4
Granular layer (dentate gyrus)	15.0 ± 3.7	12.3 ± 2.9

Values are mean ± SEM. Control, n=6; Alzheimer, n=7. *$P<0.05$, Student's t-test.

[^{3}H]-Forskolin binding sites are discretely localised in cortical and hippocampal regions of human brain. Furthermore, only selective populations of these binding sites are altered in Alzheimer's Disease.

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THE STATUS OF EXCITATORY DICARBOXYLIC AMINO ACID UPTAKE SITES IN THE CEREBRAL CORTEX IN ALZHEIMER'S AND PARKINSON'S DISEASES

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The excitatory dicarboxylic amino acids (EDAA), glutamate and aspartate are major excitatory neurotransmitters and appear to play an important role in cognitive function in the cerebral cortex. We have investigated the status of high affinity EDAA uptake sites in the cerebral cortex in Alzheimer's and Parkinson's diseases using quantitative tritium film autoradiography of ^{3}H-D-aspartate binding.

Frozen blocks of frontal cortex (Brodmann area (BA) 9, temporal cortex BA 21, 22, parietal cortex BA 7a,b and occipital cortex BA 18,19 were obtained from 5 cases of Alzheimer's disease (82±5 (SD) years, 39±21 h postmortem delay), 4 non-demented Parkinsonian patients (60±14 years, 56±14 h) and 4 demented Parkinsonian patients (74±6 years, 28±17 h). 9 control patients (78±9 years, 33±23 h) were used for the Alzheimer's and demented Parkinson's disease groups and a subset of 5 control patients (72±4 years, 43±27 h) for the non-demented Parkinson's disease group. Both control groups had senile plaques counts of <1 (expressed as the mean count from frontal, temporal, parietal and occipital cortex/ 1.3 mm^{2}), the non-demented Parkinsonian group <2, the demented Parkinsonian patients 9 and the Alzheimer group 29. Cortical neurofibrillary tangles were only present in the Alzheimer group.

Serial 25um cryostat sections were cut, thaw mounted and air-dried before storage at - 70^{0}C. The ^{3}H-D-aspartate binding assay was carried out essentially as previously described by Cross et al (1987). The sections were exposed to ^{3}H-Hyperfilm (Amersham International) together with radioactive standards (^{3}H-Microscales (RPA 501, Amersham International) for 3 weeks and then developed. Quantitative analysis of the autoradiograms was carried out using a Magiscan 2A image analysis system (Bell et al, 1987).

In the control group the parietal cortex had the lowest level of ^{3}H-D-aspartate binding (272.7±45.4 fmol/mg tissue), while the frontal, temporal and occipital areas had similar levels of binding (386.4±40.9, 413.6±63.6 and 395.4±68.2 fmol/mg tissue respectively). The overall levels of ^{3}H-D-aspartate binding were not significantly changed in either the Alzheimer's or non-demented Parkinson's disease groups. In contrast, in the demented Parkinsonian patients there was a significant reduction in binding, compared to controls, in both gyral (386.4 ±40.9 v 227.2±27.3, P=0.025, Mann-Whitney U - Test) and sulcal (418.2±31.8 v 240.9±50.0, P=0.04) regions of frontal cortex.

These results are at variance with previous reports of a reduction in ^{3}H-D-asparate binding in the temporal cortex in Alzheimer's disease (Cross et al, 1987; Cowburn et al, 1988, Procter et al, 1988). This difference may be due to variations in the extent of neurone loss in the cases studied. The selective decrease in EDAA uptake sites in the frontal cortex in demented Parkinsonian patients may be involved in the frontal lobe cognitive deficits observed in these patients (Gotham et al, 1988).

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KYNURENIC ACID LEVELS IN HUNTINGTON'S DISEASE

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Excitatory amino acids have been proposed as causative agents in the pathogenesis of the progressive movement disorder Huntington's disease (Coyle & Schwarcz 1976). One of the most favoured candidates as an endogenous excitotoxin in this disorder is quinolinic acid (Beal et al 1986); no change in the content of this agonist in the brains of patients dying with this disease could be found, however (Reynolds et al 1988).

Some time ago we suggested that a subtle change in the ratio of quinolinic acid and the closely related antagonist, kynurenic acid might be an important factor in the pathogenesis of a number of neurodegenerative disorders (Stone & Connick 1985). Following the recent demonstration of micromolar amounts of kynurenic acid in the CNS (Carla et al 1988), we endeavoured to determine the levels of kynurenic acid in the brains of patients dying with Huntington's disease.

Blocks of 100-500mg each of Human brain motor cortex were obtained from the Cambridge brain bank, together with age/sex matched controls.
Tissue was weighed and homogenised in 1ml of ethanol/0.1M NaOH (three times), with a centrifugation step between each successive extraction. Supernatants were pooled and the kynurenic acid content analysed as previously described (Carla et al., 1988).
Briefly, kynurenic acid was purified on Dowex1 x 8 and 50 x 8 ion exchange resins prior to quantification using an HPLC with reverse phase C18 column and a spectrophotometric detector at 340nm.

In the motor cortex of normal subjects the content of kynurenic acid was 85.1 ± 6.5 pmol/g (n=4). Samples of motor cortex from Huntington's disease patients contained 165 ± 17.4 pmol/g (n=5). This represents a highly significant elevation of kynurenic acid content of 194% ($P<0.01$). There was no change in the kynurenic acid content of the other brain regions analysed. The kynurenic acid content in the caudate nucleus in both control (56.8 ± 5.1pmol/g, n=5) and diseased brains (89.2 ± 17.5pmol/g, n=5) was broadly similar to that in the frontal cortex of both normal (46.3 ± 3.6pmol/g, n=5) and Huntington's brain (80.7 ± 20.7pmol/g, n=5).

This increase in kynurenic acid content is surprising, since one might predict a decrease in the concentration of an endogenous amino acid antagonist giving rise to excitotoxicity by endogenous agonists. We are currently expanding on these results in an attempt to determine whether the change in kynurenic acid is a primary effect or whether it represents a compensatory response to increase excitatory activity.

