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1P CONTROL OF TISSUE CYCLIC AMP CONTENT IN BOVINE TRACHEAL SUBMUCOSAL GLANDS

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A mucus secretory response in airway submucosal glands can be elicited both by agents which in other tissues elevate intracellular calcium levels and by agents which elevate tissue cyclic AMP content implying the involvement of two different second messenger pathways in the secretory response. We have previously demonstrated that a range of secretagogues including carbachol and histamine are able to induce an inositol phosphate response in bovine tracheal submucosal glands (Hall 1992). In this study we have examined the potential for a range of agents to elevate cyclic AMP levels in this tissue.

Bovine tracheal submucosal gland slices were prepared and [^{3}H]-cyclic AMP formation over 10 mins was quantified using the methods previously described(Hall et al 1989,Hall 1992).

Forskolin produced concentration related [^{3}H]-cyclic AMP formation (nonmaximal response; response to 1uM 6.5 $\pm$ 0.8 fold over basal, n=5). Histamine (EC_{50} 7.6 $\pm$ 1.2uM, n=3, maximum response 5.3 $\pm$ 0.8 fold, n=6) and isoprenaline (EC_{50} 20 $\pm$ 5nM, n=3, maximum response 2.9 $\pm$ 0.3 fold) both produced concentration related cyclic AMP formation in the presence of 1mM 3-isobutyl-1-methylxanthine (IBMX). The response to histamine was inhibited by cimetidine (100uM; K_A 1.3 $\pm$ 0.2x$10^6 M^{-1}$), and the response to isoprenaline was inhibited by ICI 118551 (50nM, K_A 3 $\pm$ 0.5x$10^8 M^{-1}$) indicating the involvement of H2 and beta 2 receptors in these responses (both n=3). Both the nonselective PDE inhibitor IBMX (apparent EC_{50} 133 $\pm$ 27uM, n=4, nonmaximal response) and the type IV selective PDE isoenzyme inhibitor rolipram (EC_{50} 1.4 $\pm$ 0.5uM, n=4) produced concentration related [^{3}H]-cyclic AMP formation in this tissue. The response to 1mM IBMX was 3.5 $\pm$ 0.2 fold, (n=14), and to 0.1mM rolipram 3.5 $\pm$ 0.3 fold (n=7). Neither zaprinast (0.1mM) nor the type III PDE isoenzyme inhibitor SK&F 94836 elevated cyclic AMP levels.

These results demonstrate that in bovine airway submucosal glands tissue cyclic AMP content can be elevated by stimulation of histamine H2 and beta 2 adrenergic receptors, activation of adenylyl cyclase, and inhibition of type IV PDE activity.

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Hall, I.P. (1992). Am. J. Physiol. (in press).
Hall, I.P., Donaldson, J. & Hill, S.J. (1989). Br. J. Pharmacol. 97., 603-613

2P COMPARISON OF THE EFFECTS OF TWO CALCIUM MODULATORS IN TRACHEA ISOLATED FROM NORMAL AND ALBUMIN-SENSITIZED GUINEA-PIGS

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Abnormal Ca^{2+} handling in airway smooth muscle has been suggested as a cause of the airway hyperreactivity characteristic of bronchial asthma. (Black et al, 1989). We have compared the effects of two calcium modulators on vagally-mediated constriction in trachea from normal guinea-pigs and albumin-sensitized guinea-pigs, which serve as a model for bronchial asthma. The drugs used were verapamil, a voltage-dependent Ca^{2+}-channel blocker and trifluoperazine (TFP), a calmodulin antagonist.

Tracheae, with attendant right vagus and recurrent laryngeal nerves were removed from untreated (UT), sham-sensitized (SS, saline vehicle only) or albumin-sensitized (AS, sensitizing injections i.p. and s.c. plus twice weekly inhalation of albumin for 3 weeks). The vagus nerve was stimulated at frequencies 1-50Hz for 5s with pulses of supramaximal voltage and 1ms duration. Responses were assessed as changes in intraluminal pressure (ILP) in the Krebs-filled trachea with the cyclo-oxygenase inhibitor flurbiprofen, 10^{-6}M, present.

Table 1. Percentage reduction in vagal responses at a frequency of 1Hz.(Values are mean$\pm$s.e.mean, n=5-7. Significantly different from UT *p<0.05, **p<0.01,t-test.)

	VERAPAMIL			TRIFLUOPERAZINE		
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-5}M	10^{-4}M	5x10^{-4}M
UT	44$\pm$14	72$\pm$7	76$\pm$12	47$\pm$15	68$\pm$18	93$\pm$7
SS	22$\pm$8	38$\pm$9**	62$\pm$7	22$\pm$12	43$\pm$8	94$\pm$3
AS	20$\pm$13	29$\pm$13**	45$\pm$16	17$\pm$7*	27$\pm$9*	65$\pm$8**

It can be seen from the table that verapamil attenuated vagal reponses in all 3 groups of tissues but attenuation was less marked in AS trachea. TFP also attenuated vagal responses in UT trachea but was significantly less effective in AS trachea. Similar results were obtained at other frequencies of stimulation. These results indicate that albumin- sensitization alters the response in isolated trachea to two drugs that modulate Ca^{2+} action in different ways and support the notion that abnormal calcium handling may contribute to airway hyperreactivity.

Black,J.L., Marthan,R., Armour,C.L. & Johnson,P.R.A. (1989). J. Allergy Clin. Immunol., 84, 440-447.

3P RESOLUTION OF AIRWAY OBSTRUCTION BY REDUCTION OF AIRWAY HYPERREACTIVITY

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The potassium channel opener, SDZ PCO 400, exhibited more pronounced suppression of airway obstruction in animals with overt airway hyperreactivity than in control animals (Chapman et al., 1990). This observation raises the possibility that other established (e.g. salbutamol, theophylline) and proposed (e.g. SDZ MKS 492 (8-{[1-(3,4-Dimethoxyphenyl)-2-hydroxyethyl]amino}-3,7-dihydro-7-(2-methoxyethyl)-1,3-dimethyl-1H-purine-2,6-dione-(R)), Morley et al., 1991 and Foster & Jubber, 1992) anti-asthma drugs might reduce airway obstruction by effects upon airway hyperreactivity rather than by direct spasmolysis. Guinea-pigs (450-600g) were anaesthetised with phenobarbitone (100 mg kg^{-1} i.p.) and pentobarbitone (30 mg kg^{-1} i.p.), paralysed with gallamine (10 mg kg^{-1} i.m.) and ventilated (1 Hz, 8 ml kg^{-1}) via a tracheal cannula. Airway resistance (R_L cm H_20 $l^{-1}sec^{-1}$) and compliance (C_{dyn} ml cm H_20^{-1}) were calculated from measurement of tracheal airflow and transpulmonary pressure using a digital electronic pulmonary monitoring system (PMS, Mumed Ltd., U.K.). Airway hyperreactivity to histamine was induced by three injections of PAF (43 ng kg^{-1} i.v.) at 10 min intervals. Drugs were administered i.d. or i.v. (salbutamol) and responsivity to histamine (1.0 - 3.2 μg kg^{-1}i.v.) determined at 15 min intervals thereafter (ID_{50} with confidence limits, n=10 per group; described as the dose causing 50% reduction of the bronchoconstrictor response) (Table).

Compound	Time (min)	ID_{50} normoreactive		ID_{50} hyperreactive	
AMINOPHYLLINE (mg kg^{-1}i.d.)	15	16.02	(14.3-17.96)	4.60	(3.6-5.64)
	30	16.82	(15.04-18.9)	5.57	(4.76-9.28)
	60	23.50	(21.3-25.6)	9.25	(6.31-14.31)
SDZ MKS 492 (mg kg^{-1}i.d.)	15	2.60	(1.8-3.2)	0.86	(0.5-1.0)
	30	4.38	(3.2-5.97)	1.02	(0.62-1.4)
	60	13.74	(10.2-20.4)	1.59	(1.16-2.22)
(±)SALBUTAMOL (μg kg^{-1}i.v.)	1	0.12	(0.09-0.14)	0.00044	(0.0002-0.001)
	15	4.69	(3.60-6.08)	1.88	(0.99-3.82)
	30	13.90	(10.51-18.4)	9.21	(3.43-17.77)

The capacity of ß-adrenoceptor agonists and xanthines to produce symptom relief in asthma patients when plasma concentrations are insufficient to effect relaxation of isolated smooth muscle is a long-standing anomaly. Effects of these drugs upon the expression of airway hyperreactivity at lower drug concentrations may account for the pronounced reduction of airway obstruction during asthma therapy.

Chapman, I.D., Kristersson, A., Mazzoni, L. et al.(1990) Br J Pharmacol 102: 335P.
Foster, R.W. & Jubber, A.S. (1992). Br J Clin Pharmacol (in press)
Morley, J., Chapman, I.D. & Mazzoni, L. (1991). Agents Actions. 34: 403-410.

