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POST-NATAL CHANGES IN D-2 DOPAMINE RECEPTORS IN RAT AND POST-MORTEM HUMAN BRAIN.

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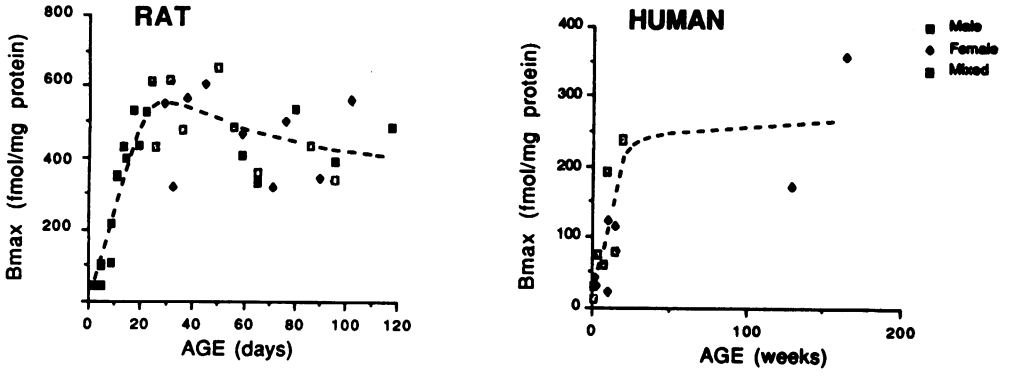
Several studies have examined changes in D-2 dopamine receptors during ageing and senescence. Relatively few studies, however, have investigated post-natal changes in D-2 receptors. Increases in D-2 receptor density during the first 1-3 months of life in rats (Hyttel, 1987) and from birth to 2 years in humans (Seeman et al., 1987) have been observed but the patterns underlying these increases have not been examined across a large number of ages. The present study describes developmental changes in D-2 receptors in rat and post-mortem human brain.

D-2 receptors were measured in Sprague-Dawley rat striatal (aged 1-118 days) or post-mortem human caudate/putamen (aged 0.5-164 weeks) membranes by saturation analysis of ^{3}H-spiperone binding as previously described (O'Boyle & Waddington, 1984). The relationships between post-natal age and D-2 receptor density are shown in figure 1. There were no changes in K_d with age.



At birth D-2 receptor densities in both rat and human brain are extremely low, being only 1/10 of the maximal values attained later on in life. In rats, B_{max} increases linearly after birth until day 20 and peak levels of D-2 receptors are achieved by post-natal day 30-40. Thereafter, B_{max} declines gradually up to day 118, the oldest age examined in this study. There was no difference in B_{max} between male and female rats of similar ages. In post-mortem human brain D-2 receptor density showed a similar linear increase from birth to 20 weeks of age. These developmental increases in D-2 receptors may be associated with the development of motor function in the young.

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We thank Dr. B. Kierse, Our Lady's Hospital for sick children, Crumlin, for human brain tissue.

NO ALTERATIONS IN BRAIN IRON, COPPER, MANGANESE OR ZINC LEVELS IN PRESYMPTOMATIC PARKINSON'S DISEASE

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Neuronal loss in Parkinson's disease (PD) is associated with a selective increase in total iron levels in substantia nigra, but a reduction in brain ferritin content, suggesting altered iron handling may occur in PD (Dexter et al, 1989, 1990). It is not known whether this represents a primary cause of dopamine cell death or whether iron accumulation is secondary to neuronal cell loss. For this reason we have determined the metal ion content of brains from control subjects who at post-mortem were found to have Lewy bodies and cell loss in the substantia nigra but who had not shown clinical symptoms of PD during life (presymptomatic PD).

Post-mortem brain tissue was obtained from 6 normal control subjects with a mean age (68.3 ± 5.7 years), death to refrigeration time (2.9 ± 0.3hr), time between death and removal of the brain (14.3 ± 1.5hr) and from 6 Lewy body positive control subjects with a mean age (68.2 ± 5.8 years), death to refrigeration time (2.7 ± 0.4hr), time between death and removal of the brain (21.5 ± 2.2hr*). Brain tissue was freeze dried, solubilized by hot acid digestion and the metal ion content measured by Inductively Coupled Plasma (ICP) spectroscopy. No differences in the total levels of copper, iron, manganese and zinc were observed when the substantia nigra, cerebellum, cerebral cortex (Brodmann area 10) and globus pallidus from normal controls and Lewy body positive subjects were compared (see Table 1).

Table 1 Total copper, iron, manganese and zinc levels in normal controls and Lewy body positive subjects.

BRAIN AREA		TOTAL METAL ION CONTENT (nmoles/g dry weight of human brain)			
		COPPER	IRON	MANGANESE	ZINC
Substantia nigra	Normal(n=6)	897 ± 111	13729 ± 1854	41 ± 9	1132 ± 86
	Lewy body +ve(n=6)	906 ± 122	13258 ± 1174	39 ± 7	1026 ± 67
Cerebellum	Normal(n=5)	498 ± 82	4066 ± 504	25 ± 2	1483 ± 109
	Lewy body +ve(n=5)	295 ± 39	3146 ± 560	24 ± 3	1177 ± 103
Cerebral cortex	Normal(n=5)	422 ± 97	4578 ± 55	24 ± 4	1273 ± 227
	Lewy body +ve(n=5)	365 ± 100	4413 ± 303	30 ± 1	873 ± 57
Globus pallidus	Normal(n=5)	385 ± 80	13443 ± 1281	48 ± 3	1461 ± 244
	Lewy body +ve(n=5)	359 ± 43	13735 ± 1151	51 ± 4	997 ± 99

Values represent mean ± SEM. *P < 0.05 compared to normal controls (Student's t test).

There appears to be no accumulation of iron in PD in the early stages of the illness. This suggests that iron accumulation does not initiate dopamine neurone degeneration. It may however, contribute to the progression of the pathology.

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EFFECT OF CARBIDOPA DOSE ON PHARMACOKINETIC AND METABOLIC HANDLING OF AN ORAL BOLUS OF L-DOPA IN RATS

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L-DOPA, when given in combination with the peripheral dopa decarboxylase (DDC) inhibitor, carbidopa, remains the treatment of choice in Parkinson's disease. Jaffe (1973) reported the optimal dose of carbidopa required to maximally inhibit systemic DDC in man to be 75mg/day, However, Cedarbaum *et al.* (1986) demonstrated enhanced L-DOPA efficacy when the dose of carbidopa exceeded the maximally effective dose. This was disputed by Contin *et al.* (1989) who found the AUC of L-DOPA and the clinical performance to be unaffected by increased dose of carbidopa. For this reason, we report the effect of increasing dose of carbidopa on the pharmacokinetic handling of L-DOPA in rats.

Male Wistar rats (306±4g) were cannulated in the carotid artery two days prior to experimentation. Rats were fasted 16h prior to drug administration. One hour prior to L-DOPA (50mg/kg po) administration, rats received carbidopa (3.125-100mg/kg ip). Blood samples were taken from the carotid cannula 15min prior to, and at intervals up to 300min after L-DOPA administration. Plasma levels of L-DOPA, 3-OMD, DOPAC and HVA were determined by HPLC with electrochemical detection.

Carbidopa pretreatment increased t1/2 and decreased Clp of L-DOPA in a dose-related manner, with maximum effect at 25mg/kg (Table 1). Carbidopa pretreatment also tended to reduce Vd in a dose-related manner. Pretreatment with carbidopa increased $AUC_{0-\infty}$ for L-DOPA and $AUC_{0-300min}$ for 3-OMD, and decreased $AUC_{0-300min}$ for DOPAC and HVA in a dose related manner, with maximum effect at 100mg/kg.

Table 1 Effect of carbidopa (CD) dose on the pharmacokinetic handling of L-DOPA (Mean±sem,*p<0.05 compared to control, Dunn's test; n=5-6)

Dose CD	t1/2	Vd	Clp	$AUC_{0-\infty}$ (mg.h/L)		$AUC_{0-300min}$ (mg.h/L)	
(mg/kg)	(h)	(L/kg)	(L/kg/h)	L-DOPA	3-OMD	DOPAC	HVA
0	0.86 ± 0.16	5.1 ± 1.1	4.1 ± 0.5	8.9 ± 1.3	10.0 ± 1.7	1.4 ± 0.2	2.9 ± 0.3
3.125	1.25 ± 0.21	3.7 ± 1.0	2.0 ± 0.2*	19.9 ± 2.0*	30.0 ± 1.7*	1.1 ± 0.2	2.5 ± 0.3
12.5	1.36 ± 0.16	3.4 ± 0.5	1.8 ± 0.3*	25.9 ± 2.9*	48.0 ± 5.0*	0.7 ± 0.1*	1.8 ± 0.2*
25	1.60 ± 0.15*	2.9 ± 0.9	1.1 ± 0.3*	39.5 ± 8.2*	72.7 ± 10.0*	0.3 ± 0.1*	1.5 ± 0.4*
100	1.50 ± 0.21	2.2 ± 0.5	1.1 ± 0.3*	44.9 ± 7.4*	88.3 ± 13.0*	0.2 ± 0.0*	1.0 ± 0.3*

Maximum bioavailability of L-DOPA was achieved after 25mg/kg carbidopa in the rat. Above this dose, peripheral decarboxylation of L-DOPA was decreased, but O-methylation to 3-OMD was increased. This suggests that, in agreement with Contin et al (1989), increasing the ratio of carbidopa:L-DOPA does not increase the bioavailability of orally administered L-DOPA if DDC is maximally inhibited.

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EFFECTS OF CHRONIC ETHANOL CONSUMPTION ON $GABA_B$ MEDIATED RESPONSES IN THE ISOLATED MOUSE VAS DEFERENS

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$GABA_B$ receptors are known to be present in a number of peripheral tissues. In the vas deferens they are responsible for the inhibitory effects of GABA and baclofen on electrically evoked twitches (Bowery et al., 1981). Several studies have indicated that changes in $GABA_A$ receptor sensitivity and number in the CNS are associated with ethanol tolerance and dependence (Taberner, 1989). Since tolerance to the direct effects of ethanol can be observed in vas deferens from mice pretreated with chronic ethanol (Hepper & Taberner, 1983), we have compared $GABA_B$ mediated responses in control and ethanol-treated LACG mice.

The ethanol drinking schedule necessary to produce tolerance and mild dependence was as described previously (Unwin & Taberner, 1980). Vasa were removed and set up singly under 250 mg tension in a 5 ml bath in Mg^{++}-free Krebs Ringer bicarbonate medium (pH 7.4) gassed with 95% O_2 5% CO_2. Isometric responses were recorded following electrical stimulation (60-100V, 0.1 Hz, 0.5 msec duration). After 60 min equilibration to establish a baseline response, drugs were added in cumulative fashion, with a 30 min washout between different drugs. IC_{50} values were derived from dose-inhibition curves fitted to the logistic equation (Barlow, 1975). Results are means ± s.e. mean from 6 animals.

Inhibition of evoked twitches was obtained with GABA (IC_{50} : 0.16 ± 0.02 μM) and baclofen (IC_{50} : 0.15 ± 0.02 μM) but not 3-aminopropane-sulphonic acid or muscimol. The maximum inhibition observed after GABA or baclofen was 35% and 18% respectively. These values were slightly reduced in ethanol dependence mice. At the same time, the IC_{50} value for GABA was significantly increased to 0.34 ± 0.07 μM ($p<0.01$, t-test). In tissue from mice after 4-6 h withdrawal, the GABA IC_{50} was 7.06 ± 1.7 μM. Ethanol applied in vitro was inhibitory but, as found previously, no significant tolerance was observed: IC_{50} control, 470 ± 48 mM; ethanol treated, 488 ± 33 mM. We conclude that peripheral $GABA_B$ receptors, like CNS $GABA_A$ receptors, are desensitized by chronic ethanol treatment and that this may account for some of the peripheral symptoms observed in ethanol dependence and withdrawal.

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CHANGES IN LONG TERM POTENTIATION IN HIPPOCAMPAL SLICES FOLLOWING CHRONIC BARBITAL TREATMENT

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The physiological basis of the tolerance and the withdrawal syndrome caused by chronic barbiturate treatment is uncertain, although decreases in GABA inhibition have been suggested. Acutely, barbiturates have been shown to potentiate GABA-mediated inhibition, to block calcium channels and to increase chloride conductance. There has been little study of the electrophysiological effects of chronic barbiturate treatment.

Male mice, TO strain, 30 - 35g, were given barbital in powdered food for seven days: 3 mg barbital per g food for two days, 4 mg/g food for two days and 5 mg/g food for three days. Controls received a matched amount of powdered food only. At the end of the seven day treatment the mice were placed in clean cages and given powdered food without barbital for 24h. Our previous studies showed that the withdrawal syndrome was maximal at this time. Hippocampal slices, 400 μm, were prepared and placed in an interface recording chamber. Extracellular recordings were made from area CA1, with Schaffer collateral/commissural stimulation, after 60 min equilibration of the slices. Threshold measurements were made every 10 min for 2h and input/output relationships established. Recordings were then made every 5 min for 60 min at 1.5 x threshold stimulation, a tetanus of 100 Hz for 1 sec applied and recording continued for another 60 min at 1.5 x threshold. Results are given as mean ± s.e.m. (n)

The slices from the barbital treated mice showed significantly lower thresholds for production of single population spikes ($P < 0.001$, analysis of variance) compared with controls, and the input/output curves were shifted to the left. After 60 min thresholds were: controls 20.2 ± 1.5 μA (5); barbital 15.2 ± 0.7 μA (5). Paired pulse potentiation was unchanged by the barbital treatment. For 20 min following the tetanus, there were no significant differences between responses in slices from barbital-treated mice and controls, but during the next 40 min the responses after the barbital treatment were significantly smaller than controls ($P < 0.01$). For example, at 10 min after tetanus, responses were: controls 109.0 ± 9.8% (5), barbital 107.6 ± 15.9% (5) of pretetanus values. At 45 min after tetanus values were: controls 112.0 ± 9.8% and barbital 99.7 ± 15.9% of pretetanus values.

The results show that the chronic barbital treatment lowered the thresholds to stimulation in hippocampal pyramidal cells. This would be consistent with decreased GABA-mediated inhibition. However, such a decrease in inhibition would be expected to increase long-term potentiation (Wigstrom & Gustafsson, 1985). This was not seen, but the maintainence of long-term potentiation was decreased by the chronic treatment. An alternative possibility to explain all these results is that chronic barbital treatment caused some of the changes that produce long-term potentiation, so that such potentation was already present at the beginning of our recordings.

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ADENOSINE ENHANCES RECOVERY OF SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPUS AFTER ANOXIA

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In the central nervous system glutamate action may be important in determining anoxic tissue damage. Adenosine reduces glutamate release, stabilises electrical activity and is released from hypoxic and hyperactive tissue. Therefore adenosine might provide some protection from anoxia.

Transverse hippocampal slices were placed on a nylon mesh in a recording bath perfused with Krebs solution from a reservoir at 25°C continually gassed with the appropriate gas mixture. A pair of laquered tungsten stimulating electrodes (125 μm separated by 100 μm) were placed on the stratum radiatum of the CA1 region of the hippocampal slices and stimuli (10 V, 0.1 ms) continuously delivered at 0.05 Hz. Synaptically evoked potentials were recorded via a single saline filled glass micropipette (approximately 2 MΩ resistance) positioned in the stratum radiatum 1 mm away from the stimulating electrodes. Recordings were made via a capacitively coupled preamplifier, digitiser and computer or oscilloscope. Experiments were performed by superfusing the slice with oxygenated solution then changing to deoxygenated solution for a predetermined period. Following this, the slice was resuperfused with oxygenated solution. To assess the effects on evoked potentials, the peak height of the response was measured 30 min after reversion to oxygenated solution and this expressed as a proportion of the height before the test or before application of adenosine. To produce anoxia, the gas in equilibration with the solution was changed from 95% O_2/5% CO_2 and 2 mM glucose to 95% N_2/5% CO_2 glucose free for predetermined periods.

The evoked responses from the preparation were a negative population epsp and a superimposed positive population spike and the form of this remained constant for several hours. The O_2 free/glucose free solution was applied for periods between 3 and 33 min to 57 slices. For periods of up to 8 min, most of the slices fully recovered and the recovery was maintained for 1 hr or more. For more prolonged exposures, the amount of recovery was inversely dependent on the exposure time. The average exposure time required for a 50% recovery was 17.6±1.8 min. In four of the slices, the evoked response transiently increased on reoxgenation followed by a decline to equilibrate out to a lower level.

In a further 28 slices, 100 μM adenosine was present 5 min before and during the period of anoxia. At any exposure time, the amount of recovery was enhanced enhanced by adenosine. If the data from 8-33 min was pooled the difference between the two sets of data had a P value of 0.0001 (using the unpaired, two-tailed t-test). The anoxic exposure time for a 50% recovery in the response was increased to 29.0±4.1 min. These results clearly showed a substantial and significant enhancement of recovery of the preparation when adenosine accompanied the period of anoxia.

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CARBACHOL-INDUCED SUPPRESSION OF THE N-WAVE IN GUINEA-PIG OLFACTORY CORTEX SLICES: WEAK ANTAGONISM BY M_3-SELECTIVE MUSCARINIC ANTAGONISTS, HHSiD AND p-F-HHSiD

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Muscarinic cholinoceptors are currently divided into M_1, M_2 or M_3 subtypes, based on their relative sensitivity to antimuscarinic compounds such as pirenzepine (Pz) and telenzepine (Tz) (M_1-selective), methoctramine and AFDX-116 (M_2-selective), or hexahydrosiladifenidol (HHSiD) and its para-fluoro-derivative (p-F-HHSiD) (selective for smooth muscle/glandular (M_3) over cardiac (M_2) receptor subtypes) (Hulme, et al., 1990). We have previously suggested that muscarinic suppression of excitatory neurotransmission in the olfactory cortex slice is mediated by presynaptic M_1 receptors located on lateral olfactory tract terminals, since this effect was competitively antagonized by nM doses of Pz or Tz, but not by methoctramine or AFDX-116 (see Bagetta & Constanti, 1990). It is conceivable however, that a component of our measured response was due to activation of M_3-type muscarinic receptors that might also be located presynaptically. We investigated this possibility by testing the effectiveness of the novel M_3-selective antagonists HHSiD and p-F-HHSiD towards the muscarinic inhibition of the N-wave in guinea-pig olfactory cortex slices maintained in vitro, using an extracellular recording method (see Bagetta & Constanti, 1990). Dose-dependent inhibition of the N-wave occurred following 2 min bath applications of carbachol (CCh; 25-150μM) as previously described. Pre-incubation of slices for 30 minutes with low doses of HHSiD (10-100 nM) or p-F-HHSiD (up to 1μM) produced no obvious inhibition of CCh responses. However, a clear reduction of CCh effects was observed in 250nM-1μM HHSiD or 10-50μM p-F-HHSiD. Longer (60 min) antagonist exposures did not lead to a further suppression of CCh responses. CCh dose-response lines were displaced in a parallel fashion by HHSiD or p-F-HHSiD, suggesting a weak competitive-type antagonism. Schild plot analysis (Schild slope constrained to unity) of pooled data yielded pA_2 values of 6.6 for HHSiD (n=9 slices) and 5.5 for p-F-HHSiD (n=9 slices) respectively. The expected pA_2 values for these antagonists acting on ileal M_3 receptors are ~ 7.9 and 7.8 respectively. In conclusion, our experiments show that muscarinic receptors involved in olfactory cortical N-wave suppression possess a low affinity for HHSiD and particularly for p-F-HHSiD. This suggests that peripheral M_3-type receptors are not significantly involved in mediating this presynaptic muscarinic response.

HHSiD and p-F-HHSiD were kind gifts from Professor G. Lambrecht (University of Frankfurt, FRG).

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501P

THE PHORBOL ESTER TPA INHIBITS MUSCARINIC RESPONSES IN RAT LACRIMAL ACINAR CELLS BUT NOT IN RABBIT SALIVARY ACINAR CELLS

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The pathway by which agonists stimulate the production of inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG) in many different cell types has been well described (Berridge, 1987), as has the role of $InsP_3$-evoked Ca^{2+} release in mediating secretion in exocrine acinar cells (Marty, 1987). However, the possible role of DAG-stimulated protein kinase C in Ca^{2+}-mediated secretion is less well understood. 12-O-tetradecanoylphorbol-13-acetate (TPA), a phorbol ester known to activate protein kinase C (Nishizuka, 1984), inhibits muscarinic responses in a number of exocrine glands. However, its effects are variable and may indicate differences in the level at which protein kinase C acts in the different cells. We have compared the effects of TPA on acetylcholine (ACh)-evoked changes in $[Ca^{2+}]$ and inositol phosphate production in acinar cell preparations from the rat lacrimal gland and the rabbit mandibular salivary gland in an effort to obtain further evidence on its mode of action in secretory epithelia.

Initial results demonstrated that ACh can elicit reversible and reproducible dose-related increases in $[Ca^{2+}]_i$ in both cell types. Incubation of the lacrimal acinar cells with 0.5uM TPA (5 minutes; 22°C) caused a decrease in the peak and plateau phases of the ACh-evoked $[Ca^{2+}]_i$ response by 50.6 (+/-7.6)% and 62.3 (+/-9.8)%, respectively. However, TPA had no significant effects on the ACh-evoked $[Ca^{2+}]_i$ response in rabbit mandibular acini. Investigations into the ability of TPA to inhibit ACh-stimulated inositol phosphate production under similar conditions demonstrated a 52.9 (+/-4.6)% reduction in rat lacrimal acini and no significant effect in the rabbit mandibular acini. These results clearly show that different exocrine acinar cells vary in their response to phorbol esters. They also indicate that in rat lacrimal acinar cells protein kinase C exerts an inhibitory action on muscarinic receptor-stimulated Ca^{2+} release at a level prior to $InsP_3$ production. This is consistent with the patch-clamp experiments of Llano and Marty (1987).

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THE DEVELOPMENT OF MICRODIALYSIS FOR THE MEASUREMENT OF THYROTROPHIN-RELEASING HORMONE IN RAT BRAIN *IN VIVO*

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Microdialysis has recently found wider application with the measurement of neuropeptide release *in vivo* (see review by Kendrick 1989). We have used the technique with a sensitive radioimmunoassay (RIA) in attempts to measure the release of thyrotrophin releasing hormone (TRH) from regions of rat antero/lateral hypothalamus.

Concentric microdialysis probes with cellulose based (CEL O.D. -200 μM) or polyacrylonitrile (PAN, O.D. -300 μM) membranes. 2 mm in length were perfused with artificial CSF (0.4 μl min^{-1}) with or without BSA (0.05% w/w) or Bacitracin (0.1% w/w) and tested for relative recovery of TRH *in vitro* at room temperature over several hours. Samples were collected in the upturned tip of an Eppendorf tube and snap frozen for later RIA. Cellulose membranes generally exhibited better relative recovery than polyacrylonitrile membranes, although considerable variation occurred both within and between probes (CEL: mean = 17.5%, range - 13-24%, n=9; PAN, mean = 10%, range = 4-16%, n=9).

Probes with CEL membranes perfused as above were run *in vitro* for approximately 60 minutes then inserted stereotaxically (Bregma: RC -1.3 mm, LAT -1.3 mm, DV -9.5 mm, Paxinos & Watson 1986) into the brain of urethane anaesthetised (1.3 g kg^{-1}) male Wistar rats (250 - 300 g) in two groups of n=10 (A and B). Group A was perfused with CSF containing BSA and no stimulus applied. Group B was perfused with CSF containing Bacitracin (0.1%) and a potassium stimulus (300 mM KCl in CSF + Bacitracin) was applied for 1 hour between 2 control periods. In all experiments samples were collected hourly following a 90 minute preliminary perfusion.

In group A no baseline levels of TRH were obtained. In group B both control and potassium stimulated release were measured in 4 animals (control release = 10.5 ± 1.8 fmol/sample (mean ± S.E.M.), potassium release - 19.6 ± 4.9 fmol/sample). For these animals probe recovery *in vitro* was 22.5 ± 3%, (mean ± S.E.M., n=4) whereas the recovery of the probes used in the remainder of the group was 11.2 ± 1.8 (n=6). This suggests the need to select probes by prior *in vitro* testing before their use *in vivo*.

This study indicates that the microdialysis technique is likely to be useful for the measurement of TRH *in vivo*, particularly if better relative recovery of peptide can be achieved and maintained. Further improvements in RIA sensitivity will also enhance the technique.

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IN VITRO STUDIES ON THE MECHANISMS BY WHICH CYTOKINES ACTIVATE THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS

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Observations that interleukins 1 and 6 (IL-1 and IL-6) stimulate the secretion of adrenocorticortrophin (ACTH) in the rat in vivo (Naitoh et al. 1988; Rivier et al. 1989) have led to suggestions that cytokines may play a role in the activation of the hypothalamo-pituitary-adrenal (HPA) axis in conditions such as septic shock. The sites at which these compounds exert their stimulatory effects are not clear. Accordingly, we have used in vitro models to examine the effects of various cytokines on the secretion of ACTH by the anterior pituitary gland and of the two major corticotrophin releasing factors, CRF-41 and vasopressin (AVP), by the hypothalamus. Hypothalamic and adenohypophyseal tissue were removed post mortem from chronically adrenalectomized rats and incubated in vitro as described previously (Buckingham & Hodges, 1977a; 1977b). Peptides released into the medium were measured by radioimmunoassay. Hypothalamic extracts (0.05-0.2 HE/ml) produced significant ($P<0.02$,n=8) concentration-dependent increases in ACTH release from the pituitary tissue but none of the cytokines tested were active in this respect. Thus, IL-1α (250 & 500pg/ml), IL-1β (50-200pg/ml) and tumour necrosis factor, TNF (312.5-1250pg/ml), alone or in combination, failed to influence significantly ($P>0.1$,n=6-7) either resting or hypothalamic extract (0.1HE/ml) - induced ACTH secretion when in contact with the tissue for 60 min, although in one instance IL-1β (200pg/ml) produced a small but significant ($P<0.05$, n=6) increase in peptide release.
Preliminary data (n=3-5) with the hypothalamus suggest that the cytokines influence the secretion of both AVP and CRF-41. IL-1α (50-1000pg/ml) and IL-6 (5-20ng/ml) produced, within 15 min, 2 to 3 fold increases in the release of AVP. TNF (625&1250pg/ml) was considerably more active in this respect and initiated approximately 30 fold increases in the secretion of the peptide. None of these responses appeared to be concentration-dependent. In contrast, IL-6 (5-20ng/ml) produced dose-dependent increases in CRF-41 release. The results support the concept that cytokines activate the HPA axis and suggest that their stimulatory effects are exerted predominantly at the level of the hypothalamus and that they may involve alterations in the release of both CRF-41 and AVP.

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504P

PHOSPHATIDYL CHOLINE METABOLISM AND CONTRACTION IN ARTERIOLAR SMOOTH MUSCLE

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Simultaneous intracellular electrical and mechanical recordings from the proximal rabbit saphenous artery have revealed that noradrenaline (NA) produces a contraction independent of any membrane depolarisation (Muir & Nally, 1989). Biochemical mechanisms by which NA could act in this tissue include hydrolysis of phosphatidylinositol 4,5 bisphosphate (PIP_2) or hydrolysis of phosphatidylcholine (PC), (see review by Loffelholz, 1989). The possibility that either or both of these mechanisms were involved in the contraction to NA in the rabbit saphenous artery was investigated.

Total inositol phosphate (IP) production from PIP_2 hydrolysis was measured in segments (2cm) of artery by the method of Akhtar & Abdel-Latif, (1984). PC hydrolysis was estimated by measuring glycerophosphocholine (GPC) choline phosphate (ChoP) and choline (Cho), using the method of Cook & Wakelam, (1989).

NA (10^{-7}-10^{-4}M) failed to raise IP content of the rabbit saphenous, but did so significantly in the rat tail artery (prepared as described by Burnstock & Sneddon, 1984). In the rabbit saphenous, however, NA (10^{-7}-10^{-4}M) produced a concentration-dependent increase in both ChoP and Cho which was abolished by prazosin (10^{-6}M). GPC levels were unaffected by these concentrations of NA. The increase in Cho levels was monophasic and maximal after 15-30s. The increase in ChoP was biphasic, with a small peak at 15-30s and a much larger increase 2-4 min later.

These results demonstrate, for the first time in this tissue, that NA utilises PC hydrolysis in this artery as a source of DAG, in the absence of PIP_2 hydrolysis. The early rise in Cho levels implies the activity of phospholipase D, although some small, simultaneous activity of phospholipase C, leading to ChoP production cannot be precluded. The later, much larger rise in ChoP, however, is likely to result from phosphorylation of Cho and does not match, in time course, the contraction evoked by NA in this tissue. The lack of production of GPC implies that involvement of phospholipase A_2 in the response to NA in this tissue is unlikely.

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505P

POST-SYNAPTIC α-ADRENOCEPTORS DO NOT MEDIATE THE RESPONSE TO FIELD STIMULATION IN SHEEP MESENTERIC LYMPHATIC VESSELS

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Bovine mesenteric lymphatic vessels have a noradrenergic innervation stimulation of which increases the frequency of spontaneous contractions. This excitatory response is converted to an inhibitory one by α- adrenergic blockers and no response persists in the presence of a combination of α and β blockers (McHale *et al* 1980). It was anticipated that sheep mesenteric vessels would have a similar innervation but in the present study this was found not to be the case.

Spontaneous isometric contractions were recorded in isolated rings of sheep mesenteric lymph duct. These contracted rhythmically at a frequency of 5.5 per min (± 2.4 S.D. n=14) and field stimulation (35 V nominal, 0.3 msec pulse width) at a frequency of 1 Hz increased this resting rhythm to 9.4 ± 4.0. This acceleration in contraction frequency was abolished by 10^{-6} M tetrodotoxin but was not blocked by 10^{-6} M phentolamine, prazosin or yohimbine. Mean frequency of contraction increased from 8 (±0.3, SEM, n=6) to 13 ± 1.0 before and from 9.2 ± 0.6 to 13.8 ± 0.7 in the presence of phentolamine. Similarly in the case of prazosin frequency increased from 6.1 ± 0.3 to 11.3 ± 1.2 before drug addition and from 7.1 ± 0.4 to 10.7 ± 1.0 in its presence (n=6). Yohimbine had similarly little effect since frequency increased from 9.5 ± 0.3 to 14.4 ± 0.7 before drug addition to 11.1 ± 0.5 to 14.3 ± 0.9 in its presence (n=7).

The effect of field stimulation was mimicked by the application of 10^{-6} M noradrenaline. Control frequency before drug addition was 5.9 ± 0.9 (S.E.M., n=4) and this was increased to 13.3 ± 1.1 during noradrenaline perfusion. In contrast to its effect on field stimulation phentolamine 10^{-6} M abolished the effect of exogenous noradrenaline (6.8 ± 0.7 contractions/min before drug addition, 6.5 ± 0.79 in the presence of both noradrenaline and phentolamine.

It can be concluded from these results that stimulation of intramural nerves in isolated sheep mesenteric lymphatics produces an excitatory effect similar to that observed in bovine vessels but in the sheep the excitation is not mediated via post-synaptic α- receptors.

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FUNCTIONAL EVIDENCE FOR THE EXISTENCE OF POSTSYNAPTIC α_2 ADRENOCEPTORS IN THE RAT ISOLATED TAIL ARTERY

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In the rat isolated tail artery, field stimulation causes a contraction that may be the resultant of at least two components (Bao et al., 1989). Evidence for such a view comes from studies in which the major, noradrenergic component, can be abolished by the alpha$_1$-adrenoceptor antagonist, prazosin, and the purinergic component can be blocked by alpha, beta-methylene adenosine triphosphate (mATP), which desensitizes the P_2-purinoceptor. The present study re-examined this hypothesis by investigating the effects of prazosin and mATP.

Wistar rats were stunned and killed by exsanguination. Segments of tail artery (3-4 mm) were attached to a fine stainless steel stimulating electrode inserted into the lumen of the vessel. The resting tension on each segment of artery was initially set at 1 g and the vessel was allowed to equilibrate in Krebs buffer (37°C, gassed with a mixture of 95% O_2/5% CO_2) for 1 hour, after which the tension was reset to 1 g and the tissue was allowed to equilibrate for a further hour. Isometric contractions were obtained to noradrenaline (NA), mATP, clonidine and field stimulation (trains of 10, 0.4 ms pulses at 10 Hz and 100 s intervals, 40 V)), administered alone and in the presence of yohimbine, prazosin or diltiazem.

Field stimulation produced motor responses that were abolished by prazosin (0.1 μM). Low concentrations of clonidine (50 nM) caused slow rhythmic contractions, which were abolished by yohimbine (10 nM) or by diltiazem (2 μM). Higher concentrations of clonidine (250 nM-5 μM) caused more rapidly-developing contractions, that were less affected by yohimbine (10 nM) or diltiazem (2 μM). Clonidine (50 nM-5 μM) inhibited motor responses to field stimulation. Prazosin (0.1 μM) also reversed the effect of clonidine on the baseline tone but unlike yohimbine, prazosin also abolished the field stimulation-induced responses. mATP (2-4 μM) produced transient contractions and increased the amplitude and reduced the duration of the field stimulation-induced motor responses. The duration of the field stimulation-induced response was reduced by diltiazem (2 μM) and yohimbine (10 nM), both of which reduced the amplitude of the response by 10%. Immediately after washing out yohimbine, the field stimulation-induced response remained elevated for 20-30 minutes. These observations suggest that in the tail artery there may be postsynaptic alpha$_2$ adrenoceptors, which operate a Ca^{2+} channel that can be blocked by diltiazem. These alpha$_2$-adrenoceptors may be activated by low concentrations of clonidine (50 nM), whilst higher concentrations of clonidine additionally activate alpha$_1$-adrenoceptors, which will mainly be responsible for the nerve-mediated, noradrenergic response, and may operate a coupling mechanism that uses intracellular rather than extracellular Ca^{2+}.

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IS 2-METHYLTHIO-ATP AN APPROPRIATE TOOL FOR THE IDENTIFICATION OF P_{2Y}-PURINOCEPTORS?

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2-methylthio-ATP (2meSATP) is a key agonist in the differential classification of P_2-purinoceptors, showing higher potency than ATP at P_{2Y}-receptors and lower potency at P_{2X}-receptors (Burnstock & Kennedy, 1985). In some systems which, according to agonist potency orders, contain P_{2Y}- receptors, 2meSATP apparently exhibits partial agonism. For example, in cultured pig aortic endothelial cells, 2meSATP produced a lower maximum response than ATP in stimulating prostacyclin production (Needham *et al.*, 1987). In principle, the analysis of partial agonists to obtain affinity and efficacy estimates provides a more quantitative means of classifying receptors than agonist potency order information. It was of interest therefore to analyse 2meSATP's agonist action on the vascular endothelium in more detail. ADPβS (Adenosine 5'-(2-thiodiphosphate)) was also studied since it too has apparently demonstrated partial agonism at the endothelial receptor (Martin *et al.*, 1985).

3mm ring segments of thoracic aorta were obtained from male Sprague-Dawley rats (330-370 g.wt.) and prepared for isometric force recordings in Krebs' solution containing indomethacin (2.8μM) and the P_1- receptor antagonist, 8-sulphophenyltheophylline (300μM), maintained at 37°C and gassed with 5% CO_2 in oxygen. Each preparation was pre-contracted with 35mM KCl and, in experiments using 2meSATP and ADPβS, 100μM α,βmeATP was included to desensitise P_{2X}-receptors. Cumulative agonist concentration-effect (E/[A]) curves were then constructed to ATP. The preparations were washed and after 45 min an E/[A] curve to a test agonist was constructed. Potency (p[A_{50}]) and intrinsic activity (α) estimates were obtained by logistic curve-fitting. Partial agonist data were analysed using the operational model (Leff *et al.*, 1990) employing ATP as reference full agonist. In some preparations an E/[A] curve to ATP was constructed in the presence of 100μM 2meSATP or ADPβS.