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THE EFFECTS OF TETRAHYDROAMINOACRIDINE ON ENDOGENOUS 5-HYDROXY-TRYPTAMINE AND DOPAMINE RELEASE FROM RAT BRAIN SLICES

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Tetrahydroaminoacridine (THA) is a potent inhibitor of acetylcholinesterase which has been reported to have therapeutic potential in Alzheimer-type dementia (ATD). However, the effectiveness of THA in ATD compared to other cholinergic agents suggests that THA may act at least in part through some undefined mechanism (Summers et al, 1986). In the present study we have examined the effects of THA on the release of endogenous 5-hydroxytryptamine (5-HT) and dopamine from rat brain slices.

Male Lister Hooded rats (200-300g) were used throughout. The release of endogenous 5-hydroxytryptamine from frontal cortex slices and endogenous dopamine from striatal slices (300μm, McIlwain prisms) was studied esentially as described by Auerbach & Lipton (1985). All incubations contained 50μM pargyline and either 5μM fluoxetine (5-HT release) or 10μM nomifensine (dopamine release). Monoamine concentrations were determined by HPLC with electrochemical detection.

The release of 5-HT from frontal cortex slices was stimulated by K^+ in a concentration-dependent manner (ED_{50} = 30mM). 100μM THA markedly potentiated 5-HT release in the presence of 1mM K^+. However in the presence of 35mM K^+ no additional effect of THA (100μM) was observed (Table 1). THA (100μM) also enhanced dopamine release in the presence of 1mM K^+. The stimulation of 5-HT and dopamine release by THA was unaffected by the omission of Ca^{2+} from the medium. The effects of THA on 5-HT and dopamine release were concentration-dependent, with a significant enhancement of dopamine release being observed at 10μM THA. The stimulation of 5-HT and dopamine release was not saturable at THA concentrations up to 1mM.

Table 1 The effects of THA on 5-HT and dopamine release

	5-HT release frontal cortex (ng/mg prot./5 min)		Dopamine release striatum (ng/mg prot./5 min)	
	1mM K^+	35mM K^+	1mM K^+	35mM K^+
Control	0.66 ± 0.03	2.89 ± 0.16	9.4 ± 1.1	64.4 ± 8.1
100μM THA	2.85 ± 0.13*	3.20 ± 0.38	54.9 ± 2.2	_____

Values are mean ± SEM; ($n \geq 3$);* $p < 0.01$ vs control

In contrast to reserpine, 100μM THA significantly enhanced 5-HT release in the absence of pargyline (control 0.08 ± 0.02; 100μM reserpine 0.15 ± 0.01; 100μM THA 1.12 ± 0.07 ng/mg prot./5min, n=3).

The present data demonstrate that THA can enhance the release of 5-HT and dopamine in vitro. Unlike the studies of Drukarch et al, (1988), the present results suggest that THA does not act through a reserpine-like mechanism.

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THE INFLUENCE OF TETRAHYDRO-9-AMINOACRIDINE ON EXCITATORY AMINO ACID NEUROTRANSMISSION IN VIVO AND IN VITRO

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Evidence is emerging that in Alzheimer's disease degeneration of some cortical excitatory amino acid (EAA) releasing neurons occurs and this is an early and clinically relevant change (see Procter & Bowen, 1988). Tetrahydroaminoacridine (tacrine), well known as an alleged drug for the palliative treatment of the disease, has been examined for effects on these neurons in vivo and in vitro.

For in vivo microdialysis studies, male Porton-Wistar rats (180-280g) were anaesthetized with chloral hydrate (400mg/kg, ip). A microdialysis probe (Carnegie Medicin, Stockholm) was implanted in the prefrontal cortex (AP 3.5, ML 2.5 from Bregma; DV 3.0mm) and was perfused with Ringer solution at a rate of 2µl/min. After 60 min perfusion, 10 min serial fractions were collected for amino acid determination by HPLC (Procter et al., 1988). In a separate group of rats (anaesthetized with sodium pentobarbitone; 60mg/kg, ip) the probe was secured in position and the rat allowed to recover for 24h before perfusion began. Tacrine was administered either centrally through the dialysis probe (1mM & 5mM) or systemically (10mg/kg, ip). The effect of tacrine on potassium evoked, calcium dependent release of aspartate (ASP) and glutamate (GLU) and D-[^{3}H] ASP uptake was assessed in cortical tissue prisms as described by Procter et al., (1988).

In conscious and anaesthetized animals, systemic administration (10mg/kg, ip) of tacrine had no effect on extracellular concentrations of ASP and GLU. In anaesthetized animals, intracortical application of only 5mM tacrine caused a significant elevation in the extracellular concentrations of ASP and GLU (168% & 370% of basal values respectively; $p<0.05$, Wilcoxon paired rank test). This elevation was insensitive to the administration of tetrodotoxin suggesting that it was independent of neuronal conductance. This is consistent with in vitro findings which indicate that over a concentration range of 50-1500µM tacrine inhibited high affinity uptake of D-[^{3}H] ASP in a dose dependent manner (IC_{50}=614±35uM). Similarly, potassium evoked, calcium dependent release of EAA's was reduced in a concentration dependent manner (Table 1).

Table 1 The effect of tacrine on K^+ evoked, Ca^{2+} dependent release of EAA's

EAA	TACRINE CONCENTRATION (µM)			
	CONTROL	50	100	1000
ASP	4.5±0.5	3.9±2.3	2.8±0.5*	0.1±0.4*
GLU	16.6±2.0	13.7±1.6	11.4±2.1*	1.3±0.3*

Values = mean (±SD) concentration of EAA (nmol/2min/mg protein), n=3
*=Significantly different from control $p<0.05$ (Mann-Whitney U test)

Effective doses of tacrine in the in vitro experiments are high compared with the concentrations likely to be achieved in the brains ofpatients (see Liston et al., 1988). It seems unlikely therefore that any beneficial effects of tacrine may be attributed to its effect on EAA neurotransmitter function. This is supported by the observation that systemic administration of large doses of tacrine had no effect on the extracellular concentration of EAA's.