4P MODULATION OF CORTICOTROPHIN (ACTH) RELEASE *IN VITRO* BY LIPOCORTIN 1 (LC-1) AND DEXAMETHASONE

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The anti-inflammatory actions of the glucocorticoids have been partially attributed to a second messenger protein, lipocortin 1 (LC1), found in abundance in many tissues (Flower, 1988). Reports that LC1 is readily detectable in freshly removed adenohypophysial tissue and that the expression of the LC1 in the anterior pituitary gland is regulated by glucocorticoids (Smith et al., 1991) suggest that the protein may also contribute to the powerful inhibitory effects of the steroids on corticotrophin (ACTH) release. Accordingly, we have studied the effects *in vitro* of LC1 and a monoclonal. anti-LC1 antibody (LC1-mAb, Zymed) on the resting and neurochemically evoked secretion of immunoreactive ACTH from freshly removed rat anterior pituitary segments and from a cultured mouse pituitary tumour cell line (ATt-20 D16/16) incubated in conditions described previously (Hadley et al., 1991, Woods et al., 1991) in the presence and absence of dexamethasone.
Hypothalamic extracts (0.05-0.20HE/ml) produced, within 30min, significant ($p<0.01$, n=6) concentration dependent increases in ACTH release from rat anterior pituitary segments. The secretory responses to a submaximal concentration of the extract (0.1HE/ml) were reduced by ≈ 60% ($p<0.01$,n=6) by preincubation of the tissue with dexamethasone (0.1μM) which also caused externalization of LC1 by the pituitary cells. The inhibitory actions of the steroid were reversed by the LC1-mAb (diluted 1:15000) but not by a correspondingly diluted control antibody (anti-spectrin alpha & beta, Sigma Chemical Co. Ltd.). Human recombinant LC1 (100fg-1ng, Biogen Inc.) had little effect on either the basal or stimulated release of ACTH although higher concentrations (10 & 100ng/ml) produced a significant increase ($p<0.01$) in basal peptide release. An N-terminal lipocortin 1 fragment ($LC1_{1-188}$, ICI plc,10pg-1μg/ml) also failed to influence basal peptide release. However, like dexamethasone, in concentrations of 100pg-100ng it reduced by ≈ 60% the ACTH response to hypothalamic extract (0.1HE/ml). In addition, it also reduced significantly ($p<0.05$) the approximately 3-fold increase in ACTH release evoked by the adenyl cyclase activator forskolin (0.1mM). In contrast, in the ATt-20 D16/16 cells, in which LC1 is not detectable, the significant ($p<0.01$) increase in ACTH release induced by a submaximal concentration of corticotrophin releasing factor-41 (CRF-41, 10nM) was unaffected by $LC1_{1-188}$ (0.10-10ng/ml). In addition LC1 mAb (diluted 1:1000 and 1:10000) failed to overcome the significant inhibitory effects of dexamethasone (0.01 μM) on the CRF-41 induced release of the peptide from the cells.
The results suggest that lipocortin 1 may contribute to the glucocorticoid induced inhibition of ACTH secretion from freshly removed rat anterior pituitary tissue.

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Flower, R.J. (1988) Br. J. Pharmacol. **94**, 987-1015
Hadley, A.J.,Flack, J.D. & Buckingham, J.C. (1991) Br. J. Pharmacol. **100**, 337
Smith, T., Smith, S., Flower, R.J. & Buckingham, J.C. (1991) Br. J. Pharmacol. **104**, 218P
Woods, M.D., Antoni, F.A. (1991) J. Endocrinol. (suppl.) **129**, No.253

5P TOPICAL STEROIDS AND ACE INHIBITORS: A COMMON LINK?

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Topical application of the anti-inflammatory glucocorticoid betamethasone inhibits the increase in skin blood flow induced in the rat by the local application of heat (Ahluwalia & Flower, 1991). The inhibitory actions of steroids in this model appeared to be produced by a mechanism independent of NO, prostanoids or the cholinergic system. We now report our further investigations into the mechanism of steroid action in this model.

The experimental procedure employing laser doppler velocimetry and application of heat (44°C) to the skin of male Wistar rats (250-350g) has already been described (Ahluwalia & Flower, 1991). Two types of experiment were performed. Firstly, the effect of a range of drugs given intravenously 30 or 5 min prior to the heat-induced response were determined. Secondly, the vasodilator properties of a range of putative mediators were tested following intradermal injection and in the presence of topical betamethasone-17-valerate (1 g rubbed into one side of the back of a rat with an 18h pretreatment) or captopril (5mg/kg, i.v.). Blood flow was measured in arbitrary perfusion units (PU). Results are calculated as maximum change in blood flow in response to heat or intradermal dilator application, the inhibition by topical steroid or drug treatment is expressed as a percentage of the change before treatment and compared to the untreated side or saline control respectively.

Methysergide or Mepyramine (6mg/kg i.v., 30 min pretreatment, n=4) were without significant effect upon the vasodilation induced by local heating. However, captopril (5mg/kg i.v., 30 min pretreatment, n=8) produced a strong inhibition (32.2±8.6% of control values, $p < 0.001$), and a similar effect was observed with ACE inhibitor, enalapril (1mg/kg, i.v., 30 min pretreatment, n=5) which reduced the response to heat to 24.4±5.3%, ($p < 0.001$). Conversely aprotinin (100,000 KIU/kg, i.v., 5 min pretreatment, n=5) potentiated the response (209.7±35.2%, $p < 0.05$). Drug vehicle controls (saline, i.v.) were without effect.

In the second type of experimental study 50ul bradykinin (50×10^{-9} mol/site) induced an increase in flow of 24.3±3.3 PU, n=6, des-Arg^9 -bradykinin (5×10^{-9} mol/site) 18.7±4.6 PU, n=4, capsaicin (1×10^{-6} mol/site) 26.5±10.0 PU, n=5 and Substance P (0.1×10^{-9} mol/site) 20.5±2.7 PU, n=6. The administration of captopril significantly inhibited the vasodilator responses to all of the i.d stimuli i.e. BK (28.6±10.4% of BK response before treatment, n=7), des-Arg^9-BK (27.7±8.9%, n=7), capsaicin (13±14.1%, n=3) and SP (6.9±4.8%, n=9). Topical betamethasone treatment had no significant effect upon the vasodilator actions of BK (n=9), SP (n=5) or capsaicin (n=6) but significantly inhibited the effects of des-Arg^9-BK (49.1±18.9%, n=6, $p < 0.05$). The steroid vehicle was without effect.

The observations that ACE inhibitors can abolish and the serine protease inhibitor aprotinin, potentiates the heat-induced vasodilation in the rat skin suggests the involvement of peptide vasodilators, the processing of which may be interfered with by these enzyme inhibitors. Of the mediators tested only the des-Arg^9-BK induced vasodilation was blocked by both topical steroid and captopril suggesting perhaps that this peptide is involved in the vasodilator response to local skin heating.

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Ahluwalia, A. & Flower, R.J. (1991). Fund. & Clin. Pharmacol., 5, 419.

6P A METHOD FOR ESTIMATING pA_2 VALUES OF COMPETITIVE ANTAGONISTS AGAINST NEURONALLY- OR IONOPHORETICALLY-RELEASED AGONISTS

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The classical method (Arunlakshana & Schild, 1959) for estimating pA_2 values of competitive antagonists requires estimation of agonist dose-ratios. More recently Mackay (1991) showed that it should be possible to measure relative pA_2 values of competitive antagonists against electrically released transmitter. It is shown here that measurement of antagonist concentration-response curves against different levels of response produced by released transmitter may provide absolute estimates of the affinity constants of competitive antagonists for the transmitter-receptor. Since for this method the concentration of agonist need not be known, it should also be useful when the agonist is applied to cells by ionophoresis.

Suppose that two constant concentrations, [A]' and [A]", of agonist A produce equal graded responses from a cell or tissue in the presence of concentrations [I]' and [I]" of antagonist I. If antagonist I competes under equilibrium conditions with agonist A then the null equation relating [I]" and [I]' is $[I]'' = (C - 1)/K_I + C.[I]'$ (Equation 1) where $C = [A]''/[A]'$ (Equation 2). Since [A]' and [A]" are constant, C is the slope and $(C - 1)/K_I$ is the intercept of a plot of [I]" against [I]'. From such a plot, K_I = (slope - 1)/intercept (Equation 3). If instead antagonist I acts as a pseudo-irreversible antagonist then although equations (1) to (3) should still apply, C would be given by $C = (1+\{1/(K_A[A]')\})/(1+\{1/(K_A[A]'')\})$ (Equation 4) where K_A is an apparent affinity constant of A for the receptor. Even then equation 4 would reduce to equation 2 if the agonist occupies only a small fraction of the receptors. When applying these equations to estimate values of K_I it is important that the antagonist should either have no effect on neurotransmitter release, uptake and metabolism or that any such effects be selectively blocked. The actions of any co-transmitter should also be selectively blocked.

These ideas have been tested by measuring concentration-response curves for prazosin, in the presence of suramin (0.8mM) and propranolol (0.3μM), acting against neuronally-released transmitter in the epididymal portion of the rat isolated vas deferens preparation. Two response levels, corresponding to unknown concentrations [A]' and [A]" of agonist, were obtained using field stimulation at different frequencies. By comparing the pairs of antagonist concentration-response curves using a computer curve-fitting technique, the pA_2 value of prazosin was estimated to be 8.83 ± 0.11 (m ± s.e.m.)(n=6). This is very similar to the value of 8.6 against exogenous noradrenaline, reported by Kenakin (1984).