The resulting potency order (p[A_{50}]'s in parentheses) was 2meSATP (6.59) > ATP (5.57) > α,βmeATP (> 4.00), apparently confirming the receptor as the P_{2Y} -type. 2meSATP and ADPβS behaved as partial agonists compared to ATP (α = 0.41 and 0.54 respectively) and the resulting affinity (pK_A) estimates were 6.30 and 5.27. However, 2meSATP at 100μM, which is 200 times higher than its apparent dissociation constant, failed to cause any rightward displacement of ATP E/[A] curves. This lack of competition between the two agonists suggests that they are not interacting with a common receptor. The same was true when ADPβS was used.

These results question the use of 2meSATP in the identification of ATP receptors at least on the vascular endothelium. They also have implications for the use of agonist potency orders in the subclassification of P_2-purinoceptors.

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508P

PHARMACOLOGICAL PROFILE OF *PHONEUTRIA NIGRIVENTER* VENOM ON RABBIT VASCULAR SMOOTH MUSCLE

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Phoneutria nigriventer is a spider responsible for most human accidents of spider bites in the Center East and South of Brazil. *Phoneutria nigriventer* venom (PNV) has been described as neurotoxic, causing activation of sodium channels (Fontana & Vital-Brazil, 1985), leading to neuromuscular blockade or release of acetylcholine and norepinephrine by the autonomic nerve endings in the guinea pig auricles (Vital-Brazil et al., 1988). Here we investigated the envolvement of both sodium and calcium channels on the PNV-induced contractions in rabbit vascular smooth muscle.

Male, New Zealand White rabbits (2-3 kg) were anaesthetised with thiopental sodium (40 mg/kg, iv) and exsanguinated via the carotid artery. Rabbit mesenteric (RbMesA), coeliac (RbCA) and pulmonary (RbPA) arteries, the vena cava (RbVC), mesenteric (RbMesV) and jugular (RbJV) veins were removed, placed in oxygenated Krebs' solution and cleared of fat and connective tissue. Spiral strips were denuded of endothelium by gentle rubbing of the luminal surface with a cotton bud and mounted in the cascade (Vane, 1964). The tissues were superfused with oxygenated (95% O_2/ 5% CO_2) and warmed (37°C) Krebs' solution at a flow rate of 5 ml/min. PNV and other agonists were given as single bolus. Antagonists were infused at a flow rate of 0.1 ml/min.

PNV (1-30 ug) produced dose-dependent contractions characterized by rapid onset and short duration in all vascular tissues assayed ($n > 10$ for each tissue). Venous tissues were more sensitive to PNV than arterial ones (see Table). Phenoxybenzamine (PBZ, 50 nM, infused for 0.5 h) did not significantly inhibit PNV-induced contractions ($n = 3$-5), although it caused a 100-1000 fold decrease in adrenaline sensitivity ($n = 5$ for each tissue).

PNV ED50 (ug)	RbVC	RbMesV	RbPA	RbMesA
Before PBZ	1.1 ± 0.3	4.2 ± 1.5	4.5 ± 1.1	5.2 ± 2.6
After PBZ	1.3 ± 0.2	9.8 ± 4.3	7.5 ± 2.1	9.6 ± 6.7

Tetrodotoxin (3.0 uM, $n = 3$), an antagonist of voltage-dependent sodium channels, did not affect PNV-induced contractions but blocked those evoked by the sodium channel agonist veratrine (100-300 ug). Nifedipine (0.1 uM, $n = 3$ for each tissue) and nicardipine (0.1 uM, $n = 3$ for each tissue) abolished BAY K8644 (0.3-3.0 nmol)-induced contractions, without affecting PNV-induced contractions.

Our results demonstrate that PNV-induced contractions on vascular smooth muscle occur independently of both voltage-dependent sodium and dihydropyridine-sensitive calcium channels activation.

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RESPONSES TO ENDOTHELIN IN HYPERTENSIVE RABBITS

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Endothelin (Et) has been intensively studied since its identification in 1988 (Yanagisawa et al). Despite this, questions remain as to its mechanism of action and role in pathophysiology. We have examined responses to Et in perinephritis hypertension in the rabbit. Animals were made hypertensive as previously described (Hamilton et al, 1987) and studied during development and in established hypertension (at 2 and 6 weeks after surgery). Pressor/depressor responses to iv Et 0.03-0.2 nmoles/kg were observed for 30 min in conscious animals. In addition, pressor responses to 0.1 nmoles/kg Et were examined before and after bolus doses of nifedipine (N) or verapamil (V) 0.1 mg/kg iv or during N infusions 0.072 and 0.096 mg/kg/hr.

MAP was increased in hypertensive animals (HT), MAP 77±5 in controls (C) compared to 97±9 and 105±17 mmHg in HT at 2 and 6 weeks post surgery. Et caused an immediate depressor response followed by a prolonged dose related pressor response which reached a maximum in 1-3 min and took 15-30 min to return to baseline. The maximum pressor response was greater in HT (Table 1), however when examined in relation to starting MAP this difference did not achieve significance.

Table 1 Increase in MAP (mmHg) 2 min after Et (nmoles/kg).

	0.03	0.05	0.1	0.2	
2 week HT	7±4	12±2*	18±8*	24±11*	
2 week C	5±2	7±3	12±3	15± 5	mean ± SD
6 week HT	12±5*	14±7	23±8*	31±12	* $p < 0.05$ vs C.
6 week C	6±2	10±4	16±4	27± 8	

N and V attenuated pressor responses to Et in HT and C but the effect was greater in HT and in many cases only a depressor response was observed. 0.1 mg/kg N decreased the response to Et at 2 min by 25±7 and 37±17 in HT and 12±9 and 10±6 mmHg in C at 2 and 6 weeks. V decreased the response by 20±18 and 1±6 mmHg in HT and C respectively at 6 weeks.

In contrast to the differential attenuation of pressor responses to Et N and V have similar effects on pressor responses to alpha adrenoceptor agonists in HT and C (Hamilton et al, 1987) suggesting differences in the mechanism of utilisation of extracellular calcium between alpha adrenoceptor agonists and Et. The differences in the effects of calcium antagonists on responses to Et in HT provides further evidence for anomalies in calcium handling in hypertension.

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EXOGENOUS HUMAN BIG-ENDOTHELIN IS CONVERTED TO ENDOTHELIN-1 IN THE RABBIT *IN VIVO*

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Endothelin-1 (ET-1) and the 38 amino acid precursor human big endothelin (Big-ET(h)) are equipotent as pressor agents in denervated rats, yet Big-ET(h) has only 1/100th of the potency of ET-1 in vitro (Kashiwabara et al., 1989). We have assessed the pharmacological activities of Big-ET(h), ET-1 and Big-ET(22-38) in vivo and monitored the changes in plasma levels of ET-1 in anaesthetized rabbits. In addition, we have assessed the in vitro effects of these peptides in guinea pig isolated perfused lungs. Rabbits were anaesthetized with sodium pentobarbitone, and a cannula placed in the left ventricle, via the right carotid artery, for measurement of left ventricular systolic pressure (LVSP) and administration of peptides and collection of blood samples (1 ml). The effects of Big-ET(h), ET-1 or Big-ET(22-38) on LVSP, ADP-induced platelet aggregation ex-vivo (Thiemermann et al., 1988) and the plasma levels of ET-1-like immunoreactivity ($ET\text{-}1_i$) were determined. The antisera for ET-1 had only 0.1% cross-reactivity for Big-ET. As in experiments with ET-1 (De Nucci et al., 1988), eicosanoid release induced by Big-ET(h) from guinea-pig isolated lungs was assessed by specific radioimmunoassays for 6-oxo-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) and thromboxane (TX) B_2 in the perfusion effluent from the lungs.

Big-ET (h) and ET-1 induced similar pressor responses (1 nmol/kg, ΔLSVP; 19 ± 4 at 5 min and 20 ± 4 mmHg at 1 min respectively). At 3 nmol/kg, Big-ET(h) inhibited platelet aggregation by 60% at 5 min, whereas 1 nmol/kg ET-1 caused 70-80% inhibition of platelet aggregation within 1 min. As with ET-1, indomethacin (5 mg/kg) both potentiated the pressor responses and abolished the anti-aggregatory effects of Big-ET(h). Big-ET(h) at 1 nmol/kg caused an increase in circulating levels of $ET\text{-}1_i$ which remained elevated for 30 min ($P<0.05$), whereas bolus injection of ET-1 (1 nmol/kg) raised $ET\text{-}1_i$ levels for only 5 min ($P<0.01$).

Infusion of Big-ET (3×10^{-7}M) induced release of PGI_2 and TXA_2 from perfused lungs (maximal release: 2.8 ± 0.3 and 3.0 ± 0.2 ng/ml respectively). Big-ET(22-38), the C-terminal product of the atypical chymotrypsin-like cleavage of Big-ET(h) (Yanagisawa et al., 1988), did not affect LVSP or circulating levels of ET-1 and did not release eicosanoids.

We suggest that Big-ET(h) has a similar pharmacological profile to ET-1 in anaesthetized rabbits, due to its gradual conversion to ET-1 in the circulation.

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MEMBRANE PERMEABILITY IS A DETERMINANT OF ARRHYTHMOGENESIS FOLLOWING ANION SUBSTITUTION

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It has been demonstrated that substitution of chloride by nitrate is a novel method of reducing ischaemia- and reperfusion-induced arrhythmias (Ridley et al, 1989). Natural membranes differ in their permeability to anions. In cardiac myocytes the order of permeability is Iodide> Nitrate> Bromide> Chloride> Methylsulphate (Hutter & Noble, 1961).

In a randomized study, 60 rat hearts (n=12/group) were perfused (Langendorff mode) either with standard chloride-containing solution (Constituents in mM, NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.0, $MgSO_4$ 1.2,$CaCl_2$ 1.4 and glucose 11.1, gassed with 95% O_2 and 5% CO_2, pH 7.4, 37°C) or with a similar solution modified by the substitution of chloride with a different anion. The incidences (%) of ventricular tachycardia (VT) and fibrillation (VF) were determined during 30 min regional ischaemia and during 10 min reperfusion (Figure 1). Hearts were excluded from the reperfusion study, and immediately replaced, if arrhythmias were already present at the time of reperfusion. Heart rate and coronary flow were recorded. Occluded zone size was measured using dye. Anion substitution of chloride profoundly altered the incidence of VT and VF. The effect did not correlate with heart rate, occluded zone size or coronary flow (all $p>0.05$) indicating a direct mechanism of action. Statistically significant reductions in ventricular arrhythmias were seen with anions with membrane permeability greater than chloride. Conversely, the impermeant anion, methylsulphate showed a tendency to promote ventricular arrhythmias (the persistent high incidence of arrhythmias at 30 min in this group made study of reperfusion arrhythmias impracticable).

In conclusion, these findings support the hypothesis that altered membrane permeability may contribute to the antiarrhythmic activity of some anion substitutions.

Fig. 1.

Anion	Arrhythmia			
	Ischaemia		Reperfusion	
	VT	VF	VT	VF
Methylsulphate	100	100	Abandoned	
Chloride (Control)	100	67	100	92
Bromide	92	8*	100	92
Nitrate	0*	0*	58*	0*
Iodide	50*	0*	75	17*

* Indicates $p< 0.05$ vs chloride group

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THE EFFECT OF DOPAMINE ON KININ RELEASE FROM RAT AND HUMAN ISOLATED RENAL CORTICAL CELLS

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In the kidney the kallikrein-kinin system is implicated in local blood pressure control and natriuresis and radioligand binding studies have localized specific kinin receptors on rat renal cortex epithelial membranes (Cox et al, 1984). Cortical dopamine receptors are also present in both rat and human kidney (Baldi et al, 1988) and it has been suggested that intra-renal dopamine participates in local vasodilatation and natriuresis. To investigate the relationship between renal kinin and dopamine we have examined the effect of dopamine on kinin release from both rat (female Wistar-Kyoto) and human isolated renal cortical cells. Cells were prepared by collagenase digestion. Superfusion was carried out in parallel teflon columns (Drury et al, 1986) at 37°C with oxygenated Krebs-Ringer bicarbonate glucose buffer containing 0.2% bovine serum albumin, at a flow rate of 0.25 ml/min. Fractions were collected over 5 minute periods. After an equilibration period of 90 minutes cells were challenged with 10 minute pulses of dopamine (10^{-10} -10^{-6}M) with 30 minute recovery periods between doses. Kinin release into the superfusate was assayed by radioimmunoassay using a monoclonal antibody. Integrated response ratios (RR) were calculated by comparing the mean basal kinin release in the three fracions before stimulation and the three fractions in the immediate recovery period, with the mean stimulated kinin release.

Using rat kidney cortical cells, after the equilibration period, basal kinin release remained stable over the next 4 hours with a Coefficient of Variation (CV) of 5.2%. In subsequent experiments stimulation with dopamine gave a bell-shaped response curve between 10^{-10} and 10^{-6} M with a significant increase at 10^{-9}M (RR:143.5±18.55,p<0.005,n=6) and a maximal response at 10^{-8}M (RR:216.7±68.61,p<0.01,n=6). Using cells from macroscopically normal surgically removed human kidney tissue, stable basal kinin release was shown in one experiment during 4 hours of superfusion (CV=7.3%). In a second experiment a similar dose-related response to dopamine was observed with maximal kinin release at 10^{-8}M.
The dose-related increase in kinin release, from both rat and human renal cortical cells, in response to dopamine suggests that kinins may play an important role in its natriuretic and vasodilatory actions.

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513P

GENTAMICIN NEPHROTOXICITY IN PRIMARY RAT RENAL PROXIMAL TUBULAR CELLS

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Nephrotoxicity is a dose limiting feature of gentamicin administration. The renal pathogenesis of gentamicin can be attributed to its selective accumulation within renal proximal tubular cells. Previous results from our laboratory have indicated that gentamicin affects ^{45}Ca transport and phospholipid metabolism in brush border membranes from rat renal cortex (Godson and Ryan 1988). The present studies concerned gentamicin toxicity in primary rat renal proximal tubular cells.

The proximal tubular cells were prepared by collagenase digestion of the rat renal cortex and isolation of the proximal fraction on a Percoll gradient. The cells were seeded into 6-well plates and maintained for the 5 days of cultures in DMEM supplemented with 10% foetal calf serum and 2 mM glutamine.

Gentamicin significantly reduced the incorporation of [^{3}H]-thymidine into the tubular cells at a concentration of 10^{-4} M or greater, at 10^{-3}M a reduction to 71 ± 12% of control values (p<0.0005) was observed. Gentamicin at 10^{-3}M significantly reduced the cell number (39.3 ± 3.1 x 104) compared to control values (72 ± 8.1 x 104, p<0.005). Gentamicin significantly reduced the incorporation of [14C]-leucine into the tubular cells at a concentration of 10^{-4}M or greater, at 10^{-3}M a reduction to 86 ± 4% of control values p<0.05 was observed. The protein content in the cells over the 5 days of culture was significantly reduced by gentamicin (p<0.005 on all days). For example, on day 2, control cell protein content was 121.4 ± 6.41 mg/ml whereas in gentamicin treated cells, protein content was 54.6 ± 2.6 mg/ml. A significant decrease in ^{45}Ca uptake into the cells was also detected at concentrations of 10^{-4}M and 10^{-3}M gentamicin. For example at 20 min control uptake was 1.713 ± 0.141 nmoles/mg protein 10^{-3}M gentamicin caused significant reduction to 0.975 ± 0.141 nmoles (p<0.0005). ^{45}Ca efflux from preloaded cells was significantly impaired by gentamicin at concentrations of 10^{-4}M and 10^{-3}M. At 20 mins control efflux was 40.8 ± 3.9% of ^{45}Ca load whereas 10^{-3}M gentamicin reduced the efflux to 67.1 ± 0.6% (p<0.0005) of the ^{45}Ca load remaining in the cells.

These results show that gentamicin, a known nephrotoxin, significantly impaired cell growth, protein content and calcium movements in primary rat renal proximal tubular cells. These primary renal cell cultures may offer a good model for mechanistic investigations of cell toxicity.

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514P

INVESTIGATION OF CISPLATIN TOXICITY IN RAT RENAL PROXIMAL TUBULAR FRAGMENTS

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Cisplatin is a widely used drug in the treatment of a variety of solid tumours. The chief dose limiting side effect of cisplatin is its pronounced nephrotoxicity which is cumulative and is characterised by necrosis of the S3 segment of the proximal tubule. In this study, the in vitro nephrotoxicity of cisplatin was examined in rats using an isolated renal proximal tubule preparation.

Proximal tubules were isolated from male Wistar rats (200-250 g) using collagenase digestion followed by mechanical sieving and differential centrifugation in 45% Percoll. The tubule preparation was characterized by marker enzymes, transport capabilities and by transmission and scanning electron microscopy. Alkaline phosphatase (AP) and gamma-glutamyltranspeptidase (gamma-GT) marker enzymes for the brush border were enriched in this preparation. Levels of AP ($p<0.05$) and gamma-GT ($p<0.05$) were significantly increased in the tubular preparation compared to the starting suspension. Na dependent hexose transport was assessed by [^{14}]C-alpha-methyl-glucopyranoside (alpha-MG) uptake. With a 0 mM concentration of sodium the uptake of [^{14}]C-alpha-MG at 20 minutes was significantly reduced ($p<0.0005$) compared to uptake in medium containing 118 mM sodium. Alpha-MG uptake was reduced by phloridzin. The release of the lysosomal enzyme N-acetyl-β-D glucosaminidase (β-NAG) was significantly increased ($P<0.05$) at concentrations of 100 μM and 1000 μM cisplatin. Cisplatin caused a dose dependent reduction in basal oxygen consumption to 51.6% of control at 16 μM and to 36.7% of control at 1000 μM ($p<0.05$). Transmission electron micrographs of tubules treated with 3.0 mM cisplatin showed enlarged mitochondria with distorted cristae. Cytoplasmic blebbing of the basement membrane indicative of an acute toxic insult was also evident Isolated proximal tubules accumulated [^{195}]Pt-cisplatin over a 5 hour period. The uptake of [^{195}]Pt-cisplatin after 90 minutes in the presence of metabolic inhibitors (30 μM) was reduced to 43.2 ± 6.2% of control. Addition of Cisplatin:Methionine complex (1:10) significantly reduced uptake of [^{195}]Pt-cisplatin ($p<0.005$) compared to control.

These results provide some insights into mechanisms of cisplatin transport and toxicity and suggest that the isolated rat proximal tubule preparation may be a useful in vitro model for the study of cisplatin nephrotoxicity.

515P

INHIBITION OF [^{3}H]-PAROXETINE BINDING BY SIBUTRAMINE, ITS METABOLITES AND OTHER ANTIDEPRESSANTS CORRELATES WITH INHIBITION OF [^{3}H]-5-HT UPTAKE

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Paroxetine, a potent and selective inhibitor of 5-hydroxytryptamine (5-HT) uptake, has been used as a radioligand for 5-HT uptake sites (Habert et al., 1985). We have compared the potency of sibutramine, its secondary (BTS 54 354) and primary (BTS 54 505) amine metabolites and various other antidepressants to inhibit [^{3}H]paroxetine binding and [^{3}H]5-HT uptake. In addition, we have determined the ability of sibutramine and these two metabolites to inhibit [^{3}H]noradrenaline (NA) and [^{3}H]dopamine (DA) uptake.

Inhibition constants (Ki's) for [^{3}H]paroxetine were measured by displacement of binding at 30pM to rat frontal cortical membranes (1 mg wet-weight/tube). Specific binding was defined by citalopram (1μM). Inhibition of synaptosomal uptake was determined at 2nM [^{3}H]5-HT or 10nM [^{3}H]NA (rat frontal cortex; 2.5 and 1.25mg/tube respectively) or 2.5nM [^{3}H]DA$_5$(rat striatum; 0.625mg/tube). Specific uptake was defined by zimeldine, desipramine and GBR 12909 (10^{-5}M), respectively.

[^{3}H]Paroxetine binding was potently inhibited by 5-HT uptake blockers such as citalopram and fluvoxamine. A good correlation was shown between the potency of sibutramine, its metabolites and other antidepressants to inhibit [^{3}H]paroxetine binding and [^{3}H]5-HT uptake (correlation 0.971; $p<0.002$). These data support the view that sites labelled by [^{3}H]paroxetine are associated with the 5-HT transporter complex (Habert et al., 1985).

Table 1 Ki values for inhibition of [^{3}H]paroxetine binding and [^{3}H]5-HT uptake

	Paroxetine	5-HT		Paroxetine	5-HT
Sibutramine	2135 ± 137	3131 ± 193	Amitriptyline	18 ± 1	49 ± 2
BTS 54 354	19 ± 1	18 ± 2	Dothiepin	31 ± 2	78 ± 5
BTS 54 505	18 ± 2	26 ± 2	Zimeldine	68 ± 6	57 ± 4
Citalopram	0.65 ± 0.04	3.5 ± 0.4	Doxepin	126 ± 14	201 ± 2
Fluvoxamine	4.2 ± 0.3	3.6 ± 0.2	Desipramine	152 ± 6	200 ± 7
Imipramine	14 ± 2	31 ± 1	Nomifensine	1256 ± 40	2260 ± 132

Ki values (nM) ± s.e. mean (n=3)

The metabolites of sibutramine more potently inhibited [^{3}H]paroxetine binding and [^{3}H]5-HT uptake than sibutramine itself. Similar results were obtained for inhibition of [^{3}H]NA and [^{3}H]DA uptake (Sibutramine, 283 ± 25; 2309 ± 104; BTS 54 354, 2.7 ± 0.3, 24 ± 1; BTS 54 505, 4.9 ± 0.4, 31 ± 2; Ki values (nM) ± s.e. mean for [^{3}H]NA and [^{3}H]DA uptake, respectively). These results support previous findings that the pharmacological effects of sibutramine are, at least in part, mediated by its secondary and primary amines (Luscombe et al., 1989), and that these two metabolites are potent NA uptake blockers in vitro.

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516P

THE EFFECT OF RS-30199 ON ANXIETY AND HIPPOCAMPAL MONOAMINE OXIDASE ACTIVITY IN THE RAT

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The 5-HT$_{1A}$ receptor ligand RS-30199 has been reported to induce anxiety in Sprague-Dawley rats tested in the elevated plus maze and modified holeboard (Redfern *et al*, 1989). In the present study, we have confirmed and extended these findings using a different strain of rat.

Male Lister Hooded rats (300-400g) were injected (i.p.) with RS-30199 (0.3-30.0 mg/kg), buspirone (2.0 mg/kg BSP), chlordiazepoxide (5.0 & 7.5 mg/kg CDP) or saline (CON). Forty minutes later, locomotor activity (Panlab Actisystem), anxiety (elevated plus maze) and hippocampal MAO activity were measured (Morinan, 1989).

At 30 mg/kg, RS-30199 reduced locomotor activity by 90%, but at 3 mg/kg there was a significant anxiogenic effect without any apparent sedation (Table 1). CDP but not BSP reduced anxiety, although none of the drugs had any effect on MAO activity (Table 1).

Table 1. Effect of the drugs on behaviour and MAO activity.

Drug	Dose (mg/kg)	Total Entries	Open/Total Entries (%)	Open/Total Time (%)	Locomotor Activity (counts)	MAO Actvity (nmol/mg/h)
CON	-	11.4 ± 0.8 (14)	26.4 ± 3.4 (14)	13.2 ± 2.3 (14)	641 ± 18 (22)	37.5 ± 3.0 (12)
CDP	5.0	7.5 ± 2.4* (6)	44.9 ± 10* (6)	17.8 ± 9.0 (6)	649 ± 18 (6)	28.2 ± 1.7 (6)
CDP	7.5	10.3 ± 1.5 (8)	40.9 ± 7.3* (8)	20.9 ± 4.2 (8)	386 ± 33* (8)	46.6 ± 4.3 (6)
BSP	2.0	8.5 ± 1.0 (6)	31.6 ± 4.4 (6)	11.9 ± 2.3. (6)	655 ± 24 (6)	35.5 ± 1.4 (6)
RS	0.3	10.1 ± 1.2 (8)	17.8 ± 5.9 (8)	10.2 ± 3.8 (8)	613 ± 37 (8)	39.7 ± 2.7 (6)
RS	1.0	12.3 ± 0.9 (6)	17.4 ± 7.1 (6)	19.4 ± 5.9 (6)	672 ± 47 (6)	37.4 ± 1.8 (6)
RS	3.0	10.3 ± 0.7 (8)	17.6 ± 5.2* (8)	7.3 ± 1.7* (8)	591 ± 18 (8)	34.1 ± 2.1 (6)

*Each value represents the mean ± s.e.m. (n) *P < 0.05 compared to CON (Dunnett's t-test)*

In conclusion, these results are in agreement with those of Redfern *et al* (1989), confirming that the effect of RS-30199 is due to anxiogenesis rather than sedation.

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517P

LOW DOSE INTERACTION OF THE 5-HT1A LIGAND GEPIRONE WITH ATROPINE ON SPATIAL LEARNING IN THE RAT

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Cholinergic and serotonergic systems have been proposed to have mutually important roles in learning and memory (Vanderwolf, 1987). The combination of a reduction in serotonin synthesis with low dose atropine results in a marked spatial memory impairment (Richter-Levin & Segal, 1989). Since the 5-HT1A receptor ligand gepirone has been found to produce deficits on a similar task (Rowan & Barrett, 1990) it was of interest to determine whether or not there would be a low dose interaction with atropine.

Male Wistar rats (250-300 g) were trained in a Morris water maze (1m diameter) to find a platform submerged in opaque water (24 - 26°C). Animals received four training trials each day for three days. Each trial consisted of a swim (maximum 2 min) and a 30s period on the platform. On the fourth day a 2 min probe test with the platform removed from the maze was videotaped and analysed. Water (1 ml/kg), gepirone (2 mg/kg) and atropine (20 mg/kg) were given i.p. 30 min before the first trial on each day. ANOVA of the log transformed data was used for inter-group comparisons. (Table 1, values are the mean ± s.e. mean, n = 12 in each group)

Table 1 Water maze performance following gepirone and atropine

	Escape latency(s) Training trial number 2	6	10	Probe test target quadrant bias (% time)	Swim speed (cm/s)
A. Water	57 ± 13	42 ± 11	22 ± 6	32 ± 3	18 ± 1
B. Gepirone + water	56 ± 12	30 ± 8	10 ± 2	28 ± 3	19 ± 1
C. Water + atropine	71 ± 14	39 ± 12	19 ± 8	25 ± 2	22 ± 1
D. Gepirone + atropine	108 ± 9	78 ± 13	61 ± 14	22 ± 2	23 ± 1

There was no significant effect of gepirone or atropine alone on the acquisition of the task whereas the combination group (D)were markedly slower in learning the hidden platform location as measured by escape latency during training trials (P < 0.05 compared to groups A, B or C). On the probe test gepirone had no effect whereas both the atropine group (C) and the combination group (D) were impaired as measured by target quadrant bias (P < 0.05 compared to group A or B). Swim speed was faster in the atropine group (C) and the combination group (D), (P < 0.05 compared to group A or B). It would appear that gepirone and atropine in combination can produce a marked deficit in the acquisition of a spatial navigation task at doses which alone had no significant effect. This supports the hypothesis of an interaction between cholinergic and serotonergic systems on spatial memory.

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518P

ANXIOGENIC EFFECTS ARE ASSOCIATED WITH YOHIMBINE BUT NOT WITH RS-15385-197 OR IDAZOXAN IN THE RAT

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RS-15385-197 is a potent α_2-adrenoceptor antagonist *in vitro* and *in vivo* which readily penetrates into the brain (Clark *et al.* 1989, Brown *et al.*, 1990). As the α_2-adrenoceptor antagonist, yohimbine, is anxiogenic in man (Holmberg & Gershon, 1961) and in rat (Handley & Mithani, 1984), we have investigated the behavioural effects of RS-15385-197, yohimbine and idazoxan in rats by observing their activity in an elevated X-maze and a partially-shaded holeboard (Redfern & Williams, 1989).

Male Sprague-Dawley rats (160-210g; 12 per group), which had been housed singly for 4-8 days before testing, were injected i.p. with drug or vehicle (1 ml/kg), and 30 min later each rat was placed for 5 min on an elevated X-maze (Redfern & Williams, 1989). The rat was then tested in a holeboard containing a small roof (12 x 15 cm) fixed 10 cm above the floor in one corner (Redfern & Williams, 1989). The rat was placed in the shaded corner and observations were made of the initial emergence latency and of general activities during forays from the shaded corner, over 12 min. In pentobarbitone-anaesthetized rats (250-440g; 6-10 per group), the ability of the 3 antagonists (administered i.p.) to block central α_2-adrenoceptors was determined by reversal of mydriasis produced by clonidine (0.3 mg/kg s.c.).

Clonidine-induced mydriasis: At 3 mg/kg i.p., RS-15385-197 and idazoxan produced a rapid, sustained reversal of the clonidine response (by 86.6 ± 2.0 and 86.2 ± 2.2% respectively, 30 min after injection) whereas yohimbine produced a gradual reversal of only 42.8 ± 12.8%. A higher dose of yohimbine (10 mg/kg) was required in order to achieve 77.1 ± 3.6% reversal of clonidine-induced mydriasis.
X-maze: Yohimbine (10 mg/kg) reduced the % time spent on the open arms (from 20.1 ± 3.2 to 8.7 ± 2.3 %; $P < 0.02$, U-test); total arm entries were reduced after doses of 3 mg/kg (from 9.5 ± 0.8 to 5.7 ± 0.9; $P < 0.02$) and 10 mg/kg (from 13.6 ± 1.1 to 5.1 ± 1.3; $P < 0.002$). RS-15385-197 (3 mg/kg) and idazoxan (3 mg/kg) did not affect any parameters in the elevated X-maze.
Holeboard: Yohimbine (3mg/kg) suppressed head dips (from 11.4 ± 1.4 to 3.6 ± 1.7; $P < 0.02$) and the number of holes explored (from 4.1 ± 0.7 to 1.8 ± 0.7; $P < 0.05$) without significantly inhibiting other activities. A higher dose (10 mg/kg) increased emergence latency (from 12.8 ± 4.4 to 73.7 ± 10.7s; $P < 0.002$) and virtually abolished all activity (e.g. forays were reduced from 8.0 ± 0.9 to 1.7 ± 0.5; $P < 0.002$). RS-15385-197 and idazoxan (both at 3 mg/kg) did not affect emergence latency or holeboard activities.

In conclusion, yohimbine reduced exploratory activity in these tests whereas idazoxan and RS-15385-197 did not, even though the doses of yohimbine used were equieffective (10 mg/kg) or less effective (3 mg/kg) at blocking central α_2-adrenoceptors. Taking the reduced open arm activity in the X-maze as an index of increased anxiety, it appears from the results of these tests that, unlike yohimbine, RS-15385-197 and idazoxan did not have anxiogenic effects. This suggests that acute blockade of central α_2-adrenoceptors is not associated with increased anxiety in the rat and that the anxiogenic effects of yohimbine therefore may be due to some other property of this drug.

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519P

THE EFFECT OF SYSTEMIC 5-CARBOXAMIDOTRYPTAMINE ON EXTRACELLULAR LEVELS OF 5-HT IN THE FREELY MOVING GUINEA-PIG

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The technique of in vivo microdialysis is well established for the measurement of neurotransmitters in the extracellular space (Ungerstedt 1984). Such experiments have been successfully employed to measure 5-HT in the anaesthetised guinea-pig (Sleight et al., 1990). The present study assessed the ability of the technique to measure extracellular 5-HT in the frontal cortex of the freely moving guinea-pig and to determine the effects of the non-selective 5-HT_1 agonist 5-carboxamidotryptamine (5-CT) (Connor et al., 1986).

Male Dunkin-Hartley guinea-pigs (300-400g) were anaesthetised with a mixture of Hypnorm (Janssen), water and midazolam (1:2:1, 8ml/kg i.p.). Dialysis probes were implanted stereotaxically into the frontal cortex using the atlas of Luparello (1967).The animals were allowed to recover for 24 hrs., and then perfused with artificial CSF. Half an hour later 4 x 20 min. samples were collected for the measurement of basal extracellular 5-HT. Animals were then given 0.01, 0.03, 0.1 or 0.3 mg/kg i.p. 5-CT, dialysis samples collected for at least a further 2 hrs., and behaviour observed.

The basal level of extracellular 5-HT was113±17 fmol/20µl (n=11). While saline administration had no significant effect on 5-HT levels, 5-CT produced a dose-related decrease with 0.3 mg/kg i.p. giving a maximal inhibition of 39±8% (n=3). 5-HT values returned to normal within 2 hrs. and there was no effect on extracellular 5-HIAA. The administration of 5-CT at all doses caused a behavioural response consisting of wet dog shakes and hind limb abduction with associated flat body posture.

These data suggest that systemically administered 5-CT can act centrally to reduce neuronal release of 5-HT. This effect is likely to be mediated via either a reduction of neuronal firing by action on the somatodendritic autoreceptor or by a direct action on the terminal autoreceptor. The occurrence of wet dog shakes suggests that 5-CT may also interact with 5-HT_2 receptors in vivo.

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520P

ACTION OF SOME TRYPTAMINE ANALOGUES THAT ARE AGONISTS AT 5-HT_1 RECEPTORS ON CENTRAL AUTONOMIC OUTFLOW IN ANAESTHETIZED CATS

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Central activation of 5-HT_{1A} receptors using compounds, e.g. 8-OH-DPAT, which are structurally unrelated to 5-HT but selective for the 5-HT_{1A} receptor, causes a hypotension which is associated with a characteristic and dose-dependent patterning of autonomic outflows (Ramage, 1990). The present experiments were carried out to determine if central administration of the tryptamine analogues 5-carboxytryptamine (5-CT), N N-di-propyl-5-CT (DP-5-CT), indorenate and 5-HT, the latter in the presence of the 5-HT_2 antagonist cinanserin, cause similar effects on autonomic outflow to those produced by 8-OH-DPAT. As these compounds have varying affinities for the various subtypes of the 5-HT_1 receptor it may be possible to discern different roles for the various subtypes in the control of autonomic outflow.

Cats, anaesthetized with α-chloralose (70 mg/kg) and pentobarbitone sodium (6 mg/kg), were artificially ventilated after neuromuscular blockade with vecuronium bromide (200 μg/kg). Simultaneous recordings were made of renal (RNA), splanchnic (SNA), cardiac (CNA) and phrenic nerve activity (PNA), blood pressure, heart rate, femoral arterial conductance (FAC), tracheal and intragastric pressure. Cumulative doses of 5HT (20 - 640 nmol/kg), 5-CT (0.625 - 160 nmol/kg), indorenate (100 - 800 nmol/kg) and DP-5-CT (2.5 - 80 nmol/kg) were given by microinjection (20 μl over 1 min) into the IVth ventricle. Cinanserin (0.1 mg/kg) was given into the IVth ventricle before the doses of 5-HT.