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AUTORADIOGRAPHY OF κ OPIOID RECEPTORS IN BASAL GANGLIA OF HUMAN BRAIN

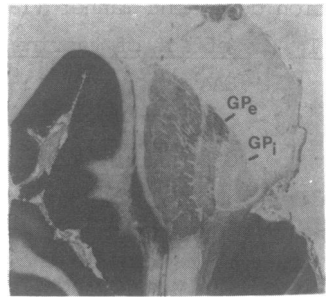
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Human brain contains mu, delta and kappa opiate receptors. Some areas have a high density of both opioid peptide immunoreactivity and receptors. An exception is the globus pallidus (GP), which contains much enkephalin and dynorphin immunoreactivity (Haber & Watson, 1985) but no binding sites for the non-selective opiate ligand [^{3}H]etorphine (Cross et al, 1987). Recently, we have shown binding of the kappa ligand [^{3}H]dynorphin$_{1-9}$ in the primate GP (Slater & Cross,1986). In this study we have used the kappa-selective ligand [^{3}H]U 69,593 (Lahti et al, 1985) to measure kappa receptors by <u>in vitro</u> autoradiography in human basal ganglia.

Blocks of brain were rapidy frozen at -40°C, sectioned (20μm), and mounted on glass slides. Sections were preincubated for 10 min at room temperature in buffer (pH 7.4) containing 12 mM HEPES, 0.5 mM NaH_2PO_4, 1 mM $CaCl_2$, 145 mM NaCl, 5 mM KCl and 2 mM $MgCl_2$. Sections were then incubated for 40 min in buffer containing 2 nM [^{3}H]U 69,593. Unbound ligand was removed by two 5 min washes in buffer at 4°C and the sections were dried and exposed to tritium film, together with standards, for 12 weeks at 4°C. Some sections were incubated with ligand and the kappa agonist U 50,488H (100nM) to define non-specific binding.

The specific binding of [^{3}H]U 69,593 in the most densely labelled part of the neocortex was 94% of the total (n=6). The neocortex had 2 bands of dense labelling in the superficial and deep layers (maximum binding was 55.4 fmol/mg, mean of 6). There was high binding in the claustrum (up to 54 fmol), but much less in binding in the putamen (19 fmol) and head of caudate (17.9 fmol). Ligand binding was present in both segments of the GP. The highest binding was in the dorsal part of the external segment (GP_e, 46 fmol), compared to 27.4 fmol in the internal pallidum (GP_i).

<u>Fig. 1 : [^{3}H]-U69,593 binding to human basal ganglia</u>



Previously we have shown a considerable mismatch between receptor distribution and opioid peptides within the GP (Cross et al, 1986; Blackburn et al, 1988). This mismatch is resolved by the identification of kappa receptors, labelled with [^{3}H]U 69,593, which are most concentrated in the external segment, an area with greater peptide immunoreactivity than the internal segment (Haber & Watson, 1985).

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ETHYLCHOLINE MUSTARD AZIRIDINIUM (ECMA) CAUSES ACTIVATION OF TRYPTAMINERGIC NEURONES IN RAT BRAIN REAGGREGATE CULTURES

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ECMA blocks the synthesis of ACh by alkylation of the high affinity carriers for choline. It has therefore been used in attempts to destroy selectively central cholinergic neurones *in vivo* and in culture. We have shown that 12.5μM ECMA causes approx 40% loss of choline acetyltransferase (ChAT) and 45% loss of muscarinic receptors within 2h of treating foetal rat whole brain reaggregate cultures. Muscarinic receptor concentrations recovered within 24-47h while ChAT activity exhibited a further decline over the subsequent 48-96h, indicating selective toxicity to cholinergic neurones (Pillar *et al*, 1988). After 25 or 50μM ECMA, however, receptor concentrations did not recover, suggesting wider cytotoxic effects. We therefore sought further information about the effects of ECMA in these cultures.

Reaggregate cultures were prepared from whole brain of 16-17 day Wistar rat foetuses, cerebellar neuronal or astrocyte monolayer cultures from 8-day old neonates, and treated, after 9 days *in vitro*, with 12.5-50μM ECMA (Pillar *et al*, 1988). Neurofilament protein was assayed by an ELISA technique (Doherty *et al*, 1984). 3-Methyoxy-4-hydroxy-phenylglycol (MHPG) and 5-hydroxyindole-3-acetic acid (5-HIAA) were assayed by HPLC with electrochemical detection, lactate dehydrogenase (LDH) spectrophotometrically by automatic analyser.

In reaggregates concentrations of neurofilament protein were significantly reduced (approx 28%) 120h after 50μM (but not after 12.5 or 25μM) ECMA. LDH release into the culture medium over 72h was significantly increased (60-160%) and directly related to dose of ECMA. 2h treatments with ECMA however caused no increase. HC-3 (40μM) did not prevent leakage over 72h. MHPG concentrations were not changed either 2 or 72h after ECMA. 5-HIAA concentrations were increased by 100-300% 2h after 12.5 to 50μM ECMA and remained 100-150% increased after 72h. In the neurone or astrocyte monolayers 50 and 25μM ECMA had no effect within 2h but caused marked loss of cells within 72h (light microscopy, 250x). At no time did 12.5μM ECMA cause any visible deleterious effect.

Since neurofilament protein is a general marker for neuronal integrity the present results confirm that 50μM ECMA is widely toxic to neurones and not specifically to cholinergic neurones. 12.5μM ECMA however does not appear to be widely neurotoxic. LDH leakage over 72h confirmed more general cellular damage at the larger concentrations. HC-3 did not prevent this latter effect consistent with a wider cytotoxicity than that confined largely to cholinergic neurones at 12.5μM ECMA. In support of this the non-cholinergic cerebellar monolayers also showed general cytotoxicity only at the larger concentrations of ECMA. Increased 5-HIAA concentrations 2h after 12.5μM ECMA, the concentration most clearly specific for cholinergic neurones, suggest blockade of ACh synthesis and release, prior to the loss of cholinergic neurones, leads to activation of 5HT-neurones. This subsides partially over the following 70h during which the cholinergic neurones degenerate. Thus, there exists in brain a functional interaction between ACh and 5HT that might be of importance during the cholinergic neuronal degeneration in Alzheimer's disease.