Arunlakshana,O. & Schild,H.O. (1959) Br. J. Pharmac.Chemother. **14**, 48-58.
Kenakin,T.P.(1984) Br.J.Pharmac. **81**, 131-141.
Mackay,D. (1991) Trends Pharmacol. Sci. **12** ,181.

7P EVIDENCE THAT PENTAGASTRIN ACTS BOTH DIRECTLY AND INDIRECTLY BY RELEASING HISTAMINE TO STIMULATE GASTRIC ACID SECRETION IN THE RAT

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Does gastrin stimulate gastric acid secretion by direct action on oxyntic cells, by releasing histamine or by being potentiated by histamine? (Black & Shankley, 1987). Studies in the mouse pointed to gastrin regulated histamine release (Black *et al.*, 1985). Guinea pig and rat are well known to vary in their sensitivity to histamine. Therefore, the effects of histamine (HA) and pentagastrin (PG) were compared quantitatively on isolated, lumen-perfused, stomach preparations from these species (Welsh, 1992) in the absence and presence of histamine H_2-receptor blockade (0.1mM tiotidine). The table shows the logistic function curve-fitting parameters (N=6/8±s.e.m.) derived from HA and PG control dose-response curves.

	guinea-pig		rat	
	HA	PG	HA	PG
$p[A_{50}]$	5.67(0.12)	8.29(0.08)	4.78(0.13)	7.39(0.11)
Maximum(ΔpH)	0.66(0.02)	0.36(0.07)	0.44(0.05)	0.42(0.06)
Slope	1.40(0.19)	0.96(0.11)	1.48(0.18)	0.89(0.10)

The loss of potency ($p[A_{50}]$) of HA in the rat is mirrored by a loss of potency of PG consistent with the idea that PG acts by releasing HA. However, in the guinea pig, PG produced only 55% of the maximum response obtained with HA, whereas, in the rat, although PG was less potent than in the guinea-pig, the maximum response was the same as that obtained with HA. Therefore, in the rat it appears that PG cannot be acting solely through the release of HA. This conclusion was supported by the finding, in the rat, but not guinea-pig, that a well-defined PG curve was obtained in the presence of H_2-receptor block.

The results could be explained by a model in which it was assumed that PG acts both directly on the oxytic cell and indirectly by releasing HA. However, it was not necessary to invoke a potentiating interaction between HA and PG at the oxyntic cell, the two effects appeared simply to add.

Black, J.W. & Shankley, NP (1987) Trends Pharmacol. Sci., 8, 486-490.
Black, J.W., Leff, P. & Shankley, NP (1987) Br. J. Pharmacol., 86, 589-599.
Welsh, N.J. (1992) PhD thesis University of London.

8P MULTIPLE CCK RECEPTOR SUBTYPES MEDIATE CONTRACTION IN GUINEA-PIG GALL-BLADDER

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Cholecystokinin contracts gall-bladder smooth muscle and this effect has been ascribed to an interaction with CCK_A receptors. However, CCK peptides produce an atypical concentration-response relationship over more than five orders of magnitude of concentration (Maubach et al., 1991), which might be consistent with an interaction at more than one receptor type. The aim of the present study was to further investigate the CCK receptor(s) that mediate contraction using the peptides; sulphated CCK-8, A71378 and A72962 (Lin et al., 1990) and also the CCK_A selective antagonist devazepide (Louie et al., 1988).

Guinea-pig gall-bladders were cut along the bile duct axis to provide two preparations. These hemi-bladders were bathed in modified Krebs solution at 37° C and gassed with 95% O_2/5% CO_2. Contractile responses were measured using isometric force transducers. Cumulative concentration-response curves (CRC) to agonists were reproducible. In antagonist studies, devazepide was equilibrated with the tissue for 30 min between CRCs.

The CCK peptides produced sustained contractions of the gall-bladder. CCK-8 and A71378 were potent agonists (EC_{50}=18 (6-56)and 3 (2-5)nM) but exhibited shallow CRCs, extending over more than five orders of magnitude in concentration. In contrast, A72962, although less potent (EC_{50}=57 (28-113)nM), produced a steep CRC which extended over only two orders of magnitude in concentration.

Devazepide inhibited contractile responses to CCK-8 and A71378 in a similar manner, producing non-parallel rightward displacements of CRCs with a greater antagonism of the responses to lower concentrations of peptides. Schild analysis of the data for CCK-8 and A71378 yielded slope values significantly greater than unity (1.5 (1.2-1.7) and 1.6 (1.3-1.8)) and pA_2 values for devazepide against these two agonists were similar (8.6 (8.4-8.9) and 8.3 (8.1-8.7)). In contrast, devazepide appeared to be a competitive antagonist of the responses to A72962, producing parallel rightward displacements of CRCs, a Schild slope not significantly different from unity and a pA_2 value of 9.3 (9-10).

These studies suggest that the interaction of CCK peptides with guinea-pig gall-bladder to produce contraction is complex. The steep CRC for A72962 and its competitive antagonism by devazepide are consistent with the view that A72962 interacts with a single receptor type which has an affinity for devazepide similar to the "classical" CCK_A receptor reported in rat pancreas (Louie et al., 1988). The shallow CRC for CCK-8 and A71378 and the nature of the antagonism produced by devazepide may be consistent with an interaction of these peptides with more than one receptor. Further studies are required using selective agonists and antagonists to evaluate this possibility.

Lin, C.W. et al., 1990, Am. J. Physiol., 258, G648-G651
Louie, D.S. et al., 1988, Am. J. Physiol., 255, G261-G266
Maubach, K.A. et al., 1991, Br. J. Pharmacol., 104, 142P

9P CHARACTERISATION OF CCK RECEPTORS IN THE CIRCULAR MUSCLE OF THE GUINEA-PIG STOMACH CORPUS

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Cholecystokinin octapeptide (CCK8S) and gastrin I cause contraction of smooth muscle cells isolated from the fundic region of the guinea-pig stomach (Collins & Gardner, 1982). Studies with the antagonists devazepide and L-365,260 against responses to gastrin I, have yielded conflicting results, with the more effective antagonist being L-365,260 in one study (Huang *et al.*, 1989), but devazepide in another (Grider & Makhlouf, 1990). In the present study we have used several agonists, and selective antagonists to attempt a characterisation of the receptors mediating contraction of a strip of circular smooth muscle from the corpus region of the guinea-pig stomach.

The stomach from 300-400g male guinea-pigs was opened along the greater curvature and pinned out in a petri-dish with the mucosa uppermost. The mucosal and sub-mucosal layers were removed by dissection to reveal the underlying smooth muscle layer. Strips of circular muscle 2 x 25mm from the corpus region were mounted under 1g tension (isometric) in organ baths and bathed in Krebs-Henseleit solution at 37°C, normally containing 5μM indomethacin.

The preparation was contracted powerfully by CCK8S (EC_{50} 9.5nM) or its non-sulphated analogue (EC_{50} 101nM), by pentagastrin (EC_{50} 13.1nM), gastrin I (EC_{50} 32.9nM) or CCK4 (EC_{50} 210nM). The responses to the agonists were not affected by tetrodotoxin or mepyramine (1μM), atropine, phosphoramidon or methysergide (10μM), or by Nω-nitro-L-arginine (100μM). The addition of indomethacin did not affect the response to agonist, but was advantageous in providing a stable baseline.

Responses to pentagastrin were antagonised in an apparently competitive manner by CI-988 (pA_2 9.5, slope 1.1) or L-365,260 (pA_2 8.3, slope 0.8) but were unaffected by up to 10nM devazepide suggesting mediation predominantly by a CCK_B/gastrin receptor. Conversely, with CCK8S devazepide was a powerful antagonist, apparently acting competitively (pA_2 9.5, slope 1.0), while CI-988 was a weak antagonist (pA_2 6.6, slope 0.8), suggesting the predominance of a CCK_A receptor in mediating contraction by this agonist.

We used the response of the selective agonist pentagastrin, in the presence of 3nM devazepide as a specific assay for stimulation of the CCK_B/gastrin receptor. When the interaction between CI-988 or L-365,260 and pentagastrin was re-examined under these conditions, the nature of the antagonism observed with L-365,260 was unaltered (pA_2 8.3, slope 0.8), while with CI-988 the antagonism was now non-competitive.

These results suggest that this novel preparation contains both CCK_A and CCK_B/gastrin receptors powerfully coupled to contraction of the smooth muscle cells, and could be a useful functional test for either receptor type.

Collins, S.M. and Gardner, J.D. (1982) Am. J. Physiol. **243**, G497-G504.
Huang, S.C., Zhang, L., Chiang, H-C.V., Wank, S.H., Maton, P.N., Gardner, J.D. and Jensen, R.T. (1989) Am. J. Physiol. **257**, G169-G174.
Grider, J.R. and Makhlouf, G.M. (1990) Am. J. Physiol. **259**, G184-G190.