DP-5-CT, 5-CT, indorenate and 5-HT caused maximum falls in BP of 44±4, 38±6, 23±8 and 22±3 mmHg respectively by the last dose. This was associated with varying degrees of sympathoinhibition but with little change in FAC. DP-5-CT, 5-CT and indorenate caused sympathoinhibition in all nerves, indorenate affecting all nerves equally, while DP-5-CT and 5-CT had a greater effect on RNA and SNA than on CNA. 5-HT caused a decrease in RNA and SNA but had no effect on CNA. DP-5-CT and indorenate also caused a bradycardia reaching a maximum of 16±4 and 33±10 beats/min respectively. Only the bradycardia caused by indorenate was weakly attenuated by atropine methonitrate (0.1 mg/kg). All drugs had little effect on phrenic nerve activity. Cinanserin had little effect on the above variables except to cause a small increase in FAC.

These results support the view that activation of central 5-HT_1 receptors causes a differential sympathoinhibition, with cardiac nerve activity being the least sensitive and renal nerve activity being the most sensitive.

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521P

SHAPE CHANGES: A NOVEL METHOD FOR ASSESSING THE EFFECT OF AGONISTS AND ANTAGONISTS ON HUMAN PLATELETS?

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Agonists interact with specific platelet receptors, causing shape change followed by aggregation. Low concentrations of agonists, such as adenosine diphosphate (ADP), 5-hydroxytryptamine (5-HT) and collagen, only induce a shape change which involves the transformation of platelets from a discoid to a spherical form.

Platelet shape change in platelet rich plasma (PRP) has previously been investigated by monitoring light transmission/scattering in the presence of EDTA to prevent platelet aggregation (Born, 1970). We devised a sensitive procedure to investigate agonist-induced platelet shape change in the presence/absence of antagonists. The method involves the stimulation of stirred PRP (1000 rpm, at 37 °C, for 30 s), the fixation of the platelets with glutaraldehyde (4% w/v in saline) and the measurement of platelet count and median platelet volumes (MePV) using a Coulter Counter ZM coupled to a Coulter Channelyzer 256 (resolution: ±0.07 fl).

5-HT and ADP stimulated platelet shape change (PSC) *in vitro*, in a dose-dependent manner with significant (P <0.004) increases in MePV occurring at 0.1 μM for both agonists (control=5.46±0.44 fl, 5-HT=5.98±0.50 fl, ADP=5.55±0.38 fl, n=27). Near maximal ADP and 5-HT-induced PSC was achieved with 0.4 μM and 1 μM (6.15±0.50 fl and 6.07±0.50 fl, respectively). Noradrenaline, at relatively high concentrations (1 μM) also increased the MePV (5.55±0.40 fl, n=12), whereas adrenaline was ineffective (5.40±0.45, n=12). The simulta-neous addition of ADP (0.1 μM) + 5-HT (0.01 μM) caused a synergistic increase in MePV. At all these concentrations of agonists there was no platelet aggregation as measured by free platelet count. All samples were obtained from healthy male and female volunteers.

We also assessed the effect of naftidofuryl oxalate (NAF), a drug used in the treatment of peripheral vascular disease. NAF, at concentrations of 50 and 100 μM, has been shown to inhibit platelet aggregation induced, *in vitro*, by 5-HT and ADP respectively (Davies & Steiner, 1988). Using the above methodology, we observed significant inhibition of 5-HT-induced PSC following the incubation of PRP with 3.125 μM NAF for 5 min. In contrast, ADP- and collagen-induced PSC was significantly inhibited at concentrations >50 μM. PSC induced with the combination of ADP + 5-HT was significantly inhibited at concentrations as low as 1.56 μM NAF.

In conclusion, we have devised a sensitive method for investigating agonist-induced shape change, a phenomenon which precedes platelet aggregation. Using this methodology, NAF appears to be at least 16 times more potent as a 5-HT blocker when compared to ADP or collagen. This is considerably more discriminating than with platelet aggregation methodology (2 fold difference). Furthermore, our results show that the inhibition of PSC by NAF occurs at therapeutic concentrations (≈4 μM) of NAF.

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AN EP_3-RECEPTOR MAY MEDIATE PROSTAGLANDIN E-INDUCED POTENTIATION OF AGGREGATION IN HUMAN PLATELETS

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The pro-aggregatory action of PGE_2 on human platelets often appears as a potentiation of second phase aggregation (Shio & Ramwell, 1972), but enhancement of primary aggregation has also been reported (Andersen *et al.*, 1980). It has been suggested that a discrete PGE (EP-) receptor mediates these effects (Andersen *et al.*, 1980) and that negative coupling to adenylate cyclase may be involved (Ashby, 1988). In this communication we report on the potencies of seven PGE analogues as potentiators of aggregation in human platelets and as inhibitors of the field-stimulated guinea-pig vas deferens (an EP_3 preparation; Coleman *et al.*, 1987) (Table 1). Small reversible waves to PAF (10 - 30 nM) were obtained in human PRP in the presence of the TP-receptor antagonist GR 32191 (1000 nM) and the DP-receptor antagonist BW A868C (200 nM). PGE analogues were added 2 min before PAF: PC_{200} is the concentration required to double the size of the PAF response.

Table 1 Equi-effective molar ratios (EMR) for PGE analogues on guinea-pig vas deferens and human platelets (n = 4)

Prostanoid	Vas deferens EMR	Platelet EMR	Comments on platelets
Sulprostone	1.0 (IC_{50} = 0.2 nM)	1.0 (PC_{200} = 20 nM)	
5,6-Dihydro sulprostone	0.80	1.3	
16,16-Dimethyl PGE_2	0.96	1.2	
Misoprostol	3.8	18	
MB 28767	5.4	16	
PGE_2	7.5	?	inhibition often seen (IP agonism)
17-Phenyl-ω-trinor PGE_2	47	110	shallow log dose-response curve
11-Deoxy PGE_2-1-alcohol	73	?	inhibition often seen (IP agonism)

In washed platelet suspensions the pro-aggregatory action of sulprostone was not blocked by 12 μM AH 6809, suggesting that an EP_1-receptor is not involved (Coleman *et al.*, 1985) and this is supported by the low potency relative to sulprostone of 17-phenyl-ω-trinor PGE_2, a potent EP_1 agonist (Lawrence *et al.*,1989). In spite of the difficulties associated with the high sensitivity of human platelets to IP-receptor agonists, the data suggest that an EP_3-receptor may mediate the potentiation of aggregation.

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CHLORIDE TRANSPORT IN HUMAN PLACENTAL BRUSH BORDER MEMBRANE VESICLES

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A major component of chloride transport in epithelial cells is mediated by chloride channels. The possibility of chloride transport defects in cystic fibrosis has stimulated interest in such chloride channels. Membrane vesicles prepared from epithelial cells serve as a useful model to study regulation of chloride channels. In this study we have prepared brush border membrane vesicles (BBMV) using a magnesium precipitation technique, (Ganapathy et al. 1985) from human term placenta. We have used these membrane vesicles to study chloride channels and the effects of some inhibitors.

The purity of the BBMV was assessed using the marker enzymes, alkaline phosphatase was enriched 25.3 ± 4.8 (n=4) and gamma-glutamyltranspeptidase was enriched 53.4 ± 8.9 (n=5) over the homogenate. Transmission and scanning electron microscopy showed the vesicles to be closed spherical structures of varying sizes, with the brush border membrane facing outwards. Chloride uptake into the BBMV was measured in the presence of an outwardly directed Cl gradient (Landry et al., 1987). Using this system, we have shown a time-dependent accumlation of ^{36}Cl within the vesicles. Initial uptake at 30s was found to be 8.6 ± 0.6 nmol Cl/mg protein, rising to 17.1 ± 0.8 nmol Cl/mg protein by 12 min. By 40 min this value had decreased to 11.6 ± 0.7 nmol Cl/mg protein (mean ± s.e.mean; n=6). Addition of 5 μM valinomycin during the uptake reaction resulted in a discharge of accumulated isotope. When valinomycin was added after 2 min, the uptake was reduced from 17.2 ± 3.6 nmol Cl/mg protein before addition to 7.6 ± 1.6 nmol Cl/mg protein after addition. The effect of N-phenylanthranilic acid and 4,4'-diisothiocyanostilbene-2,2'-disulphonic acid (DIDS) on Cl uptake was examined. Both drugs showed dose dependent inhibition, the $I.C._{50}$ values being 0.83 ± 0.35 μM for NPA and 3.45 ± 0.37 μM for DIDS (mean ± s.e.mean; n=3). Linoleic acid also inhibited Cl uptake with $I.C._{50}$ of 4.59 ± 0.51 μM.

These results show that BBMV prepared from human placenta can be used as a model to study Cl transport. The results with valinomycin confirmed that the uptake was dependent on a membrane potential. The Cl transport system was sensitive to NPA and DIDS, both of which have been shown to inhibit Cl transport in other systems to a similar extent. Since the basic defect in cystic fibrosis is known to be a defective regulation of epithelial chloride channels, this system may be useful in the study of regulation of Cl channels in both normal and cystic fibrosis tissue.

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524P

MODULATION OF THE BRADYKININ-STIMULATED RELEASE OF PROSTACYCLIN FROM ENDOTHELIAL CELLS

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Vascular endothelial cells release the vasodilator substances prostacyclin (PGI_2) and endothelium deri relaxing factor (EDRF) in response to a range of natural stimuli, such as bradykinin (Crossman et al., Kondo et al., 1989). In this study we have investigated whether EDRF can modulate release of PGI_2 fro cultured endothelial cells. The effects of nitroprusside, which mimics the action of EDRF (Kondo et a 1989) and L-N^G-nitroarginine (NOARG) which inhibits EDRF formation (Moore et al., 1990) have been exam the basal and bradykinin stimulated release of PGI_2 from the bovine aortic endothelial cell line AG476

AG4762 cells (passage 16-22) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fo calf serum and 1.5mM glutamine and plated out onto 15mm multiwells. Confluent cells were incubated fo min in warm, gassed DMEM containing 0-100nM bradykinin in the presence or absence of 100μM nitroprussi 100μM NOARG. The release of PGI_2 was then determined by radioimmunoassay (Crossman et al., 1987).

Bradykinin produced a dose dependent release of PGI_2. The maximal release obtained was ~350% of basal release. The EC_{50} for bradykinin was ~3nM. In the presence of nitroprusside, the maximal response to bradykinin was reduced by ~40%. There was no effect of nitroprusside on bradykinin EC_{50}. In the pres NOARG, the maximal response to bradykinin was increased by ~30% and there was no effect of NOARG on th obtained for bradykinin. Nitroprusside and NOARG had no effect on basal release of PGI_2.

The bradykinin stimulated release of PGI_2 from cultured endothelial cells is inhibited by nitroprussid enhanced by NOARG. This suggests a role for EDRF in the feedback control of PGI_2 release. Whether th release of other vasodilator compounds is similarly affected remains to be determined. The mechanism apparent effect of EDRF is unclear. EDRF and nitrovasodilators stimulate guanylate cyclase in endothe cells (Martin et al., 1988), suggesting that PGI_2 release might be inhibited by cGMP. However, Martin al., (1989) have reported that changes in cGMP levels do not affect PGI_2 release. Thus these effects might involve a cGMP independent mechanism.

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525P

A COMPARISON OF THE THROMBOXANE RECEPTOR LIGANDS [^{125}I]-PTAOH AND [^{3}H]-SQ29548 BINDING IN CULTURED BOVINE AORTIC ENDOTHELIAL CELLS

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Thromboxane A2 (TXA2) is a potent vasoconstrictor and an effective inducer of platelet aggregation. W previously shown that the stable TXA2 mimetic U46619 initiates the release of prostacyclin (PGI2) from cultured endothelial cells (Hunt et al., 1989). PGI2 is antiaggregatory and a vasodilator, so this re may be important as a feedback control mechanism modulating TXA2 activity. In this study we have atte to characterise TXA2 receptor binding sites on an endothelial cell line, AG4762, using two established receptor ligands [125I]-PTAOH (Swayne et al., 1988) and [3H]-SQ29548 (Hedberg et al., 1988).

Binding assays were carried out as described previously (Hunt et al., 1990), using washed membranes fr AG4762 cells (passage 16-22) at 50-100μg protein/tube. Non-specific binding of 0.1nM [125I]-PTAOH was defined using 10μM U46619 and that of 2.5nM [3H]-SQ29548 using 100μM BM13505.

Total binding of [125I]-PTA-OH was typically ~2000dpm sample of which 35% was specific. In self compe studies I-PTAOH displaced all the specific binding of [125I]-PTAOH, with nH=1, Kd=0.4nM Bmax=23 fmols/ protein. The TXA2 antagonists SQ29548, BM13505 and BM13177 (Swayne et al., 1988) displaced all the sp binding of [125I]-PTAOH; BM13505 IC50=30±12nM (mean±s.e.m., n=3), SQ29548 IC50=2.1±0.6μM (n=6), BM131 IC50=0.1mM (n=2). In all cases the antagonist displacement curves were consistent with binding to a homogeneous population of sites. Total binding of [3H]-SQ29548 was typically ~260dpm/sample; 45% was specific. In self competition studies with SQ29548, nH=1, Kd=2.5nM, and Bmax=26 fmols/mg protein. Th antagonists again displaced all the specific binding and appeared to recognise a single population of SQ29548 IC50=4±2nM (mean±s.e.m. n=3), BM13505 IC50=198±56nM (n=3) and BM13177 IC50=13μM (n=2).

[125I]-PTAOH and [3H]-SQ29548 labelled high affinity binding sites in AG4762 cell membranes. The dens both these sites appeared to be the same and both populations of sites were homogeneous with respect t antagonist binding. However the order of potency of the antagonists is different for the two ligands. order of potency obtained in [125I]-PTAOH studies agrees with that previously identified in functional studies on this cell line (Hunt et al., 1990), whilst that described for [3H]-SQ29548 agrees with func and binding studies carried out on platelets by Swayne et al., (1988). The reason for these differenc at present unclear. Whether the 'specific' binding of either ligand represents a bonafide TXA2 recept endothelial cells remains to be established.

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526P

INDUCTION OF THROMBOXANE BIOSYNTHESIS IN GUINEA-PIG EOSINOPHILS STIMULATED WITH LEUKOTRIENE B_4

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Leukotriene B_4 (LTB_4) stimulates respiratory burst in human neutrophils and in guinea-pig eosinophils, increases IgE-receptor expression and IgE-mediated cytotoxicity in human eosinophils and is found in bronchoalveolar lavage fluid and sputum from asthmatic subjects. There is, however, little information as to whether LTB_4 can also promote the biosynthesis of cyclo-oxygenase products in eosinophils. As TXA_2 may play a role in the pathogenesis of bronchial asthma, experiments were designed to determine whether LTB_4 stimulates TXA_2 production in eosinophils.

Eosinophils were harvested from the peritoneal cavity of human serum-treated male guinea-pigs and purified on discontinuous Percoll gradients. Cells with a purity greater than 96% and viability greater than 99% were resuspended in HEPES-buffered physiological salt solution containing 1.6 mM $CaCl_2$, equilibrated at 37°C (5 min) and then challenged (10 min) with either vehicle, LTB_4, PAF, serum-opsonised zymosan (SOZ) or the calcium ionophore calcimycin. The amount of TXB_2, the stable metabolite of TXA_2, released in to the bathing buffer was measured by RIA. Hydrogen peroxide generation was used as an index eosinophil activation and was measured fluorimetrically by horse-radish-catalysed oxidation of scopoletin (Root *et al.*, 1975).

LTB_4 (1 uM for 5 min) stimulated the biosynthesis and release of immunoreactive TXB_2 (from 0.84 ± 0.21 to 9.4 ± 1.8 pmol 10^6 $cells^{-1}$, n = 5) from guinea-pig eosinophils. This effect of LTB_4 was concentration-dependent (EC_{50}: 164 ± 10.8 nM, n = 5) with maximum stimulation (*ca.* 12-fold) of prostanoid synthesis occurring at 1 uM LTB_4. The ability of LTB_4 to generate TXB_2 was rapid ($t_{1/2}$: *ca.* 12.5 s), transient and *non*-competitively antagonised by the selective LTB4 blocking drug U-75302 (6-(6-(3-hydroxy-1E, 5Z-undecadien-1-yl)-2-pyridinyl)-1,5-hexanediol; Morris *et al.*, 1988). LTB_4 (1 uM) was significantly more effective than PAF (1 uM) and SOZ (500 ug) at promoting TXB2 release and *ca.* equieffective with calcimycin (1 uM). Pre-treatment of eosinophils with the cyclo-oxygenase inhibitor, flurbiprofen (8 uM for 5 min), abolished LTB_4-stimulated TXB_2 release but did not antagonise the ability of LTB_4 to generate hydrogen peroxide at any concentration examined.

It is concluded that LTB_4 is an effective stimulant of TXA_2 generation in guinea-pig eosinophils which is mediated through an interaction with specific LTB_4-receptors. Furthermore, LTB_4-stimulated hydrogen peroxide generation in these cells is not dependent upon the concomitant generation of cyclo-oxygenase products.

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527P

POTENTIATION OF FMLP-INDUCED PLASMA EXUDATION BY PGE ANALOGUES: EFFECT OF THE THROMBOXANE RECEPTOR ANTAGONIST GR 32191

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We have recently demonstrated that potentiation of bradykinin (BK)-induced plasma exudation by PGE_2 in the skin of anaesthetised rabbits (measured by leakage of ^{125}I-albumin) may have two components (Armstrong *et al.*, in press). The high potency (1-10 ng) component may be mediated by an EP_3 receptor and appears to be unrelated to dilatation, yet accounts for 50% of the maximum potentiation achieved with PGE_2 (1000 ng). PGE_2 not only potentiates directly-acting mediators of inflammation, such as BK, but also leucocyte-dependant mediators such as the chemotactic peptide FMLP (Wedmore & Williams, 1981). We have compared the ability of the EP_3 agonist M&B 28,767 to potentiate oedema induced by intradermal injection of FMLP (50 picomoles/site) with PGE_2, misoprostol and butaprost. M&B 28,767 has considerable thromboxane (TP) receptor agonist activity and so its true potency could only be assessed after TP receptor block by GR 32191 (2 mg/kg i.v.).

Table 1 Interaction of PGE analogues and FMLP on plasma exudation (ml of exudate) in rabbit skin (n = 4 or more, ± s.e.m.)

Intradermal injection	Control rabbits			GR 32191-treated rabbits		
	0	1 ng	1000 ng	0	1 ng	1000 ng
FMLP + saline	9.0 ± 1.0			15.0 ± 1.3		
FMLP + PGE_2		12.8 ± 0.8	19.8 ± 2.1		17.7 ± 1.1	45.8 ± 7.3
FMLP + Misoprostol		13.0 ± 1.3	23.9 ± 3.5		19.1 ± 1.3	31.4 ± 10.6
FMLP + M&B 28,767					17.6 ± 1.9	16.9 ± 2.6
FMLP + Butaprost		11.1 ± 1.1	19.4 ± 2.7			

In control animals, PGE_2, misoprostol and butaprost showed a similar ability to potentiate FMLP-induced exudation, the effect being most marked at 100-1000 ng doses. The marked effect of PGE_2 at 1 ng in combination with BK is absent with FMLP. M&B 28,767 and sulprostone (data not shown) do not significantly potentiate FMLP, suggesting that EP_3 agonists cannot potentiate FMLP probably due to their inability to induce dilatation in rabbit skin. GR 32191 pre-treatment had no effect on BK-induced exudation or potentiation by PGE_2 but enhanced both FMLP-induced exudation and potentiation, particularly by PGE_2. A similar profile was seen with the NSAID, flurbiprofen (2 mg/kg i.v.). We suggest that FMLP induces a thromboxane-dependent constriction (see Crowell *et al.*, 1989); after TP-receptor block by GR 32191 dilatation could result in increased exudation.

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528P

THE ANTI-INFLAMMATORY EFFECTS OF INHALED SALMETEROL AND SALBUTAMOL IN GUINEA-PIG LUNG

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ß-Adrenoceptor agonists inhibit both increased vascular permeability and oedema formation (Green, 1972; Persson, 1987), but the extent of this inhibition is limited by the short duration of action of these agents. The present report compares the new long-acting, $ß_2$-adrenoceptor agonist, salmeterol (salm; Bradshaw et al, 1987), with the shorter-acting compound, salbutamol (salb), against histamine-induced plasma protein extravasation (PPE) in guinea-pig lung following aerosol administration.

Guinea-pigs received an intracardiac injection of iodinated human serum albumin (0.5µCi;0.3 ml) under isoflurane anaesthesia. On recovery, varying doses of the $ß_2$-adrenoceptor agonists or vehicle were administered by inhalation as described by Ball et al, 1987. At intervals after dosing, guinea-pigs were placed in a perspex chamber and exposed to nebulised histamine (H; 0.5mg.ml^{-1}) for 30 sec, followed by a further 30 sec exposure in the chamber. After 30 min, the animals were killed, plasma samples prepared and the lungs lavaged with 2x10ml heparinised (10U.ml^{-1}) phosphate buffered saline. The radioactive content of an aliquot of plasma and 5ml of lavage fluid (BALF) were determined, and PPE was calculated as µl plasma .ml^{-1} BALF. Histamine challenge increased the albumin content of BALF from 0.80 µl.ml^{-1} (95% C.L. 0.60-1.08, n=10) to 5.26 µl.ml^{-1} (4.82-5.72, n=13). Salm (0.001-1.0 mg.ml^{-1}) and salb (0.001-1.0 mg.ml^{-1}), 30 min prior to H, caused a dose-related inhibition of PPE. Salm and salb were approximately equipotent in this respect, 0.1 mg.ml^{-1} reducing H-induced PPE to 1.08 µl.ml^{-1} (0.92-1.28, n=6) and 1.32 µl.ml^{-1} (1.26-1.38, n=6) respectively. However, while the duration of action of salb was short (1-2h), that of salm was substantially longer (6-8 h). Inhibition of PPE was not secondary to bronchodilatation since an equi-bronchodilator dose of PGE_2 (0.1 mg.ml^{-1}) had no effect. Pretreatment of guinea-pigs with propranolol (1mg.kg^{-1} s.c.) abolished the inhibition of H-induced PPE by salm.

The experiments described show that inhaled salm and salb inhibit H-induced PPE in guinea-pig lung at bronchodilator doses (Ball et al, 1987). However, the duration of action of salm is markedly longer than that of salb. In man, salmeterol results in at least 12 hr bronchodilatation, and unlike shorter-acting $ß_2$-adrenoceptor agonists, may also provide a clinically useful anti-inflammatory action in bronchial asthma.

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529P

GLUCOCORTICOID INDUCTION OF RAT GROWTH HORMONE GENE PROMOTER FUNCTION

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Previous studies in this laboratory (Treacy and Martin, 1987) resulted in the characterisation of cell-type independent glucocorticoid inducible enhancer activity in the proximal sequences (-523 to -12) of the rat growth hormone gene promoter. We have now investigated the role of two 15 bp sequence elements within this promoter, which closely resemble glucocorticoid response elements (GREs) in other genes (Beato, 1989), in mediating this glucocorticoid responsiveness.

Two sequences (-111/-97) and (-250/-264), within the rat growth hormone promoter, resemble GREs. Small deletion mutations were generated in either or both of these elements by oligonucleotide directed mutation using a method based on the gapped duplex DNA approach (Kramer et al., 1984). The generation of the desired mutations was confirmed by DNA sequence analysis. The ability of the mutated rGH promoter fragments to mediate glucocorticoid induction of gene expression was then tested in a series of transfection studies: CV-1 cells, which are glucocorticoid receptor deficient were co-transfected with a glucocorticoid receptor expression plasmid and a plasmid containing the wild-type rat growth hormone promoter fragment (or one of the three mutation) cloned upstream of a basal promoter/reporter gene (chloramphenical acetyl transferase (CAT)) construct. The glucocorticoid inducibiltiy was determined by the ability of dexamethasone (10^{-6} M) to induce expression of CAT enzyme activity in the transfected CV-1 cells. The wild type rGH promoter sequences mediated dexamethasone induction of CAT gene expression ($p<0.05$). However, mutation of both GRE-like sequences or the (-111/-97) sequence, alone, resulted in a loss of glucocorticoid responsiveness. Glucocorticoid inducibility ($p<0.01$) was still seen with the promoter fragment bearing the mutation in the (-264/-250) sequence.

These results suggest that the GRE-like element (-111/-97) in the rat growth hormone promoter is necessary for the mediation of glucocorticoid responsiveness by this gene promoter.

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530P

MODULATION OF IL-8 GENERATION IN THE PRE-MONOCYTIC CELL LINE, U937

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U937 cells are a continuous line of human cells of committed monocytic origin (Larrick, J.W. et al 1980; Ward, S.G. & Westwick, J. 1988). We have investigated the effects of agents which modulate GTP binding proteins and protein kinase C (PKC) to explore the signal transduction mechanisms involved in lipo-polysaccharide (LPS) and cytokine stimulated interleukin-8 (IL-8) generation.

A sensitive and specific ELISA (Ceska, M. et al 1989) was used to quantitate extracellular IL-8. Additions of LPS (0.1-100ng/ml), interleukin-1α (IL-1α) (0.1-10ng/ml) and to a lesser extent tumour-necrosis factor TNFα (3-30ng/ml) produced a dose-related generation of extracellular IL-8. U937 cells may be transformed into monocyte like cells using the following transforming agents, di-methyl sulfoxide (DMSO) 1.25% and di-butryl cyclic-AMP (cAMP) 0.8mM.

Untransformed, DMSO-transformed and cAMP-transformed U937 cells were all responsive to the above stimului. Pre-treatment of U937 cells with pertussis toxin (100ng/ml) for 4h produced approximately 80% inhibition of sub-optimal LPS, IL-1α and TNFα induced generation of IL-8. In contrast incubation for with cholera toxin (100ng/ml) for 4h produced a 10-40% enhancement of IL-8 generation induced by LPS, IL-1α or TNFα. Additions of the phorbol ester tetra-phorbol acetate (TPA, 0.1-30nM), but not 4α phorbol produced a marked stimulation of IL-8 formation. However, the addition of LPS or IL-1α did not modify TPA induced IL-8 formation. Staurosporine (0.1-3μM) the non-selective PKC inhibitor, produced a dose related inhibition of basal, LPS and TNFα stimulated generation of IL-8. Low dose (0.1-0.3μM) staurosporine doubled the IL-1α-induced formation of IL-8, although 1-3μM staurosporine produced a significant inhibition.

These results indicate that GTP binding proteins and PKC have a regulatory role in the generation of IL-8 derived from U937 cells.

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531P

α,β-METHYLENE-ADP POTENTIATES ATP RESPONSES IN THE RABBIT EAR ARTERY

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ATP, which is susceptible to degradation by ectonucleotidases, produces a transient contraction of the rabbit ear artery (REA) via P_{2X}-receptors. However, as in many P_{2X}- systems, ATP exhibits a very low potency ($p[A_{50}]$ 3.14) compared to stable analogues such as α,β-meATP ($p[A_{50}]$ 6.47) (O'Connor *et al.*, 1990). α,β-meADP is an inhibitor of 5'-nucleotidase, the enzyme which converts adenosine monophosphate to adenosine. Attempts have been made to use this compound to inhibit ATP metabolism (Pearson *et al.*, 1980, Morishita & Furukawa 1989) but these were apparently unsuccessful. We have studied the effect of α,β-meADP on responses to both stable and unstable purines in the REA.

Experiments were conducted using isolated rings of endothelium denuded ear artery from NZW rabbits. All contractions were expressed as a % of the maximum response to the standard P_{2X}- agonist α,β-meATP. Concentration effect (E/[A]) curves to α,β-meATP, β,γ-meATP, ATP and ADP were constructed alone and following 60 min incubation with α,β-meADP (30μM).

α,β-meADP caused a large leftward displacement ($\Delta p[A_{50}]$ 1.88 ± 0.17 n=5) of the E/[A] curve to ATP and also changed the response from a phasic to a tonic contraction. In contrast, α,β-meADP resulted in only small leftward displacements of the E/[A] curves to α,β-meATP, β,γ-meATP and ADP ($\Delta p[A_{50}]$ 0.15 ± 0.13 n=5, 0.41 ± 0.14 n=5, and 0.30 ± 0.15 n=6 respectively), and had no effect on the kinetic nature of the contraction in each case.

In contrast to the findings of Morishita & Furukawa (1989) we found that α,β-meADP had a profound potentiating effect on the response to ATP. In addition it produced only slight potentiation of the effects of the stable analogues α,β-meATP and β,γ-meATP. However, the effects of ADP, which like ATP is unstable, were not potentiated by α,β-meADP. These results imply that the effect on ATP was not due to inhibition of 5'-nucleotidase. The reduction in the fade of the contraction to ATP, induced by α,β-meADP, may explain the marked leftward displacement of the E/[A] curves (Leff, 1986). However, this seems unlikely as the contractions to both β,γ-meATP and ADP, which produce rapidly fading responses, remained unchanged by pretreatment with α,β-meADP.

This selective potentiation and stabilisation of ATP responses by α,β-meADP in the rabbit ear artery requires further investigation to elucidate the mechanism.

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532P

THE MECHANICAL AND BIOCHEMICAL INTERACTIONS OF INDIVIDUAL CO-TRANSMITTERS IN RAT TAIL ARTERY

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Sympathetic nerves innervating blood vessels may release more than one excitatory substance, noradrenaline (NA) and ATP or a closely related substance - the phenomenon of co-transmission (Burnstock 1976). While both substances play a role in transmission, the extent of their interaction in contraction remains unclear. We have investigated the interaction between noradrenaline (NA) and α,β, methylene ATP (α,β,MeATP) - a stable analogue of ATP - in contraction and on phosphatidylinositol - 4,5 bisphosphate (PIP_2) hydrolysis in rat tail arteries a tissue which exhibits co-transmission (Sneddon & Burnstock, 1985). PIP_2 hydrolysis is of major importance in NA-induced contractile responses (Abdel-Latif, 1986).

Changes in perfusion pressure produced by electrical field stimulation (N.S. 0.5ms 1-16 Hz supra maximal voltage 20 s), exogenous NA (10^{-6}-10^{-4}M) and α,β,MeATP (10^{-7}-10^{-5}M) were measured (Muir & Wardle, 1989). PIP_2 hydrolysis evoked by NA (10^{-6}-10^{-4}M) and ATP (10^{-7}-10^{-5}M) was estimated in segments (1cm. 5mg wet wt.) of artery by measuring total inositol phosphate (IP) production (Akhtar & Abdel-Latif, 1984).

N.S. increased perfusion pressure, this was largely (80%) blocked by prazosin (10^{-6}M) but unaffected by prior, repeated (5 times) addition of α,β,MeATP (10^{-6}M) in a concentration sufficient to desensitise purinoceptors. This confirmed that the contractile response to N.S. was largely adrenergic. Consistently, NA (10^{-6}-10^{-4}M) increased perfusion pressure to only 80% of that evoked by N.S. and increased IP accumulation. α,β,MeATP (10^{-7}-10^{-5}M), however, increased perfusion pressure to only 20% of that evoked by N.S. and did not increase IP accumulation. PIP_2 hydrolysis, therefore, may underlie stimulus-contraction coupling for NA but not that for α,β,MeATP. Moreover, α,β.MeATP at a concentration (10^{-5}M) which produced near maximal contractile responses, when acting alone, failed to potentiate NA-stimulated increases in perfusion pressure or IP accumulation.

These results demonstrate that, despite releasing two excitatory substances, sympathetic nerve-evoked vasoconstriction in rat tail arteries is largely adrenergic. No major contribution of the co-released ATP in either vasoconstriction or in the associated biochemical responses, was detected. Since no interaction was detected on either contraction or PIP_2 hydrolysis, the site and mechanisms responsible for the co-operative effect of NA and ATP in this tissue remain to be elucidated.

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533P

ALTERATIONS IN SENSITIVITY, PHOSPHOINOSITIDE HYDROLYSIS AND CYCLIC NUCLEOTIDE SYNTHESIS IN ISOLATED AORTIC RINGS FROM SPONTANEOUSLY HYPERTENSIVE RATS

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Some studies have reported that isolated blood vessels from hypertensive rats are supersensitive to vasoconstrictors, whilst others have reported subsensitivity. Conflicting results with vasorelaxants have also been reported (Winquist et al., 1982; Bohr & Webb, 1988). Such discrepancies might reflect differences in the model of hypertension used or in the method of recording the responses of isolated vessels. This study examined the sensitivity of isolated aortic rings from spontaneously hypertensive rats (SHR) to noradrenaline (NA) and to sodium nitroprusside (SNP). In addition, the ability of NA to increase phosphoinositide (PI) hydrolysis and of SNP to increase cyclic guanosine monophosphate (cGMP) synthesis was examined in vessels from SHR and from Wistar Kyoto (WKY) controls.

Blood pressures of conscious SHR (190 ± 2, mm Hg (mean ± s.e. mean), n = 10) and WKY rats (109 ± 3, n = 10), measured by the tail cuff method, were significantly different ($0.01>P>0.001$, Student's t-test). Isolated aortic rings (3-4 mm) were suspended under an initial resting tension of 1 g in 25 ml organ baths, containing Krebs buffer at 37°C. Concentration-response curves to NA (10^{-7}-10^{-3} M) and to SNP (10^{-9}-10^{-6} M) in the presence of NA (EC_{75}) were obtained in rings from SHR and WKY rats. NA-induced PI hydrolysis was measured in aortic rings, labelled with (myo-^{3}H)-inositol (8 μCi ml^{-1}) and then incubated in Krebs buffer containing lithium chloride (10 mM) and NA for 1 hour. Prior to measuring PI hydrolysis of cGMP synthesis, aortic rings were homogenised in Potter-Elvehjem homogenizers. Inositol phosphates were extracted, separated chromatographically on Dowex formate resin and measured in a liquid scintillation counter. In separate experiments cGMP was extracted in trichloracetic acid (TCA) and washed with water-saturated ether. These extracts were neutralised with 200 mM sodium acetate buffer and their cGMP content was measured by radioimmune assay.

The sensitivity of aortic rings to NA was lower in SHR rings than in WKY rings. This reduced sensitivity in contractile responses was reflected in a corresponding reduction in NA-induced PI hydrolysis (WKY: 610 ± 44, n = 7; SHR: 537 ± 17, n = 14 (dpm g^{-1} tissue), $0.01>P>0.001$) at high concentrations of NA (10^{-3} M). In aortic rings from SHR, inhibitory responses to SNP were increased, particularly at high concentrations (10^{-7} M) and this increased sensitivity to SNP was reflected in a corresponding enhancement in the ability of SNP to increase cGMP levels (WKY: 2050 ± 550, n = 6; SHR: 6200 ± 600, n = 6 (cGMP pmol g^{-1} tissue), $0.01>P>0.001$). In aortic rings from SHR, concentration-dependent changes in sensitivity to NA and SNP occur and these are reflected in corresponding changes in the ability of NA to induce PI hydrolysis and of SNP to increase cGMP synthesis.

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534P

DIFFERENCES IN INOSITOL PHOSPHATE ACCUMULATION IN RESPONSE TO NORADRENALINE IN TAIL ARTERIES FROM SPONTANEOUSLY HYPERTENSIVE RATS

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An enhanced contractile response to nerve stimulation in several arteries from hypertensive rat animal models is now well established (Muir & Wardle, 1989). Although presynaptic mechanisms were initially implicated in this phenomenon (Vidal et al, 1986), a postsynaptic site has now been emphasised (Muir & Wardle, 1989). Postsynaptic changes may involve alterations in signal-transduction systems including the polyphosphoinositide (PPI) cycle, already associated with the contractile response to noradrenaline (NA) (Abdel-Latif, 1986). Accordingly, the present investigation was an attempt to determine whether any alteration in PPI metabolism in response to NA could be detected in a hypertensive rat model.