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TWO TYPES OF HIGH AFFINITY CHOLINE TRANSPORT IN DEVELOPING RAT BRAIN DETECTED BY ETHYLCHOLINE MUSTARD AZIRIDINIUM (ECMA)

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Hemicholinium-3 (HC-3,1μM) sensitive high affinity choline transport (ChT) is the rate limiting step in the synthesis of ACh from extracellular choline. Accumulation of [^{3}H]choline mediated by this mechanism, or the membrane binding of [^{3}H]HC-3, is potentially therefore a marker for cholinergic nerve terminals in brain. However, after transport of this kind choline is less extensively converted into ACh in developing brain than in mature samples (Kotas & Prince, 1987). This suggests ChT is not confined to cholinergic nerve terminals, particularly in developing brain. ChT into synaptosomes or fine slices from mature brain is inhibited irreversibly by ECMA but that into erythrocytes is not (Pedder & Prince, 1987; Uney & Marchbanks, 1987). We therefore estimated the proportion of ChT that is into cholinergic nerve terminals, in developing and mature brain, from the effects of depolarisation using K^+ and from the sensitivity of ChT to inhibition by ECMA.

Fine slices (McIlwain tissue chopper, 0.1 x 0.1 x approx 1mm) of hippocampus or cerebral cortex were prepared from 8-11 day old neonates or from 3 month old male Wistar rats. [^{3}H]Choline transport and [^{3}H]ACh synthesis were measured before and after depolarisation with K^+ (35mM) using 1μM HC-3 to define high affinity transport (Kotas & Prince, 1987). ECMA was prepared and used as described by Pedder & Prince (1987).

Prior depolarisation of immature hippocampus increased ChT by 78.4% ($\pm$ 19.4) and by 237.7% ($\pm$ 41.2) in mature samples. Depolarisaton also increased the proportion of the transported choline that was converted into ACh in immature hippocampus (25.3% $\pm$ 2.1 increased to 45.5% $\pm$ 2.6) but not in mature samples (75.8% $\pm$ 1.6 to 81.3% $\pm$ 1.3). At both stages of development 76.9-82.2% of the additional choline accumulated, after depolarisation, was converted into ACh. Very similar results were obtained with comparable samples of cortex. 5 min reactions with 20-30μM ECMA completely abolished [^{3}H]ACh synthesis in immature as well as in mature hippocampus or cortex. ChT was also completely inhibited in mature hippocampus, but in immature samples 32.2% $\pm$ 3.7 was resistant even to 100μM ECMA. In comparable samples of cortex 55% of ChT was resistant to ECMA.

Thus in developing brain 1μM HC-3 does not uniquely define the high affinity choline transport subserving ACh synthesis, ie, it does not specifically identify cholinergic nerve terminals. In 8-11 day old rats 32-55% of HC-3 sensitive transport is into a compartment not synthesising ACh. The remaining 45-68% provides for the synthesis of ACh and is stimulated by tissue depolarisation, ie, it is into cholinergic nerve terminals. This latter compartment becomes increasingly dominant during development, so that in mature brain the specificity of HC-3 sensitive transport is complete. Within this neuronal compartment 76-82% of the transported choline is converted into ACh, at both stages of development, indicating that neither choline acetyltransferase nor acetylCoA is rate limiting in the synthesis of ACh, even in developing brain. ECMA specifically inhibits the high affinity transport of choline that is into this compartment and that provides for the synthesis of ACh. ECMA is therefore a more accurate marker for cholinergic nerve terminals in developing brain than is HC-3.

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NERVE GROWTH FACTOR (NGF) ENHANCES CHOLINERGIC SYNAPTIC FUNCTION IN RAT WHOLE-BRAIN REAGGREGATION CULTURES

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In addition to its well-known role in the survival and regulation of specific properties of sympathetic and sensory neurones in the peripheral nervous system (Levi-Montalcini, 1982), Nerve Growth Factor (NGF) has a neurotrophic role on cholinergic neurones in the CNS. In vivo studies have demonstrated significant increases of choline acetyltransferase (ChAT) activity in both the normal developing and lesioned, adult rat CNS following NGF treatment (see Gnahn et al, 1986; Kromer, 1987; Honegger & Lenior, 1982; Hefti et al, 1985). We have previously reported that although low concentrations of NGF (5 ng/ml) do not reverse an ECMA-induced cholinergic lesions in rat whole brain reaggregate cultures (Atterwill et al, 1988) it does induce a modest increase in ChAT activity in the control cultures. We have now further examined the dose-responsiveness of this effect on both ChAT activity and the muscarinic cholinergic receptor.

Foetal rat whole brain reaggregate cultures were prepared as described previously (Atterwill et al, 1988) in a serum-supplemented (S+) culture medium. NGF (7S subunit, Sigma Chemical Co) was added (5 or 50 ng/ml) at 8 days in vitro (DIV) and thereafter every 48 hours until the cultures were harvested at 15 DIV. ChAT activity and muscarinic receptor binding (mAChR-defined as the specific binding of [^{3}H] quinuclidinyl benzilate, QNB, in the presence of 100 μM atropine) were then measured. In some experiments ECMA (12.5 μM) was also added at 8 DIV in order to lesion the cholinergic neurones.

Compared with it's reported effect at 5 ng/ml where 45-50% increases in ChAT were found (Atterwill et al, 1988) NGF at 50 ng/ml (8-15 DIV) produced much larger increases in ChAT activity (Control = 68.5, NGF treated = 344.3 pmols/min/mg protein; $P<0.001$). Similarly, although no effect on [^{3}H] QNB binding was seen at 5 ng/ml NGF, exposure to 50 ng/ml NGF increased muscarinic receptor binding by approx. 100%. When added to the reaggregates at the same time as ECMA, NGF (50 ng/ml) was unable to reverse the ECMA-induced loss of ChAT activity.

We have, therefore, now shown that high concentrations of NGF (50 ng/ml) are able to produce large elevations in ChAT activity in developing reaggregate cultures of rat whole brain. This agrees with previous reports where the ED_{50} in cultured septal cholinergic neurones was 10 ng/ml (Hefti et al, 1985). It has been shown for the first time that NGF treatment evelates muscarinic receptor binding in developing brain reaggregates in agreement with its reported action in cultured rat phaeochromocytoma (PC12) cells (Jumblatt & Tischler, 1982). This action has a different concentration-dependence to actions on ChAT since no effects on the receptor were seen at 5 ng/ml NGF. It also demonstrates a neurotrophic action on the central cholinergic synapse rather than just the presynaptic nerve ending. The apparent failure of even high concentrations of NGF to reverse the ECMA-induced cholinergic lesion in vitro (despite a residual pool of ChAT in the treated cells) may be due to alkylation of NGF receptor proteins precluding retrograde NGF transport to and action on nuclei in the cholinergic perikarya.