10P TREATMENT OF EXPERIMENTAL ACUTE PANCREATITIS BY THE BRADYKININ ANTAGONIST HOE 140

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The long-acting bradykinin antagonist, HOE 140 (DArg-[Hyp^3-Thi^5-DTic^7-Oic^8]-bradykinin, Lembeck *et al.*, 1991), abolished the fall in rabbit blood pressure induced not only by bradykinin but also by kallikrein, demonstrating that HOE 140 can also block the effects of endogenously released kinins. It was therefore used in an experimental model of acute pancreatitis.

Hyperstimulation of the exocrine pancreas of anaesthetized rats with the cholecystokinin analogue, caerulein (8 nmol kg^{-1}, infused i.v. within 120 min), resulted in a massive oedema of the pancreas, increased serum activities of amylase and lipase, and sustained hypotension due to loss of plasma volume. Ultrastructural changes of the pancreas induced by this treatment are similar to those seen in human acute pancreatitis (Willemer *et al.*, 1990).

Pretreatment with HOE 140 (100 nmol kg^{-1}, s.c.) abolished the pancreatic oedema and the hypotension. These effects of caerulein can, therefore, be attributed to the action of endogenous kinins released in the pancreas. The oedema was also significantly inhibited when HOE 140 was injected up to 25 min after the start of the caerulein infusion.

The caerulein-induced increases in the serum activities of amylase and lipase were augmented by HOE 140. Prevention by HOE 140 of the kinin-mediated oedema allows the pancreatic enzymes to leave the tissue without hindrance and, thus, will diminish subsequent pathological events within the pancreas.

These actions of HOE 140 provide the pharmacological basis for its clinical trial in acute pancreatitis.

Lembeck, F., Griesbacher, T., Eckhardt, M., Henke, S., Breipohl, G. & Knolle, J. (1991) *Br. J. Pharmacol.* **102**, 297-304.
Willemer, S., Bialek, R., Köhler, H. & Adler, G. (1990) *Histochemistry* **95**, 87-96.

11P COMPARISON OF THE EFFECTS OF HOE 511 AND CICLAZINDOL ON RESPONSES TO BRL 38227 IN A VARIETY OF SMOOTH MUSCLES

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We have reported previously that ciclazindol, an agent which induces weight loss in man (Greenbaum & Harry, 1980) and modifies diet-induced thermogenesis in experimental animals (Rothwell et al., 1981), antagonizes the action of the potassium (K) channel opener, BRL 38227 in a variety of smooth muscles (Morita et al., 1991; Noack et al., 1992). HOE 511, (6-chloro-8b-hydroxy-1-methyl-2-methylimino-1,3,4,8b-tetrahydro-2H-indeno[2,1-d]thiazole hydrochloride) was found to reduce food intake in experimental animals (Geisen & Granzer, unpublished observations). Such an observation raises the possibility that HOE 511, like ciclazindol, can block K channels in smooth muscle. We have therefore compared the effects of HOE 511 with those of ciclazindol on some of the actions of BRL 38227 in rat portal vein, bladder and aorta.

Rat portal veins and segments of rat bladder were set up for isometric tension recording in Krebs solution at 37° C and the ability of HOE 511 or ciclazindol to modify the relaxant actions of BRL 38227 was studied using a cumulative protocol. Identical tissues were also loaded with ^{86}Rb and the efflux of this isotope was evaluated using standard techniques (Edwards et al., 1991). In bladder and portal vein, HOE 511 (1-10 μM) produced non-competitive antagonism of BRL 38227-induced relaxation. These inhibitory effects of HOE 511 were reversed following washout. In contrast, aminophylline-induced relaxation of bladder segments was unaffected in the presence of HOE 511 (10 μM). In portal vein and aorta, BRL 38227 (5 μM)-induced stimulation of ^{86}Rb efflux was antagonized by HOE 511 (10-30μM) in a concentration-dependent manner. Similar antagonistic effects of ciclazindol (0.03-3 μM) against BRL 38227-induced relaxation of bladder and portal vein and against BRL 38227-induced stimulation of ^{86}Rb-efflux from portal vein and aorta were also observed.

These findings show that HOE 511 is a K-channel blocker with actions qualitatively similar to those of ciclazindol. Such blockade may be related to the ability of HOE 511 and ciclazindol to modify food intake in experimental animals.

Edwards, G., Henshaw, M., Miller M. & Weston, A.H. (1992) Br. J. Pharmacol., **102**, 679-686.
Greenbaum, R. & Harry, T.U.A. (1980) J. Pharmacol. Ther., **3**, 82-83.
Morita, T., Edwards, G., Andersson, P., Greengrass, P., Ibottson, T., Newgreen, D.T., Weston, A.H. and Wyllie, M.G. (1992) J. Physiol., **446**, 365P.
Noack, Th., Edwards, G., Deitmer, P., Greengrass, P., Morita, T. Andersson, P-O., Criddle, D., Wyllie, M.G. & Weston, A.H. (1992) Br. J. Pharmacol., **106**,17-24.
Rothwell, N.J., Stock, M.J. & Wyllie, M.G. (1981) Br. J. Pharmacol., **74**, 539-546.

12P ROLE OF K^+-CHANNEL OPENING IN SALMETEROL-INDUCED RELAXATION OF TRACHEALIS MUSCLE

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In order to investigate the role of K^+-channel opening in salmeterol-induced relaxation of airways smooth muscle, segments of guinea-pig trachea were set up for recording tension changes as described by Foster et al. (1983). Log concentration/relaxation curves for isoprenaline, salbutamol and salmeterol were constructed in the absence and presence of charybdotoxin (100nM). The toxin increased spontaneous tone and shifted the log concentration/effect curves for isoprenaline, salbutamol and salmeterol to the right (2.8, 2.9 and 4 fold, respectively) without reducing the maximal effects. Exposure of tracheal segments to K^+-rich (80mM), iso-osmolar Krebs solution increased tissue tone. In the presence of the K^+-rich medium, the log concentration/relaxation curves of isoprenaline, salbutamol and salmeterol were rightward shifted 5.6, 26.3 and 22.4 fold respectively. The maximal effect of isoprenaline was reduced by 18% and the maximal effects of salbutamol and salmeterol were reduced by 45% and 39% respectively.

Microelectrode recording of membrane potential changes in guinea pig trachealis was performed by the method of Dixon & Small (1983). The relaxant effect of isoprenaline (0.1μM) was accompanied by suppression of spontaneous electrical slow waves and by marked cellular hyperpolarisation (14.7 ± 1.4mV, measured after 4 min drug contact; n=6). The relaxant effect of salbutamol (1μM) was accompanied by less marked hyperpolarisation (9.7 ± 1.6mV, measured after 5 min drug contact; n=6). The relaxant effect of salmeterol (0.1 and 1μM) was accompanied by very minor hyperpolarisation (4.3 ± 2.9 mV and 4.8 ± 3.1mV, measured after 14 min drug contact; n = 8 and 9 respectively). Strips of bovine trachealis were preloaded with $^{86}Rb^+$ prior to monitoring the efflux of the radiotracer as described by Longmore et al. (1991). Isoprenaline, salbutamol and salmeterol (0.1, 1 and 10μM) were added to the medium after the initial loss of tracer from the rapidly-exchanging compartment. Salmeterol did not affect $^{86}Rb^+$ efflux but isoprenaline and salbutamol both increased the efflux rate constant. Propranolol (1μM) did not itself influence the efflux rate constant but inhibited the increase in the $^{86}Rb^+$ efflux rate constant induced by isoprenaline.

The antagonism of salmeterol by charybdotoxin suggests that the opening of large, Ca^{2+}-dependent K^+-channels may contribute to the mechanisms by which salmeterol relaxes trachealis muscle. However, the failure of K^+-rich Krebs solution to ablate the relaxant action of salmeterol suggests that K^+-channel opening (with consequential cellular hyperpolarisation and inhibition of Ca^{2+} influx through voltage operated Ca^{2+}-channels) cannot be crucially important for the relaxant activity of salmeterol. Compared with salmeterol, the greater ability of isoprenaline to hyperpolarize trachealis muscle and to promote $^{86}Rb^+$ efflux may reflect the greater intrinsic efficacy of isoprenaline at β_2-adrenoceptors (Dougall et al., 1991). Alternatively it may be that K^+-channel opening depends on the activation of β_1-rather than β_2-adrenoceptors. Experiments are planned to help distinguish between these possibilities.

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Dixon, J.S. & Small, R.C. (1983) Br. J. Pharmacol., **79**, 75-83.
Dougall, I.G., Harper, D., Jackson, D.M. & Leff, P. (1991) Br J. Pharmacol., **104**, 1057-1061.
Foster, R.W., Small, R.C. & Weston, A.H. (1983) Br. J. Pharmacol., **79**, 255-263.
Longmore, J., Bray, K.M. & Weston, A.H. (1991) Br. J. Pharmacol., **102**, 979-985.