The tail artery, a tissue in which NA produces a contractile response, was chosen for this investigation and was dissected by the method of Holman & Surprenant (1980). Age-matched Wistar Kyoto (WKY) and spontaneously-hypertensive rats (SHR) were used to compare the normal and diseased states. Changes in PPI metabolism stimulated by NA in artery segments (1 cm, 5 mg wet.wt) were estimated by measuring inositol phosphate (IP) accumulation by the methods of Akhtar & Abdel-Latif, 1984.

NA (10^{-6}-10^{-3}M) increased total IP accumulation in both SHR and WKY rats. No significant difference was found in either the maximal response or the EC_{50} values between these two populations. However, analysis of the individual IP's which contributed to the total revealed differences between SHR and WKY rats. NA (10^{-6}-10^{-3}M) increased inositol trisphosphate (IP_3) accumulation in both SHR and WKY rats. This increase was significantly greater at each effective concentration of NA in SHR rats, the maximum response being 2-fold greater. In contrast, NA (10^{-6}-10^{-3}M)-stimulated increases in both inositol bisphosphate (IP_2) and inositol monophosphate (IP_1) were not significantly different between SHR and WKY rats.

These results demonstrate an alteration in PPI metabolism in the tail artery of spontaneously hypertensive rats giving rise to an enhanced IP_3 accumulation in response to NA. Since IP_3 is involved in calcium mobilisation, an enhanced accumulation of this inositol phosphate could be partly responsible for the enhanced vascular contractile response to NA in the hypertensive state. This alteration in PPI metabolism may therefore be a factor in the development of the diseased state.

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535P

EFFECTS OF RESERPINE AND 6-HYDROXYDOPAMINE ON FIELD STIMULATION-INDUCED RESPONSES OF THE RAT ISOLATED TAIL ARTERY

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Field stimulation of the rat isolated tail artery produces a motor response that is predominantly noradrenergic but may also include a non-adrenergic component, mediated by adenosinetriphosphate (ATP) and possibly an unidentified "substance X" (Bao et al., 1989). Evidence for co-transmission in this blood vessel is indirect and is provided by experiments, in which alpha$_1$-adrenoceptor antagonists do not inhibit the field stimulation-induced responses completely but the residual response is blocked by alpha, beta-methylene adenosine triphosphate (mATP), which desensitizes purinoceptors. In such studies, where the smooth muscle is used to detect the released transmitter, the identification of the transmitter(s) depends on the specificity and concentration of the drugs used to block the various components of the motor response. This study re-examined the nature of neurotransmission in the rat tail artery by investigating the effects of drugs on field stimulation-induced responses of this vessel. Responses of arteries from rats pretreated with reserpine to deplete or 6-hydroxydopamine (6-OHDA) to destroy the sympathetic nerves were also examined.

Tail artery rings (3-4 mm) from Wistar rats were attached to fine stainless steel electrodes on a perspex rod. An initial resting tension of 1 g was applied to each ring, which was allowed to equilibrate for 2 hours in Krebs buffer (37°C, gassed with a 95% O_2/5% CO_2 mixture). Isometric contractions were obtained to field stimulation (0.5,2,20 Hz, pulse width 0.4 ms, 10 pulses, 70 V) or noradrenaline (NA, 0.01-100 μM), administered alone and in the presence of prazosin (0.1 μM), ATP (1-100 μM) or mATP (2 μM). Arteries from rats pretreated with 6-OHDA (2 x 25 mg kg^{-1} day 1 and 2 x 100 mg kg^{-1} day 3) were examined on days 4-5 and from rats pretreated with reserpine (0.5 mg kg^{-1} for 3 days) were examined on days 4-5.

In arteries from control rats, field stimulation produced motor responses that were abolished by tetrodotoxin (TTX, 0.1 μM) and prazosin (0.1 μM). ATP (1-100 μM) produced no response and mATP (2-4 μM) produced small contractions that quickly showed tachyphylaxis and did not affect field stimulation-induced responses. Field stimulation of arteries from rats pretreated with reserpine, either produced no response or caused contractions that were little affected by prazosin, mATP or TTX, suggesting that these contractions, like the similar contractions obtained in the denervated vessels from 6-OHDA-pretreated rats, were not neurogenic.

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536P

ADRENALINE AND NORADRENALINE ACTIVATION OF α_2-ADRENOCEPTORS IN HUMAN RESISTANCE ARTERIES

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Postjunctional α_2-adrenoceptors have been identified in human subcutaneous resistance arteries using the α_2-agonist BHT933 (Nielson 1989) and noradrenaline (NA) (Nielson 1990). This study sought to compare the actions of NA and adrenaline (Ad) and try to establish the relative contribution of α_1- and α_2-adrenoceptors to the contractile response.

In vitro studies were undertaken on human subcutaneous resistance arteries (internal diameter 160-550μm) in a microvascular isometric myograph. 13 resistance arteries were obtained from 11 patients undergoing surgery and mounted as ring segments bathed in Krebs solution at 37°C, bubbled with 95% O_2; 5% CO_2. The vessels were allowed to equilibrate and then set at a normalised internal circumference as previously described by Mulvany & Halpern (1977). They were then exposed to 118 mM potassium depolarising solution (KDS) and NA (10μM). Vessels which failed to produce an effective active pressure of 100 mmHg were discarded. Schild studies were performed, exposing the arteries to five sequential cumulative dose response curves (DRCs) to either NA or Ad in the presence of cocaine (1 μM) and propranolol (1 μM). Between each DRC there was an minimum incubation period of 10 minutes. The first DRC acted as a control to determine the reactivity to either NA or Ad. All subsequent DRCs took place in the presence of doxazosin (1 μM) to determine the presence of α_2-adrenoceptors. The 3rd to 5th DRC took place in increasing concentrations of yohimbine (30, 100, & 300 nM). pA_2 and pD_2 values were determined to establish potency.

Doxazosin reduced the maximal contraction to NA by 53 ± 11% and the maximum to Ad by 40 ± 16%. Control pD_2 values for NA and Ad were 6.7 ± 0.4 and 7.0 ± 0.4 respectively. In the presence of doxazosin these were slightly reduced to 5.9 ± 0.8 and 6.5 ± 0.4. Schild plot analysis calculated pA_2 values for yohimbine against NA as 8.3 and against Ad as 7.9. (The percentage reductions in the maximum are expressed as means ± s.e.m.)

The equivalent yohimbine pA_2 values indicate that NA and Ad activate α_2-adrenoceptors equally in human resistance arteries. The small effect of doxazosin on the pD_2 values suggests that the majority of the reponse to NA and Ad is due to α_2-adrenoceptors in arteries of this size. It was noted that doxazosin elicited an apparent non-competitive action as it did not shift the DRCs to the right but reduced the maximum.

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537P

FURTHER INVESTIGATION OF SUBTYPES OF PRE- AND POSTJUNCTIONAL α_2 ADRENOCEPTORS IN THE PERIPHERY

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We have recently shown that the prejunctional alpha-2 adrenoceptors of rat vas deferens and rat atrium resemble the alpha-2 ligand binding sites in human platelet and rat kidney, respectively (Connaughton & Docherty, 1990), and can therefore be tentatively identified as alpha-2A and alpha-2B, respectively (Bylund, 1988). We now investigate subtypes of functional alpha-2 adrenoceptors prejunctionally in rat submandibular gland and postjunctionally in human saphenous vein.

In rat isolated submandibular gland, antagonist potency was assessed as the ability to produce 30% potentiation of stimulation-evoked overflow of tritium (EC_{30}) in tissues pre-incubated with [3H]-noradrenaline. In human saphenous veins obtained from varicose vein surgery, antagonist potency was assessed as a K_B or pA2 from the shift in the contractile potency of noradrenaline.

In rat submandibular glands, yohimbine and ARC 239 had EC_{30} values of 7.35±0.88 and 5.95±0.95 (-log M, n=3 each), respectively, while prazosin (1μM) significantly increased the basal outflow of tritium so that an EC_{30} could not be calculated but was <6.5. The low potency of ARC 239 and prazosin, both absolute and relative, suggest that the receptor resembles the alpha-2A subtype.

In human saphenous vein, yohimbine and prazosin had pA2's of 7.40 (95% limits of 6.75-8.03) and 6.45 (5.83-7.59) against noradrenaline, while ARC 239 had a K_B of 7.54±0.98. The high potency of ARC 239 and prazosin seem to rule out the possibility that this receptor resembles the alpha-2A subtype. However, the correlation for a series of 9 antagonists between antagonist K_B in human saphenous vein and antagonist Ki at alpha-2B binding sites in rat kidney yielded a relatively poor correlation (slope=0.52, r=0.65, n=9), so that these receptors do not seem to resemble the alpha-2B binding site.

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538P

8-OH-DPAT DISCRIMINATES BETWEEN SUBTYPES OF α_2 ADRENOCEPTOR

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The 5-HT-1A receptor ligand 8-OH-DPAT (Middlemiss & Fozard, 1983) has been reported to be an alpha-2 adrenoceptor antagonist in guinea-pig submucous nerves (Crist & Surprenant, 1987). We have examined the actions of 8-OH-DPAT at subtypes of alpha-2 adrenoceptor.

In ligand binding studies, Ki values were obtained for the displacement by 8-OH-DPAT and other agents of 3H-yohimbine binding to human platelet (alpha-2A) and rat kidney (alpha-2B) membranes (see Connaughton & Docherty, 1990). In functional studies, the actions of 8-OH-DPAT at prejunctional alpha-2 adrenoceptors in rat atrium were assessed as an EC_{30} (concentration producing 30% increase of stimulation-evoked release of tritium in tissues pre-incubated with 3H-noradrenaline, and actions at postjunctional alpha-2 adrenoceptors were assessed as a K_B from the shift in the contractile potency of noradrenaline in human saphenous vein.

Yohimbine had similar affinity for the alpha-2A ligand binding sites of human platelet and the alpha-2B ligand binding sites of rat kidney, with Ki values of 8.04+0.10 and 7.93+0.07, respectively, whereas 8-OH-DPAT showed higher affinity for alpha-2B (6.41+0.08) than for alpha-2A (5.44+0.04).

In rat atrium, yohimbine and 8-OH-DPAT had prejunctional EC_{30} values of 7.89+0.05 and 6.37+0.17, so that 8-OH-DPAT was approximately 30 times less potent than yohimbine. In human saphenous vein, yohimbine and 8-OH-DPAT had K_B values (-log M) of 7.37 and 5.40 so that yohimbine was approximately 100 times more potent than 8-OH-DPAT.

In conclusion, 8-OH-DPAT shows moderate potency as an antagonist at prejunctional alpha-2 adrenoceptors in rat atrium and as a ligand at alpha-2B ligand binding sites, and so shows selectivity for alpha-2B over alpha-2A ligand binding sites.

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539P

β-INHIBITION OF SPONTANEOUS RHYTHM BUT NOT CONTRACTILITY IN SMOOTH MUSCLE OF THE SHEEP LYMPH NODE

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Stimulation of β-adrenoceptors in the smooth muscle of lymphatic ducts decreases the frequency and force of spontaneous contractions (Allen et al, 1986). This is evident either when the intramural nerves are stimulated in the presence of an α-adrenoceptor antagonist or when isoprenaline is applied alone (McHale et al, 1980). Similarly intravenous isoprenaline in conscious sheep depresses lymph flow by inhibiting lymphatic pumping (McHale & Thornbury, 1986). Sheep lymph nodes are also spontaneously contractile and are known to possess a noradrenergic innervation (Thornbury et al, 1990). It was the purpose of this study to assess the role of β-adrenoceptors in the modulation of both spontaneous and evoked contractions in the smooth muscle of the lymph node capsule.

Strips 2mm wide and 30mm long were dissected from the hilar region of sheep para-intestinal lymph nodes. Measurements were made of isometric tension in a water-jacketed organ bath maintained at 37°C and continually perfused with Krebs solution gassed with 5% CO_2, 95% O_2. Spontaneous contractility was rather less regular than in lymphatic vessels but when isoprenaline 10^{-7}M was added to the perfusate frequency of contraction decreased in a manner similar to that found in lymph ducts. In a summary of seven such experiments mean frequency was approximately halved during drug addition (from 9.4 ± 1.1, SEM, before to 5.3 ± 1.3) and remained depressed for up to 30 min thereafter. In contrast isoprenaline in doses as high as $3x10^{-7}$M did not relax contractures induced by increasing external K+ and indeed at higher doses caused a contraction. Neither could any evidence be found of β-inhibition when the intramural nerves were stimulated by an applied electric field since the response to the latter stimulus was not significantly potentiated by 10^{-6}M propranolol. These results suggest that β-adrenoceptors modify pacemaking in sheep nodal smooth muscle without depressing contractility. This is in contrast to the response in bovine lymph vessels where both are depressed.

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540P

EFFECTS OF PROPAFENE ON ^{45}Ca MOVEMENTS AND CONTRACTILE RESPONSES IN RAT ISOLATED VASCULAR SMOOTH MUSCLE

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Propafenone (P) is a class Ic antiarrhythmic drug which also inhibited the slow inward Ca current in cardiac tissues (Delgado et al., 1985). However, the possibility that the calcium antagonist properties of P may be responsible for the relaxation of vascular smooth muscle has not been studied yet. Therefore, the present study was undertaken to examine the effects of P on contractile responses and ^{45}Ca uptake in isolated rat aortic strips and portal veins.

Aortic strips and vein segments from Sprague-Dawley rats were incubated in Krebs-Henseleit solution (KS). Techniques employed to estimate ^{45}Ca uptake in aortic strips were as previously described by Barrigón & Tamargo, 1986.

P, 10^{-7}M and 10^{-4}M, inhibited the spontaneous myogenic activity in portal vein segments. Thus, at 10^{-5}M, P inhibited the amplitude of contractions by 44.6 ± 9.9% (n = 7) and at 5 x 10^{-5}M it suppressed the spontaneous activity. In aortic strips P, 10^{-7}M - 10^{-4}M, inhibited the contractions induced by high K (80 mM) and noradrenaline (NA, 10^{-5}M), the IC_{50}s values for P depression being 2.5 ± 0.7 x 10^{-6}M and 8.7 ± 0.8 x 10^{-6}M, respectively. Contractile responses induced by addition of Ca (1-5 mM) to 0Ca high-K KS were also inhibited by P (IC_{50} = 2.5 ± 0.8 x 10^{-6}M). In another group of experiments, the effects of P were studied on the phasic contractile responses induced by NA in strips previously incubated in 0Ca KS, then to 2.7 mm Ca KS ("Ca-loading") for 5 min and finally in 0Ca KS for 2-3 min. At 10^{-6}M and 5 x 10^{-6}M, P when applied after "Ca-loading" inhibited by 29.7 ± 3.3% ($p < 0.001$) and 81.0 ± 1.7% ($p < 0.001$), respectively, the magnitude of the NA-induced phasic contraction. At 5 x 10^{-6} M and 10^{-5}M, P significantly decreased ^{45}Ca uptake stimulated either by KCl from 0.523 ± 0.02 to 0.378 ± 0.01 ($p < 0.05$) and 0.299 ± 0.18 mmol/kg aorta ($p < 0.001$) and by NA from 0.506 ± 0.024 to 0.361 ± 0.013 ($p < 0.01$) and 0.279 ± 0.012 mmol/kg aorta ($p < 0.001$). However, P had no effect on ^{45}Ca uptake in resting, non-stimulated aortic strips.

These results indicated that in isolated rat aortae and portal vein P inhibited Ca entry through voltage- and receptor-operated channels as well as Ca release from intracellular stores. As a consequence it would reduce the concentration of intracellular free Ca available at the contractile apparatus for vascular smooth muscle contraction.

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541P

EFFECTS OF NAFENODONE ON GUINEA-PIG ATRIAL AND VENTRICULAR MUSCLE FIBRES

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Cardiotoxicity is frequently observed after overdosage and during therapeutic use of tricyclic antidepressants. In an effort to minimize undesirable cardiovascular effects, new antidepressants have been recently developed. The present study was made to determine the effects of nafenodone (N) on the electromechanical properties of isolated guinea-pig atrial and ventricular muscle fibres.

Guinea-pig atria and papillary muscles were incubated in Tyrode solution. Transmembrane action potentials were recorded through glass microelectrodes. Slow responses were elicited by isoprenaline (10^{-6}M) or histamine (3 x 10^{-5}M) in fibres incubated in 27 mM K solution.

In right atria N, 10^{-8}M - 10^{-4}M, decreased the rate (IC_{50} : 1.1 ± 0.8 x 10^{-5}M) and amplitude of contractions (IC_{50} : 5.2 ± 0.8 x 10^{-6}M) and lengthened the sinus node recovery time. In left atria driven at 1 Hz, N, imipramine (IMI) and desipramine (DMI) caused a negative inotropic effect, the IC_{50}'s being 1.0 ± 0.8 x 10^{-5}M, 1.0 ± 0.2 x 10^{-6}M and 2.9 ± 0.8 x 10^{-6}M, respectively.

At concentrations $> 10^{-6}$ M N had no effect on resting membrane potential but it decreased ($p < 0.05$) the amplitude and Vmax of the upstroke both in atrial and ventricular muscle fibres. In atrial fibres N at concentrations $\geq 10^{-5}$M lengthened ($p < 0.05$) the action potential duration (APD). The lengthening of the APD was accompanied by a significant prolongation of the effective refractory period (ERP). In ventricular muscle fibres N slightly shortened the APD values ($p > 0.05$), while the ERP was slightly prolonged and thus, at 10^{-5}M and 5 x 10^{-5}M it increased the ERP/APD ratio. IMI and DMI were more potent to inhibit amplitude and Vmax of the action potential and following exposure to 5 x 10^{-5} M of both drugs atrial and ventricular fibres became inexcitable.

N shifted the dose-response curve of Ca downwards and to the right and decreased the amplitude of the slow contractions induced by histamine (IC_{50} : 4.5 ± 0.7 x 10^{-6}M). At concentrations between 10^{-5} M and 10^{-4} M it reduced the amplitude and Vmax and shortened the APD of the slow action potentials induced by isoprenaline, while exposure of ventricular fibres to 5 x 10^{-5}M IMI and DMI suppressed the slow action potentials and contractions.

In conclusion, the present results indicated that N exerted in isolated guinea-pig atria and ventricular muscle fibres less cardiodepressant effects than IMI and DMI.

542P

THE DEVELOPMENT OF AN *IN VITRO* ANIMAL MODEL TO EXAMINE MYOCARDIAL NORADRENALINE UPTAKE: PRELIMINARY RESULTS USING A SHORT DURATION ANGIOTENSIN II INFUSION

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Previous in vitro studies have shown that angiotensin II (AII) inhibits myocardial noradrenaline (NA) uptake (Peach et al, 1969). More recently Sumners and Raizada (1986) reported that AII had a biphasic effect on NA uptake, being stimulatory in the short term and inhibitory in the long term. As angiotensin converting enzyme inhibitors have been shown to lower plasma NA and prolong patient survival in chronic heart failure (Cleland et al, 1984) our aim was to develop an in vivo animal model to examine the effect of AII on myocardial NA uptake.

Male Sprague-Dawley rats (250-400g) were anaesthetized with Urethane (25% w/v; 6ml/kg) and jugular, femoral and carotid catheters inserted. The carotid catheter was connected via a transducer to a chart recorder to allow monitoring and recording of both heart rate and blood pressure. After anaesthesia the rats received a bolus dose of [^{3}H]-NA (10 µCi/kg) again through the jugular line, the rats were then sacrificed by anaesthetic overdose at varying times (1, 2, 5 10, 20 mins) after the bolus of [^{3}H]-NA. The hearts were then removed, blotted, weighed and homogenized in ice-cold 0.4N perchloric acid before counting in the scintillation counter. In order to test that the model was capable of detecting uptake inhibition by known uptake inhibitors, preliminary experiments were performed in which the rats received 2mg/kg of both desipramine (an uptake-1 inhibitor) and corticosterone (an uptake-2 inhibitor). This was administered in a bolus injection through the jugular line thirty minutes prior to the [^{3}H]-NA. In experiments where AII was used, the AII was infused through the femoral line for 10 minutes before the bolus [^{3}H]-NA was given and continued until the animal was sacrificed 3.5 mins later.

The uptake of NA was significantly ($p<0.001$) inhibited in the presence of desipramine and corticosterone showing that the model was valid and capable of detecting uptake inhibition by known uptake inhibitors. The addition of the blockers reduced the uptake from 186329 ± 7465 dpm/g wet weight tissue to 48852 ± 10846 after 5 minutes. In the experiments with short duration AII infusions we found that a low subpressor dose (20 ng/kg/min) produced no significant change in the myocardial uptake of NA over the control situation (saline only infused). However when a higher (pressor) dose of AII (80 ng/kg/min) was infused a significant ($p<0.0001$) increase in the amount of [^{3}H]-NA taken up by the heart was found. The level of [^{3}H]-NA rose from 138608 ± 4350 dpm/g wet weight tissue in the control to 239698 ± 19965 in the 80 ng/kg/min infusion. This increase in the myocardial uptake is likely to result from the increased afterload placed on the heart by the pressor effect of AII. The increased afterload may cause the [^{3}H]-NA to be retained within the heart cavity for a longer period of time before expulsion thereby allowing more [^{3}H]-NA to be taken up by the myocardium.

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543P

EFFECT OF LEUKOTRIENE D_4 ON THE RESPONSES OF CYSTEINYL LEUKOTRIENES AND U46619 IN GUINEA-PIG HEART

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The cysteinyl leukotrienes (LTs), C_4 and D_4, are potent vasoconstrictors of the vasculature supplying the cardiac muscle. If LTs have a role in coronary vascular disease in man, antagonists selective against LTs in the coronary vessels may be of therapeutic benefit.
We have investigated the actions of four new LT antagonists which are selective for LTD_4 in guinea-pig (GP) airways: ICI 198,615 (Snyder *et al.* 1987), SK+F 104,353 (Hay *et al.* 1987), CGP 45715A (personal communication), MK-571 (Jones *et al.* 1989) on LT-induced responses in GP isolated hearts.

Hearts from male Dunkin-Hartley GPs (350-450g) were perfused using the modified Langendorff technique with Krebs buffer gassed with 95% O_2, 5% CO_2.The hearts were perfused at a constant pressure (PP) of 40mmHg and the variable coronary flow (CF) was measured by means of a drop counter. The coronary vascular resistance (CVR) was determined by: peak PP/peak CF - basal PP/basal CF. Bolus doses of the agonists: LTD_4, LTC_4, U46619 were given and the antagonists were continually present in the perfusing buffer.

Both ICI 198,615 (3-30nM) and SK+F 104,353 (10-100nM), at similar concentration ranges to those required to inhibit the effects of LTD_4 in GP airways, antagonised the cysteinyl LT-induced vasoconstrictor responses in GP isolated heart. However, ICI 198,615 selectively antagonised only the responses to LTD_4 whereas both the LTD_4- and LTC_4-induced vasoconstriction was inhibited by SK+F 104,353. No effect was observed by either compound on the responses to U46619. CGP 45715A (30-100nM) and MK-571 (5.5-18.5µM) appeared to have no specific antagonistic effect against LTs in the GP heart, with the responses to U46619 also being inhibited. MK-571 had a greater effect on the responses induced by U46619 than those observed with the cysteinyl LTs.

Therefore, these LTD_4 receptor antagonists have a different spectrum of activity in the GP heart from that observed in the GP airways.

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544P

EFFECTS OF SIMVASTATIN ON PLASMA CHOLESTEROL AND FAT DEPOSITION AND ENDOTHELIUM DAMAGE IN LARGE ARTERIES FROM CHOLESTEROL-FED RABBITS

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Hypercholesterolemia is a major risk factor for cardiovascular disease. The HMG CoA-reductase inhibitor simvastatin (S) lowers plasma cholesterol in man (Mol et al, 1986) and attenuates cholesterol deposition in large arteries in animals (Ishida et al, 1990).

The aim of this study was to compare effects of S on plasma cholesterol, fat deposition and endothelium damage in arteries from cholesterol-fed rabbits. Animals were fed a 0.3% cholesterol diet plus 0 (controls) 1, 5 or 10 mg/kg/day S. Plasma cholesterol and triglyceride, mean arterial pressure (MAP), heart rate (HR) and weight were measured pretreatment and after 2, 4 and 8 weeks. Animals were killed and thoracic aorta and mesenteric artery removed for staining with sudan red for fat deposition and toluidene blue for endothelium damage. Similar increases in MAP and weight were observed in all groups. HR was slightly but significantly decreased in rabbits fed S, 5 and 10 mg/kg/day, being 192±18, 183±22, 178±19 and 177±15 beats/min at 8 weeks in the 0,1,5 and 10 mg/kg/days groups respectively.

There was a dose-related decrease in plasma cholesterol in S treated animals which reached significance with 10 mg/kg/day. Triglyceride levels were not altered by S. Both plasma cholesterol and fat deposition in the arteries varied widely between rabbits and although sudan red staining appeared to be reduced in the aorta from S treated rabbits, this did not reach statistical significance. There were no treatment effects on sudan red staining in mesenteric artery. In contrast toluidine blue staining was significantly reduced in both aorta and mesenteric artery with 10 mg/kg/day S. There was no relationship between plasma cholesterol and sudan red or toluidene blue staining in individual animals. Neither plasma cholesterol nor lipid deposition was related to weight.

TABLE: Effect of S on plasma cholesterol, sudan red and toluidene blue staining.

	Plasma cholesterol mmol/L		% sudan red		% toluidene		
S mg/kg/day	0 weeks	8 weeks	aorta	mesenteric	aorta	mesenteric	
0(n=7)	1.0±0.3	20.0±8.7	46±28	26±14	61±23	58±25	
1(n=4)	0.9±0.1	18.0±3.5	30±18	21±20	52±12	37±12	mean ± SD. * significantly lower
5(n=7)	0.8±0.2	14.8±11.7	26±28	15± 9	52±18	31±19	than controls using ANOVA ($p < 0.05$)
10(n=6)	0.9±0.5	*6.1±4.4	16±14	22± 5	*25± 7	*17±13	

In summary S attenuated the increase in plasma cholesterol and endothelium damage in cholesterol-fed rabbits. However there was a poor correlation between plasma cholesterol and fat deposition or endothelium damage in arteries from individual rabbits.

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545P

EFFECT OF MORPHINE AND RUTHENIUM RED ON A C-FIBRE EVOKED BLOOD PRESSURE REFLEX IN THE ANAESTHETISED RAT

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Close intra-arterial injection of capsaicin into the rat hind paw produces a reproducible fall in blood pressure (Donnerer and Lembeck, 1983) mediated by excitation of polymodal C-units which can be excited repetitively at low doses (Szolcsanyi, 1988). In the present study we investigated the effect of morphine infusion on this reflex and subsequent reversal with naloxone. We also studied the effect of Ruthenium Red, an inorganic dye which inhibits transmembrane and mitochondrial Ca^{++} transport and antagonises capsaicin-induced Ca^{++} uptake in dorsal root ganglion cells (Wood et al, 1988) and also protects sensory fibres from capsaicin desensitisation (Maggi et al, 1988).

Sprague Dawley rats were anaesthetised with sodium pentobarbitone (50mg/kg, i.p.). The left carotid artery was cannulated for measurement of blood pressure and the left epigastric artery cannulated retrogradely for injections into the femoral artery. Morphine was infused via a jugular cannula and in some preparations the contralateral femoral vein was cannulated for control injections. Injection of capsaicin (0.1nmol, intra-arterially) every 10 minutes caused a reproducible fall in blood pressure over several hours. At this dose it produced no systemic effects when injected intravenously. In confirmation of the reflex nature of the response section of the saphenous and sciatic nerves or application of local anaesthetic (10% procaine) abolished the depressor response. Infusion of morphine (75µM, 100µl/min i.v. for 1.75h) blocked the capsaicin evoked depressor reflex. Subsequent infusion of naloxone (1mM, 100µl/min) reversed this effect within 20 minutes. Ruthenium Red (10nmol) when co-injected with capsaicin abolished the reflex. It was ineffective at a dose of 1nmol.

The efferent pathway of this reflex consists of a loss of sympathetic vasoconstrictor tone with the reflex centre located in the brain stem (Donnerer and Lembeck, 1983). Morphine is most probably acting at the level of the spinal cord to inhibit substance P release from central terminals of sensory fibres (Donnerer, 1989). The Ruthenium Red block of the capsaicin-evoked response in the in vivo preparation described here is consistent with previously reported findings with in vitro preparations or in the isolated perfused rabbit ear (Amman and Lembeck, 1989). This block is specific for capsaicin as the response to bradykinin or acetylcholine is unaffected (Amman and Lembeck, 1989). This preparation therefore provides a useful in vivo model for the study of capsaicin and capsaicin analogues.

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546P

PHARMACOKINETICS AND METABOLISM OF 4-AMINOPROPIOPHENONE IN THE RAT

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4-Aminopropiophenone (PAPP) is a methaemoglobin forming cyanide antidote (Bright, 1987) whose action is reputedly dependent on the formation of 4-(N-hydroxyamino)propiophenone (Graffe et al, 1964).

In the studies ^{14}C-carbonyl labelled PAPP was used in male rats and ring-labelled PAPP was used in female rats. For excretion studies 5 mg/kg PAPP was administered orally to 4 male and 4 female rats housed singly in metabolism cages for separate collection of urine and faeces. Urine was collected over the first 6 h and subsequent 18 h after dosing and faeces and expired air over the first 24 h period. Urine, faeces and expired air were then collected over 24 h periods until 120 h had elapsed from dosing. At 120 h animals were killed by cervical dislocation, the cages were washed and washings, carcasses, urine and faeces stored at -20°C. For estimation of plasma concentrations, PAPP (5 mg/kg) was administered to separate groups of 4 male and 4 female animals. Blood was sampled at 15 min intervals for 1 h and then at 2,3,5,7,10,30,48,72,96 and 120 h thereafter. The samples were centrifuged in heparinised tubes and then stored at -20°C. Total radioactivity was measured in the collected samples and the chemical nature of radioactive components in plasma, erythrocytes, urine and faeces was studied using a combination of TLC, HPLC, mass spectrometry and proton magnetic resonance spectroscopy (60 MHz) to elucidate the metabolism of PAPP.

In the excretion studies, males and females excreted 57.6±11.4% and 46.4±10.3% of the dose respectively in the urine in 6 h and 24.4±10.6% and 42.1±11.7% in the next 18 h. Over the 120 h period of the study, 83.7±0.4% and 92.2±4.1% was excreted in the urine of males and females (results $\bar{x}$±SD). The remaining dose was found in the faeces and expired air. Peak levels of plasma radioactivity were seen at 15 min in the males and 1 h in the females. 4 major components were seen in methanolic extracts of rat plasma: about 10% of the radioactivity was accounted for by unchanged PAPP, 40% N-acetyl PAPP and 25% N-acetyl-p-aminobenzoic acid. Additional, chromatographically very polar material could not be identified. Urine contained 3 major components, PAPP (10%) and N-acetyl-p-aminobenzoic acid 60%. Polar material, accounting for about 30% was shown by enzyme treatment to be in part a conjugate of PAPP. A minor component in both sexes was N-acetyl PAPP, while N-acetyl-p-aminophenol was a minor component in the urine of females; it was not observed in males due to the position of the radiolabel. It was concluded that the major route of metabolism in the rat was N-acetylation.

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547P

COMPARISON OF THE HEPATIC CLEARANCE OF (±) RP 49356 AND ITS ENANTIOMERS IN THE ISOLATED PERFUSED LIVER

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The extent of hepatic clearance of the racemate RP 49356 1RS,2RS - [N-methyl-2 (3-pyridyl) -2-tetrahydrothiopyran - carbothioamide-1-oxide], a novel antihypertensive agent, and its enantiomers (-) RP 52891 and (+) RP 61499 has been studied in the isolated perfused rat liver. A perfused liver can be a suitable model for determining differences in the hepatic clearance of a racemate and its enantiomers and for predicting the extent of first pass metabolism in vivo (Silver and Dauterman, 1989)

Isolated livers were prepared by the method of Miller (1973). After a 60 minute equilbration period, compounds (3 mg in DMSO) were added to the perfusate. Thereafter, sampling of perfusate occurred over a period of 120 minutes. Samples were centrifuged and the supernatants stored at -20°C until analysed.

Samples were initially assayed by a non-chiral h.p.l.c. method with internal standardisation, following extraction into dichloromethane containing 1% IPA at neutral pH. Extracts were evaporated to dryness under nitrogen at 50°C and reconstituted in 100µl of the mobile phase (methanol:water, 1:1 v/v) for analysis. The analytical column was a 250 x 4.6mm LiChrosorb 'Select B' cartridge (5µm, C8) protected by a 4 x 4mm C18 (7µm) guard cartridge. A flow rate of 1.0 ml/min was used and the compounds were detected by their U.V absorption at 275 nm. The racemisation of the enantiomers and any changes in the isomer ratio of the racemate with time were investigated using a chiral h.p.l.c. method which utilised a 250 x 4.6mm cellulose triphenylcarbamate column with a flow rate of 1.0 ml/min (mobile phase - IMS:hexane, 3:7 v/v).

Low hepatic clearance of (±) RP 49356, (-) RP 52891 and (+) RP 61499 occurred giving mean values of 0.024 ± 0.003, 0.0027 ± 0.003 and 0.019 ± 0.003 ml $min^{-1}g^{-1}$ liver respectively. The values were calculated for periods of equivalent bile flow rate (0.6 - 0.85 µl min^{-1} g^{-1} liver) and revealed that the (-) enantiomer was removed from the perfusate at a rate about 30% greater than the (+) enantiomer. Mean half-lifes for the removal of RP 52891 and RP 61499 were 195 and 300 minutes respectively. At the end of each perfusion with RP 49356, there was a mean decrease of about 14% in the quantity of RP 52891 present in the perfusate. As anticipated on structural grounds, there was no interconversion of RP 52891 and RP 61499.

It can be concluded that RP 49356 is likely to undergo some degree of stereoselective hepatic clearance which could influence its pharmacological activity and duration of action.

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548P

AMELIORATION OF ACUTE RENAL FAILURE WITH 8-CYCLOPENTYL-1, 3-DIPROPYLXANTHINE: DOSE DEPENDENCY

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8-Cyclopentyl-1, 3-dipropylxanthine (CPX) is a selective A_1 adenosine antagonist *in vivo* and at a dose of 0.1 mg kg^{-1} it ameliorates glycerol-induced acute renal failure (ARF) in the rat (Kellett *et al*, 1989). In this study the beneficial effects of a range of CPX doses were compared. ARF was induced in male Wistar rats by an i.m. injection of 50% glycerol in saline (10 ml kg^{-1}) after which animals were treated with CPX (0.03, 0.1 or 0.3 mg kg^{-1} i.v.) and treatment was repeated 12, 24 and 36h later. An additional group of rats was injected with glycerol and received no further treatment. Renal function was assessed by measuring plasma urea and creatinine levels and clearances of [^{3}H]-inulin (C-IN) and [^{14}C]-p-aminohippurate (C-PAH).