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IS TOLERANCE TO THE CNS EFFECTS OF AMINOGLUTETHIMIDE IN THE MOUSE ASSOCIATED WITH DEPENDENCE?

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Tolerance to the CNS-depressant effects of the aromatase inhibitor, aminoglutethimide (AG) occurs in breast cancer patients receiving the drug repeatedly. The phenomenon also occurs in mice where a functional component is involved (Ahmad et al. 1987). In view of this and because the drug may be abruptly withdrawn from patients not responding to AG, it was considered useful to examine whether there was any indication of physical dependence in mice tolerant to AG. In this study, barbitone was a positive control for the induction of dependence (Kaneto et al. 1986).

Male albino mice (22g; n=7) received oral doses twice daily of either 0.75% w/v carboxymethylcellulose (control), babritone or AG for 2 weeks. In the first and second weeks the doses were 100 and 200mg/kg respectively for both drugs. Such a regimen has previously induced dependence on barbitone (Kaneto et al. 1986) and tolerance to AG (Abusrewill et al. 1986). Hypersensitivity to leptazol-induced seizures at the end of the chronic dosing schedule was employed as an index of dependence (Flint & Ho, 1980) and three parameters were measured (% survival, latency to onset of tonic extensor convulsion and scoring of seizure severity; Abusrewill et al. 1986).

Table. Response of mice to leptazol following barbitone and AG withdrawal

Pretreatment[1]	Leptazol response[2]		
	% survival	Latency to convulsion[3]	Total convulsion severity score
Vehicle (control)	86	600 ± 0	27
Barbitone	29	231 ± 69	40
AG	100	600 ± 0	11

[1]Pretreatment as given in text. [2]Leptazol 100mg/kg i.p. 18h after last pretreatment dose; values from n=7. [3]Seconds (mean ± SEM).

Results presented in the Table show that barbitone-pretreated mice, in comparison with control animals, had an increased sensitivity to leptazol (lowered survival, shorter latency to convulsion, increased severity score) 18h after the final dose of barbitone. Such hypersensitivity was also present for at least 72h after the termination of the barbitone-dosing schedule. In addition, the log dose-response line for latency to leptazol-induced convulsion was significantly ($P<0.05$) shifted to the left by barbitone-pretreatment. In contrast, convulsion latency was unaltered, survival slightly increased and severity score halved in the AG-treated mice 18h after the last dose of the drug. As the AG level in plasma and brain at this time was 3.5µg/ml and 4.5µg/g respectively, it was considered that the effects of leptazol might have been influenced by a mildly anticonvulsant concentration of AG. However, 36h after the final dose of AG, the drug was not detectable in plasma or brain. Leptazol-challenge of AG-pretreated mice at this time and at 48 and 72h, elicited responses identical with those obtained in controls. It is concluded that, under the conditions of the experiment, AG does not share the ability of barbitone to induce a dependence phenomenon. As the $t_{0.5}$ of AG is longer in humans than mice (Dalrymple & Nicholls 1988), it seems unlikely that a withdrawal syndrome would be precipitated in patients in whom the drug is stopped after a prolonged course of treatment.

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METHOCTRAMINE SELECTIVITY ANTAGONISES THE MUSCARINIC HYPERPOLARISATION OF RAT SUPERIOR CERVICAL GANGLIA IN VITRO

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It has been shown that muscarinic agonists can evoke two responses on the rat superior cervical ganglion (Newberry and Priestley, 1987). The M1-mediated depolarising response often masks a preceding hyperpolarisation activated by cardiac-like M2 receptors. Methoctramine, a polymethylene tetramine, has been shown to antagonise cardiac M2 receptors in preference to M1 receptors (Giraldo et al, 1988). We have tested the antagonist potency of methoctramine against the two ganglionic muscarinic responses.

DC potential changes evoked by 1 min applications of (±)muscarine were recorded from isolated superior cervical ganglia using a grease-gap technique (Newberry and Priestley, 1987). Concentration-response relationships were determined in a medium containing 2.5mM $CaCl_2$, 3mM K^+ and 0.1 μM TTX. To facilitate the observation of the hyperpolarising response to 1 μM muscarine the medium was changed to contain 0.1 mM $CaCl_2$ and 0.3 μM pirenzepine. Methoctramine (Research Biochemicals Inc.) was superfused for at least 1 hr before determining the following. Dose-ratios (DR) against the depolarising response were determined at the level of half the response to 3 μM muscarine (at this concentration the dose-response curve normally reaches a plateau). The pA_2 was estimated from the equation: $pA_2=\log_{10}(DR-1)-\log_{10}$(molar concentration of methoctramine). The concentration required to inhibit the hyperpolarisation by 50% (IC_{50}) was determined in separate experiments ($pIC_{50}=-\log_{10}(IC_{50})$).

The most prominent effect of 0.3 μM methoctramine was to increase the maximum depolarisation evoked by muscarine, mean (range, n) = 1.6 (1.4-1.8, 6), with little rightward shift of the concentration-response curve. The increase in the maximum was still seen with 3 μM methoctramine but the curve was clearly shifted to the right. Dose-ratios produced by both concentrations gave estimated pA_2 values that were not significantly different, mean (± s.e.m., n) = 6.5 (0.1,10). Against the hyperpolarising response, methoctramine was more potent, mean pIC_{50} = 7.5 (0.1,6).

The estimated pA_2 value against the depolarisation and the pIC_{50} against the hyperpolarisation are in good agreement with the binding affinities (pK 0.5) for methoctramine in the rat cortex (M1, 6.59) and rat heart (M2, 7.45) (Giraldo et al, 1988). These data support our suggestions about the receptors involved in the two ganglionic responses and extend the reports of the selective antagonist action of methoctramine. The increase in the maximum depolarisation, also seen with gallamine (Newberry and Priestley, 1987), could be explained by the selective antagonism of the underlying hyperpolarisation.