13P THE EFFECT OF LEMAKALIM, A POTASSIUM CHANNEL OPENER, ON THE ATP-MODULATED POTASSIUM CHANNELS IN RAT ISOLATED HYPOTHALAMIC NEURONES

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The ventromedial nucleus (VMN) of the hypothalamus plays a major role in the regulation of feeding and appetite (Morley, 1980). Certain neurones within this nucleus are glucose-receptive, with increased extracellular glucose producing cellular depolarisation and increased firing of action potentials (Ono *et al.*, 1982). This glucose-induced excitation has been shown to be mediated by the closing of ATP-sensitive potassium (ATP-K^+) channels (Ashford *et al.*, 1990). Recently, potassium channels which are activated by increasing levels of intracellular ATP (ATP-act K^+ channels) have been demonstrated in glucose-sensitive neurones isolated from the VMN and basomedial regions of the hypothalamus. The potassium channel opener, lemakalim, has been shown to be active in a number of physiological preparations and is the subject of clinical evaluation in a number of disease states (Robertson and Steinberg, 1990). In this present study we have examined the influence of lemakalim on both types of ATP-modulated potassium channel contained within VMN neurones.

Inside-out and cell-attached patch recordings were obtained from VMN neurones acutely dissociated with enzymatic treatment and trituration from hypothalamic slices from male Sprague-Dawley rats (1-2 months of age), as described previously by Ashford *et al.*, (1990). Patch pipettes contained in mM: 140 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES (pH 7.2). The bath solution contained normal physiological saline (in mM); 135 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES pH 7.2 for cell-attached recordings and, for inside-out patches, (in mM): 140 KCl, 0.9 $CaCl_2$, 1 $MgCl_2$, 1 EGTA, 10 HEPES pH 7.2.

The ATP-K^+ channel can be isolated from glucose-receptive cells in the VMN, whereas the ATP-activated K^+ channel is found in patches excised from neurones which respond to elevated extracellular glucose with the activation of a K^+-channel in cell-attached patches (Ashford et al, 1990., and Treherne, Rowe & Ashford, unpublished). Lemakalim, at a range of concentrations between 30-100μM, had no effect on the rate of firing of any neurones isolated from the VMN, as measured by cell-attached recordings. In the isolated patch, the ATP-K^+ channel (140pS) is inhibited almost fully by ATP at concentrations exceeding 1mM. Lemakalim, at a range of concentrations from 30-100μM, had no effect on the P_{open} or conductance of the channel in the presence (0.5mM and 3mM) or absence of ATP, and regardless of membrane potential. The ATP-act K^+ channel (135pS) is activated by ATP in a manner similar to the channel described previously in rat cultured cortical cells (Ashford and Treherne, 1989). This channel was examined in a similar way, either activated in the presence of 5mM ATP (P_{open} of approximately 0.80) or barely open (P_{open} 0.02) in the absence of any ATP, and lemakalim, at concentrations ranging from 30-100μM, was without effect on this channel also. It can be concluded that the ATP-modulated potassium channels found in the basomedial or ventromedial hypothalamus are unaffected by the K^+-channel opener lemakalim. It remains to be seen whether other structurally diverse potassium channel openers can modulate the nucleotide-sensitive K^+-channels in this brain region

Ashford M.L.J., Boden P. & Treherne J.M.A. (1990) Pflugers Arch. **415.**, 479-483.
Ashford M.L.J. & Treherne J.M.A. (1988) J Physiol. **409**, 54P
Morley J.E. (1980). Life Sci. **27**,, 355-368.
Ono T., Noshimo H., Fukuda M., Sasaki K., Muramoti K.I. & Oomura Y. (1982) Brain Res., **232**, 494-449.
Robertson D.W. & Steinberg M.I. (1990) J Med Chem. **33.**, 1529-1541.

14P ALINIDINE PREVENTS HYPOPERFUSION-INDUCED REDISTRIBUTION OF MYOCARDIAL FLOW IN ISOLATED RAT HEART AS ASSESSED BY FLUORESCENT MICROSPHERE TECHNIQUE

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The antianginal actions of alinidine seem to be due to its bradycardic effect at the sinus node. However, an additional cardioprotective action of the drug is independent of heart rate. In electrically paced isolated rat heart low flow perfusion induces lactacidosis and a loss of high-energy phosphates as well as contractility. Alinidine maintains aerobic metabolism and mechanical function under these conditions (Streller & Walland, 1990). Low flow perfusion induces, possibly by involvement of endogenous adenosine, myocardial flow redistribution which compromises the endocardium in particular. As alinidine exerts adenosine antagonistic effects (Lang et al., 1988) we wanted to test by means of a new fluorescent microsphere technique (Weihs & Walland, 1991) whether the compound prevents flow redistribution as well.
Langendorff heart preparations from male rats, 350 g body weight, were perfused with Tyrode solution (37° C) at a pressure of 80 cm H_2O and paced electrically at 5 Hz. Left ventricular pressure was measured with a latex balloon catheter and coronary flow by collection of perfusate over periods of 5 min. After 15 min of adaptation perfusion pressure was reduced resulting in a reduction of left ventricular maximum pressure to approx. 33 % in 8 control hearts. In another group of 7 hearts low flow perfusion was continued after 5 min with medium containing 45.6 μM alinidine. After a total of 65 min low flow perfusion 20000 fluorescent microspheres (Fluoresbrite Carboxylate Yellow-Green, 10 μm diameter, Polysciences, St. Goar, FRG) were infused over 1 min into the heart. Nine min later the hearts were flushed with 15 mM EGTA for relaxation followed by 10 % formaldehyde for fixation and the ventricles were cut transversally into 52 μm slices with a freezing microtome. Photographs were taken from 3 slices/heart with a photomacroscope with fluorescence equipment. The left ventricular wall was subdivided on the enlarged print into equally wide zones (EPI 1, EPI 2, ENDO 1, ENDO 2) which were evaluated planimetrically. The number of beads within each zone was counted and the density calculated (Weihs & Walland, 1991).
In the low flow group coronary flow decreased continuously during low pressure perfusion. Contractility ceased in 6 hearts and contracture developed in four of these. In the presence of 46.5 μM alinidine coronary flow and contractility were maintained in a statistically significant manner during the observation period. The ENDO 2/EPI 1 quotient of microsphere densities amounted to 1.88 ± 0.280 (SEM) in control hearts perfused at 80 cm H_2O for 60 min. Low flow perfusion resulted in an ENDO 2/EPI 1 quotient of 0.32 ± 0.147 in the absence and 0.88 ± 0.096 in the presence of alinidine ($P = 0.0083$).
These results indicate that flow redistribution in response to hypoperfusion is also detectable in rat heart and the cardioprotective effect of alinidine is parallelled by reduction of flow redistribution.

Streller, I. & Walland, A. (1990) Basic Res. Cardiol. **85**, 71 - 77
Lang, U., Traunecker, W. & Walland, A. (1988) Naunyn-Schmiedeberg's Arch. Pharmacol. **337**, R68
Weihs, H. & Walland, A. (1991) Naunyn-Schmiedeberg's Arch. Pharmacol. **343**, R62

15P THE ADENOSINE ANTAGONIST 8-(p-SULFOPHENYL)THEOPHYLLINE FAILS TO ABOLISH ISCHAEMIC PRECONDITIONING IN ISOLATED RAT HEARTS

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(introduced by M.J. Curtis)

A brief period of ischaemia (5 min) and reperfusion (5 min), prior to a longer period of ischaemia and reperfusion improves the final recovery of the heart - despite the greater cummulative ischaemic time. Adenosine has been implicated in this phenomenon of ischaemic preconditioning (Lui et al., 1991). We investigated the effects of the adenosine antagonist 8 (p-sulfophenyl)theophylline (8-SPT) on preconditioning in isolated perfused working rat hearts with whole heart ischaemia. After 20 min of aerobic perfusion (bicarbonate buffer gassed with 95%O_2+5%CO_2, 37°C) the function of the hearts (n=6/group) was assessed in terms of aortic flow, coronary flow, cardiac output and heart rate. This was followed by 10 min Langendorff perfusion (control group, C1) or 5 min ischaemia and 5 min Langendorff reperfusion (preconditioned group, P1). All hearts were then subjected to 21 min of ischaemia and 35 min reperfusion (15 min Langendorff reperfusion plus 20 min working heart perfusion) before function was again assessed. In parallel studies, the above protocol was repeated with 8-SPT (10μM) present in the perfusate throughout the experiment (C2, P2). This concentration of 8-SPT was shown in preliminary rat heart studies to inhibit the negative chronotropic effects of bolus injections of adenosine. The results (Figure 1) show that 8-SPT had a depressive effect on the recovery of aortic flow after ischaemia in both the control and preconditioned hearts (C1 vs C2 and P1 vs P2). Mean coronary flow recovery during reperfusion was less in 8-SPT treated controls than non treated controls (79±3% C1 vs 66±10% C2), however, this was not statistically significant. Further, although 8-SPT (10μM) in the perfusate had a depressive effect on the absolute recovery from ischaemia compared to non drug treated controls, 8-SPT failed to abolish the protection provided by ischaemic preconditioning and these hearts still showed a significantly improved functional recovery of aortic flow (Figure 1, C2 vs P2) and coronary flow (66±10% C2 vs 89±4% P2) compared with controls. These results do not support the view that adenosine is the principle mechanism of ischaemic preconditioning in the isolated perfused working rat heart.