TABLE 1

	Plasma Urea (mg dl^{-1})		Plasma Creatinine (mg dl^{-1})		C-IN (ml min^{-1} $100g^{-1}$)	C-PAH (ml min^{-1} $100g^{-1}$)
	0h	48h	0h	48h	48h	48h
Group 1 No treatment	38 ± 2	247 ± 18	0.66 ± 0.08	4.51 ± 0.70	0.24 ± 0.05	0.89 ± 0.14
Group 2 CPX (0.03 mg kg^{-1})	59 ± 2	214 ± 29	0.55 ± 0.02	2.53 ± 0.52	0.35 ± 0.06	1.16 ± 0.18
Group 3 CPX (0.1 mg kg^{-1})	45 ± 1	97 ± 10^{**+}	0.62 ± 0.02	1.11 ± 0.12**	0.54 ± 0.06*	1.84 ± 0.20**
Group 4 CPX (0.3 mg kg^{-1})	49 ± 1	103 ± 13^{**+}	0.59 ± 0.02	1.14 ± 0.17**	0.66 ± 0.09^{**+}	1.94 ± 0.15^{**+}

Mean ± s.e. mean (n = 12); * P < 0.05; ** P < 0.01 relative to group 1; + P < 0.01 relative to group 2 (ANOVA).

Rats given 0.03 mg kg^{-1} CPX showed small improvements in renal function, but these changes were not statistically significant when compared to the untreated group (Table 1). By contrast, treatment with either 0.1 or 0.3 mg kg^{-1} CPX significantly improved renal function. However, there was no appreciable difference in the beneficial effects afforded by 0.1 or 0.3 mg kg^{-1} CPX. These results show that in glycerol-induced ARF the protective effect produced by CPX is restricted to a narrow dose range.

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549P

MODULATION OF INTERFERON ALPHA 2c RECEPTOR EXPRESSION IN HUMAN BREAST CANCER CELL LINES BY ESTRADIOL, PROGESTINS AND ANTI-PROGESTINS

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Recombinant interferon alpha 2c (IFN) increases estrogen receptor (ER) expression in ZR-75-1 human breast cancer cells and sensitises them to the anti-proliferative effects of the anti-estrogen tamoxifen, (van den Berg et al 1987). We have been unable to demonstrate induction of ER in a tamoxifen resistant, ER negative, variant line, ZR-75-9a1, (van den Berg et al 1989). In this study we have investigated the influence of estradiol, progestins and anti-progestins on IFN receptor expression. We have also examined the relationship between IFN receptor and steroid hormone receptor expression in ZR-75-1 cells, the tamoxifen resistant variant and an estrogen independent subline (ZR-PR-LT). The latter line lacks binding sites characterisic of the Type 1 ER but expresses elevated levels of progesterone receptor (PGR), (van den Berg et al 1990).

IFN was labelled with 125-Iodine using the Iodogen reaction and purified by HPLC to a specific activity of 298Ci/mmol. Binding of 125-I IFN to whole cells at 4^{O}C was determined in the presence and absence of excess cold IFN. ZR-75-1 cells contained 1498±541 IFN receptors/cell which fell to 802±447 receptors/cell during a 5 day exposure to 10-9M estradiol (E2). This E2 induced reduction in IFN receptor expression was accompanied by a 6-fold increase in PGR concentration. There was no significant reduction in IFN receptor expression in ZR-PR-LT cells associated with their elevated basal PGR content. However, a 6 day exposure of ZR-LT-PR cells to the progestin Medroxyprogesterone acetate or the anti-progestin RU.38.486, which results in a marked down regulation of PGR in these cells, also results in a 30 -50% increase in IFN receptor expression. A similar inverse relationship between IFN and PGR receptor expression is apparent in the ZR-75-9a1 line which lacks PGR and expresses the highest concentration of IFN receptors (3443±389 sites/cell). This increased receptor expression is not associated with increased sensitivity of the tamoxifen resistant cells to the anti-proliferative effects of IFN.

We conclude that E2 and IFN appear to have opposite effects on the expression of each others' receptor in ZR-75-1 cells suggesting that an ER/PGR/IFN receptor axis exists.This proposal is supported by the observations that downregulation of PGR in the estrogen independent cell line is accompanied by an increase in IFN receptor expression and that IFN receptor content is highest in the tamoxifen resistant cell line lacking detectable PGR. The failure of IFN to induce ER in this line is not the result of failure to express IFN receptor.

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PROGESTIN/ANTI-PROGESTIN ACTION TOWARDS HUMAN BREAST CANCER CELL LINES DIFFERING IN THEIR PROGESTERONE RECEPTOR CONTENT

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Progestins such as Medroxyprogesterone acetate and Megestrol acetate have been traditionally used as second or third line treatment in hormone responsive breast cancer, although response rates have been reported equal to those for the anti-estrogen tamoxifen in unselected groups of patients. Recently novel anti-progestins such as RU 38.486 (Mifepristone) and ZK 98.299 have shown promising activity in experimental breast cancer, (Schneider et al 1989). The mechanisms by which progestins/anti-progestins exert their direct anti-proliferative effects on breast cancer cells are poorly understood.

We have studied the effects of the progestins Medroxyprogesterone acetate (MPA) and ORG 2058 and the anti-progestins RU 38.486 and ZK 98.299 on progesterone receptor (PGR) expression by and proliferation of ZR-75-1 human breast cancer cells and variants differing in their steroid hormone receptor profiles. The relative affinities of progestins/anti-progestins for PGR in an oestrogen (E2) independent variant of the ZR-75-1 line (ZR-PR-LT, van den Berg et al 1990) expressing high basal levels of PGR were: ORG 2058 = 100, MPA = 200, RU 38.486 = 44, ZK 98.299 = 30. A 5 day exposure of ZR-75-1 cells to 10-9M E2 in the presence of 10-9M MPA resulted in an almost complete blockade of E2 induction of PGR, whilst RU 38.486 (10-9M) lowered induced PGR concentration by 50%. ZK 98.299 (10-9M) had no effect on E2 induction of PGR. Progestins/anti-progestins also showed marked differences in their ability to down regulate PGR in the estrogen independent variant, ZR-LT-PR. MPA and RU 38.486 were equipotent, reducing PGR levels by 50% at a concentration of 10^{-10}M, (IC_{50}). IC_{50}s for ORG 2058 and ZK 98.299 were $5x10^{-7}$M and 10^{-6}M respectively. MPA was the most potent anti-proliferative agent in all cell lines tested, but the ZR-PR-LT line was only marginally more sensitive than the parent line despite expressing a 20-fold higher basal PGR concentration. Surprisingly, the growth inhibitory response to MPA, RU 38.486 and ZK 98.299 was greatest in a tamoxifen resistant variant (ZR-75-9a1, van den Berg et al 1989) lacking detectable PGR.

These results suggest that there is a poor relationship between affinity for PGR and ability to down-regulate the receptor - an indication of receptor activation. The reason for the marked differences in the ability of these agents to down-regulate PGR is not known, but it is of interest that the two most potent agents, MPA and RU 38.486, possess intrinsic glucocorticoid/anti-glucocorticoid activity respectively. Our results further suggest that PGR status will not accurately predict anti-proliferative response to these agents.

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TAMOXIFEN INHIBITS THYROTROPIN RELEASING HORMONE-INDUCED EXPRESSION FROM A MINIMAL RAT PROLACTIN PROMOTER

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Earlier studies from this laboratory (Whelan and Martin, 1987) indicated that the triphenylethylene antioestrogen, tamoxifen, suppressed oestradiol induced increases in prolactin mRNA levels in rat pituitary tumour cells in culture (GH_3 cells). In addition, these studies demonstrated that tamoxifen also suppressed thyrotropin releasing hormone (TRH) induced increases in prolactin mRNA levels. These findings suggested that tamoxifen may have some activities that are not mediated through the oestrogen receptor. Other groups (eg. Lam (1984)) have also demostrated that tamoxifen can impair the action of elements of signal transduction cascades. We have now sought to demonstrate the inhibition of TRH induction of expression from a rat prolactin gene promoter construct that is nonresponsive to oestrogen, after transfection into GH3 cells. GH3 cells were maintained in monolayer culture. They were transfected with plasmids containing rat prolactin gene promoter fragments linked to a reporter gene encoding the bacterial enzyme, chloramphenical acetyl transferase (CAT). The ability of hormones to induce expression from the rat prolactin promoter fragments was analysed by measuring the levels of CAT enzyme activity in GH_3 cell extracts at the end of the treatment period (48 h). All procedures have been previously described (Schuster et al., 1988).

When transfected into GH_3 cells, a construct prPrl(-1953)CAT containing the most proximal 1.95 Kb of prolactin promoter sequence was induced by oestradiol (10^{-8} M), as judged by expression of CAT enzyme ($p < 0.05$). The induction was suppressed by tamoxifen (10^{-5} M) ($p<0.05$). An identical construct containing only the most proximal 75 bp of the rat prolactin gene promoter (prPrl (-75) CAT) was no longer inducible by oestrogen. Expression from prPrl (-75) CAT was, however, significantly, induced by TRH (10^{-7} M) ($p<0.05$) and this induction was completely suppressed by tamoxifen (10-5 M) ($p<0.05$). Tamoxifen alone had no effect on CAT gene expression.

These findings provide further evidence that tamoxifen inhibits TRH induction of prolactin gene expression by a mechanism that may not involve the oestrogen receptor.

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552P

METABOLIC INTERACTIONS *IN VITRO* BETWEEN ALBENDAZOLE AND IVERMECTIN

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A combination of the macrocyclic lactone ivermectin (IVM) and a benzimidazole carbamate has been proposed for the treatment of onchocerciasis. Inhibition of hepatic drug oxidation has been demonstrated with these classes of compounds (Ward *et al.* 1985, Miura *et al.* 1989). The efficacy of ABZ is limited by poor bioavailability. Any metabolic interaction resulting in increased plasma and/or tissue concentrations of ABZ or its active sulphoxide metabolite (ABS) may be beneficial. We have evaluated the metabolic interactions between IVM and ABZ in rat liver microsome preparations and the isolated perfused rat liver (IPRL). In microsomes, ABS production was inhibited non-competitively by IVM as evidenced by no change in Km but a progressive fall in Vmax with increasing concentrations of IVM. In the IPRL although there was a significant increase in the AUC of ABS after co-administration of ABZ and IVM ($p < 0.01$ unpaired t-test) the pharmacokinetic parameters of ABZ were unchanged, in contrast to results obtained in microsomes. ABZ had no effect on the disposition of IVM. The conversion of ABZ to ABS is mediated by P-450 and FAD dependent processes, whereas the conversion of ABS to ABZ sulphone is mediated only by P-450 (Rolin *et al.* 1989). Selective inhibition by IVM of P-450 isozymes offers one explanation of this apparent contradiction. The increase in plasma ABS in the presence of IVM without a corresponding decrease in ABZ concentrations might improve efficacy due to the higher concentrations of pharmacologically active compounds.

Table 1: Summary of the Pharmacokinetic Parameters of ABZ and ABS in the IPRL Clearance (Cl); apparent terminal elimination Half-life (t½); Volume of Distribution (Vd); and Area Under the Curve (AUC)

Compound	Co-administration	AUC_{0-4} ($\mu g.min\ ml^{-1}$)	$AUC_{0-\infty}$ ($\mu g.min\ ml^{-1}$)	Cl ($ml\ min^{-1}$)	t½ (min)	Vd (ml)
ABZ	-	117 (20)	128 (17)	7.9 (1.2)	48 (18)	512 (211)
ABZ	IVM	105 (20)	108 (20)	9.5 (1.8)	33 (11)	425 (125)
ABS	-	531 (114)	-	-	-	-
ABS	IVM	790 (174)	-	-	-	

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553P

COMPARISON OF OPIOID BINDING IN BRAIN, CEREBELLUM AND SPINAL CORD OF NEW ZEALAND WHITE RABBITS

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Although three types of opioid receptors, μ, δ and κ, have been identified using binding assays, in vitro bioassays, electrophysiological recordings and behavioural experiments, there is increasing evidence that the non-mu, non-delta binding of non-selective opioid ligands is heterogeneous (Zukin et al., 1988; Hunter et al., 1989). In the present investigation, the κ-binding of [^{3}H]-U-69593 was compared with the non-mu, non-delta binding of [^{3}H]-bremazocine, [^{3}H]-ethylketazocine and [^{3}H]-diprenorphine in brain, cerebellum and spinal cord obtained from New Zealand white rabbits.

The μ-sites were labelled with [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin, the δ-sites with [^{3}H]-[D-Pen^2,D-Pen^5]enkephalin and the κ-sites with [^{3}H]-U-69593. The non-mu, non-delta binding of [^{3}H]-bremazocine, [^{3}H]-ethylketazocine and [^{3}H]-diprenorphine was determined in the presence of unlabelled [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin and [D-Ala^2,D-Leu^5]enkephalin to prevent μ- and δ-binding.

The proportions of μ : δ : κ sites were 51:31:19 in rabbit brain, 84:6:10 in rabbit cerebellum and 88:0:12 in rabbit spinal cord. In rabbit brain, the binding capacity found with [^{3}H]-U-69593 was 2.28 ± 0.16 pmol g^{-1} (n=6) whereas that of [^{3}H]-bremazocine after suppression of μ- and δ-binding was 4.44 ± 0.84 pmol g^{-1} (n=4). In the cerebellum,the binding capacity of [^{3}H]-U-69593 was 1.14 ± 0.08 pmol g^{-1} (n=5) and the non-mu, non-delta binding capacity of [^{3}H]-bremazocine was 1.58 ± 0.08 pmol g^{-1} (n=3). In spinal cord the binding capacities were 0.42 ± 0.05 pmol g^{-1}(n=4) and 1.09 ± 0.14 pmol g^{-1}(n=3) respectively.

Thus in rabbit brain, cerebellum and spinal cord [^{3}H]-U-69593 labels less opioid sites than does [^{3}H]-bremazocine after suppression of μ- and δ-binding. These findings will be compared to those obtained with [^{3}H]-ethylketazocine and [^{3}H]-diprenorphine.

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554P

ANTIDEPRESSANT INHIBITORY ACTIVITY IN THE ABDOMINAL CONSTRICTION ASSAY IS COMPETITIVELY BLOCKED BY OPIOID ANTAGONISTS

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Some antidepressants possess clinical efficacy in analgesic therapy. They also produce antinociception in rats (Isenberg et al., 1984) which is only partially naloxone-reversible when higher doses of the opioid antagonist are employed. The present study examines the possibility that amitryptiline [AMI] and (+) oxaprotiline [(+)OXPN] (Waldmeier et al., 1982) produce graded antinociceptive effects in the abdominal constriction test which may be competitively blocked by low doses of the opioid antagonists naloxone and MR2266 (Millan, 1989).

Male ICI-GB1 (WSP) mice (20 ± 2g; n = 8) were pretreated with antidepressants at 30min and naloxone or MR2266 (both at 0.5mg/kg sc) at 5 min before 1% acetic acid (ip). Abdominal constrictions were evaluated over 20 min and both AMI and (+) OXPN produced linear dose related inhibitory actions in the assay. These dose-response relationships were shifted rightwards in a parallel manner by both antagonists.

Table 1.

Blockade of antidepressant activity in the abdominal constriction assay

	ED_{50} (mg/kg)		
	Alone	Naloxone	MR2266
AMI	2.6 (1.9 - 9.61)	7.8[a] (5.8 - 17.7)	27.2[a] (5.2 - 39.9)
(+) OXPN	14.8 (6.7 - 39.1)	91.0[b] (85.9 - 155.6)	56.9[b] (15.0 - 109.4)

Results are expressed as means (95% confidence limits) and dose-response lines analysed using ANCOVA followed by Neuman-Keuls test. [a, b] $P < 0.01$ from antidepressant alone

Since both antagonists completely blocked the inhibitory actions of the antidepressants in this test, it might by hypothesised that the antidepressants directly interact with opioid receptors (Isenberg et al., 1984) and/or release opioid peptides (Sacerdote et al., 1987) to play a more important role in inhibiting this type of nociceptive stimulus.

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555P

ARE OPIOID RECEPTORS OR α_2-ADRENOCEPTORS INVOLVED IN BENZODIAZEPINE-INDUCED HYPOTHERMIA IN MICE?

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Benzodiazepines (BDZ) decrease body temperature in mice (Taylor et al., 1985). Other compounds which produce hypothermia in these animals include opioid (Rosow et al., 1980) and α2-adrenoceptor agonists (e.g. UK 14,304; Bill et al., 1989). In the current study we have examined whether the hypothermia induced by the BDZ agonist loprazolam involves the release of endogenous opioids or noradrenaline using the opioid antagonist naltrexone and the selective α2-adrenoceptor antagonist RX811059 (Doxey et al., 1985). Body temperatures of male TO mice, 25-35g, were recorded at 15 min intervals using a rectal probe (inserted 2 cm) and digital thermometer (BAT-12, Sensortek). Drugs were dissolved in 0.9% saline - except for the BDZ antagonist Ro 15-1788 (Taylor et al., 1985) which was suspended in Tween 80 solution - and administered i.p. Antagonists were injected 15 min after loprazolam and 15 min before morphine or UK 14,304. Treatment group means (n=8) were compared using ANOVA and Dunnett's test. The decrease in body temperature produced by loprazolam (3 mg/kg) was significantly attenuated by 10 mg/kg of Ro 15-1788 (vehicle 37.9±0.1; L 36.1±0.3*; L+Ro 37.7±0.2§ at t=15 min) but not altered by either naltrexone (0.3 mg/kg) or RX811059 (0.5 mg/kg). The antagonists did not modify body temperature themselves. However, naltrexone and RX811059 blocked the hypothermia induced by morphine (30 mg/kg) and UK 14,304 (1 mg/kg) respectively. Thus, the thermoregulatory effects of BDZ do not appear to be mediated through either opioid receptors or α2-adrenoceptors. The results with naltrexone contrast with studies implicating endogenous opioids in the effects of benzodiazepines on food intake - a behaviour which like body temperature is under hypothalamic control (Cooper, 1983).

Treatment (mg/kg i.p.)	Body temperature °C (mean±SEM) Baseline	15 min	60 min
Vehicle	37.3±0.1	37.8±0.2	37.8±0.2
Loprazolam 3	37.5±0.1	35.7±0.1*	36.1±0.1*
+Nalt 0.3	37.5±0.1	36.0±0.3*	36.2±0.3*
+RX811059 0.5	37.5±0.2	36.3±0.2*	36.1±0.1*
Vehicle	37.1±0.2	37.4±0.1	37.4±0.1
Morphine 30	37.3±0.2	35.9±0.3*	35.4±0.4*
+Nalt 0.3	37.3±0.2	37.2±0.2§	37.4±0.2§
Vehicle	36.8±0.2	37.2±0.2	37.4±0.2
UK14,304 1	36.4±0.3	35.5±0.1*	32.9±0.4*
+RX811059 0.5	36.5±0.2	37.5±0.3§	36.9±0.3§

*P<0.05-v-vehicle; §P<0.05-v-drug control group

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HISTAMINE H2 ANTAGONISTS AND NOCICEPTION IN THE MOUSE

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Considerable evidence suggests that histamine is involved in nociception. Centrally- but not peripherally-acting H1 antagonists have been shown to potentiate antinociception induced by morphine in mice (Freeman, Sturman & Tang, 1989). The existence of H2 receptors in the c.n.s. has been demonstrated and stimulation of them in the rat dorsal raphe area has produced antinociception (Glick & Crane, 1978). The recent development of centrally-penetrating H2 antagonists facilitates further investigation into histamine's role in nociception. Groups of ten female TO mice were pretreated with antihistamine or vehicle and tested for response to nociceptive stimuli before and after morphine injection as previously described (Freeman & Sturman, 1989). Neither zolantidine nor cimetidine (H2 antagonists which penetrate the c.n.s. to varying degrees) nor ranitidine (a non-c.n.s. penetrating H2 antagonist) alone affected responses to nociceptive stimuli,and only cimetidine potentiated morphine's effects,possibly nonspecifically due to its p450 inhibitory action(Table 1)

Table 1

Pretreatment ($mg\ kg^{-1}$ s.c.)		Morphine sulphate ($mg\ kg^{-1}$ s.c.)	Median Latency Hot plate (30 min)	Tail clip (30 min)
Cimetidine	(200)	5	15.7 **	1.0
Cimetidine	(100)	5	9.6	2.0
Zolantidine	(10)	5	13.1	1.0
Zolantidine	(5)	5	11.6	2.0
Ranitidine	(100)	5	15.6 NS	1.5
Ranitidine	(50)	5	10.9	1.5
Cimetidine	(200)	10	20.0 NS	30.0 *
Cimetidine	(100)	10	14.2	1.5
Zolantidine	(10)	10	15.7	2.0
Zolantidine	(5)	10	10.5	8.5
Ranitidine	(100)	10	13.3	7.5
Ranitidine	(50)	10	18.2 NS	30.0 NS

Significantly different from control by Kruskal Wallis analysis *$p<0.05$, ** $0.02<p<0.05$

Thus although the H1 receptor appears to influence the nociceptive response, these results suggest that there is no evidence of an involvement of H2 receptors in these models.

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MEASUREMENT OF STIMULATED ENDOGENOUS 5-HT RELEASE IN RAT BRAIN SLICES USING FAST CYCLIC VOLTAMMETRY

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We have used fast cyclic voltammetry (FCV) to measure 5-HT release *in vitro* using methods previously described (Palij, et al., 1988, 1990). Rat brain slices (350μm) containing the dorsal raphe nuclei (DRN) or caudate putamen (CPu) were superfused with oxygenated ACSF at 32°C. Stimulating electrodes were placed centrally in the DRN and 80μm below the surface. 5-HT release was monitored with a carbon fibre microelectrode 100 to 200μm from the stimulating electrodes. 500ms trains of square waves (0.1ms; 50Hz; 20V) were applied every 5 minutes. Reproducible evoked release of 5-HT could be measured for 6hr. Concentrations of 5-HT greater than 10^{-8}M were readily detectable. Omission of calcium from the perfusion medium reversibly abolished the electrochemical signal. Ro 4 1248 (10^{-7}M and 10^{-6}M) irreversibly attenuated the signal in a concentration-dependent manner. The effects of 5-HT uptake inhibitors on evoked 5-HT overflow and reuptake are shown in table 1. Benztropine (10^{-6}M) had no effect on the signal detected in the DRN while in the same bath there was a large increase in the evoked DA overflow detected in slices of CPu (0.39±0.1μM to 1.22±0.4μM; n=3)

Table 1 — Effects of 5-HT blockers (all 10^{-6}M) on evoked 5-HT overflow and reuptake.

		Citalopram	Clomipramine	Fenfluramine	Fluvoxamine
Peak 5-HT release (nM±sem (n))	Control	58±13 (4)	63±11 (5)	84±8 (3)	55±10 (3)
	+Drug	142±16 (4)*	122±23 (5)*	112±14 (3)	92±17 (3)
$t_{1/2}$(seconds±sem (n))	Control	4.9±0.5 (4)	6.3±1.1 (5)	4.5±1.0 (3)	3.8±0.3 (3)
	+Drug	7.4±1.1 (4)*	13.2±1.6 (5)*	11.3±3.9 (3)*	7.8±1.1 (3)*

* $P<0.05$ (paired Student's t-Test). $t_{1/2}$: time in seconds for the overflow to decline to 50% maximum.

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EFFECTS OF THE PUTATIVE 5-HT$_{1A}$ AGONIST ANXIOLYTIC, IPSAPIRONE, ON BENZODIAZEPINE WITHDRAWAL IN RATS

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Two studies investigated the effects of ipsapirone in animals being withdrawn from the benzodiazepine (BZ) chlordiazepoxide (CDP). Rats were injected b.i.d. for 21 days with saline or CDP at doses up to 40 mg/kg/injection. Subsequently, controls continued to receive the treatment administered previously, other subjects received saline during CDP withdrawal. Further subjects received ipsapirone (3, 10 or 30 mg/kg b.i.d.) during withdrawal. Withdrawal indices recorded were body weight and food intake. Significant withdrawal signs were seen after CDP withdrawal, food intake and bodyweight measures fell and then recovered. At the high dose of 30 mg/kg (b.i.d.) ipsapirone potentiated CDP withdrawal in both studies. Potentiation of withdrawal was not seen in animals treated with ipsapirone at 3 and 10 mg/kg (b.i.d.). In a third study, ipsapirone induced a conditioned taste aversion, a possible index of drug-induced "malaise", at doses as low as 7.5 mg/kg. Therefore, a possible explanation for the potentiation of CDP withdrawal in subjects treated with high doses of ipsapirone was that drug-induced "malaise" reduced food intake and body weight, rather than ipsapirone causing a true potentiation of BZ withdrawal. However, in a fourth study we showed that the ipsapirone treatment regime that potentiated BZ withdrawal (30 mg/kg b.i.d.) did not significantly reduce food intake or body weight, suggesting that high doses of ipsapirone potentiate CDP withdrawal by a mechanism that does not simply involve nonspecific "malaise". The most plausible mechanism to account for the observed potentiation of withdrawal involves actions of the ipsapirone metabolite (1-(2-Pyrimidinyl)-Piperazine) on alpha$_2$-adrenoceptor systems, which have been implicated in BZ withdrawal. However, the precise mechanism involved in the observed potentiation of BZ withdrawal remains uncertain at present. At no dose was there any evidence that ipsapirone attenuated BZ withdrawal, it is therefore likely that patients being withdrawn from BZs will inevitably experience withdrawal if treated with ipsapirone as an anxiolytic; and that if treated with very high doses of ipsapirone (and related agents) withdrawal signs may be exacerbated. These data may be related to clinical reports that buspirone-type anxiolytics are poorly tolerated by patients with a history of BZ treatment (Ashton et al. 1989; Harto et al. 1988; Schweizer et al. 1986).

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559P

THE ABILITY OF 5-HT$_{1A}$ LIGANDS TO MODIFY FEEDING BEHAVIOUR IN THE COMMON MARMOSET (*CALLITHRIX JACCHUS*)

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It is now well established that 5-hydroxytryptamine (5-HT) neurons are involved in the control of feeding behaviour (Blundell, 1984; Nicolaidis, 1986). The role of 5-HT appears to be inhibitory and generally 5-HT agonists and drugs which release 5-HT decrease food intake in both animals and man. It was therefore unexpected when Dourish *et al.* (1985) showed that the novel 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) increased food intake in non-deprived rats. However this effect is consistent with the inhibitory role of 5-HT as this drug has been shown to decrease serotonergic neurotransmission via an agonist action on 5-HT$_{1A}$ autoreceptors in the raphe nuclei (Dourish *et al.*; Hutson *et al.*, 1986). The present studies investigate the ability of 8-OH-DPAT, gepirone and buspirone to modify firstly a model of free feeding, and secondly a model of satiated feeding in the common marmoset (Callithrix jacchus).

The studies used adult, non-food deprived marmosets weighing 330±20g. For free feeding studies marmosets were transferred to individual testing cages (identical to their home cages and to which they had been habituated) 45 min following subcutaneous treatment with vehicle (saline 1ml kg^{-1}), 8-OH-DPAT (10-100μg kg^{-1}), gepirone (0.25-1.0mg kg^{-1}), or buspirone (0.25-1.0mg kg^{-1}). Animals were presented with 25±0.1g of highly palatable food (syrup coated brown bread cubes), and the quantity consumed in a 10 min test period was recorded.

For satiated feeding studies marmosets were presented with 15±0.1g of the same palatable food in their home cages. Immediately following the satiating period marmosets received either drug or vehicle. Following a 45 min pretreatment marmosets were transferred to the test cages and presented with a further 15±0.1g of food. The quantity consumed in a 10 min test period was recorded.

8-OH-DPAT (10-100μg kg^{-1} s.c.) and gepirone (0.25-1.00mg kg^{-1}) both failed to modify free feeding, but both caused a decrease in satiated food intake. 8-OH-DPAT (100μg kg^{-1}) reduced food intake from 4.88±1.23g, vehicle, to 1.68±1.00g. Gepirone (1.00mg kg^{-1}) reduced food intake from 4.45±1.16g, vehicle, to 1.66±0.85g. Buspirone (0.25-1.00mg kg^{-1} s.c.) failed to modify food intake in both models.

These data suggest that 5-HT$_{1A}$ receptor ligands may have an inhibitory effect in a model of satiated feeding in the marmoset.

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560P

5-HT_3 RECEPTOR AGONIST EFFECTS OF ZACOPRIDE IN THE FERRET

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Zacopride is a potent and selective antagonist of 5-HT3 receptors (Smith et al 1988) which has been shown to be effective against the emesis caused by cytotoxic agents in dogs and man, (Cohen et al 1989 ; Smith et al 1986). In the ferret however, zacopride was found to produce retching and vomiting at doses of 10-100 µg/kg i.m. We have investigated the cause of this emetic effect , using the 5-HT induced Bezold-Jarisch (B-J) reflex in the urethane anaesthetised ferret as an indicator of 5-HT_3 receptor activity.

Zacopride is a racemic mixture of *R* and *S* stereoisomers, both of which are 5-HT_3 receptor antagonists. In urethane anaesthetised rats *S*-zacopride was approximately 3 fold more potent than *R*-zacopride against the 5-HT induced B-J reflex. We have shown that *S*-zacopride but not *R*-zacopride (at doses of 10-100 µg/kg i.m.) possessed emetogenic activity in the ferret. The emesis produced by both zacopride and by its *S*-enantiomer was blocked by prior administration (using a 15 min pretreatment time) of the 5-HT_3 antagonist, ondansetron (1 mg/kg i.m.) but not by atropine (0.1-1.0 mg/kg i.m.).

In the urethane anaesthetised ferret a B-J reflex was elicted by 5-HT (10 µg/kg i.v.) administered via a jugular venous cannula.The carotid artery was cannulated to record blood pressure and heart rate. The B-J reflex was quantified by measuring the change in heart period (H.P.) from predose over 4 consecutive beats, starting at the point of apnoea. 5-HT induced a mean increase in H.P. of 703 ±113 ms which was blocked by zacopride (3 µg/kg i.v.). Zacopride itself evoked a pronounced increase in H.P. in this preparation, an effect which was investigated further using its two enantiomers.

S-zacopride (0.3 µg/kg i.v.) evoked a B-J-like response with a mean increase in H.P. of 7232 ±3530 ms. This was approximately 15 fold that produced in the same animals by 5-HT (10 µg/kg i.v.). A subsequent B-J reflex response to 5-HT was not attenuated following the administration of this dose of *S*-zacopride. The responses to both 5-HT and *S*-zacopride were blocked by the 5-HT_3 antagonist ondansetron (30 µg/kg i.v.). In contrast, *R*-zacopride, when administered over the dose range 1-100 µg/kg showed no inherent agonist activity but produced a dose dependent inhibition of both the 5-HT and the *S*-zacopride induced increases in H.P

Neither zacopride or its enantiomers produced a B-J reflex in the urethane anaesthetised rat.

In conclusion, the *S*-enantiomer of zacopride appears to possess 5-HT_3 receptor agonist properties in the ferret which are blocked by both the *R*-enantiomer of zacopride and by ondansetron.

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561P

ONDANSETRON DOES NOT AFFECT HEMICHOLINIUM-3 INDUCED DISRUPTIONS IN A T-MAZE REINFORCED ALTERNATION TASK

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There is considerable evidence that cognitive performance critically depends on the normal function of the cholinergic system (Perry 1986). When this is disrupted by scopolamine, 5-HT_3 receptor antagonists (e.g. ondansetron) can inhibit the impairments (Costall et al. 1990). Hemicholinium-3 (HC-3) infused intracerebroventricularly (ICV) persistently also disrupts performance of the T-maze task (Costall et al. 1989). Here we report on the effects of 5-HT_3 receptor antagonists on the HC-3 disturbance in cognition.

HC-3 (25µg/day for 9 days, dose chosen from preliminary observations) was infused persistently into the lateral ventricles of Lister hooded rats via chronically indwelling cannulae stereotaxically located (Ant. 5.8, Vert. 2.5, Lat. ±2.0) and coupled to subcutaneously implanted Alzet osmotic minipumps.

Rats were trained on a food reinforced alternation task using an elevated T-maze (see Costall et al. 1989, 1990). Food was withdrawn 2 days prior to the test, and throughout testing animals were food deprived for 23hr/day (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 favoured 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 while the other 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.

HC-3 (2.5µg/day) disrupted performance, reducing correct responses (compared to control) over the 9 day test period [$F(4.16) = 32.6$ $P<0.01$].

The 5-HT_3 receptor antagonists, ondansetron at 10ng/kg i.p. b.d. and zacopride at 10ng/kg i.p. b.d., failed to influence the HC-3 impairments, e.g. control, HC-3, HC-3 + drug % correct responses were, respectively, on day 9, 89.9, 54.4, 52.2 (ondansetron) and 86.7, 51.1, 42.2 (zacopride). In contrast, arecoline (30mg/kg/day i.p.) was shown to significantly attenuate the hemicholinium response (82.2, 45.5, 62.4 respectively, as above).

The present data indicate that the 5-HT_3 receptor antagonists do not influence the cognitive impairments caused by HC-3 implying that the action of 5-HT_3 receptor antagonists to improve cholinergic transmission may be a result of an interaction with the release mechanisms of the cholinergic neurone.

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562P

ONDANSETRON ATTENUATES THE SCOPOLAMINE DEFICIT IN A WATER MAZE TASK

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The muscarinic receptor antagonist scopolamine has been widely used to produce experimental cognitive deficits in both animals and man. Since a disruption in central cholinergic function has traditionally been associated with age related memory disorders (Bartus, 1982) scopolamine was used in the present studies to produce an experimental deficit in performance of a water maze task. Previous studies have shown that the 5-HT_3 receptor antagonist ondansetron attenuates a scopolamine-induced deficit in a T-maze task (Barnes et al., 1990). The present studies were designed to investigate the effects of ondansetron on the scopolamine induced deficit of a water maze task (Morris, 1984).

Male hooded Lister rats (250-300g, Bradford strain) were trained in a 2 day paradigm in the water maze. On day 1 each rat was pretreated with test compound or vehicle and placed on the island (located 2cm below the surface of the water) for 30 s immediately before testing commenced. The island was kept in a constant position for each rat (the position was randomised and balanced across the groups) but each rat began the trial at a different corner in the pool (the positions balanced across the groups). A training trial began with the animal being lowered into the pool, close to and facing the corner designated for the trial. The timer and tracking device was started and the time recorded for the animal to escape from the water onto the platform. The rat remained on the platform for 2s before being placed in the pool for trial 2. On each trial the rat was allowed a maximum 100s to find the island and the latency to find the island was noted. If the rat failed to find the island within 100s it was placed on the island for 10s. Each rat received 6 trials on day 1. A 7th trial with a black, visible island was also carried out to ensure no visual disturbances were influencing performance. The same procedure was carried out on day 2 on which drug effects were usually detected. Thus, increase in escape latency was attenuated by arecoline, 5.0mg/kg i.p., (the latencies being control, scopolamine, scopolamine + arecoline 26.0, 58.0 and 40.0s respectively, scopolamine response $P<0.05$ compared to control and arecoline + scopolamine $P<0.05$ compared to scopolamine). Ondansetron caused similar changes to arecoline (on day 2 latencies vehicle, scopolamine, scopolamine + ondansetron, 10μg/kg, were respectively 28, 45 and 35s).

These are preliminary data that support previous observations using the rat T-maze in which ondansetron inhibited scopolamine impairment in cognitive performance. The data further support a role for 5-HT_3 receptors in the modulation of cholinergic deficits in cognition

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563P

THE ABILITY OF BTG 1501 TO INHIBIT THE SUPPRESSED BEHAVIOUR ASSOCIATED WITH WITHDRAWAL FROM DRUGS OF ABUSE

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BTG 1501 has been shown to release suppressed behaviour in rodent and primate tests in a manner consistent with that of an anxiolytic agent (Costall et al., 1990a).