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A STUDY OF TACHYKININ RECEPTORS IN THE RAT VAS DEFERENS

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Stimulation of tachykinin receptors in the rat vas deferens enhances stimulus-evoked contractions. Classification of tachykinin receptors in this tissue suggests that they are of the NK-2 type. Stimulation of these receptors in other tissues leads to an increase in inositol phospholipid metabolism. The present report seeks to characterise the second messenger response using a variety of agonists and antagonists in vas deferens.

Inositol phosphate hydrolysis was examined in cross chopped (150μm) slices of rat vas incubated in Krebs bicarbonate buffer, pH 7.4 containing 10mM LiCl; BSA (1mg/ml); bacitracin (0.1mg/ml); trypsin inhibitor (0.04mg/ml); phosphoramidon (1μM) and [^{3}H]-inositol 2μCi/ml. After 45 min incubation at 37°C drugs were added and the incubation continued for a further 30 min. The reaction was terminated by the addition of 1ml chloroform and the labelled inositol phosphates separated by anion exchange chromatography (Berridge, 1982). Under these conditions 724 ± 64 dpm were incorporated as [^{3}H]-inositol-monophosphate. Addition of 3μM eledoisin gave a significant increase in the amount of radioactivity incorporated (3277 ± 341 n = 5). Single vasa deferentia were mounted in a 3ml organ bath in the same buffer at 37°C and field stimulated with single biphasic pulses delivered at 0.15Hz. In both series of experiments potencies were compared by reference to eledoisin.

Table 1

	Contraction		[^{3}H]-inositol phosphate	
Drug	EC_{50}μM	Range	EC_{50}μM	Range
Neurokinin A	0.15	0.13- 0.21	0.22	0.12- 0.04
Kassinin	0.26	0.15- 0.30	0.04	0.02- 0.08
Eledoisin	0.50	0.15- 1.44	0.29	0.17- 0.49
[Glp,D-Pro^9]SP(6-11)	0.66	0.49- 1.06	0.22	0.19- 0.26
Substance P	13.60	5.49-19.74	7.89	4.29-14.83

The results showed a good correlation between the two functional assays (r = 0.9). Furthermore the order of potency of agonists was consistent with an NK-2 receptor classification with [Glp^{6}D-Pro^9]SP being active while the L-Pro isomer of the compound was virtually inactive on both systems as was DiMe-C7. Substance P was about 20 times less active than eledoisin and substance P methyl ester was inactive (EC_{50} greater than 100μM). Two cyclic antagonists L-659,837 and L-659,877 which have a high affinity for NK-2 receptors (McKnight et al, 1988) were competitive inhibitors of both assays. The pA_2 values for antagonism of the contractile response and the formation of [^{3}H]-inositol monophosphate were 6.8 and 7.3 for L-659,837 and 8.0 and 8.77 for L-659,877 respectively. This emphasises the similarity of the two systems.

Thus tachykinin-induced contractions in the rat vas deferens appears to involve stimulation of the inositol phospholipid second messenger pathway following occupation of NK-2 receptors.

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STOICHIOMETRY OF THE INHIBITION OF STIMULUS-SECRETION COUPLING BY ANAESTHETICS

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General anaesthesia has been regarded as a classical example of non-specific drug action. This view is supported by the well-documented correlation between anaesthetic potency and lipid solubility (Miller et al., 1972). Detailed analysis of anaesthetic action, however, has suggested that certain anaesthetics interact with physiological processes in a highly specific way (Franks & Lieb, 1988). Here we present evidence that at least some anaesthetics inhibit catecholamine secretion in bovine adrenal chromaffin cells by a saturable process.

Table 1. Hill coefficients for anaesthetic inhibition of catecholamine secretion and Ca^{++} influx

	Carbachol stimulation			K^+ - stimulation		
	n	K_I	r	n	K_I	r
Catecholamine secretion						
Pentobarbitone	1.00	44	0.94	1.03	136	0.97
Halothane	1.7	248	0.88	1.55	1011	0.95
Methoxyflurane	1.75	172	0.90	1.36	1140	0.78
Ca^{++} influx						
Pentobarbitone	0.76	49	0.88	0.97	159	0.96
Halothane	1.13	253	0.85	1.55	860	0.97
Methoxyflurane	1.43	355	0.85	1.34	1467	0.99

n is the Hill Coefficient, K_I the binding constant for inhibition and r the correlation coefficient.

Pentobarbitone and the volatile anaesthetics halothane and methoxyflurane inhibit the Ca^{++} influx and catecholamine secretion evoked by carbachol and potassium depolarisation (Pocock & Richards, 1987, 1988). The carbachol-evoked secretion is more sensitive to inhibition than that evoked by high K^+. Moreover, the potency of the agents in inhibiting catecholamine secretion correlates closely with their lipid solubility. Hill plots of the dose response curves yield coefficients between 1 and 2 (see Table 1). This suggests that the anaesthetics bind to saturable sites to cause inhibition of secretion, although for different agents different numbers of molecules must be bound to target sites to bring about a given level of inhibition.

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THE ANTICONVULSANT ACTIONS OF OCTANOIC AND DECANOIC ACIDS

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Interest in the potential anticonvulsant effects of octanoate and decanoate was initiated by the findings of Haidukewych et al (1982) who demonstrated a correlation between plasma levels of these compounds and protection against epileptic seizures. This was observed in children successfully treated with the medium chain triglyceride (MCT) diet, after failure of conventional drug therapy. This study was designed to investigate the possible anticonvulsant effects of these metabolites of the MCT diet in mice and to determine whether there was indeed a correlation between plasma levels of octanoate and deconaote and protection in mice.

Anticonvulsant activity was measured using one of two tests, the maximal electroshock test (platinum ball and clip ear electrodes; 15mA, 100Hz for 0.2sec) or the pentylenetetrazol (PTZ) test (15mg ml^{-1} in saline administered by intravenous infusion at 0.3ml min^{-1} until a clonic seizure was evoked).