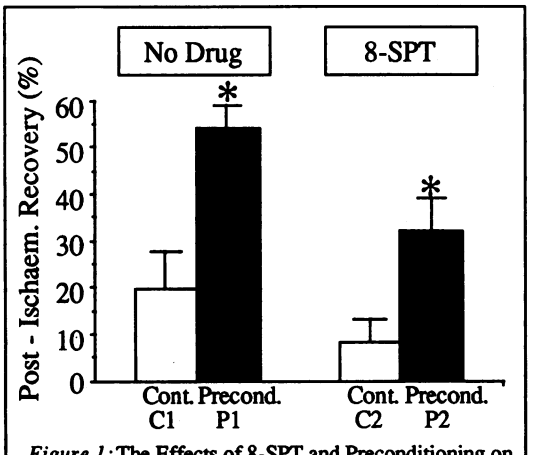

Figure 1: The Effects of 8-SPT and Preconditioning on Recovery of Aortic Flow. Data are mean ± sem. *P<0.05 compared to the respective control (ANOVA).

Liu, G.S., Thorton, J., Van Winkle, D.M., Stanley, A.W.H., Olsson R.A., & Downey, J.M. (1991) *Circulation* **84**, 350-356

16P KCl-INDUCED AUGMENTATION OF α_1-ADRENOCEPTOR PRESSOR RESPONSES IN THE RAT MESENTERIC VASCULAR BED (MVB) IS THROMBOXANE-DEPENDENT

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Previous studies have demonstrated that augmentation of noradrenergic pressor responses in the MVB by prostaglandin E_1, E_2 (Malik et al, 1976; Tobian & Viets, 1970) and angiotensin II (Kondo et al, 1977). More recently we reported that phenylephrine (PE)-induced pressor responses were enhanced following KCl administration (Holden et al, 1991), via an unknown, possibly endothelium-dependent mechanism. This communication describes experiments to resolve the mechanism underlying this KCl effect.

The MVB was prepared using a modified McGregor (1965) technique, whereby the isolated MVB was perfused in vitro (2mL/min) with warmed (37°C), gassed McEwen's solution. Changes in perfusion pressure were detected by a Bell & Howell physiological pressure transducer and displayed on a Grass Polygraph (79E). Drugs were administered either as a bolus (≤ 100μL) every 4 min, or as a constant infusion.

Following a KCl-induced dose-response curve (1-300μmol) the dose-response curve to PE (10pmol - 300nmol) was moved to the left with an associated enhanced maximal response, potentiating the maximal increase in perfusion pressure from 57±5mmHg (n=13) prior to KCl, to 117±17mmHg (n=8, P<0.001) following KCl. In the presence of indomethacin (5μM), which did not alter either PE or KCl pressor responses per se, KCl failed to induce a significant potentiation of PE pressor responses.

To examine the possibility of thromboxane (Tx) A_2 mediated enhancement of PE responses a series of experiments were carried out in the presence of the novel TxA_2 receptor antagonist ICI192605 (Brewster et al, 1988). ICI192606 (10μM), which did not effect pressor responses to either PE or KCl, inhibited the potentiation of PE responses by KCl. In support of the supposition that TxA_2 mediates the potentiation of PE pressor responses the stable Tx mimetic U46619 (0.1μM), a sub-threshold concentration, also augmented pressor responses, which were readily antagonised by ICI192605 (1μM).

Our results demonstrate that the MVB responds to KCl-induced contractions by releasing a factor, possibly endothelium-derived TxA_2, which is capable of modulating vascular α_1-adrenoceptor-mediated responses.

Brewster, A.G., Brown, G.R., Foubister, A.J., Jessup, R. & Smithers, M.J. (1988) Prostaglandins **36**, 173-178
Holden, K., Munby, J. & Weetman, D.F. (1991) J.Pharm.Pharmacol., **43**, 2P
Kondo, K., Manku, M.S., Horrobin, D.F., Boucher, R. & Genest, J. (1977) Clin.Sci.Mol.Med., **53**, 233-239
Malik, K.U., Ryan, P. & McGiff, J.C. (1976) Clin.Res., **39**, 163-168
McGregor, D.D. (1965) J.Physiol. (Lond), **177**, 21-30
Tobian, L. & Viets, J. (1970) Fed.Proc., **29**, 387

17P ATP DEPLETION AFTER OXIDANT EXPOSURE IN HUMAN VASCULAR ENDOTHELIAL CELLS

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The vascular endothelium is a target for oxidant induced damage which is thought to play a contributory role in a number of pathological conditions. Oxidant effects on cellular sites such as proteins, membranes and DNA may ultimately lead to cell death. Depletion of cellular ATP is often one of the earliest events following oxidant attack and can lead to a perturbation of cellular function. The aim of this study was to examine the importance of ATP depletion in the endothelial injury response to the oxidant, hydrogen peroxide (H_2O_2).

Human umbilical vein endothelial cells (HUVEC) were isolated and cultured as previously described (Jaffe et al, 1973). Confluent monolayers were exposed to bolus administration of H_2O_2 for a treatment period of five hours. Lytic injury was assessed by the release of lactate dehydrogenase (LDH) and ATP levels were determined using a lucin-luciferase bioluminesence method.

Lytic injury, in response to H_2O_2, showed both a time and dose-dependent (100 μM-500 μM) increase over the treatment period with a lag phase of 3 hours before any significant changes were observed. Depletion of endothelial ATP was also dose dependent within this concentration range, at 250 μM H_2O_2 ATP levels fell from 17.66 ± 0.89 in control cells to 1.35 ± 0.28 nmol/mg protein in treated cells (mean ± SD $p < 0.01$). However in contrast to cell lysis, ATP levels declined rapidly (within 1 hour) after exposure and remained at this low level. The decline in ATP was prevented by treatment with the H_2O_2 scavenger, catalase, and also dimethylthiourea (DMTU) which we have previously shown to be an effective H_2O_2 scavenger in this system (Millican et al, 1991). ATP depletion was also attenuated by 3-aminobenzamide (3AB; 5mM), an inhibitor of the enzyme poly (ADP-ribose) polymerase. The results indicate that ATP depletion is an early event in response to H_2O_2-induced injury. The protective effect of 3AB suggests that the mechanism for this depletion is in part due to activation of poly (ADP-ribose) polymerase, an enzyme known to be activated by DNA damage. Our results contradict previous studies using HUVEC where 3AB did not affect ATP depletion in response to xanthine/xanthine oxidase induced injury (Andreoli, 1989). This study does however agree with more recent reports using bovine pulmonary artery endothelial cells (Kirkland, 1991).

Andreoli SP (1989) Pediatr. Res. **25**, 97-101
Jaffe EA, Nachman RL, Becker RL and Minick CUR (1973) J. Clin. Invest. **52**, 2745-2756
Kirkland JB (1991) Biochim. Biophys. Acta **1092**, 319-325
Millican SA, Webster J and Wallace HM (1992) Br. J. Pharmacol. in press

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18P THE EFFECT OF 8-CYCLOPENTYL-1, 3-DIPROPYLXANTHINE ON *E. coli* ENDOTOXIN-INDUCED RENAL DYSFUNCTION

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We have previously shown that treatment with 8-cyclopentyl-1,3-dipropylxanthine (CPX), an A_1 selective adenosine antagonist, ameliorates glycerol and cisplatin-induced acute renal failure in the rat (Kellett *et al.*, 1989; Knight *et al.*, 1991). Adenosine may play a significant role in the impairment of renal function produced by bacterial endotoxins (Churchill *et al.*, 1987). Consequently this study has determined whether CPX can attenuate acute renal dysfunction induced by E. coli endotoxin.

Mean arterial blood pressure (MAP) and renal function were measured in rats (250-300g) anaesthetized with thiobutabarbitone (180 mg kg^{-1} i.p.), 30 min before and up to 75 min after i.v. injection of E. coli endotoxin (20 mg kg^{-1}) or saline (1.0 ml kg^{-1}). In some experiments CPX (0.1 mg kg^{-1} i.v.) or its vehicle (1.0 ml kg^{-1} i.v.; 1.0% DMSO, 0.75% 1M NaOH in 0.9% saline) were administered immediately following endotoxin injection. Renal function was assessed by measuring urine flow, urinary Na^+ excretion and [^{3}H] inulin clearance to estimate glomerular filtration rate (GFR) over 15 min periods. Results are given as mean ± s.e. mean.

Endotoxin administration evoked an immediate fall in MAP of 28 ± 3 mm Hg (n=8) which had returned to pretreatment levels 1-2 min later. Injection of endotoxin produced a fall in GFR from 1.3 ± 0.35 (n=8) to 0.10 ± 0.03 (n=6) ml min^{-1} $100g^{-1}$ 75 min later. This reduction in GFR was accompanied by a pronounced decrease in urine flow rate, such that 75 min after endotoxin administration urine flow had declined from 7.0 ± 1.2 (n=8) to 0.31 ± 0.09 (n=7) μl min^{-1} $100g^{-1}$. In addition Na^+ excretion diminished from 2.3 ± 0.7 (n=8) to 0.17 (n=2) mmol min^{-1} $100g^{-1}$ (only 2 rats produced sufficient urine to measure Na^+). However, fractional sodium excretion ($FE_{Na}+$) declined to a lesser extent, decreasing from 1.1 ± 0.36 (n=8) to 0.50 (n=2) % 75 min after endotoxin. The values for GFR and urine flow following endotoxin injection were significantly ($P<0.01$) lower than the corresponding values noted in control rats 75 min after saline injection.