Using a mouse black:white test box, an 'anxiogenesis' of withdrawal has also been observed following cessation of treatment with drugs of abuse, alcohol, nicotine and cocaine. The present studies were therefore carried out to determine whether BTG 1501 had a capacity to inhibit the adverse behavioural phenomena associated with withdrawal from these drugs of abuse. The light:dark test, briefly, comprised a two-compartment box, one compartment being painted white and brightly-lit, the other being painted black and dimly lit. Naïve mice were placed individually into the centre of the brightly-lit compartment which they normally find aversive. Thus, after a 7-10 sec delay mice will move from the light to the dark compartment where most exploratory behaviour is exhibited.

Groups of mice (n = 5) received alcohol (8% w/v for 14 days in their drinking water), nicotine (0.1mg/kg i.p. b.d. for 7 days) or cocaine (1.0mg/kg i.p. b.d. for 14 days). During treatment with these drugs of abuse mice showed reduced aversion for the brightly-lit compartment of the black:white box, which is consistent with an anxiolytic profile of activity (latency for the initial move from the white compartment was delayed for some 15-28 sec for all compounds, $P<0.001$). In contrast, 8-48 hr following cessation of alcohol intake or treatment with nicotine or cocaine, the normal suppression of behaviour in the novel white compartment was enhanced, and animals moved within 2 sec into the black compartment ($P<0.001$). Naïve animals were used on all test days and behaviour was analysed from remote video recordings.

Groups of mice withdrawn from alcohol, nicotine or cocaine were given BTG 1501, 10mg/kg i.p. b.d., during the periods of withdrawal and the behaviour of separate groups of mice was analysed 8, 24, 48, 96 and 240 hr after withdrawal from the drugs of abuse. In each situation, the first dose of BTG 1501 was given at the actual time of withdrawal (last dose of nicotine or cocaine, or time of removing alcohol). BTG 1501 was shown to prevent the enhanced suppression of behaviour, interpreted as anxiogenesis, during the periods of withdrawal from alcohol, nicotine or cocaine. Thus, latencies of withdrawn animals (<1 sec-2 sec) were delayed for some 15-30 sec ($P<0.001$), and this was associated with increased time spent in the light compartment with increased rears and line crossings in the light.

It is suggested that BTG 1501, which presents with a novel anxiolytic profile, also prevents the behavioural consequences of withdrawal from chronic treatment with drugs of abuse.

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564P

THE ANXIOLYTIC ACTION OF BTG 1501 REVEALED IN RODENT AND PRIMATE TESTS

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BTG 1501 (3-[3-(dimethylamino)propyl]-3-(3-methoxyphenyl)-4,4-dimethyl-2,6-piperidinedione,monohydrochloride) was tested in 4 tests for anxiolytic potential using both rodent and primate species.

Mice (male, Albino BKW) were placed in the centre of a brightly-lit environment in a two-compartment box having a dark section simultaneously available (Costall et al., 1988). Normally mice move rapidly (5-10 sec) from the light to the dark where they spend most time exploring. This suppressed behaviour is released by known anxiolytic agents such that animals spend more time in the white environment (latency delayed for 15-25 sec). BTG 1501 (0.1μg/kg-100mg/kg s.c.) also released the suppressed behaviour (latency 17-28 sec, $P<0.001$) and more time was spent exploring the light compartment.

Male Sprague-Dawley and Lister hooded rats were used in the social interaction (SI) and elevated X-maze test respectively. For SI pairs of unfamiliar rats were placed in a brightly-lit arena and time spent in SI determined (Costall et al., 1988). Normally, under these test conditions, rats spend the majority of a 10 min test period avoiding each other. Known anxiolytic agents enhance active SI. This was also observed for animals treated with BTG 1501 (10ng/kg-100mg/kg, SI increased from 50-70 sec to 155-200 sec., $P<0.001$). In the X-maze animals were placed facing an open arm in a situation where 2 closed and 2 open arms were available for exploration. Normally rats spend most time in the closed arms. An anxiolytic agent will release this suppressed behaviour such that rats spend more time on the open arms, particularly in the furthermost section (Costall et al., 1989). BTG 1501 caused a maximum effect indicative of anxiolytic action in this test at 0.01mg/kg i.p. Thus, time spent on the open arms increased, particularly on the end sections of the open arms (15-25 sec increased to 40-55 sec, $P<0.001$). All rodent tests used remote video recordings, $n = 5-6$. Oral efficacy was confirmed for BTG 1501 in the rodent tests. Sedation was not observed in any test.

Common marmosets confronted with a human threat normally retreat to the cage back and exhibit characteristic postures. Under the influence of an anxiolytic agent marmosets spend more time forward and posturing is reduced (Costall et al., 1988). At 0.1μg/kg-1mg/kg s.c. BTG 1501 increased time forward in a 2 min test from 20-25% to 45-55%, and reduced postures from 8-12 to 2-7 ($P<0.001$, $n = 4$).

In all tests described BTG 1501 caused changes in behavioural profiles consistent with those determined for known anxiolytic agents. It is therefore proposed that BTG 1501 may present as a novel non-sedative anxiolytic agent which is orally active over a wide dose range.

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565P

USE OF THE STONE MAZE AS A TEST OF COGNITIVE FUNCTION IN THE RAT

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The Stone maze or multiple unit T-maze (Stone, 1929) has previously been used as a test of reference memory in the rat (Knowlton, 1985) and is distinct from models which use spatial cues, such as the Morris water maze. The present experiment investigated the use of the Stone maze and the effect of scopolamine on Stone maze performance which is of particular interest since a cholinergic deficit has been documented in dementia and memory disorder (Bartus, 1982).

The apparatus consisted of a large square perspex maze (144cm x 144cm) having inner walls forming alleys 12cm wide and 42cm high. The maze had only one correct route from the enclosed start box, to a food reward with fourteen blind ending error points between. Four perspex guillotine doors separated the maze into five equal areas. The measurement of learning ability was taken as the time required to reach the food reward and the number of errors made in deviating from the correct route. The experiments were carried out in white light, in a sound proofed room, with no extra maze cues.

Male Lister Hooded rats, food deprived to 85% of normal body weight received either scopolamine (0.125-1.0mg/kg i.p. b.d.), methylscopolamine (0.5mg/kg i.p. b.d.) or vehicle (0.9% saline) throughout the period of testing. The rats were given one 5 min exposure to the maze each day for fifteen days. The rat was placed in the start box and allowed access to the maze. The time (s) taken to reach the food reward, and the number of errors (maximum 14) were scored for each rat on each day of testing. To prevent retracing, the doors were lowered as the rat passed through the maze.

Scopolamine (0.25-1.0mg/kg) increased the latency to complete the task. 1.0mg/kg scopolamine increased the latency from 14.7±0.95s to 176.1±39.05s ($P<0.05$ ANOVA) and the number of errors increased from 2.1±0.2 to 4.5±0.9 ($P<0.1$ ANOVA) on day 9 of testing. Methylscopolamine (0.5mg/kg), a selective peripheral muscarinic antagonist, had no effect on maze performance. Pupillary diameter was measured to indicate the peripheral effects of scopolamine and dilatation was observed at 0.125-1.0mg/kg. However, methylscopolamine (0.5mg/kg) which induced maximal pupillary dilatation failed to influence performance of the task.

These results suggest that the Stone maze is a suitable apparatus in which to train rats to perform a task. Scopolamine produced a learning deficit in that it increased the latency to perform the task and number of errors made. It is concluded that the Stone maze may be a suitable model in which to study some aspects of cognitive function.

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566P

BEHAVIOURAL AND BIOCHEMICAL EFFECTS OF BILATERAL COMMON CAROTID ARTERY OCCLUSION IN THE RAT

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Following the induction of focal or global cerebral ischaemia many animals suffer gross neurological deficits which compromise behavioural testing. We propose a simple yet effective method for the induction of global cerebral ischaemia following which the rat is able to perform in a behavioural test which has previously been used to demonstrate the learning ability of rats, the Morris water maze (Morris, 1984).

Bilateral occlusion (45 min) of the common carotid arteries was performed in male Lister Hooded rats under ketamine anaesthesia (100mg/kg i.p.) with sham operated animals acting as controls. For 7 days following surgery the animals were examined to assess their neurological status and were given a "neurologic deficit score" (max. 274) according to a revised schedule of Cahn et al. (1988). Behavioural assessment was performed on days 7-16 in the Morris water maze. Using a fixed island position for individual animals, each animal performed six trials per day for a 10 day period; escape latency was used as a measure of learning ability and the animal's swim speed was used as an indication of locomotor activity. Biochemical analyses were made of cholineacetyltransferase (ChAT) activity (Fonnum, 1975) in the amygdala, entorhinal cortex, frontal cortex, hippocampus, septum, striatum and tuberculum olfactorium on tissue taken from animals subjected to 10 days training (initiated at 7 days post-op) and on tissue which was taken 7 days after occlusion or sham operation.

The escape latency was significantly increased in the occluded group when compared to the sham operated controls throughout the test period (54.6±5.3s and 8.3±1.3s on day 4 of training with n = 10 and 7 respectively, $P<0.001$ ANOVA). Whilst a consistent and significant neurologic deficit was observed in the occluded rats as compared to the sham operated animals (7.9±0.2 and 0.2±0.1, n = 10 and 7 respectively, $P<0.001$ ANOVA), no significant changes in locomotor activity were recorded, hence the apparent reduction in learning ability is not directly related to changes in locomotor activity. The biochemical assays revealed no significant changes in ChAT activity at the end of the 10 day training period. However the analysis performed 7 days after occlusion revealed a significant reduction in ChAT activity (sham vs occluded) in the striatum and tuberculum olfactorium (779.0±64.8 reduced to 465.5±50.5nmol/min/mg protein and 504.9±52.3 reduced to 352.2±20.0nmol/min/mg protein respectively, $P<0.05$, n = 8, T-test)

The present method would appear to produce a reproducible deficit in learning ability in the Lister Hooded rat. Lower activities of ChAT in the striatum and tuberculum olfactorium at the start of testing warrant further investigation.

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567P

EFFECTS OF BARBITURATES ON THE RELEASE OF [^{3}H]NORADRENALINE FROM RAT BRAIN SLICES

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A wide variety of chemically dissimilar general anaesthetics share the ability to potentiate the effects of GABA receptor stimulation. However, adrenergic mechanisms may also have a role in anaesthesia, possibly via modulation of cortical arousal, wakefulness and processing of sensory stimuli (Mason & Angel, 1983). A previous report (Jones, 1990) described preliminary results that indicated that high concentrations of methohexitone decrease the K^+-stimulated release of [^{3}H]noradrenaline from rat cerebral cortex slices. Effects at lower concentrations were not clear. As part of the further investigation of these effects the present experiments compare the actions of three barbiturate anaesthetics on the release of [^{3}H]NA. The results are presented in Table 1.

Table 1 Effects of barbiturates on K^+-stimulated [^{3}H]NA efflux

Concentration	Methohexitone	Pentobarbitone	Thiopentone
10^{-7} M	108.7 ± 17.6	-	-
10^{-6} M	70.7 ± 10.0	70.5 ± 18.0	70.7 ± 23.0
$3x10^{-6}$ M	96.9 ± 23.7	81.6 ± 23.6	110.3 ± 13.3
10^{-5} M	56.3 ± 7.7**	120.1 ± 11.8	119.0 ± 7.0
$3x10^{-5}$ M	129.3 ± 19.0	81.3 ± 11.2	53.4 ± 9.0*
10^{-4} M	54.0 ± 8.6*	66.5 ± 13.3	34.8 ± 9.4**
$3x10^{-4}$ M	33.6 ± 8.3*	16.6 ± 2.7*	13.8 ± 1.3**
10^{-3} M	6.5 ± 1.8**	4.8 ± 1.5*	4.5 ± 0.4**

Results are % control, mean ± s.e. mean, n = 4-11. *$p < 0.05$, ** $p < 0.01$, Student's t test.

At the highest concentrations studied, all three barbiturates significantly decreased [^{3}H]NA release in a dose-dependent manner. As would be expected, thiopentone appears to be more potent that pentobarbitone. However, although clinically methohexitone is more potent than thiopentone there is no obvious correlation with inhibition of [^{3}H]NA efflux. This may be due to the different concentration-response profile seen at lower concentrations with methohexitone, as compared to thiopentone and pentobarbitone. The effects seen may be due to multivariate processes and further studies will focus on possible component mechanisms.

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568P

CHANGES IN DIHYDROPYRIDINE BINDING IN THE CEREBRAL CORTEX FOLLOWING CHRONIC BARBITAL TREATMENT

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We have previously shown that long-term ethanol treatment increased the number of central binding sites for dihydropyridines, thought to represent voltage-sensitive calcium channels. Their possible causal role in ethanol dependence was suggested by the prevention of the development of ethanol tolerance, the ethanol withdrawal syndrome and the upregulation of binding sites by chronic administration of dihydropyridine calcium channel antagonists, concurrently with ethanol (Dolin & Little, 1989; Whittington & Little, 1989). Ethanol and barbiturate withdrawal syndromes show many similarities, both types of compound block calcium channels acutely, and a calcium channel antagonist gave protection in barbital withdrawal (Rabbani & Little; 1990). We have therefore now investigated the effects of chronic barbiturate treatment on central dihydropyridine binding.

Male mice, TO strain, 30 - 35g, were given barbital in powdered food for seven days: 3 mg barbital per g food for two days, 4 mg/g food for two days and 5 mg/g food for three days. Controls received a matched amount of powdered food only. At the end of the seven day treatment the mice were placed in clean cages and given powdered food without barbital for 24h. Our previous studies showed that the withdrawal syndrome was maximal at this time. The mice were killed by cervical dislocation and the cerebral cortices removed and frozen. Tissues were homogenised in Tris HCl 50 mM, centrifuged and the pellet washed twice more before duplicate binding determinations using [^{3}H]-nitrendipine. Tubes were incubated for 45 min at 25°C and the binding terminated by filtration through Whatman GFB filters. Nonspecific binding was defined by displacement with cold nimodipine (1 µM).

The tissues from the barbital treated mice showed an 82% increase in Bmax values for dihydropyridine binding, compared with concurrently treated controls. Values: controls 525 ± 67; barbital treatment: 953 ± 135 fmol/mg protein ($P < 0.01$ by analysis of variance; n = 22, 19 respectively). The Kd values were also increased by the barbital treatment: controls 5.89 ± 0.82 nM; barbital 11.83 ± 2.38 nM ($P < 0.02$ by analysis of variance; n = 22, 19).

The results suggest that the number of voltage-sensitive calcium channel was increased by chronic barbital treatment. The pattern differed from that after chronic ethanol treatment in that the affinity of the sites was decreased. Barbiturates have been shown to have a small affinity for these binding sites (Harris et al; 1985), but the barbital concentration in the CNS would have been very low after 24h withdrawal (P.V.Taberner, personal communication). This type of calcium channel may be involved in the barbiturate withdrawal syndrome.

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569P

INSULIN ACTION ON 2-DEOXYGLUCOSE UPTAKE IN NORMAL AND DIABETIC CBA/CA MICE FOLLOWING *IN VIVO* AND *IN VITRO* ETHANOL

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Previous studies have indicated that chronic ethanol treatment in mice increases glucose uptake into brain, muscle, and white adipose tissue (Connelly et al., 1987); the effect being greater in hyperglycaemic mice (C57) than in normals (LACG). Since insulin-stimulated glucose uptake in the periphery is reduced in both streptozotocin-diabetic mice and in non-obese diabetic mice as a result of decreased insulin sensitivity (Goto et al., 1988), it was of interest to compare the effects of chronic ethanol in normal and insulin resistant obese diabetic CBA/Ca mice (Connelly & Taberner, 1989).

Normal and diabetic male CBA/Ca mice were from the Bristol colony. Ethanol (24% v/v) was administered in the drinking water over 4-6 weeks. The uptake of the non-metabolizable substrate 2-deoxy-[^{3}H]-glucose (2-DG) into isolated soleus muscles was measured as described by Cuendet et al. (1976). Briefly, soleus muscles were dissected, weighed, then preincubated for 2h at 37°C in Krebs-Ringer bicarbonate solution containing 2% defatted bovine serum albumin to remove endogenous insulin. One muscle was used to measure basal uptake; the other was incubated in the presence of 2.5 mIU/ml insulin. 2-DG (10 mM, 1 µCi) was then added. After 15 min the muscles were removed, rinsed in saline, and dissolved overnight in NaOH prior to liquid scintillation counting.

Normal untreated CBA/Ca mice showed higher basal and insulin-stimulated 2-DG uptake compared to LACG and C57 strains. Chronic ethanol treated mice showed a significant increase in basal 2-DG uptake over control levels (means ± SEM (n), nmoles/mg wet wt/hr): untreated lean, 3.85 ± 0.34 (20); ethanol treated lean, 5.56 ± 0.46 (8) ($p<0.01$, t-test). Insulin-stimulated uptake was significantly increased ($p<0.01$) in the ethanol-treated group; untreated, 5.22 ± 0.55 (9); ethanol treated, 8.49 ± 0.58 (8). Obese CBA/Ca mice showed significantly lower uptake of 2-DG compared to lean animals, but did not respond to the chronic ethanol treatment. 2-DG uptake in control obese mice was: basal, 3.73 ± 0.14 (8); insulin-stimulated, 3.85 ± 0.14 (8). After chronic ethanol, the rates were: basal, 4.07 ± 0.19 (8); insulin-stimulated, 4.22 ± 0.39 (8). Ethanol (0.3 - 100 mM) in vitro had no effect on basal or insulin-stimulated uptake. Similarly, acute ethanol (2.5 g/kg I.P.) was without effect on the uptake rate. In conclusion, ethanol appears to increase insulin-stimulated 2-DG uptake only when given chronically, and does not potentiate the action of insulin in an insulin-resistant animal model of diabetes.

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570P

DOES CENTRAL ADMINISTRATION OF THE $GABA_B$ RECEPTOR AGONIST BACLOFEN PRODUCE HYPERPHAGIA IN RATS?

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We have previously demonstrated that intracerebroventricular (ICV) administration of the GABA-B agonist baclofen will produce a short lasting hyperphagia in satiated pigs (Ebenezer and Baldwin, 1989). More recently we have shown that systemic administration of low doses of baclofen to rats will cause a short-term stimulation of feeding (Pringle and Ebenezer, 1990). The present study was undertaken to investigate the effects of ICV administration of baclofen on food intake in rats.

Male Wistar rats (280-350g, n=10) were implanted under equithesin anaesthesia with guide tubes directed at the lateral cerebroventricle for subsequent chronic ICV administration of drugs. After recovery from surgery, the rats were given 6-8 daily training sessions during which time they were placed separately in cages where they had free access to water. The rats were allowed to habituate to the cage for 30 min before being presented with food for 2 h. The food was placed in a shallow cylindrical container in the centre of the cage. The animals were not food deprived. During the experimental sessions the rats were injected ICV with either physiological saline (control) or baclofen solution (0.1, 1, 2.5 and 5 nmol) at the end of the 30 min acclimatization period. The amount of food consumed was measured 15 min, 30 min, 60 min and 120 min after vehicle or drug administration.

Baclofen (0.1 - 5 nmol ICV) caused a dose-related increase in food intake in these rats. The 0.1 nmol dose had no significant effects on feeding compared with control data. In contrast, the higher doses of baclofen increased feeding ($P < 0.01$ in each case) during the first 30 min after administration. For example, the rats (n=9) ate 3.81 $\pm$ 0.26 g of food during the first 30 min after baclofen (5 nmol) compared with 0.18 $\pm$ 0.63 g after saline. Eating usually began about 1-2 min after administration, and this effect was particularly spectacular with the 5 nmol dose. All rats given baclofen (5 nmol) showed signs of ataxia and other motor side effects about 10 min after administration, and these effects were also noted in some of the rats given the 2 nmol dose. Nevertheless, the rats kept on eating despite these motor abnormalities. They eventually went into a sleep-like state for up to 2 h. The effects of baclofen (5 nmol) on feeding could be abolished by pretreating the animals with the GABA-B antagonist phaclofen (40 nmol, ICV). Furthermore, phaclofen pretreatment almost completely prevented the motor side effects associated with 5 nmol of baclofen.

The results of this study extend previous findings in the pig (Ebenezer and Baldwin, 1989), and show that ICV administration of baclofen produces a hyperphagic response in rats by acting at central GABA-B receptors.

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571P

INTERACTION OF GABA AGONISTS AND GENERAL ANAESTHETICS ON IONIC CURRENTS OF TRANSMITTER RELEASING AXONS OF RAT OLFACTORY CORTEX

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The interaction of some general anaesthetics with the GABA receptors involved in inhibitory transmission has been well documented. Many authors have presented evidence indicating that general anaesthetics act by reducing excitatory transmitter release. Presynaptic GABA receptors are thought to be present on some central axons (Cain & Simmonds, 1982; Steel & Scholfield, 1985) and anaesthetics could interact with endogenous GABA to activate these receptors and reduce excitatory transmission. In the present study, by using a suction electrode technique, we have measured the electrical activity generated in unmyelinated axons which give rise to transmitter releasing varicosities *en passant*.

Slices of rat olfactory cortex of 150-250 µm thickness were placed in a recording bath through which Krebs solution flowed. A suction electrode was connected to a current amplifier and by applying positive polarisation to the tissue, a crude estimation of the Na and K currents generating the action potential could be obtained (Scholfield, 1989). Effects on axonal Ca currents could be observed by blockade of the K-currents in these axons (Scholfield, 1988).

Pentobarbitone (0.1 to 5 mM) progressively depressed the Na, K and Ca currents in a dose-related manner. In the presence of the $GABA_A$ antagonist, bicuculline (10 µM), the depressant action of pentobarbitone was reduced (the ID_{30} concentration increased from 0.72±0.13 to 2.3±0.5 mM). Axonal Na, K and Ca currents were depressed by the $GABA_A$ agonist, muscimol (0.5-5 µM), by up to 78±5%. Further increases in muscimol produced no further depression. Muscimol enhanced pentobarbitone action: the ID_{30} concentration for pentobarbitone was decreased from 0.72±0.13mM to 0.1±0.03mM. Bicuculline reduced and muscimol enhanced alphaxalone sensitivity whereas halothane and ketamine were unaffected. The $GABA_B$ agonist, baclofen, had no effect on any of the axonal currents, nor did it affect the depressant action of pentobarbitone.

These results confirm the presence of presynaptic $GABA_A$ receptors on the transmitter releasing axons and that activation of these can depress axonal Na, K and Ca currents. Furthermore, the activation of these receptors (by muscimol) enhances the depressant actions of some general anaesthetics. This can partly explain the effect of some general anaesthetics at excitatory synapses.

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572P

ALTERATIONS OF DOPAMINE D2 RECEPTOR DENSITY FOLLOWING UNILATERAL INFUSION OF MPTP IN THE COMMON MARMOSET

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Following peripheral MPTP treatment, the density of dopamine D2 receptors has been reported to be unchanged in the common marmoset (Jenner et al., 1986) and increased in the cynomolgus monkey (Joyce et al., 1985). The present study investigates the acute and chronic effects of a unilateral intranigral infusion of MPTP on striatal dopamine D2 receptors in the common marmoset, and considers whether changes in D2 receptors correlate to the behavioural recovery observed in such animals (Jenner et al., 1986; Gerrard, unpublished data).

Male or female common marmosets (*Callithrix jacchus*) weighing 350-400g were subjected to a unilateral infusion of MPTP (20 μg/24h) or vehicle (0.9% w/v NaCl) into the substantia nigra (see Barnes et al., 1987). The MPTP infusion was continued until severe parkinsonism motor deficits were apparent (7-14 days). The animals were killed by decapitation at various time intervals after the MPTP treatment and the caudate putamen were dissected out. Saturation studies were carried out using [^{3}H]spiperone (11 concentrations between 0.005-5.0 nmol/L); non-specific binding was defined by 10 μmol/L (-)sulpiride.

Table 1. Effects of unilateral intranigral infusion of MPTP on [^{3}H]spiperone binding to the caudate putamen of the common marmoset at various time intervals following withdrawal of MPTP treatment.

		Post-infusion times					
	Side	Vehicle	1D	3M	5M	8M	18M
B_{max}/Kd	Ipsilateral	504±74/36±3	308±24*/36±1	381±45/34±4	561±95/39±1	443/35	378/36
	Contralateral	438±61/33±3	363±44/36±1	388±32/35±3	458±60/38±4	510/33	455/42

Data are the mean ± S.E.M. (n = 3-5) or mean (n = 2), D = day, M = month. B_{max} is represented as fmol/mg protein and Kd as pmol/L. Significant difference in B_{max} values compared to the respective vehicle value is indicated by *P<0.05 (Mann-Whitney U test).

The unilateral intranigral administration of MPTP caused a significant reduction in the density of [^{3}H]spiperone binding sites in the ipsilateral hemisphere at day 1 after the termination of MPTP treatment. No significant changes over all other post-infusion times were apparent in the ipsilateral or contralateral hemispheres. The results suggest that the initial reduction in ipsilateral striatal dopamine D2 receptors induced by MPTP infusion is reversible and that behavioural recovery does not appear to be the consequence of the development of total striatal dopamine D2 receptor supersensitivity.

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573P

THE EFFECT OF HALPERIDOL PRETREATMENT ON VASOPRESSIN, OXYTOCIN AND CORTISOL RELEASE IN SHEEP BY CHOLECYSTOKININ (CCK)

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Substances, such as CCK, that cause nausea in mammalian species with emetic reflexes also produce increases in plasma vasopressin but not of oxytocin (see Ebenezer et al; 1989). These results suggest that plasma vasopressin levels may be used as a marker for nauseogenic agents. Recently, we showed that administration of CCK to sheep, at a dose level that inhibits feeding but does not cause emesis, increased plasma levels of vasopressin (Ebenezer et al; 1989). As it has been demonstrated that dopamine antagonists have antinausea and antiemetic properties (Niemegeers, 1982), we decided to investigate the effects of the dopamine antagonist haloperidol on the CCK-induced release of vasopressin. We also measured plasma cortisol to provide an indication of non-specific stress.

Adult Clun forest sheep (n=10, 5M, 5F, mean b.wt. 64 kg) were used. The sheep were injected with the following: physiological saline followed by saline (control), haloperidol (80 μg/kg) followed by saline; saline followed by CCK (1 μg/kg); haloperidol (80 μg/kg) followed by CCK (1 μg/kg). The 1st injection was given s.c. 60 min before the 2nd injection (given i.v.). Blood samples were taken by jugular venepuncture before (-10, 0 min) and after (5, 10, 20 min) the 2nd injection. The plasma was analyzed by radioimmunoassay for vasopressin, oxytocin and cortisol concentrations as described previously (see Ebenezer et al; 1989).

Administration of CCK resulted in a transient but significant elevation of vasopressin levels after 5 min (148% ↑ from base-line; $P<0.05$) with no effect on oxytocin levels. There were also significant increases in cortisol levels starting 5 min after CCK injection, with maximal increases occurring at 10 min when plasma levels were approximately 170% above baseline ($P<0.001$). These results are consistent with those reported previously (Ebenezer et al; 1989). In contrast, pretreatment with haloperidol almost completely prevented the increases in plasma vasopressin concentrations that were evident when CCK was administered alone. Interestingly however, haloperidol pretreatment had no effects on CCK-induced cortisol release. Saline or haloperidol plus saline had no effects on the release of these hormones.

The results of this study indicate that haloperidol pretreatment causes a dissociation between the effects of CCK on cortisol (non-specific stress) and vasopressin (possible marker for nausea) release. Furthermore, these results suggest that CCK may cause the release of vasopressin via a dopaminergic mechanism. It is not possible at present to speculate as to whether blocking the increase in plasma vasopressin will lead to a decrease in the nauseogenic properties of CCK.

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574P

REVERSAL OF CCK-INDUCED HYPOLOCOMOTION IN THE MOUSE BY THE CCK-A ANTAGONIST DEVAZEPIDE BUT NOT BY THE CCK-B ANTAGONIST L-365,260

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Intraperitoneal (i.p) injection of cholecystokinin octapeptide (CCK) reduces locomotor activity in rodents (Crawley et al, 1981). It is unclear, however, whether this response is due to a peripheral or a central action of the peptide and whether it is mediated by CCK-A or CCK-B receptors. This study addressed both of these issues by comparing the effects of i.p. and intracerebroventricular (i.c.v.) injection of CCK on locomotor activity and determining the ability of the CCK-A antagonist devazepide (Evans et al, 1986; Chang & Lotti, 1986) and the CCK-B antagonist L-365,260 (3R(+)-N(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3 -yl)-N1-(3-methylphenyl)-urea) (Bock et al, 1989; Lotti & Chang, 1989) to block these effects. Male BKTO mice (20-25g) were housed in groups of four prior to testing. On test day they were randomly assigned to treatment groups and injected with devazepide (0.001-1.0 mg/kg), L-365,260 (0.0001-0.1 mg/kg) or 0.5% methylcellulose vehicle (s.c) 30 min prior to cholecystokinin octapeptide sulphated (i.p. or i.c.v). After the second injection the mice were placed in individual Perspex boxes fitted with 4 pairs of infrared photocells linked to a CUBE microcomputer system. Locomotor activity was measured as number of photocell interrupts.

Devazepide and L-365,260 had no intrinsic effect on activity. CCK dose dependently reduced levels of activity when administered i.p. [$F(3,28) = 14.1$, $P<0.0001$] and i.c.v. [$F(4,44) = 4.56$, $P<0.005$]. The minimum effective doses for reducing activity were 10 ug/kg (i.p.) i.e. 0.2 ug in a 20g mouse, and 3.5 ug (i.c.v.). In mice treated with CCK i.p. activity was reduced within 5 min of injection, whereas after i.c.v.infusion of CCK the latency to onset of the sedative response was 15 min. Devazepide at a dose of 0.1 mg/kg reversed the sedative effect of 10 ug/kg CCK injected i.p. [$F(4,118) = 4.95$, $P<0.005$] and of 3.5 ug CCK injected i.c.v. [$F(3,84) = 4.95$, $P<0.005$]. In contrast, L-365,260 had no effect on hypolocomotion induced by centrally or peripherally administered CCK.

These findings indicate that CCK injected i.p. or i.c.v. induces hypolocomotion via an action at CCK-A receptors. Larger doses of CCK were required to reduce locomotion when injected i.c.v. than when injected i.p. Furthermore the latency to onset of the sedative response was longer following i.c.v. CCK than after i.p. CCK. These observations suggest that hypolocomotion induced by i.c.v. CCK may be caused by leakage of the peptide to the peripheral circulation (see Passaro et al, 1982) and subsequent activation of CCK-A receptors.

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575P

BEHAVIOURAL AND NEUROCHEMICAL EVIDENCE FOR AN INTERACTION OF CCK WITH D1 DOPAMINE RECEPTORS

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Cholecystokinin co-exists with dopamine in a subpopulation of mesolimbic neurones and has been shown to modulate brain dopamine transmission (Crawley, 1988). Brain dopamine receptors are heterogenous and have been classified into two types namely D-1 and D-2 receptors (Kebabian and Calne, 1979). It is known that CCK modifies the behavioural respnse to a mixed D-1/D-2 agonist such as apomorphine (Crawley, 1988). To date, however, the effects of CCK on the response to a selective D-1 agonist have not been examined. D-1 dopamine receptor stimulation produces a characteristic pattern of behaviour and increases the formation of cAMP by stimulating the enzyme adenylate cyclase (Waddington and O'Boyle, 1989). In the present study, we examined the effects of CCK on the behavioural responses and the increase in cAMP overflow induced by the selective D-1 agonist SKF 38393.
For behavioural studies, male SD rats (300-400g) were habituated for 2.5h to Perspex observation boxes. CCK (0.001-10.0 ug/kg i.p.) was injected 10 min prior to (±)-SKF 38393 (10-80 mg/kg s.c.). Beginning 75 min after injection of SKF 38393, animals were observed for a 15 min period and behaviour recorded using a keypad interfaced to a CUBE microcomputer. Striatal cAmp overflow was measured in vivo, in a separate group of rats, by intracerebral dialysis, as previously described (Hutson and Suman-Chauhan, 1990).
Preliminary studies revealed that 20 mg/kg SKF 38393 was the minimum dose required to induce the characteristic D-1 agonist syndrome (Waddington and O'Boyle, 1989). Therefore, this dose of the drug was used for the CCK interaction studies. CCK attenuated SKF 38393-induced mouth movements at doses of 0.01-1.0 ug/kg (ANOVA, CCK main effect: $F(5,142) = 2.45$, $P<0.05$) and SKF 38393-induced sniffing at doses of 1.0 -10.0 ug/kg (ANOVA, CCK main effect: $F(5,142) = 2.70$, $P<0.03$) but had no effect on grooming induced by the drug. Similarly, CCK (1-10 ug/kg i.p.) attenuated the increase in cAMP overflow in striatal dialysate induced by SKF 38393.
These results provide the first evidence of a functional interaction between CCK and D-1 dopamine receptors. As the mouth movement response induced by SKF 38393 has recently been proposed as an animal model of tardive dyskinesia (Rosengarten et al, 1982) our data suggest that CCK (or a CCK agonist) may be a potential therapy for this disorder. Interestingly, CCK attenuates mouth movements induced in rats by withdrawal from chronic neuroleptic treatment (Stoessl et al, 1989). Furthermore, there is preliminary clinical evidence that the CCK analogue ceruletide alleviates symptoms of tardive dyskinesia in schizophrenic patients (Nishikawa et al, 1986).

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576P

EVIDENCE THAT THE ANXIOLYTIC-LIKE EFFECTS OF THE CCK ANTAGONISTS DEVAZEPIDE AND L-365,260 IN THE ELEVATED PLUS-MAZE PARADIGM IN RATS ARE MEDIATED BY CCK RECEPTORS

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Recent data indicate that cholecystokinin (CCK) antagonists induce anxiolytic-like effects in two animal models of anxiety, conditioned suppression of drinking and the light/dark exploration box (Hendrie and Dourish, 1990a,b; Dourish et al, 1990). The present study was designed to examine the effects of three CCK antagonists L-365,260, devazepide and L-365,031 in an another animal model sensitive to benzodiazepine anxiolytics, the elevated-plus maze paradigm (Handley and Mithani, 1984).
Male Sprague Dawley rats (250-300g) were individually placed in the centre of an elevated-plus maze for a single 5-min session. During the test session, the number of entries into and the time spent in the open and closed arms were recorded for each rat. To validate the elevated-plus maze as an anxiety model, the effects of diazepam (0.5, 1 and 2 mg/kg, i.p., 30 min before testing) were studied in the first experiment. In experiments 2, 3 and 4, we examined the effects of the CCK antagonists L-365,260, devazepide and L-365,031 (0.01-100ug/kg in all cases, s.c., 30 min before testing). In all studies, 0.5% methylcellulose (i.p. or s.c. as appropriate, 30 min before the test) was used as a vehicle control. In an additional experiment the effects of diazepam, L-365,260 and devazepide on exploration in a four hole box were examined to determine whether the effects observed in the maze were due to an increase in exploratory behaviour.
Vehicle-treated rats exhibited a low percentage of entries into the open arms and spent significantly less time in the open than in the closed arms ($p < 0.01$). Diazepam significantly increased the percentage of entries into and the time spent in the open arms ($p < 0.05$ for all doses), as previously described (Handley and Mithani, 1984). In subsequent experiments with CCK antagonists, diazepam (2 mg/kg) was used as a positive control and was observed to increase the total arm entries. L-365,260 dose-dependently increased the percentage of entries into and the time spent in the open arms, significant effects being observed at 1 and 10 ug/kg ($p < 0.01$ and 0.05, respectively). A dose of 1 ug/kg of L-365,260 also increased the total number of arm entries. Devazepide significantly increased the percentage of entries into the open arms at 1 and 10 ug/kg ($p < 0.01$ and 0.05, respectively). The percentage of time spent in the open arms was increased only by 1 ug/kg, a dose which significantly increased the total number of arm entries. L-365,031 had no effect on any of the parameters measured in the test. Diazepam (2 mg/kg), L-365,260 and devazepide both at a dose of 1ug/kg did not increase the number and the duration of nose pokes and the number of rears in the four hole box model of exploration. Thus, the present results suggest that devazepide and L-365,260 have anxiolytic-like properties in the elevated-plus maze paradigm. Both L-365,031 and devazepide are selective CCK-A antagonists whereas L-365,260 is a selective CCK-B antagonist (Freidinger, 1989). Since devazepide and L-365,260 but not L-365,031 induced anxiolytic-like effects in the elevated-plus maze paradigm, the results indicate mediation of the behavioural response by CCK-B receptors.