Initial experiments following a single oral dose or parenteral injection were disappointing as no protection could be seen. However a study of the pharmacokinetics of octanoate and decanoate, in mice, revealed extremely short half lives of 2.8 and 3.3 minutes respectively. Mice were therefore given intravenous infusions of octanoate (56-112mg ml^{-1}; 12.5µl min^{-1} for 60 minutes) resulting in high steady state plasma levels of 200-600µg ml^{-1}. 12 mice infused with 112mg ml^{-1} sodium octanoate were subjected to the maximal electroshock test. 75% of these mice were protected against seizures. All mice that had plasma levels of octanoate greater than 400µg ml^{-1} were protected. To determine convulsive threshold further groups of mice were infused with 86 or 112mg ml^{-1} sodium octanoate (n=16 and 5 respectively) and tested with PTZ. A significant correlation was demonstrated between minimum convulsant dose of PTZ and both plasma and brain levels of octanoate (r=0.82; r=0.77 respectively).

Similar experiments were carried out with intravenous infusion of decanoate (90mg ml^{-1}, 12.5µl min^{-1} for 30 minutes). The results revealed that 30% of mice were protected against electroshock but no protection could be seen using PTZ as the convulsive stimulus.

These results, in contrast to those of Keane et al (1983) and Loscher & Nau (1985), show that both octanoate and decanoate possess anticonvulsant action, the former being more potent. A correlation between plasma levels of octanoate and protection was also clearly demonstrated. That octanoate can protect animals from seizures induced by these two methods is a new finding and interesting in that similar plasma levels are effective against both stimuli; many anticonvulsants show up better in one test than the other.

Whether octanoate or decanoate are themselves responsible for the anticonvulsant effect of the MCT diet remains unclear. Plasma levels in protected mice were higher than those in chidren (Haidukewych et al 1982) but this may not in itself be significant. Further metabolic products of the MCT diet and of octanoate or decanoate may also be involved.

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DIFFERENCES IN TRANSLATION PRODUCTS DERIVED FROM NORMAL AND DYSTROPHIC HUMAN RETINAL mRNAS

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Retinitis pigmentosa (RP) is the name applied to a group of hereditary diseases in which the retina slowly degenerates. There is no known treatment that can slow the progress of RP and the disease is a major cause of blindness in young people.

The genetic lesions which underlie RP are most probably expressed in messenger RNAs (mRNA) present in the retina, and in the present study, we have analysed these mRNAs in an attempt to determine which are expressed abnormally in diseased eyes.

Human eyes supplied by the U.K. Transplant & RP Donor Scheme were dissected and the retinas frozen in liquid nitrogen. Total cellular RNA was extracted from the retinas using cesium chloride ultracentrifugation. The RNA was then translated *in vitro* using rabbit reticulocyte lysate and incorporating ^{35}S-labelled methionine. The translation products were subsequently analysed on a 2D-SDS polyacrylamide gel electrophoresis system (pI range 4-7) and subjected to autoradiography. The post-mortem time varied from 1 to 10 days but preliminary experiments suggested that the mRNAs in ocular tissue were relatively stable.

RNA was extracted from the retinas of two RP sufferers (ages: 72 and 65 years; both believed to have suffered from recessive disease) and four control retinas. The latter were obtained from patients with no known retinal disease and were aged 11, 22, 52 & 56 years.

Comparison of autoradiographs prepared from the mRNAs of control and RP retinas consistently revealed differences in five polypeptides. Of these, two were produced in reduced amounts whilst three were increased in the autoradiographs from the RP mRNA. These polypeptides have not yet been identified. However, it is likely that the reduction in expression is secondary to photoreceptor loss as one of the reduced polypeptides is almost certainly opsin. The three peptides showing increased expression were at: 48kD, pI 5.4; 39kD, pI 5.5; 21kD, pI 5.6. Densitometric analysis revealed that the most enhanced polypeptide (48kD, pI 5.4) was increased about 250 fold compared with controls. This polypeptide has been tentatively identified as glial fibrillary acidic protein (GFAP). This probably reflects increased expression of GFAP in the Muller fibres of RP retinas. These glial cells do not normally produce GFAP but are known to do so in disease or following injury to the retina. We have not yet identified the remaining two enhanced species. Increased expression of lower molecular weight polypeptides has not been shown in other CNS tissues undergoing gliosis. It is possible that these changes in RP retinal polypeptide expression are specific to the disease.

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STEREOSPECIFIC BINDING OF MYO-INOSITOL (1,4,5)TRISPHOSPHATE TO RAT CEREBELLUM AND BOVINE ADRENAL CORTEX: EVIDENCE FOR HETEROGENEITY

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There is now much evidence that the second messenger inositol(1,4,5)trisphosphate ($Ins(1,4,5)P_3$) interacts with a specific 'receptor' which is linked to a Ca^{2+} channel associated with the endoplasmic reticulum or a more discrete and specialised structure - the 'calciosome'.

Binding studies have revealed a very high density of sites in rat cerebellum that possess identical stereo- and positional specificity to the Ca^{2+} release sites (Willcocks et al. 1987; Strupish et al. 1988). We present evidence here that the $Ins(1,4,5)P_3$ binding sites in bovine adrenal cortex could represent a different sub-type of the $Ins(1,4,5)P_3$ 'receptor'.

A membrane fraction of rat cerebellum and bovine adrenal cortex was prepared in a 'cytosol-like' buffer (mM: NaCl 20; KCl 100; EDTA 1; BSA 1 mg/ml; Tris-HCl 20; pH 7.7) and binding of $[^3H]D\text{-}Ins(1,4,5)P_3$ (20 Ci/mmole) was performed at 4° for 10 min using centrifugation to separate bound and free ligand as previously described (Willcocks et al. 1987).