GFR 75 min after endotoxin and CPX injections was 0.48 ± 0.09 ml min^{-1} $100g^{-1}$ (n=8) which was significantly ($P<0.05$) higher than GFR in endotoxin/vehicle-treated rats (0.16 ± 0.02 ml min^{-1} $100g^{-1}$; n=5). In comparison to endotoxin/vehicle-treated animals, CPX administration to rats injected with endotoxin resulted 75 min later in a significantly elevated urine flow (5.1 ± 1.1, n=8 vs 1.5 ± 0.39, n=7 μl min^{-1} $100g^{-1}$; $P<0.05$) and urinary Na^+ excretion (0.51 ± 0.11, n=8 vs 0.19 ± 0.04, n=6 mmol min^{-1} $100g^{-1}$; $P<0.05$). There was no significant difference ($P>0.05$) in $FE_{Na}+$ between vehicle and CPX treatments in endotoxin-injected rats.

The data show that CPX produced a significant amelioration of endotoxin evoked falls in GFR, urine flow and Na^+ excretion. The results support the proposal of Churchill *et al.*(1987) that adenosine plays a role in endotoxin nephrotoxicity.

Churchill, P.C., Bidani, A.K. & Schwartz, M.M. (1987) *Am. J. Physiol.* **253**, F244-F250.
Kellett, R., Bowmer, C.J., Collis, M.G. & Yates, M.S. (1989) *Br. J. Pharmac.* **98**, 313-318.
Knight, R.J., Collis, M.G., Yates, M.S. & Bowmer, C.J. (1991) *Br. J. Pharmac* **104**, 1062-1068

19P EFFECTS OF ANGIOTENSIN AT_1 OR AT_2 RECEPTOR BLOCKADE ON BASAL RENAL FUNCTION AND ON THE RENAL EFFECTS OF ANGIOTENSIN II (Ang II) IN THE ANAESTHETISED DOG

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Angiotensin AT_1 and AT_2 binding sites are present in the kidney (Chang & Lotti, 1991). This study assessed the relative importance of AT_1 and AT_2 receptors in mediating the renal effects of Ang II in vivo. We examined the effects of the AT_1 receptor antagonist, GR117289 (Robertson et al., 1991; 0.5mg/kg + 1μg/kg/min i.v.), and the AT_2 receptor ligand, PD123177 (20μg/kg/min intra-renal artery; i.r.a.) on basal renal function, and on the renal effects of Ang II infusions (i.r.a.) in pentobarbitone-anaesthetised dogs.

The following were measured or derived: mean aortic blood pressure (MBP), left renal artery blood flow (RBF) and renal vascular conductance (RVC), urine output (U_V), glomerular filtration rate (GFR), and absolute ($U_{Na}V$) and fractional (FE_{Na}) sodium excretion. When basal values were stable, GR117289 or PD123177 were administered and their effects monitored over three, 15min periods. Ang II (1-300ng/kg/min i.r.a.; 15min each dose) was then infused and its effects compared with those seen in antagonist-free dogs.

Table 1: Effects of GR117289 or PD123177 (n=5-6) on renal function 45min post-dose.

	MBP	RVC	RBF	U_V	$U_{Na}V$	GFR	FE_{Na}
GR117289	-12.0±4.1*	+40.2±10.5*	+20.6±5.2*	+25.5±7.7	+25.4±9.9	+4.0±4.7	+21.0±11.0
PD123177	+0.8±1.6	-6.0±7.0	-5.6±6.1	+15.0±5.1	+19.1±3.8	+9.3±6.2	+2.3±5.8

Values are mean percentage change ± s.e.m. from basal measurements. * P<0.05 vs time matched saline controls (n=4); Dunnett's test.

GR117289 caused both renal vasodilatation and a sustained fall in MBP (Table 1). Despite its vasodepressor effect, GR117289 tended to cause diuresis and natriuresis, although these changes were not statistically significant. PD123177 did not significantly change renal haemodynamic or excretory function. In the absence of antagonist, Ang II (1-300ng/kg/min i.r.a., n=5) caused marked, dose-related decreases in RVC, RBF, U_V, $U_{Na}V$, FE_{Na}, and GFR. In the presence of GR117289, mean Ang II dose-response curves for these renal parameters were displaced some 12-38 fold to the right of those obtained in antagonist-free dogs. PD123177 did not antagonise the effects of Ang II on renal excretory function. Similarly, PD123177 did not antagonise renal vasoconstrictor responses to low doses of Ang II (1-10ng/kg/min); however, PD123177 did significantly inhibit vasoconstrictor responses to higher doses of Ang II (30-300ng/kg/min), causing a 4-fold rightward displacement of the mean Ang II dose-response curve on RVC.

These results suggest that the renal haemodynamic and excretory effects of endogenous and exogenous Ang II are mediated primarily by angiotensin AT_1 receptors. However, the modest antagonism exerted by the AT_2 ligand, PD123177, against Ang II-induced renal vasoconstriction may indicate a functional role for AT_2 receptors in the renal vasculature. This warrants further investigation.

Chang, R.S.L. & Lotti, V.J. (1991). Life Sci., **49**, 1485-1490.
Robertson, M.J., Middlemiss, D.M., Ross, B.C., Drew, G.M., Scopes, D.I.C. & Dowle, M.D. (1991). Br. J. Pharmacol., **104**, 300P.

20P RENAL Na^+-H^+ EXCHANGER ACTIVITY, ALPHA-ADRENOCEPTORS AND THE CONTROL OF DOPAMINE OUTFLOW

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The vectorial transport of sodium plays an important role in the renal formation of dopamine by tubular epithelial cells (Soares-da-Silva, 1992). The flux of sodium across tubular epithelial cells is largely dependent on Na^+-K^+ ATPase activity, whereas the Na^+-H^+ exchanger has a major role in the transport of sodium into these cells. Previous studies have shown that inhibition of the Na^+-H^+ antiport by ethylisopropylamiloride (EIPA) results in an increased accumulation of newly-formed dopamine in rat kidney slices; this effect, however, appears to be dependent on a reduced ability of the amine to leave the cell. The aim of the present study is to determine the importance of the Na^+-H^+ exchanger in the control of dopamine outflow in rat kidney slices loaded with L-DOPA. For this purpose inhibitors (EIPA and amiloride) and activators (alpha$_1$- and alpha$_2$-adrenoceptor agonists) of the Na^+-H^+ exchanger (Gesek & Schoolwerth, 1990) were used. Slices of rat renal cortex were preincubated for 30 min in warm (37ºC) and gassed (95% O_2 and 5% CO_2) Krebs' solution and incubated for further 15 min with (50 μM) L-DOPA. The total amout of L-DOPA decarboxylated into dopamine was neither affected by EIPA (1, 5 and 10 μM) and amiloride (5, 10 and 50 μM) nor by the alpha$_1$- and alpha$_2$-adrenoceptor agonists used, respectively phenylephrine (0.1, 0.5 and 1.0 μM) and U.K. 14,304 (10, 50, 100 and 500 nM). The fractional outflow of newly-formed dopamine was, however, significantly decreased (58% to 78% reduction) by EIPA (5 μM and 10 μM) and only slightly reduced by amiloride (50 μM). Both U.K. 14,304 and phenylephrine were found to increase in a concentration-dependent manner the fractional outflow of dopamine. The effects of the alpha$_1$- and alpha$_2$-adrenoceptor agonists were blocked by selective antagonists, respectively, prazosin (1 μM) and yohimbine (100 nM). EIPA (10 μM) was also found to antagonize the effects of U.K. 14,304 and phenylephrine. Submaximal concentrations of U.K. 14,304 (50 nM) plus submaximal concentrations of phenylephrine (0.5 μM) were found to produce a synergic increase in the outfow of dopamine. The effects of U.K. 14,304, phenylephrine and the synergic effect of U.K.14,304 plus phenylephrine were antagonized by dybutiryl cyclic AMP (250 μM), forskolin (10 μM) and pertussis toxin (1 μg/ml). A synergic increase in the outflow of dopamine was also observed between U.K.14,304 and the protein kynase C activator phorbol 12,13-dibutyrate (500 nM); this synergic effect was completely abolished by d-sphingosine (1 μM). d-sphingosine also abolished the synergic effect observed with U.K. 14,304 plus phenylephrine. No synergic effect was found to occur between phenylephrine and phorbol 12,13-dibutyrate (500 nM) In conclusion, it is suggested that the Na^+-H^+ exchanger has a major role in the outward transport of newly-formed dopamine in tubular epithelial cells. The synergic activation of the Na^+-H^+ antiport by alpha$_1$- and alpha$_2$-adrenoceptor agonists appears to involve, respectively, the generation of phosphoinositides and inhibition of adenylate cyclase activity; the inhibitory effect of dybutiryl cyclic AMP on the facilitatory effect of phenylephrine may be related to the inhibition of phospholipase C as a result of the increased intracellular levels of cyclic AMP (Neylor & Summers, 1988).