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BLOCKADE OF CCK-B RECEPTORS BY L-365,260 INDUCES ANALGESIA IN THE SQUIRREL MONKEY

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Blockade of CCK receptors has been shown to enhance opiate analgesia in a variety of pain models in rodents, primates and humans (Baber et al, 1989; Dourish et al, 1990). A novel series of non-peptide CCK antagonists has recently been developed which has helped to clarify the role of CCK receptor subtypes in the mediation of CCK/opiate interactions. Thus, it was demonstrated that the rank order of potency of these antagonists to enhance morphine analgesia (L-365,260 > devazepide > L-365,031) correlates with their relative affinities for the CCK-B receptor (Dourish et al, 1989). This suggested mediation of CCK/opiate interactions in rodents by CCK-B receptors. To date there have been no reports that CCK antagonists have intrinsic analgesic properties in rodents or primates. In the present study we determined the effects of the potent and selective CCK-B antagonist L-365,260 in the squirrel monkey tail withdrawal test, and observed that the compound induces analgesia in this model. Six male squirrel monkeys (Saimiri Sciureus) were habituated to sitting in Perspex chairs with their tails suspended in cool (35°C) water. Pain threshold was measured as the latency to remove the tail from warm (55°C) water. L-365,260 [3R(+)-N(2,3-dihydro-1-methyl-2-oxo-phenyl-1H-1,4-benzodiazepin-3-yl)-N1-(3-methylphenyl)-urea] was suspended into 0.5% carboxymethyl cellulose and injected i.p. 30 min prior to testing. Tail withdrawal latencies were measured at 30 min intervals for 120 min beginning immediately after injection of the drug. L-365,260 significantly elevated tail withdrawal latencies [L-365,260 main effect: $F(7,35) = 3.97$, $p < 0.001$] and induced significant analgesia across a 1000-fold dose range from 100ng/kg to 100ug/kg (see Table 1) . Levels of analgesia were significantly raised throughout the two hour test period.

Table 1 Effect of L-365,260 on Tail Withdrawal Latency in The Squirrel Monkey

Dose/kg	Vehicle	1ng	10ng	100ng	1ug	10ug	100ug	1mg
Mean	1.62	3.13	4.70	4.30*	9.78*	9.04**	9.02**	6.44
S.E.M.	0.28	0.91	1.28	0.96	1.71	1.18	1.37	1.41

Data are mean latencies (S.E.M.) for 6 monkeys during a 120 min test. Significant differences from vehicle were determined by planned contrasts following significant ANOVA, * $p < 0.05$, ** $p < 0.01$.

These data provide the first evidence that blockade of CCK receptors induces analgesia in primates. Previous studies have indicated that the selective CCK-A antagonist devazepide has no intrinsic analgesic activity in this test (Dourish et al, 1990). This suggests that the analgesic action of L-365,260 is mediated by its action on CCK-B receptors.

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578P

LACK OF EVIDENCE FOR CHEMICAL COMPLEXATION BETWEEN NEUROTENSIN AND DOPAMINE

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It is recognized that central administration of neurotensin produces neuroleptic-like effects but the mechanism of action of the peptide has not been clearly established (Jolicoeur et al., 1985; Nemeroff, 1986). However a recent in vitro study reported that neurotensin could block the action of dopamine by forming a complex with the amine (Adachi et al., 1990). We have investigated this proposed mechanism of action by examining the effect of NT on the dopamine signal recorded in vitro using the method of differential pulse voltammetry with carbon fibre micro-electrodes. If neurotensin binds to dopamine in the solution, a diminution of the dopamine peak height and/or a shift in its oxidation potential would be observed after the addition of neurotensin.

The working electrodes were made from single 12 µM carbon fibres electrically pre-treated as previously described (Sharp et al., 1984). A working, reference, (Ag/AgCl) and auxiliary (silver wire) electrode were immersed in a solution of artificial cerebrospinal fluid (pH 7.4) containing dopamine (10^{-5}M). After recording the dopamine oxidation peak every 2.5 min for 20 min, saline or neurotensin (10^{-4}M to 10^{-6}M) was added and voltammograms recorded every 2.5 min for a further 30 min and both the dopamine oxidation potential and itd peak height were measured before and after the addition of neurotensin. The dopamine peak height after the addition of neurotensin was compared with the mean height of ther peak obtained from the last scans before addition of the peptide. The results were analysed using ANOVA and individual differences were assessed by means of Dunnett's test *$p<0.05$ (n=4/groups).

The height of the dopamine (10^{-5}M) oxidation peak was attenuated after the addition of the neurotensin (10^{-5}M = 38% and 10^{-4}M = 65%) into the solution while saline had no effect. However when the electrode was removed from the solution for 20 min just before the addition of neurotensin, there was no reduction in the height of the dopamine peak at the first measurement after replacing the electrode in the solution but the peak was reduced in subsequent recordings. Moreover, the oxidation potential (the time to reach the maximum height for the dopamine peak, 33.8 sec ± 0.19) was not changed by the presence of neurotensin (10^{-5}M) (33.9 sec ± 0.19). Therefore, the reduction of the dopamine peak was caused by an effect of neurotensin on the recording propeties of the electrode rather than a real diminution of the free dopamine in the solution. Together, these results do not support the recent hypothesis of a chemical complexation between neurotensin and dopamine being involved in the pharmacological effects of the neuropeptides.

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579P

THE PHARMACOLOGY OF NEUROTENSIN AND NEUROTENSIN ANALOGUES ON DOPAMINE NEURONES IN THE RAT SUBSTANTIA NIGRA PARS COMPACTA

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Autoradiographic studies indicate that high densities of neurotensin (NT) binding sites occur on dopamine neurones in the substantia nigra and ventral tegmental area and electrophysiological studies have shown that NT excites dopamine neurones in the substantia nigra (Pinnock 1985; Seutin, et al 1989). In this study, we have compared the effects of NT to those of NT1-11, NT1-8, Acetyl NT8-13, NT8-13, NT9-13, LANT6-the avian derivative of neurotensin (LANT6) and Neuromedin N (Nn), which is spliced from the same mRNA as NT (Minamino et al 1984) and kinetensin. We have also investigated the actions of thyrotropin releasing hormone (TRH) on NT activity since TRH has been inferred to antagonise NT induced responses in a multiplicity of physiological and behavioural situations (Nemeroff et al 1979).

The preparation of, and extracellular recording from, slices of rat brain containing substantia nigra was as described previously (Pinnock, 1985). Slices were perfused at 4 mls/min with ACSF at 37°C . Drugs were applied directly dissolved in ACSF.

Neurotensin excited 74/79 dopamine neurones on which it was tested. The neurotensin analogues LANT6, Nn, NT 8-13, Acetyl NT8-13 and NT9-13 when applied at submicromolar concentrations, all excited neurotensin sensitive neurones. Kinetensin, NT 1-8 and NT 1-11 (10µM) were inactive when applied to these neurotensin sensitive neurones. Exposure of the preparation to 10µM TRH (n=3) had no effect on the response to submaximal doses of neurotensin. TRH had no action of its own.

The results of this study show that NT9-13 contains within it the amino acid sequence required to excite dopamine neurones. Peptides such as neuromedin-N and avian neurotensin in which the arginine at position 9 has been substituted by isoleucine and asparagine respectively are active on neurotensin sensitive neurones. Substitution of the arginine at position 9 with histidine and the isoleucine at position 12 as in the peptide kinetensin results in loss of biological activity. The lack of effect of TRH on neurotensin responses suggest that the behavioural actions of TRH as a neurotensin antagonist do not extend to a physiological or pharmacological blocking action at the single neurone level in the rat substantia nigra.

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580P

BINDING OF NEUROTENSIN ANALOGUES TO NEUROTENSIN RECEPTORS FROM RAT BRAIN

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Neurotensin (NT), a tridecapeptide originally isolated from bovine hypothalamus (Carraway & Leeman, 1973), is widely distributed in mammalian brain. Specific NT binding sites have been identified in the central nervous system and in the gastrointestinal tract of mammals (Kitabgi *et al.*, 1985). The C-terminal hexapeptide comprises the minimum structure necessary for many of the biological actions of NT. We have synthesised several analogues of this region and in each have replaced isoleucine-12 by N-methylisoleucine (MeIle) to produce derivatives with potentially increased metabolic stability (Aldalou et al., 1990). In this report we present data on the specific binding of NT and one of these analogues to plasma membranes from rat brain.

NT and NAc-NT (8-13) were purchased from Peninsula Laboratories (St. Helens, U.K.). The peptide NAc-[MeIle^{12}]NT (8-13) was synthesised by solid phase methods as previously described (Aldalou et al., 1990). Plasma membranes were prepared from 3 rat brains by a modification of the method described by Nelson *et al.* (1986) in 10 mM Tris/HCl buffer, pH 7.4, containing 1.0 mM $MgSO_4$, 1.0 mM dithiothreitol and 40 mg/L bacitracin. [3-[^{125}I]iodotyrosyl^3]-NT (2000Ci/mmole) was purchased from Amersham (U.K.) . Routinely, 100µl of the membrane suspension were mixed with an aliquot (100µl) of radiolabelled NT in the presence or absence of NT or an analogue and the mixture was incubated for 60 min at 37°C. The final concentrations of peptides were in the range $8x10^{-11}$ -$8x10^{-7}$ M. The tubes were then centrifuged for 30 min at 4000 r.p.m, and the pellet was washed with buffer at 4°C and recentrifuged. The radioactivity remaining in the pellet was determined using a gamma counter. Specific binding was found to be 89% of total binding.

The radiolabelled NT was displaced from the membrane suspension by NT, with an ID_{50} value of 9.1 and by NAc-NT (8-13) and NAc-[MeIle^{12}]NT (8-13) with IC_{50} values of 8.25 and 8.35 respectively. It seems, therefore, that MeIle does not cause a significant decrease in affinity for receptor binding.

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581P

BINDING OF NEUROKININ A ANALOGUES TO NK-2 RECEPTORS FROM GUINEA-PIG BRAIN

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The tachykinins, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), have been identified in the central nervous system (Kimura *et al.*, 1983). Three different classes of tachykinin-binding sites have been characterised and named NK-1, NK-2 and NK-3, showing binding preferences for SP, NKA and NKB respectively (Burcher, 1989). We have previously reported the syntheses of some analogues of NKA, two of which were specific antagonists of the action of NKA on guinea-pig tracheal smooth muscle (Guthrie *et al*, 1990). We now report that these two compounds displace [^{125}I]-NKA bound to isolated membranes from guinea-pig brain .

The peptides 1) [Ala^5, Aib^8, Leu^{10}]NKA (2-10), and 2) [Ala^5, Aib^8, Leu^{10}]NKA were synthesised by solid phase methods as previously described (Guthrie *et al.*, 1990). [^{125}I]-NKA (2000 Ci/mmole) was purchased from Amersham (U.K.). Male guinea-pigs (250-300 g) were sacrificed by decapitation. Brains were rapidly removed and the cerebellum was discarded. Plasma membranes were prepared according to a modified procedure of Viger *et al.* (1987). Routinely, 100 µl of the membrane suspension were mixed with an aliquot (100 µl) of [^{125}I]-NKA (35000 cpm) in the presence or absence of NKA or an analogue and the mixture was incubated for 20min at 25°C. The final concentrations of analogues were in the range 10^{-11} - 10^{-5} M. At the end of the incubations, the tubes were centrifuged for 30 s at 10,000 g, and the pellet was washed once with the incubation buffer. The radioactivity remaining in the pellet was measured using a gamma counter. Under these conditions, specific binding was found to be 68% of total binding.

Bound [^{125}I]NKA was displaced from the membrane suspension by NKA, with an ID_{50} value of 9.0 and by peptides 1 and 2, both with an IC_{50} value of 8.5. Substance P and peptides not related to NKA did not significantly displace the label. Many antagonists of tachykinin activity have been reported, but generally they have much reduced receptor affinity and also lack specificity (Burcher, 1989). Peptides 1 and 2 are highly specific antagonists of NKA with receptor affinity almost equal to that of NKA.

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NERVE GROWTH FACTOR RECEPTOR BINDING IN NORMAL AND ALZHEIMER'S DISEASE BASAL FOREBRAIN

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Alzheimer's disease (AD) is the most common cause of dementia in the elderly and is characterised by memory loss and a progressive global impairment of intellect. It involves loss of central cholinergic function associated with degeneration of cholinergic neurones in the nucleus basalis of Meynert (nbM) in the basal forebrain.

β-Nerve growth factor (β-NGF) has been shown to induce choline acetyltransferase (ChAT) activity and promote growth and survival of cholinergic neurones in vivo and in vitro. Infusion of β-NGF has been shown to prevent death of septal neurones after fimbrial fornix transection and to reverse spatial memory impairment and shrinkage of cholinergic neurones in a sub-population of aged rats. Colocalization studies have shown that the majority of basal forebrain cholinergic magnocellular neurones are β-NGF receptor positive in human brain. Receptor binding studies on neoplastic cells and chick embryo sensory ganglia cells, have suggested that there are two sub-types of the β-NGF receptor, a fast dissociating low affinity (1nM) and a slow dissociating high affinity (20-50pM) low capacity receptor.

The aim of this study was to measure the dissociation constants and capacities of β-NGF receptors in membrane preparations of basal forebrain from AD and age-matched normal subjects. [^{125}I]NGF was found to bind in a specific fashion indicative of a single receptor and is not displaced with μM concentrations of cytochrome C, insulin or epidermal growth factor (EGF). Steady state saturation analysis of basal forebrain membranes from normal and AD with radiolabelled NGF revealed one binding site which corresponded to the low affinity site previously reported in other species. There was no significant difference in mean Kd or B-max between normal and AD brain tissue. The mean Kd values ± S.E.M. for normal and AD basal forebrain membranes were 3.02nM ± 0.93 and 3.67nM ± 1.16, the mean B-max values were 0.548 ± 0.143 and 0.516 ± 0.147 fmoles/μg membrane protein. In AD, mean ChAT values were reduced from 68.6± 9.86 to 16.4 ± 5.50 pmoles acetylcholine produced /μg protein /minute.

These results show that there is no decrease in receptor number or affinity between AD and normal basal forebrain and in agreement with studies mentioned above this suggests that NGF may have a therapeutic role in AD.

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SEPARATION OF THE *IN VIVO* VOLTAMMETRIC OXIDATION PEAK OF SEROTONIN, 5-HYDROXY-INDOLACETIC ACID AND URIC ACID: A NUMERICAL METHOD VERSUS NORMAL RECORDINGS

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The use of numerical methods for the analysis of in vivo voltammetric signals has been demonstrated recently for the mathematical separation of dopamine (DA) and its metabolite 3,4-hydroxyphenylacetic acid (DOPAC), components of the "catechol peak" or Peak 2 as obtained with 12 μm diameter carbon fibre micro-electrodes (CFE) associated with differential normal pulse (DNPV) voltammetry (G.-Mora *et al.*, 1988). Voltammetric Peak 3, seen in vivo with specifically pretreated CFE (Crespi *et al*, 1984) is the result of the oxidation of a mixture of serotonin (5HT), its metabolite 5-hydroxyindolacetic acid (5HIAA) and uric acid (UA) all having an oxidation potential (Ox-P) between +250 and +300 mV (Crespi *et al.*, 1983 & 1984). We have here investigated the specific Ox-P of UA, 5HIAA and 5HT using classical paper recordings and the computerised numerical method simultaneously. With both of these analytical procedures we observed that in conscious freely moving rats prepared for DNPV in the striatum (G.-Mora *et al.*, 1988) injection of the monoamine oxidase inhibitor (MAO-I) pargyline (75 mg/kg i.p. n=4) reduced Peak 3 to approximately 60% of pre-injection control values. In addition, this treatment, which is responsible for the disappearance of 5HIAA from the extracellular fluid of conscious rats (Crespi *et al.*, 1984) was followed by a shift in the Ox-P of Peak 3, of +8 to 10 mV at its beginning with a smaller change of +4 to 6 mV at its top. Two hours later, injection of allopurinol (15 mg/kg i.p.) further decreased the size of Peak 3 down to approximately 10% of its initial size within 90/120 min., thus confirming that striatal Peak 3 is mainly determined by the oxidation of a mixture of extracellular 5HIAA and UA (allopurinol blocking the formation of this purine catabolite) (Crespi *et al.*, 1983) and that these two compounds have a very similar, but possibly separable Ox-P, when the numerical system is applied. After the removal of UA via allopurinol, a larger shift of the Ox-P than that recorded after pargyline alone (which removes 5HIAA) was also seen: +15/25 mV and +10/20 mV at the beginning and top of the signal respectively. At that moment, injection of 5HTP (15 mg/kg i.p.), which stimulates the synthesis and release of 5HT, doubled the size of the signal without any change in the potentials, suggesting that the peak remaining after MAO-I and allopurinol treatments is mainly due to the oxidation of extracellular 5HT whose Ox-P seems then more clearly distinguishable from that of UA and 5HIAA.

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584P

EFFECTS OF CALCIUM CHANNEL-BLOCKING DRUGS ON VERATRIDINE-EVOKED RELEASE (OVERFLOW) OF GLUTAMATE FROM CEREBELLAR SLICES OF WISTAR RATS

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The ability of veratridine to evoke neurotransmitter release is well recorded (see Nicholls, 1989). We have shown that veratridine increased glutamate release from cerebellar slices in calcium-free medium and that this was followed by a further increase when the slices were returned to calcium-replete (2mM) medium (Dickie and Davies, 1990). At least three types of voltage-sensitive Ca^{2+}channels have been identified (L-, N- and T-type) (Tsien *et al*, 1988). In this study we have investigated the effects of the Ca^{2+}channel blocking agents amiloride (T), verapamil (L) and w-conotoxin (N & L) on veratridine-evoked glutamate release.

Parasaggital slices (250μm) 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.

Stimulation of the slices was carried out by perfusion with a one-minute 'pulse' of veratridine ($1x10^{-5}$ M) in Ca^{2+}-free ACSF, which produced a significant increase in glutamate release ($p<0.001$), followed by another significant increase ($p<0.001$) when the slices were perfused with Ca^{2+}-replete ACSF 5 minutes post-stimulation. The Ca^{2+} channel blocking agents were added either with the veratridine pulse or with the Ca^{2+}-replete ACSF. When added with the Ca^{2+}-replete ACSF w-conotoxin (1μM) and verapamil (10μM) did not affect glutamate release. Verapamil, however, significantly inhibited release when added with veratridine ($p<0.001$). Amiloride (100μM), when added with the veratridine pulse, significantly increased Ca^{2+}-evoked release ($p<0.05$).

These results show that veratridine can stimulate glutamate release in Ca^{2+}-free conditions, and that this can be inhibited by verapamil, probably by a blocking action on Na^{+} channels (Nachshen and Blaustein, 1979). The Ca^{2+}-evoked release was unaffected by w-conotoxin and verapamil, and was increased by amiloride. This suggests that neither N-, L- or T-type Ca^{2+} channels are involved. The increased release observed in the presence of amiloride is probably due to an effect on Na^{+} exchange mechanisms.

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585P

MICROINJECTIONS OF DLH INTO THE RAT PERIAQUEDUCTAL GREY MATTER INDUCE A DOSE-DEPENDANT DEFENCE RESPONSE ATTENUATED BY MIDAZOLAM

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Chemical stimulation of the dorsal periaqueductal grey matter using the excitatory amino acid D,L Homocysteic acid (DLH), (Hilton & Redfern 1986) elicits a series of cardiovascular, autonomic and behavioural effects, collectively termed the defence reaction This response is in part under the modulatory control of GABA (Schenberg *et al* 1983). The present study determined whether there is a relationship between the dose of DLH, the magnitude of the defence reaction and its attenuation by midazolam.

Rats anaesthetised with a halothane, N_2O/O_2 mixture had stainless steel guide cannulae chronically implanted 2mm above the DPAG (AP -6.07, ML +0.2, DV -3.0 relative to bregma). Seven days post surgery the animals were placed in a circular arena and received a microinjection (0.2µl) of saline or 2, 4, 8 or 16 nmol DLH (pH 7.5) into the DPAG, and the resulting behaviour recorded using visual observation and contrast computer tracking. Each animal was given the four doses over eight days, 48 hours later this process was repeated following administration of midazolam (1mg/kg, i.p.) 20 minutes before DLH. Subsequently, rats were anaesthetised with urethane and blood pressure and heart rate measured, and the effect of DLH (40nmol) alone and 20 min after midazolam recorded. The injection site was verified with dye.

Administration of DLH into the DPAG resulted in behaviour characteristic of the defence reaction (jumping, running and vocalisation) the duration of which was dose dependant (37.1±2.8 s. with 2nmol to 145.6±8.6 s. with 16nmol, n=10). The number of jumps and revolutions around the arena were also dose dependant and none of the behaviours were observed in saline controls. DLH significantly increased blood pressure and heart rate in the anaesthetised rat (+41 ±5mmHg^{-1} and +63.±9 bpm). Midazolam significantly shifted the behavioural dose response to the right (ANOVA $F=34.11$ $p<0.001$) and attenuated the cardiovascular components (+16 ±5mmHg^{-1} and +22±5bpm). These results demonstrate that DLH placed in the DPAG induces the defence reaction dose dependently and that both the behavioural and cardiovascular components are partially antagonised by the benzodiazepine midazolam.

S.R.G.B. is a SERC CASE student in collaboration with ICI Pharmaceuticals.

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CHARACTERISATION OF THE NMDA RECEPTOR THAT STIMULATES RELEASE OF α-MELANOCYTE-STIMULATING HORMONE FROM THE RAT HYPOTHALAMUS

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α-Melanocyte-stimulating hormone (α-MSH), a tridecapeptide derived from processing of the pro-opiomelanocortin (POMC) precursor, is synthesised in perikaria of the arcuate nucleus of the hypothalamus and is postulated to be a neurotransmitter or a neuromodulator. Release of central α-MSH is under both dopaminergic and serotoninergic control, and the presence of a glutamergic influence on the POMC neurone has been identified by the demonstration of N-methyl-D-aspartic acid (NMDA)-stimulated release of α-MSH (Tiligada & Wilson, 1990). In this study we have further characterised the NMDA receptor mediating this latter response.

Two hundred and fifty μm thick coronal slices of hypothalamus obtained from 150-200g male Wistar rats were superfused with oxygenated artificial cerebrospinal fluid (aCSF) at 37°C and 0.5ml/min under an atmosphere of humidified 95% O_2/ 5% CO_2. Two minute samples of superfusate were collected commencing 30min after the start of the superfusion, frozen immediately and freeze-dried at -25°C. The dried samples were reconstituted and their content of α-MSH determined by radioimmunoassay (Wilson & Morgan, 1979). The response to zero, 1 and 5mM Mg^{2+} ion concentrations, to ketamine and to the NMDA glycine site antagonist 1-hydroxy-3-amino-pyrrolidone-2 (HA-966) were investigated by comparing the total release of α-MSH over a 10min period following the administration of a 4min pulse of 10^{-4}M NMDA in the absence and then the presence of the drug or modified aCSF. Responses to the second pulse of NMDA were compared to the second of two sequential NMDA pulses in unmodified aCSF.

Superfusion of hypothalamic slices with a 4min pulse of NMDA resulted in a significant ($p<0.001$, one-way analysis of variance) increase in release of α-MSH. A second pulse of NMDA in normal aCSF (1mM Mg^{2+}) produced a stimulation of α-MSH release that was not significantly different ($p>0.05$) from that to the first pulse, being 107±8% (±s.e.mean) of the first pulse. When slices were superfused with Mg^{2+}-free aCSF, there was no significant change ($p>0.05$) in either basal or NMDA-stimulated release of α-MSH. However, NMDA-stimulated peptide release did not return to basal release levels as in control experiments but remained elevated until the end of the experiment. Treatment of slices with aCSF containing high (5mM) Mg^{2+} ion concentrations caused a significant reduction in both basal ($p<0.001$) and NMDA-stimulated ($p<0.001$) release of α-MSH. Ketamine 10^{-4}M similarly inhibited both basal ($p<0.001$) and NMDA-stimulated ($p<0.001$) release. Superfusion of slices with 10^{-4}M HA-966 produced no significant change ($p>0.05$) in either basal or NMDA-stimulated release of α-MSH.

These data demonstrate that the NMDA receptor that mediates the stimulatory glutamergic action on release of α-MSH from the rat hypothalamus shares features in common with other central NMDA receptors being blocked by high Mg^{2+} ion concentrations and by ketamine. We were unable to show the presence of glycine potentiation of the NMDA receptor response by blockade of endogenous glycine with HA-966.

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THE EFFECT OF CHRONIC ADMINISTRATION OF COCAINE ON LOCOMOTOR ACTIVITY AND ON A COGNITIVE TASK: EVIDENCE FOR DIFFERENTIAL DEVELOPMENT OF TOLERANCE AND SENSITIZATION

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It is well established that chronic administration of cocaine results in a progressive increase in locomotor activity and stereotyped behaviour in rodents (Post and Rose, 1976). The development of paranoid psychosis as a result of chronic cocaine administration has been presented as evidence of a similar effect in humans (Angrist, 1983). It has been suggested that this 'behavioural sensitization' may be due to an increase in dopaminergic transmission as a result of repeated stimulation of dopaminergic pathways (Peris et al., 1990). It might be expected that the development of sensitization would result in a progressive deterioration of the ability to perform complex tasks while under the influence of cocaine. Somewhat surprisingly, the development of sensitization is often paralleled by a development of behavioural tolerance, provided that the initial behavioural disruption results in reinforcement loss (Woolverton et al., 1978). We examined these paradoxical effects of chronic cocaine administration in rats by monitoring their locomotor activity and their behaviour on a complex cognitive task, delayed-matching-to-position (DMTP). In order to obtain food reward, rats in the DMTP task were required to press a 'sample' lever in an operant chamber, and to choose to press it again, after a variable delay, when it was presented alongside a second lever. Initially, a 10 mg/kg dose of cocaine induced a significant deficit in the total number of trials completed indicating a behavioural deficit, and in the number of trials successfully completed, indicating a cognitive deficit. However, by the third administration of this dose, there was no evidence of impairment on either measure compared to vehicle treated rats, demonstrating not only the development of behavioural tolerance, but also the development of tolerance to the cognitive disrupting effects of cocaine. In contrast, the locomotor activity measure yielded evidence of sensitization rather than tolerance. When the rats that had been tested on the DMTP test were given a fourth dose of 10 mg/kg cocaine, they showed a significantly greater elevation in locomotor activity than a separate group of rats injected with either 10 or 30 mg/kg of cocaine for the first time. These results are interpreted as indicating that cocaine disrupts cognitive as well as behavioural processes and that tolerance can develop to each. However, it remains to be determined whether reinforcement loss is a necessary requirement for the development of tolerance to the cognitive disrupting effects of the compound.

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588P

THE ANGIOTENSIN CONVERTING ENZYME INHIBITORS ENALAPRIL AND CAPTOPRIL INHIBIT APOMORPHINE-INDUCED ORAL STEREOTYPY

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Recent studies have suggested a functional interaction between the brain renin-angiotensin system and dopaminergic neurotransmission. Angiotensin II injected intracerebroventricularly potentiates apomorphine-induced stereotypy (Georgiev *et al*, 1984) whereas the angiotensin converting enzyme (ACE) inhibitor captopril attenuates the response (Sudilovsky *et al*, 1989). We have now compared the effects of the ACE inhibitors enalapril and captopril on apomorphine-induced stereotypy to those of the classical neuroleptic haloperidol.

In the first study the effects of captopril on apomorphine-induced behaviour were compared to those of haloperidol. Rats were habituated to observation boxes for 45 min and injected with 10 mg/kg captopril i.v., 0.2 mg/kg haloperidol i.p. or appropriate vehicle. The animals were returned to the boxes for 60 min (captopril) or 20 min (haloperidol) and injected with 0.03, 0.3, or 3 mg/kg apomorphine or vehicle (0.5mg/ml ascorbate in saline) s.c. The duration and frequency of cage crossing, grooming, rearing, sniffing, immobility, licking and gnawing were recorded for 30 min, beginning immediately after the second injection, using a keypad linked to a BBC microcomputer. Captopril had no effect on apomorphine-induced sniffing but significantly decreased apomorphine-induced gnawing and licking. Similarly, haloperidol had no effect on apomorphine-induced sniffing but blocked the licking and gnawing response.

In the second study dose response curves for inhibition of apomorphine-induced oral stereotypy by two ACE inhibitors (captopril and enalapril) were determined. Rats were allowed to habituate to observation boxes for 45 min and injected with 1, 3, 10 or 30 mg/kg captopril i.v. or 1, 3, 10, 30 or 100 mg/kg enalapril i.v or s.c. or saline vehicle. The animals were returned to the boxes for 50 min and then injected with 1 mg/kg apomorphine or vehicle s.c. Behaviour was recorded for a period of 10 min beginning 10 min after the second injection. Captopril (3 and 10 mg/kg i.v.) attenuated oral stereotypy induced by apomorphine, but was ineffective at any other dose. Similarly, enalapril injected s.c. blocked apomorphine-induced gnawing at doses of 10 mg/kg and 30 mg/kg. Enalapril injected i.v. attenuated apomorphine-induced gnawing at 3 and 10 mg/kg, and abolished the response at 30 mg/kg.

The selective blockade of apomorphine-induced oral stereotypy by haloperidol observed in this study is consistent with a previous report by Ljungberg and Ungerstedt (1978) that 0.2 mg/kg haloperidol blocks apomorphine-induced gnawing but has no effect on apomorphine-induced locomotion and sniffing. Furthermore, the results suggest that the effect of ACE inhibitors on apomorphine-induced stereotypy are similar to those of a classical neuroleptic as captopril and enalapril selectively blocked apomorphine-induced oral stereotypy.

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589P

INHIBITION OF HISTAMINE RELEASE FROM HUMAN BASOPHILIC LEUKOCYTES BY THE PROTEIN KINASE C INHIBITOR STAUROSPORINE

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Mast cells and basophilic leukocytes are involved in allergic/pseudoallergic reactions and local inflammatory responses, mediated by the release of histamine. Cross-linking of cell-bound IgE-molecules and hence cross-linking of IgE-receptors ($FC_\epsilon RI$) leads to a variety of cellular signal transduction processes, involving phosphoinositol hydrolysis, Ca^{2+} - influx, and protein kinase C (PKC). We therefore performed experiments to study the influence of PKC-inhibition on histamine release from basophils.

Suspensions of human basophils were obtained from healthy donors by dextran-sedimentation. Cells were challenged in HEPES-buffer with goat anti-human IgE in different dilutions for 30 min at 37° C. Cells were preincubated with different concentrations of the PKC-inhibitor staurosporine ($1x10^{-9}$-$1x10^{-5}$M) for 15 min before being triggered with anti-IgE. Histamine was measured spectrofluorometrically using an autoanalyzer.

Thousand-, hundred-, and then-fold dilution of the anti-IgE stock solution yielded a histamine release of 8.7±2.1%, 43.2±2.0%, and 35.1±2.4%, respectively (n=10). Preincubation with staurosporine exerted concentration-dependent inhibition of the IgE-mediated histamine release at an optimal dilution of anti-IgE (hundred-fold, table 1).

Table 1 Inhibition of IgE-mediated histamine release by staurosporine (in %, n=5)

	Concentration of staurosporine (M)				
	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
anti-IgE :	15.3±9.6	20.1±7.9	67.5±8.7	88.0±3.9	81.0±7.2

The present results show a strong inhibition of histamine release mediated through the $FC_\epsilon RI$ by staurosporine. The IC_{50} was 0.047 μM. Staurosporine is a potent PKC-inhibitor (Tamaoki *et al.*, 1986). Our data suggest that PKC is involved in the stimulus-secretion coupling following cross-linking of IgE-receptors on the basophil surface.

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590P

VAGALLY-RELEASED ACETYLCHOLINE INHIBITS SYMPATHETIC NEUROTRANSMISSION IN THE GUINEA-PIG TRACHEA

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In the guinea-pig, parasympathetic and sympathetic nerves lie in close proximity and have opposing effects on the airway smooth muscle (Jones et al 1980). We have previously reported inhibition of pulmonary sympathetic neurotransmission by exogenously applied muscarinic agonists via activation of prejunctional muscarinic M_3 heteroreceptors (Pendry & Maclagan 1989). In the present experiments, the effect of endogenously released acetylcholine on relaxations evoked by stimulation of the pulmonary postganglionic sympathetic nerves was investigated.

The guinea-pig trachea with the right sympathetic nerve trunk, and both vagi and recurrent laryngeal nerves attached was mounted in oxygenated Kreb's solution containing indomethacin ($5x10^{-6}$M) and maintained at 37 °C. Increases or decreases in intraluminal pressure (ILP) reflected contraction or relaxation, respectively, of the trachealis smooth muscle. When the ILP had been raised with the stable thromboxane analogue U46619 (10^{-8}-$3x10^{-6}$ M), stimulation of the sympathetic nerve trunk (40Hz, V_{max}, 0.2ms, 5sec) evoked relaxations which were directly related to tone but reproducible at any given ILP.

Sympathetic nerve-induced relaxations were reduced in the presence of concurrent submaximal vagal stimulation (20Hz, 0.2ms for 3 or 4.5mins) in 50% of the preparations. The involvement of postjunctional physiological antagonism was excluded by stimulating the vagi at parameters which caused no increase in ILP. In addition, neuropeptides released from nonadrenergic noncholinergic nerves which run in the vagal bundle did not appear to be involved, as the neuropeptides substance P, vasoactive intestinal peptide, neurokinin A and endothelins did not significantly alter sympathetic nerve-induced relaxations.

The anticholinesterase, physostigmine (10^{-8}-10^{-6}M) induced an increase in tone of the guinea-pig tracheal tube preparation and potentiated the postjunctional contractile action of acetylcholine (10^{-5} M). In contrast, physostigmine (10^{-6}M) inhibited sympathetic nerve-induced relaxations; for example, at an ILP level of 300mmH_2O sympathetic relaxation in the absence and presence of physostigmine was 145± 22 and 42 ± 12 mmH_2O respectively (x±s.e.m., n>5; P<0.005). The muscarinic antagonist, atropine (10^{-7} M) antagonised both the contractile action of physostigmine on airway smooth muscle and its inhibitory effect on sympathetic nerve-induced relaxation.