Analysis of saturation binding data obtained by isotopic dilution yielded the following data. Bovine adrenal cortex: K_d 3 ± 1 nM, B_{max} 591 ± 66 fmol/mg protein. Rat cerebellum: K_d 23 ± 5 nM, B_{max} 10.2 ± 2.55 pmol/mg protein. The ability of the D- and L- enantiomers of $Ins(1,4,5)P_3$, D-L inositol(1,4,5)trisphosphorothioate (a synthetic, metabolically stable analogue of $Ins(1,4,5)P_3$, Strupish et al. 1988). L-α-glycerophospho-D-myo-inositol-4,5-bisphosphate ($GpIP_2$), ATP and heparin (porcine, Na^+ salt), a potential $Ins(1,4,5)P_3$ receptor antagonist (Cullen et al. 1988) were examined for their ability to inhibit specific $Ins(1,4,5)P_3$ (1-2 nM) binding in each membrane preparation. Although both preparations possessed sites with marked stereospecificity, the apparent affinity of the trisphosphates and $GpIP_2$ was about ten-fold higher in the adrenal preparation. In contrast, heparin possessed significantly higher affinity for the cerebellar membranes. It remains possible that there are differences in the accessibility of heparin to adrenal cortical membranes, however this difference coupled to an apparent lower affinity of the phosphates in the cerebellum may indicate receptor heterogeneity.

	Rat Cerebellum	Bovine Adrenal Cortex
	(IC_{50} ± SEM (M))	
$D\text{-}Ins(1,4,5)P_3$	5.6 (± 0.2) x 10-8	4.0 (± 0.5) x 10-9
$L\text{-}Ins(1,4,5)P_3$	1.0 (± 0.1) x 10-4	3.1 (± 0.2) x 10-5
$DL\text{-}Ins(1,4,5)P(S)_3$	1.3 (± 0.2) x 10-6	2.7 (± 0.2) x 10-7
$GpIP_2$	1.8 (± 0.2) x 10-6	1.7 (± 0.2) x 10-7
ATP	7.9 (± 0.7) x 10-4	4.3 (± 0.8) x 10-4
Heparin µg/ml	16 ± 6	51 ± 6

Data analysed using 'ALLFIT' (De Lean et al. 1978).

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COMPUTER ASSISTED INSTRUCTION IN PHARMACODYNAMICS

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Simulation programs reproducing laboratory experiences offer, when coupled with appropriately challenging problem sets, good possibilities for the teaching of pharmacology. If the main objective is to provide students with the skills to interpret the effects of drugs on isolated tissues or on whole animals, then a computerprogram, either or not with an interactive video component, is a good alternative for the use of animals in practical classes. As has been shown with the "Simulated Guinea-pig Ileum Program", it is possible to provide a record of responses of the 'guinea-pig ileum' when agonists (with or without antagonists) are added to the 'tissue bath' (1). The responses are quantitatively correct and relative potencies and pA values can be calculated.

The computer assisted instruction in pharmacodynamics is a courseware program consisting of a package of 8 lessons accessed from a menu. The programs are implemented on the IBM-PC, using TENCORE authoring language.

The program provides a realistic record, simulating the trace obtained during exposure of the guinea pig ileum to an agonist, or an agonist together with a (non-)competitive antagonist. Students are encouraged to practise correct experimental design. Interactive instruction affords the students to make their own log-dose effect curve, Lineweaver-Burk plot or Arunlakshana-Schild plot. Quantitative correct drug parameters can be calculated. A tutorial help menu provides test and feedback to the student. The 'isolated ileum' can also be used as a bioassay to identify the nature of an unkown drug or to calculate the strength of an unknown solution of a drug with a four-point assay, using Latin squares. With statistical methods and variance analysis students will learn to express the concentration with the 95 % confidence limits.

The program has been used by medical students since 1984 and later on also for the education of technical assistants. It provides a useful teaching aid that can reinforce instruction in pharmacology.

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QUALITATIVE COMPUTER SIMULATIONS OF ORGAN BATH EXPERIMENTS

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Computer simulation of pharmacological experiments can provide a useful adjunct to undergraduate practical classes. Such simulations can be used in various ways, for example as an aid to learning the theory behind an experiment or, in some cases, as a replacement for the experiment itself (Hughes, 1988; Brown & Dewhurst, 1988). We have developed a program which simulates qualitatively the effects of a variety of agonists and antagonists on a series of different tissues.

The program, which is menu-driven, is available in compiled Turbo Basic (Borland) and requires an IBM PC (XT or AT), or compatible machine, and an appropriate printer. Five tissues (rabbit ileum, stimulated guinea pig vas deferens, stimulated guinea pig atrium, guinea pig trachea, and rabbit trachea) are displayed simultaneously in colour or monochrome. Seven agonists - noradrenaline, adrenaline, isoprenaline, acetylcholine, nicotine, histamine and 5-hydroxy-tryptamine - are offered with or without appropriate antagonists. The flexible structure of the program is such that it is easy to expand the options available by adding other drugs. Other menu options allow investigation of the effects of known drugs on a single selected tissue. Alternatively, an unknown drug can be selected randomly by the computer and identified by use of antagonists and comparisons with known drugs. A hard copy can be produced after each run if required.

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USING A GENERAL-PURPOSE CONTOUR-PLOTTING PROGRAMME FOR INTERPRETING AND PLANNING EXPERIMENTS ON DRUG INTERACTIONS

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Drug combinations often produce effects which are complex and not directly predictable from those of their constituents. The results can usually be plotted as isobol diagrams which consist of lines joining points of equal effects, similar to geographical contours (Loewe, 1928; Rushton and Steinberg, 1967).

We have recently investigated the use of contour-plotting software, both for constructing such diagrams from results of rodent behaviour tests and to help in the planning of future experiments (Davies et al, 1988).

We have now further explored the potential of this software and have identified both its advantages (speed and versatility) and areas where more specialised algorithms would be of benefit. The software (Butland, 1979) has extensive facilities for the processing of non-gridded data into isobolograms and for the subsequent sectioning of the solid models produced. This can be useful in predicting the effects of untried dose combinations.

The use of the software will be demonstrated on data obtained from chlordiazepoxide-dexamphetamine and chlordiazepoxide-clenbuterol combinations, and the resulting isobols will be compared with those obtained by standard plotting methods. Ways will be suggested in which the unique properties of the software may aid the modelling of effects of drug combinations which do not lend themselves to simple mathematical description. We shall also propose solutions to problems for which general purpose software of this kind is inappropriate.

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ANTAGONISM OF THE INTOXICATING EFFECTS OF ETHANOL BY THE BENZODIAZEPINE RECEPTOR LIGAND R_o 19-4603

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THE EFFECT OF THE EPITHELIUM ON RELAXATION OF CANINE BRONCHI IS DEPENDENT ON CONTRACTILE TONE

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