These results suggest that, in the guinea-pig trachea, acetylcholine released from pulmonary parasympathetic nerves inhibits sympathetic neurotransmission via an action on muscarinic receptors, situated prejunctionally on the sympathetic nerve terminals. Anticholinesterase drugs potentiate this effect and have a sympathoinhibitory action, in contrast to muscarinic M_3 antagonists, which have a sympathoexcitatory effect in the airways (Pendry & Maclagan, 1989).

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THE EFFECT OF POTASSIUM CHANNEL OPENING DRUGS ON GUINEA-PIG PULMONARY NERVES

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The potassium channel opening drug cromakalim prevents bronchoconstriction induced by various spasmogens in animals and man. Hall & Maclagan (1988) reported that cromakalim inhibits transmission in pulmonary cholinergic nerves due to an effect at either the ganglia or the final nerve terminals. In the present experiments, the location of the neural effect was studied. Contractions of the isolated guinea-pig tracheal tube, measured as a change in intraluminal pressure (ILP), were elicited by either preganglionic vagal nerve stimulation (PGS) via a ring electrode or by transmural stimulation (TMS; 30 V, 30 Hz, 0.2 ms pulse width, 5 s duration, 40 s interval) of postganglionic cholinergic nerves in the presence of hexamethonium (50 μM). Indomethacin (5 μM) was present throughout to abolish prostaglandin-induced tone.

Cromakalim (3.2 to 12.8 μM) did not significantly affect the dose-related rise in tracheal tone caused by the spasmogens acetylcholine, histamine or U46619. Table 1 shows that cumulative addition of cromakalim (0.1 to 26 uM) caused inhibition of TMS-induced increases in ILP, reaching a maximum inhibition of 53.25 ± 3.25 % at 26 uM. Cromakalim also inhibited TMS-induced contractions when hexamethonium and indomethacin were omitted, in contrast to the results reported by McCaig and Dejonkeere, (1989). The inhibitory effect of cromakalim was significantly greater on responses elicited by PGS, reaching a maximum of 65 ± 1 % at 26 μM. The active (+) enantiomer lemakalim (BRL38227; 0.01 to 26 μM) was more potent than cromakalim, and caused 100% inhibition of PGS and TMS at 12 μM. Glibenclamide (Glib; 20 μM), a specific blocker of ATP-operated K^+ channels, significantly reduced the inhibitory action of cromakalim on contractions elicited by PGS and TMS (P <0.005).

Increase in ILP (mm H_2O) to transmural (TMS) and preganglionic (PGS) stimulation.

	Control	Cromakalim 26 μM	%Inhibition
TMS	155.0±39.3	82.2±20.8	53.3±3.3[a,b]
TMS + Glib	102.0±68.1	76.7±54.5	25.1±4.5[b]
PGS	158.0±45.3	102.0±29.4	65.0±1.0[a,c]
PGS + Glib	126.7±27.9	83.6±21.0	34.5±6.0[c]

Mean ± sem, n = 5 a,b,c - P <0.005

Nonadrenergic noncholinergic (NANC) relaxant responses of the trachea were elicited by TMS in the presence of hexamethonium (50 uM), atropine (1 μM) and propranolol (1 μM) when the tone was raised to 200 mmH_2O with histamine. Cromakalim caused variable facilitation of these NANC-mediated relaxations of the trachea. PGS in the presence of atropine (1 μM), propranolol (1 μM) and histamine did not induce a relaxant response of the trachea, indicating that NANCergic fibres enter the trachea below the cervical vagal nerve trunk.

These results suggest that cromakalim inhibits transmission both at the ganglionic synapse and at the final terminals of pulmonary cholinergic nerves, and a proportion of the K^+ channels involved appear to be ATP-sensitive. Cromakalim, however, causes facilitation of NANC-mediated relaxations. In contrast, pulmonary noradrenergic nerves are unaffected (Pendry & Maclagan, 1990).

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COMPARISON OF THE BRONCHODILATOR PROPERTIES OF RO 31-6930, LEMAKALIM AND SALMETEROL IN THE GUINEA-PIG

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Ro 31-6930 2-(6-cyano-2,2-dimethyl-2H-1-benzopyran-4-yl)pyridine-1-oxide is a potassium channel opener with bronchodilator properties (Paciorek et al., 1989). The aim of this study was to further evaluate the bronchodilator properties of Ro 31-6930, in comparison with lemakalim (Arch et al., 1988) and salmeterol (Bradshaw et al., 1987) following i.v. and inhaled administration in the guinea pig.

Guinea pigs were anaesthetised with urethane (1.75 g kg^{-1} i.p.). Drugs were administered via a jugular vein and mean arterial pressure (MAP) measured from a carotid artery. Animals were respired with air (10 ml kg^{-1} body weight: 55 min^{-1}), and pulmonary inflation pressure (PIP) was measured from a side arm of the tracheal cannula. Bronchoconstriction was evoked by $\approx ED_{65}$ doses of 5-HT (2-20 μg kg^{-1} i.v.) or by construction of dose response curves to 5-HT (1-300 μg kg^{-1} i.v.). Test agents were administered cumulatively i.v. or by inhalation form a DeVilbiss ultrasonic nebulizer via the tracheal cannula. In a separate series of experiments lung resistance (R_L) and dynamic lung compliance (C_{dyn}) were measured using a Buxco model 6 Pulmonary Analyser. A submaximal dose of 5-HT (2-20 μg kg^{-1} i.v.) which increased R_L by $\approx$250% and decreased C_{dyn} by $\approx$70% was determined. Test agents were then administered cumulatively i.v. 10 min prior to each 5-HT challenge. Values presented are mean ± s.e. mean, n = 4-5.

Ro 31-6930, lemakalim and salmeterol evoked dose related inhibition of the submaximal increases in PIP with ID_{50} values of 36.8 ± 11.1, 169 ± 44.0 and 7.6 ± 2.9 μg kg^{-1} i.v. respectively. Inhaled Ro 31-6930 (0.1, 0.3 and 1.0 mg ml^{-1}: 2 min) lemakalim (0.3 and 1.0 mg ml^{-1} 2 min) and salmeterol (0.03 and 0.1 mg ml^{-1}: 2 min) evoked dose related inhibition of the response curve to 5-HT which was most marked following the lowest doses of 5-HT (43.2-100%) and least at the highest doses of 5-HT (15.9-75.3%). Mean pre-inhalation MAP was 30.6 ± 1.2 mmHg (n = 28). Ro 31-6930 reduced MAP by 7.9 ± 1.2, 7.6 ± 1.7 and 5.4 ± 1.5 mmHg and lemakalim by 7.1 ± 1.2 and 7.2 ± 2.4 mmHg respectively. Ro 31-6930, lemakalim and salmeterol evoked dose related inhibition of the effects of 5-HT on R_L and C_{dyn} (ID_{50} values: 7.4 ± 1.5 and 14.2 ± 3.7; 103.4 ± 25.6 and 157.8 ± 33.6; 4.2 ± 1.9 and 4.9 ± 2.0 μg kg^{-1} i.v. respectively).

Ro 31-6930 is a potent bronchodilator which is effective by both the i.v. route and by inhalation.

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593P

COMPARATIVE EFFECTS OF ENDOTHELIN-1 AND LEUKOTRIENE D_4 ON HUMAN AND GUINEA-PIG AIRWAY PREPARATIONS

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Endothelin – 1 (ET – 1) is a newly isolated 21 – amino acid peptide with potent vasoconstrictor and bronchoconstrictor properties (Yanagisawa *et al.* 1988). Although the possible role of this peptide in physiological and pathological processes is unknown, its presence in bronchial epithelial cells suggests that ET – 1 could affect adjacent bronchial smooth muscle tone. In the present study we have compared the effects of ET – 1 with those of leukotriene (LT)D_4 on strips of guinea – pig lung parenchyma (GPP), human lung parenchyma (HLP) and human bronchi (HBr).

GPP were prepared from the major lobes of lungs from male Dunkin – Hartley guinea pigs and HLP and HBr from human lung tissue. The tissues were superfused in series in a cascade system with Tyrode solution (5ml/min) at 37°C. LTD_4 and ET – 1 were administered as bolus injections. FPL 55712 (1.9μM), indomethacin (2.8μM), WEB 2086 (10nM) and a mixture of antagonists to ACh, histamine, 5 – HT and α – and β – adrenoceptors were given as a continuous infusion. In addition GPP were also used in conventional organ baths. Following stimulation with LTD_4 and ET – 1 the bath fluids were assayed for TxB_2 and 6 – keto – $PGF_{1\alpha}$ by RIA.

ET – 1 (10 – 1000 pmol) induced dose – related contractions of GPP and was approximately 10 – 30 times less active than LTD_4 (1 – 30 pmol). The contractile response elicited by ET – 1 had a long duration of action and was unaffected by antagonists to ACh, histamine, 5 – HT, PAF and LTD_4. However these responses were attenuated in the presence of indomethacin. In organ bath, equiactive doses of ET – 1 (100nM) and LTD_4 (1nM), induced the release of 303±51 and 1171±131 pg/ml TxB_2 and 75±23 and 237±35 pg/ml 6 – keto – $PGF_{1\alpha}$ respectively. The release of both cyclooxygenase products was completely inhibited by indomethacin. ET – 1 (10 – 300 pmol) also contracted HLP and was approximately 10 times more active than LTD_4 on this preparation. In contrast this peptide was less active than LTD_4 on HBr. The action of ET – 1 on both preparations of human airways was not inhibited by indomethacin.

These results show that ET – 1 causes a long lasting contraction of GPP which is mediated in part by the release of TxB_2, as has been previously shown for LTD_4. In contrast the action of ET – 1 on both HLP and HBr was not mediated by the release of cyclooxygenase products and like LTD_4, appears to be a direct effect on both vascular and bronchial smooth muscle in these preparations.

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DOSE-RELATED EFFECTS OF ALPHA, BETA-METHYLENE ATP ON CANINE NASAL VASCULAR AND AIRWAY RESISTANCES

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Sympathetic stimulation contracts nasal venous vessels via some non-adrenergic and non-cholinergic mechanisms (Lung & Wang, 1989). ATP has been shown to act as a co-transmitter in sympathetic neuro-transmission (Burnstock & Warland, 1987). This study demonstrates the action of alpha beta-methylene ATP, a potent P_{2x}-purinoceptor activator, on the nasal vascular and airway resistances.

In sodium pentobarbitone anaesthetized dogs (n=6), nasal vascular resistance (Rnv) was measured by constant-flow perfusion of the main artery to the nasal mucosa and nasal airway resistance (Rna) by monitoring the transnasal airway pressure with constant airflow through the nasal cavities. Alpha, beta-methylene ATP was infused intra-arterially via the perfusion circuit at the rate of 0.1 ml min^{-1}.

Table 1

Doses of drug ($ug\ kg^{-1}min^{-1}$)	Rnv (% change)	Rna (% change)
0.05	+2±1.2	-2±1.1
0.5	+18±4.3*	-12±3.2*
5	+40±4.7*	-24±3.8*
50	+50±4.6*	-28±4.5*

Results are means±s.e.means of % change from controls. * $P < 0.05$.

Alpha, beta-methylene ATP increased Rnv and decreased Rna, suggesting contraction of both arteries and veins. Hence, it seems likely that ATP is involved in the non-adrenergic non-cholinergic sympathetic control of the nasal blood vessels.

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595P

ADENOSINE-INDUCED BRONCHOCONSTRICTION IN THE CONSCIOUS GUINEA-PIG

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Bronchoconstrictor responses to adenosine (ADO) have been reported in asthmatic patients (Cushley et al., 1983) and in vitro in isolated preparations taken from sensitised guinea-pigs (Thorne & Broadley, 1988, 1990). This study investigates the in vivo effects of ADO in normal and sensitised conscious guinea-pigs measured using a whole body plethysmographic technique.

Male Dunkin-Hartley guinea-pigs were sensitised to ovalbumen (OA) by i.p. injections 14 days (5mg in 0.1ml of water for injections) and 12 days (10mg) before the first exposure to adenosine. Groups of six animals individually inhaled nebulized saline or solutions of carbachol or adenosine in saline for 1 min. Immediately afterwards, specific airways conductance (sGaw) was measured by constant volume plethysmography (Griffiths-Johnson et al. 1988).

In control (untreated) animals, saline caused an increase in sGaw peaking at 27±7% at 5 min. ADO (1mg/ml) caused a greater increase in sGaw of 34.8±8% at a 15 min peak, suggesting bronchodilatation, which remained elevated after 90 min. A similar effect was observed when repeated after 7 days. Carbachol (0.02mg/ml) caused a decrease in sGaw, indicative of bronchoconstriction. The peak reduction (18±5%) occurred after 5 min and returned to baseline after 60 min. This constriction was slightly reduced when repeated a week later.

In sensitized guinea-pigs, ADO (1mg/ml) induced a bronchoconstriction, the peak reduction of sGaw ocurring (24±4%) after 15 min. The degree of bronchoconstriction was no greater when exposed to a higher concentration (3mg/ml) of adenosine (sGaw reduced by 18±5% after 15 min). Both doses induced similar responses when repeated 7 days later indicating that the tachyphylaxis demonstrated in vitro (Thorne & Broadley, 1988) was reversed within 7 days.

These results demonstrate that an ADO-induced bronchoconstriction may be observed in vivo, like in vitro, by prior sensitisation with ovalbumen.

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596P

9-METHYL ADENINE ANALOGUES ARE SELECTIVE A_1 ADRENOCEPTOR ANTAGONISTS

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Most adenosine receptor antagonists are xanthine derivatives, many are also cyclic nucleotide phosphodiesterase inhibitors. Adenosine receptor antagonists based upon the structure of the natural ligand, 7-deazo-9-phenyladenines, have been shown in binding studies to have low affinities at both A_1 and A_2 receptors (Daly *et al* 1988a) and N^6-substituted 9-methyladenines have also previously been investigated to a limited extent (Daly *et al* 1988b). N^6-substitution enhanced affinity for the A_1 receptor. We have studied the effects of a series of N^6-substituted 9-methyladenines upon responses elicited by 5' (N-ethyl)carboxamidoadenosine (NECA) in guinea pig left atrium (A_1-receptor assay) and guinea pig taenia caecum (A_2-receptor assay). The unsubstituted compound had no affinity for either the A_1 or the A_2 receptor, however all of the N^6-substituted compounds behaved as simple competitive antagonists at the A_1 receptor and exhibited selectivity for this site. A pK_B value of 5.26±0.10 (Schild slope=1.15±0.18) was estimated for the pentyl substituted compound N-0837 (N^6-(3-pentyl)-9-methyl adenine) and this compound was shown to interact with 8-phenyltheophylline in a manner consistent with simple competition indicating that both compounds act at the same site. The most potent compounds in the series were N^6-(endo-2-norbornyl)-9-methyl adenine (N-0861: pK_B= 6.28±0.09 Schild plot slope=1.09±0.07) and N^6-cyclopentyl-9-methyl adenine (N-0840: pK_B=6.17±0.11 Schild plot slope=0.95±0.08). These compounds were inactive in the A_2-receptor assay at 30 and 100uM respectively. For comparison, 1,3-dipropyl, 8-cyclopentylxanthine (DPCPX) was found to have a pK_B of 8.61 in the A_1-receptor assay. However, in the A_2-receptor assay DPCPX produced a complex mixture of antagonism and potentiation. A pA_2 of 6.8 was estimated indicating an A_1/A_2 selectivity of about 60-fold. Therefore, although of lower affinity than DPCPX, the N^6-substituted compounds are more A_1 selective and should be useful probes in the classification of adenosine receptors.

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597P

THE INFLUENCE OF ELECTRICAL FIELD STIMULATION ON GABA-INDUCED ILEAL CONTRACTIONS

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γ-aminobutyric acid (GABA) and muscimol cause a transient contraction of ileum which is antagonised by bicuculline, tetrodotoxin and atropine, suggesting an action on $GABA_A$ receptors to release acetylcholine from cholinergic nerves (Kerr & Ong, 1986). When investigating this GABA-mediated contraction, we noted that it could be inhibited by a preceding period of electrical field stimulation (EFS) and here we describe some of the characteristics of this inhibition.

Segments of guinea-pig ileum were suspended in Krebs Henseleit solution (37°C, 95% O_2/5% CO_2). Consistent submaximal isometric contractions were elicited in response to applications of GABA (0.1mM). Tissues were then subjected to EFS (0.1Hz, 0.2msec, supramaximal voltage) for 2-40 min and the subsequent (test) responses to GABA (0.1mM) were recorded immediately after stimulation. Periods of EFS of 10-40 min caused significant inhibition of GABA contractions (n = 8), the extent of the inhibition increasing with increasing periods of EFS. After 20 and 40 min periods of EFS, test responses to GABA were 52±3% and 12±2% respectively of control responses. Test responses to GABA were not inhibited by a 2 min period of EFS, or by a 2-40 min EFS-free interval between control and test response, or when tissues were washed between the period of EFS and the application of the test dose of GABA. A 20 min period of EFS caused similar and significant inhibition over the entire concentration-response curve for GABA (0.01-1mM, n = 6). Increasing the frequency of EFS (0.2, 0.4Hz) significantly, and in a frequency-dependent manner, increased the inhibition of submaximal GABA responses observed with all periods of EFS: a 20 min period of EFS at 0.4Hz virtually abolished the subsequent response to GABA (0.1mM) (n = 4).

To establish whether this phenomenon of EFS-provoked inhibition was specific for GABA responses, experiments were conducted where submaximal control and test responses to muscimol, acetylcholine, dimethyl 4-phenylpiperazium iodide, nicotine, 5-hydroxytryptamine, histamine and KCl were examined (n ≥6). EFS inhibited test responses to muscimol and to GABA similarly, but was without effect on responses to the other agents. Antagonists or synthesis inhibitors for a range of putative neurotransmitters or modulators were unable to protect the responses to GABA from the inhibitory influence of EFS (n ≥6, equilibration period 20 min). Agents used included theophylline, naloxone, indomethacin, propranolol, prazosin, yohimbine, methysergide, ritanserin, ascorbic acid, glutathione, catalase and superoxide dismutase.

The results suggest that EFS of guinea-pig ileum releases a substance or initiates a process which selectively inhibits $GABA_A$-receptor mediated contractions. The effect is time- and frequency-dependent, is reversed by washing and is not susceptible to a range of antagonists for putative neurotransmitters or modulators.

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598P

DIFFERENCES IN THE MECHANISMS OF ACTION BETWEEN EXEPANOL AND 5-HT_3 AND 5-HT_4 RECEPTOR AGONISTS TO CAUSE CONTRACTIONS IN THE GUINEA-PIG ILEUM

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Exepanol is a benzoxepine derivative that can facilitate muscle contraction in the oesophagus and other regions of the gastrointestinal system (Wolf, 1985). Its mechanism of action is not known and in the present study we investigate a 5-HT_3/5-HT_4 receptor involvement.

Isolated ileal segments (2cm) (taken approximately 9cm from the ileo-caecal junction) from female guinea pigs (Dunkin-Hartley, 250-500g) were suspended under 1g tension in oxygenated (95% O_2/5% CO_2) Krebs-Henseleit solution routinely containing methysergide (1μM). A 50mM potassium solution was administered for 3 min, washed out and the tissues allowed to equilibrate for 1 hour; 2 bolus doses of 5-hydroxytryptamine (5-HT) (10^{-6}M) were then added 15 min apart before the non-cumulative concentration response curves to agonist drugs were established using a 1 min contact time and an 11 min dose cycle. All experiments were repeated at least once using 4 tissues per experiment.

The administration of the 5-HT_3 receptor agonist 2-methyl-5-HT (10^{-7}-10^{-5}M) caused concentration related contraction responses (maximum changes in tissue tension 1.8-2.5g). Following the work of Craig & Clarke (1990), the prior (30 min) and continuing exposure of tissues to a bathing solution containing 2-methyl-5-HT (10^{-5}M) abolished the contraction responses to subsequent challenge with 2-methyl-5-HT (10^{-7}-10^{-5}M); responses to exepanol (10^{-7}-10^{-4}M) and renzapride (10^{-7}-10^{-5}M) were not antagonised. In subsequent experiments using Krebs-Henseleit solution containing methysergide (1μM) and the 5-HT_3 receptor antagonist ondansetron (5μM), the administration of the 5-HT_4 receptor agonist 5-methoxytryptamine (5-MOT) (10^{-7}-10^{-5}M) caused concentration related contraction responses (maximum changes in tissue tension 1.3-1.4g). Desensitisation of the 5-HT_4 receptors using a high concentration of 5-MOT (10^{-5}M) prior to (30 min) and a continuing exposure, antagonised the contractions induced by 5-MOT (10^{-7}-10^{-5}M) and renzapride (10^{-7}-10^{-5}M) but not exepanol (10^{-7}-10^{-4}M). The 5-HT_3 and 5-HT_4 receptor antagonist ICS 205-930 (10μM) antagonised the contractions induced by renzapride and 5-HT but not by exepanol.

Selective 5-HT_3 or 5-HT_4 receptor desensitisation or blockade antagonised the contractions induced by 5-HT_3 and 5-HT_4 receptor agonists respectively, but all such treatments failed to modify the contractions induced by exepanol. It is concluded that exepanol does not mediate its effects via a 5-HT_3 or 5-HT_4 receptor mechanism in the guinea pig ileum.

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599P

RELAXANT EFFECTS OF PINACIDIL ON THE LONGITUDINAL MUSCLE OF THE RAT ILEUM

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The potassium channel opening agents have been shown to cause relaxation in various smooth muscle preparations, particularly vascular (Hamilton and Weston, 1989). In the present study we investigated the relaxant effects on rat ileum of pinacidil, as a representative of this group of agents, and the antagonism of the relaxation by tetraethylammonium (TEA) and glibenclamide (GBC).

Longitudinal muscle-myenteric plexus preparations were derived from the distal ileum of male rats and suspended under 1g tension in Krebs' solution (37°C, 95% O_2/5% CO_2). Following equilibration, isometric contractions were elicited in response to 20mM KCl (approximately 75% E_{max}). At the plateau of contraction, pinacidil (PIN, 0.1-13μM) or vehicle was applied cumulatively. Relaxation was expressed as % reversal of the initial contraction. The procedure was repeated following incubation of the tissue for 20 minutes with a single concentration of TEA (0.3-3mM) or GBC (30nM-3μM) or antagonist vehicle.

PIN produced a concentration dependent relaxation, complete reversal of contraction occurring with higher concentrations. The apparent IC_{50} value was 0.9±0.09μM (n = 15) when the vehicle for PIN was ethanol/water and 1.02±0.11μM (n = 5) when the vehicle was 0.5M HCl. However, it was noted that tissues exposed to vehicle alone relaxed during the experiment. Both TEA and GBC caused parallel rightward shifts of the PIN concentration-response curve whilst having no effect on the action of the vehicle. The apparent pA_2 values were 2.92±0.5 (n = 13) for TEA and 6.86±0.18 (n = 14) for GBC (mean ± 95% confidence limits). GBC was without direct effect on the tissue while TEA caused a small increase in baseline tension which was concentration dependent, the highest concentration of TEA (3mM) causing an increase <20% of the KCl contraction. To ascertain whether this contractile effect could offset the action of PIN in a non-specific manner, theophylline (6.4-800μM) was used to relax KCl contractions: TEA was without antagonist effect (n = 5).

Contractile responses to electrical field stimulation (0.1Hz, 5msec pulse width, supramaximal voltage) were reduced in a concentration-dependent manner by PIN (0.1-13μM). Higher concentrations of PIN virtually abolished responses to stimulation: the vehicle (ethanol/water) was without effect. The IC_{50} for PIN in these experiments was 1.17±0.16μM (n = 6).

The IC_{50} values reported here for PIN are comparable with those quoted for other smooth muscles and indicate no marked difference in potency to inhibit nerve-mediated or direct muscle responses in intestinal muscle. Similarly the antagonist potencies for TEA and GBC are comparable to those reported for other types of smooth muscle (Cavero *et al.*, 1989; Wilson *et al.*, 1988).

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600P

AN EXAMINATION OF THE CONTRIBUTION MADE BY THE CONSTITUENTS OF PEPPERMINT OIL (PO) TO THE RELAXATION OF GASTROINTESTINAL SMOOTH MUSCLE

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Peppermint oil (PO) is used in the therapy of Irritable Bowel Syndrome. The efficacy of this natural substance is attributed to its ability to reduce muscle spasm and the constituent thought to be responsible for this action is menthol. While menthol is present in PO, and relaxes gut smooth muscle in vitro (Taylor et al., 1984), many of the other constituents of PO have not been tested. Here we have examined the activity of PO in the guinea-pig taenia coli (gptc) and guinea-pig colon, and have investigated the activity of most of the known constituents of PO in the (gptc). Male guinea-pigs (250-400g) were killed, and either taenia-coli or proximal colon removed into Krebs. Preparations were mounted in organ baths containing Krebs solution, gassed with 5% O_2/95% CO_2. The taenia and colon preparations were suspended under 1 and 2g isometric tension respectively. Taenia relaxations were assessed as the % inhibition of carbachol (100-300nM) contractions and colon relaxations as % inhibition of spontaneous contraction frequency and mean amplitude. PO relaxed the taenia in a concentration dependent manner (IC_{50} 22.1 µg/ml). At IC_{50} concentrations, the relaxation persisted for at least 20 mins. PO also inhibited spontaneous activity in the colon. Mean contraction amplitude (IC_{50} 30.7 µg/ml) was inhibited more readily than contraction frequency (IC_{50} 84.6 µg/ml).

Table 1

Constituent	IC_{50} gptc relax. Mean±SEM (µM) (n=4)	Approx. amount in PO
Jasmone	4.2 ± 0.5	<0.1%
3-octanol	24.9 ± 3.1	2.0%
neomenthol	88.1 ± 32	6.0%
pulegone	105.0 ± 18	0.9%
isomenthone	125.0 ± 18	9.0%
menthol	154.0 ± 24	43.0%
cineole	237.0 ± 41	3.0%
menthone	473.0 ± 1-3	19.0%
menthyl acetate	696.0 ± 220	4.0%
(-)limonene	>1000	
(+)limonene	>1000	1.0%

When the relative potencies of the constituents of PO (Table 1) are considered with the data on the proportion of the constituents present, it appears that menthol, due to its abundance in the oil, is likely to be the constituent responsible for much of the relaxant activity. This observation therefore supports the previously held suggestion that menthol is the active ingredient of PO (Taylor et al., 1985).

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601P

A METHOD FOR DETERMINING BLADDER OR GUT SELECTIVITY AND POTENCY OF ANTIMUSCARINIC COMPOUNDS IN THE CONSCIOUS MOUSE

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Antimuscarinic agents showing selective receptor interaction for the urinary bladder over other systems (eg. gut, salivary glands, CNS) may have a potential use in urinary incontinence. We report here a procedure for testing the effects of compounds on the urinary tract and gastro-intestinal function in the conscious animal.

Male, Albino, CD1 mice (25-27g) starved for four hours prior to the experiment, received 0.5ml charcoal suspension orally (2% charcoal in 0.5% gum tragacanth in distilled water) and an i.v. injection (tail vein) of 0.2ml compound or vehicle. Twenty minutes after dosing, the mice were killed by cervical dislocation, a laparotomy performed and the bladder was removed and weighed. The gut was removed and the distance travelled by the charcoal measured. The endpoint for an effect on bladder was urinary retention (Tita et al, 1988) and data are presented as the dose producing 50% of the maximal attainable increase in weight of bladder plus contents (200mg). Impairment of gut motility was measured as a reduction in charcoal transit (ED_{50} = 50% reduction). Table 1 shows the effects of a range of antimuscarinic compounds in this model.

All compounds with the exception of dicyclomine and secoverine display bladder versus ileum selectivity which is consistent with radioligand binding studies (^{3}H-QNB; [Nilvebrandt and Sparf, 1983]). However, secoverine was more active on the gut than the bladder in vivo. Further, dicyclomine actually caused slight gastro-intestinal stimulation. The apparent divergence of functional and radioligand binding data for these agents might be due to either pharmacokinetics or affinity for muscarinic receptor subtypes. It remains, however, that the model described here can be used for the simultaneous assessment of muscarinic antagonists on gastrointestinal and urogenital function and might thus be useful in the search for tissue-selective antimuscarinic agents.

Table 1

	BLADDER ED_{50} (mg/kg)	GUT ED_{50} (mg/kg)
ATROPINE	0.26	3.0
PIRENZEPINE	2.0	>10
4-DAMP	0.9	>5
PROPANTHELINE	0.11	4.0
DICYCLOMINE	2.6	stimulation
SECOVERINE	>10	3.5

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602P

INFLUENCE OF NON-SMOOTH MUSCLE ELEMENTS ON BRADYKININ-INDUCED CONTRACTIONS OF GUINEA-PIG GALLBLADDER *IN VITRO*

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Bradykinin (BK) stimulates bicarbonate secretion by guinea pig isolated gallbladder and is more potent when applied mucosally than serosally (Baird & Margolius, 1989). This suggests a principal site of action of BK in the epithelial layer. The aim of this study is to determine the effect of epithelium removal on contractile responses of gallbladder smooth muscle to BK and to other agonists. Furthermore, since BK is known to stimulate sensory nerve endings in gallbladder (Ordway & Longhurst, 1983) the possibility that BK stimulation of gallbladder smooth muscle contraction involves neurons was examined.

Tension was recorded isotonically from gallbladder strips suspended in tissue baths containing Krebs-Henseleit solution gassed with 95% O_2 / 5% CO_2 at 37°C. Matched preparations were obtained from individual female Dunkin-Hartley guinea pigs and the mucosal layer of one of each pair was removed by dissection. Complete separation of smooth muscle and epithelial layers was assessed by histological examination. Drugs were added sequentially to the bathing solution. Differences between groups of results were compared by paired t-test. Full concentration-response curves were obtained for BK, prostaglandin (PG) E_2 and acetylcholine (ACh) in matched pairs of intact and epithelial-denuded strips of gallbladder smooth muscle and EC_{50} values (μM) were calculated for each agonist.

Table 1.

	BK (n=6)	PGE_2 (n=7)	ACh (n=5)
+EPI	0.34 ± 0.09	0.13 ± 0.05	1.09 ± 0.35
-EPI	0.32 ± 0.11	0.09 ± 0.04	0.96 ± 0.45

EC_{50} values (mean ± s.e.m.) were not significantly different in intact (+EPI) and epithelial deprived (-EPI) preparations.

In separate experiments to determine whether nerves are involved in the response of intact gallbladder preparations to BK, neurogenic contractile responses were obtained to electrical field stimulation (EFS; 30V.,1ms.,50Hz., for 20s) via platinum field electrodes. The BK antagonist [$Thi^{5,8}$,$DPhe^7$]-BK (6μM) which reduced responses to 0.1μM BK by 32.8 ± 8.2% (n=6, $P<0.05$) did not alter contractions induced by EFS which, in the presence of the antagonist were 108.5 ± 5.6% of control responses. Tetrodotoxin (TTX, 1μM) which reduced the response to EFS by 90.2 ± 3.3% (n=7, $P<0.05$) had no effect on responses to BK (0.1μM) which, in the presence of TTX were 110.5 ± 11.4% of responses in untreated control tissues (n=7).

The influence of non-smooth muscle components on BK-induced contraction of gallbladder has been studied. Responses to BK were unaffected by epithelial removal suggesting that this peptide, as well as the other agonists, has a direct action on smooth muscle cells which is not detectably altered by mucosal elements. Also, since BK-induced contractions were not affected by TTX and [$Thi^{5,8}$,$DPhe^7$]-BK did not alter EFS-induced contractions, it appears unlikely that neurons are involved in this action of BK on gallbladder smooth muscle in vitro.

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603P

EFFECTS OF CIMETIDINE HYDROCHLORIDE ON SUBMANDIBULAR BLOOD FLOW AND SECRETION IN DOGS

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Little attempt has been made to investigate the action of H2 antihistaminic drugs on salivary secretion. This study demonstrates the action of cimetidine hydrochloride, a H2 antihistamine, on the blood flow and secretion of the submandibular gland.

In sodium pentobarbitone anaesthetized dogs (n=6), we measured with electromagnetic and Doppler flow sensors the submandibular arterial inflow (Qa) from the first glandular branch of the facial artery and salivary secretion (Qs) from the submandibular duct (Lung & Wang, 1990). Cimetidine was given intra-arterially, at the rate of 0.1 ml min^{-1}, via a catheter retrogradely inserted into the facial artery.

Table 1

Doses of drug (mg $kg^{-1}min^{-1}$)	Qa (ml min^{-1})	Qs (ml min^{-1})
0.25	4.5±0.43	0
0.5	6.0±0.40*	0.1±0.05
2.5	13±1.2*	0.5±0.06*
5	16±1.6*	1.0±0.09*

Results are means±s.e.means. * $P < 0.05$, when compared with resting flow.

The resting arterial inflow was 4.0±0.42 ml min^{-1}. Cimetidine infusion caused salivary secretion and an increase in blood flow. Atropine (1 mg, i.a.) abolished the secretory response and signficantly weakened the vascular response. Thus, cimetidine, apart from a direct vasodilatatory action, induces salivary secretion and vasodilatation via a cholinergic mechanism.

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604P

ADENOSINE INHIBITS ACID PRODUCTION BY HUMAN ISOLATED PARIETAL CELLS

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Pharmacological studies have shown that adenosine (Aden) can exhibit both stimulatory and inhibitory effects. In a human glioma cell line, Aden stimulated adenylate cyclase (Clark & Seney, 1976), yet inhibited cAMP formation in human sub-cutaneous adipocytes (Ohisalo et al, 1983). These effects are mediated via stimulatory (Ra/A_2) and inhibitory (Ri/A_1) receptors respectively. The present study has investigated the effect of Aden on human isolated parietal cells (PC).

Biopsies were taken from the gastric fundus of patients during routine endoscopy. Approximately 50mg of pooled tissue was collected from three or four subjects. Exclusion criteria were age >60 years, overt inflammation of the mucosa, tumour, and medication with proton-pump inhibitors. All drug treatment was suspended at least 14 hours prior to examination. A PC suspension (17% PC) was prepared following sequential pronase and collagenase digestion and filtration. Cells showed >85% viability by trypan blue. Cell suspensions (300ul total volume) were incubated for 140 mins in 96 well microtitre filtration plates (Millipore) and maintained in an oxygenated and heated ($37^{o}C$) chamber. The [^{14}C] aminopyrine uptake (APU) technique was used as an index of acid production by the PC. The incubation was terminated by simultaneous vacuum filtration of the wells, and APU assessed by scintillation counting of the filters upon which the cells were sedimented.

Histamine (10nM-100uM) stimulated APU in a concentration-dependent manner (EC50 2.4uM, max response 56.8 $\pm$ 10.3% increase over basal. Mean $\pm$ s.e.mean, n=6). Aden (10,100uM, 1mM) inhibited the maximum response to histamine by 24.5%, 33.7% and 43.1% respectively. Downward displacement of the curve suggested non-competitive antagonism. Theophylline (Theo), a non-specific Aden receptor antagonist, was without effect on basal or histamine-stimulated (10uM) APU. However, Theo (100nM-100uM) antagonised the inhibitory response to 1mM Aden on maximally-stimulated APU (100uM histamine). Dipyridamole (10,100uM) and 2-deoxyaden (10nM-10uM), Aden uptake inhibitor, and P-site agonist respectively, were without effect on basal or stimulated cells.

In conclusion, adenosine has been shown to inhibit histamine-stimulated APU in human isolated PC. Theophylline overcame the inhibition by adenosine. This effect appeared to be independent of phosphodiesterase inhibition since theophylline alone did not alter APU. It is suggested that adenosine inhibits acid production by human isolated PC by interaction at a specific Ri adenosine receptor.

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