Gesek, F.A. & Schoolwerth, A.C. (1990) Am.J.Physiol.,258,F514-F521.
Neylor, C.B. & Summers, R.J. (1988) Eur.J.Pharmacol.,148,441-444.
Soares-da-Silva, P. (1992) Br.J.Pharmacol.,105,1P.
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21P VARYING $[K^+]_{ext}$ REVEALS TWO COMPONENTS TO ACETYLCHOLINE (ACh)- AND HISTAMINE (H)-INDUCED VASODILATATION IN THE PERFUSED RAT MESENTERIC ARTERIAL BED (MAB)

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Endothelium-dependent vasodilatation of the isolated perfused MAB is insensitive to methylene blue and oxyhemoglobin (Furchgott et al, 1986; Adeagbo & Malik, 1990); it is also insensitive to superoxide dismutase (Bhardwaj & Moore, 1988). The relative contribution of endothelium-derived nitric oxide (EDNO) and endothelium-derived hyperpolarizing factor (EDHF) to ACh and H vasodilatation was examined by studying the effects of nitro-arginine methyl ester (L-NAME), and alterations in membrane potential resulting from changing $[K^+]_{ext}$.

Mesenteric arteries of male Sprague Dawley rats (200-300 g) were isolated according to McGregor (1965) and perfused with Krebs solution (37°C) at 5 ml/min using a peristaltic pump (Microperplex, LKB Bromma, model 2132) and bubbled continuously with 95% O_2/5% CO_2 mixture. Changes in perfusion pressure were recorded via a Statham pressure transducer coupled to a Grass polygraph (model 7E). The ability of ACh or H to relax arteries preconstricted following infusion with either 0.1-0.5 μM cirazoline, or 60 mM K^+ depolarizing Krebs solution containing 10 nM prazosin and 1 μM tetrodotoxin, was evaluated.

ACh (10^{-8}-10^{-5}M) and H (10^{-7}-10^{-4}M) each elicited concentration-dependent relaxations of cirazoline-preconstricted MAB. Relaxations were rapid in onset, with complete reversal of tonus occurring at 10^{-5}M (ACh) and 10^{-4}M (H). Infusion of 50 μM L-NAME enhanced cirazoline tonus from 65.0 ± 3.8 to 138.4 ± 4.2 mm Hg; ACh and H vasodilator responses were not affected by the infusion except that the responses appeared more transient in nature. Both the ACh and H responses obtained during L-NAME infusion were abolished by either 0.5 μM apamin or 5 mM tetraethylammonium, and in the case of ACh, also by graded concentrations of p-fluorohexahydrosilaphenidol (10-100 nM) but not pirenzepine. These results suggest that ACh initiated vasodilatation via the release of an EDHF, which activates Ca^{2+}-activated K^+ channels via M_3 muscarinic cholinoceptors. In arteries, where tonus was maintained with an infusion of excess K^+ Krebs solution, ACh- or H-induced vasodilatation, which was abolished by L-NAME (5 μM) or methylene blue (10 μM), followed a slower time course and at 10^{-3}M of either agonist, the maximal reversal of tonus attained were 26.4 ± 1.2% and 29.6 ± 2.2% (n=6) respectively.

These data suggest that ACh and H mediate vasorelaxation of the MAB via the release of an EDHF and that a vasodilatory contribution from EDNO is of lesser importance.

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Adeagbo, A.S.O. & Malik, K.U. (1990) Br. J. Pharmac. **100**, 427-434.
Bhardwaj, R. & Moore, P.K. (1988) Br. J. Pharmac. **95**, 835-843.
Furchgott, R.F. et al. (1986) Blood Vessels **23**, 69.
McGregor, D.D. (1965) J. Physiol. **177**, 21-30.

22P EVIDENCE FOR NITRIC OXIDE AS THE INHIBITORY NEUROTRANSMITTER IN ISOLATED RABBIT ANOCOCCYGEUS

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In the rat and mouse anococcygeus nitric oxide (NO) has been implicated as the non-adrenergic non-cholinergic (NANC) inhibitory neurotransmitter responsible for relaxation in response to nerve stimulation at low frequencies (Gibson et al., 1989; Gillespie et al., 1989). We investigated NO as the inhibitory neurotransmitter in rabbit anococcygeus using L-nitroarginine methyl ester and nitro L-arginine (NO-ARG) to inhibit NO synthesis. L-arginine (L-arg) and D-arginine (D-arg) were used to examine substrate reversal.

Adult rabbits were killed by i.v. injection of an overdose of pentobarbitone. The anococcygeus muscles were mounted between parallel platinum electrodes in Krebs solution maintained at 36.5 ± 0.5°C. Contractile responses were recorded using an isometric transducer. Adrenergic and cholinergic transmission were blocked by guanethidine (10^{-5}M) and atropine (10^{-6}M) respectively. All relaxation responses were expressed as % reduction in tone induced by addition of 10^{-6}M histamine. NANC responses were obtained by electrical stimulation at a frequency of 1-2Hz (0.5ms, supramaximal voltage) for 20 seconds at 2 minute intervals. Statistical analysis was by Student's paired t-test.

Nerve stimulation at 1-2Hz produced rapid relaxation in all tissues. The mean reduction in tone was 86.9 ± 2.5% (mean ± s.e.mean, n=21).The relaxation to nerve stimulation in the presence of NO-ARG (10^{-4}M) was significantly reduced, to 47.6 ± 5.6%, n=21 ($p<0.001$). L-nitroarginine methyl ester (10^{-4}M) produced a similar inhibition of the nerve mediated relaxations (from a control value of 64.8 ± 5.3% to 35.8 ± 4.0% in the presence of the drug, $p<0.05$). The effects of L-arg and D-arg in the presence of NO-ARG were examined. Addition of L-arg (10^{-4}M) partially reversed the inhibitory effect of NO-ARG on nerve mediated relaxations. D-arg (10^{-4}M) however did not reverse the inhibitory effect of NO-ARG, as shown in Table 1.

TABLE 1: Effect of NO-ARG, L-arg and D-arg on nerve mediated relaxation of isolated rabbit anococcygeus

CONTROL	NO-ARG (10-4M)	L-arg (10-4M)	D-arg (10-4M)
93.6 + 2.7%	46.3 + 7.0% **	67.8 + 8.1% *	-
75.0 + 3.6%	47.3 + 4.6% **	-	45.0 + 6.8%

(n=6, ** = $p<0.001$, * = $p<0.005$ by paired Student's t-test)

Addition of exogenous ATP ($5x10^{-5}$M)produced a reduction in tone similar in magnitude to that seen on nerve stimulation. (88.7 ± 4.8%, n=6). This reduction in tone was unchanged in the presence of 10^{-4}M NO-ARG (88.7 ± 4.1%, n=6), indicating that NO-ARG did not have any non-selective effect on relaxation of the smooth muscle. These results suggest that inhibition of NO synthesis results in reduction in the nerve mediated relaxations in the rabbit anococcygeus. This reduction can be partly reversed by addition of further substrate. These results support the proposal that NO is an inhibitory neurotransmitter in rabbit anococcygeus.

Gibson, A., Mirzazedeh, S, Al-Swayeh, O.H., Chong, N.W.S., & Moore, P.K. (1989) Br. J Pharmacol. **99**, 940P
Gillespie, J.S., Liu, X., & Martin, W. (1990) *Br. J. Pharmac* **98**, 1080-1082

23P EFFECT OF INHIBITING NITRIC OXIDE SYNTHASE ON RELAXATIONS TO ISOPRENALINE IN ISOLATED RAT MESENTERIC RESISTANCE ARTERIES

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There is recent evidence that vascular smooth muscle relaxation to β-agonists can be affected by inhibition of nitric oxide (NO) synthase. Gardiner et al (1991) found that L-N^G-nitro arginine methyl ester (L-NAME) attenuated the hyperaemic vasodilatation to salbutamol in the rat hindquarters circulation *in vivo*. This evidence suggests that release of NO from the vascular endothelium and subsequent activation of soluble guanylate cyclase in the smooth muscle cell may play a role in the relaxation of some vascular beds to β-agonists. β-agonists are conventionally held to relax vascular smooth muscle by activating adenylate cyclase and increasing intracellular cAMP. Thus β-agonists may relax vascular smooth muscle by both cAMP and cGMP dependent processes. In this study the effects of L-NAME upon isoprenaline induced relaxations of isolated rat mesenteric resistance arteries have been investigated.

Male Wistar rats 225-250g were killed by cervical dislocation and mesenteric resistance arteries (normalised internal diameter 332±4.7 μm n=45) were dissected and mounted on a small vessel myograph (Mulvany & Halpern, 1977). Arteries were preconstricted with potassium (K^+) to give approximately 50% of maximum tension and a concentration response to isoprenaline carried out. After incubation with 10^{-4}M L-NAME, a further concentration response to isoprenaline was performed in the presence of L-NAME. Initially it was found that L-NAME potentiated the K^+ precontraction and so the concentration of K+ was reduced to give equivalent tension to the previous contraction. Concentration responses to K^+ with and without L-NAME were carried out to clarify the effect of NO synthase inhibition of K^+ induced tension.

Isoprenaline relaxed the arteries in a dose dependent manner (pEC_{50} 8.03±0.40 M ; maximum relaxation 66.79%±2.43 n=7). L-NAME inhibited the maximum relaxation to isoprenaline (66.79%±2.43 v 27.64%±7.64 $p<0.001$ paired t-test n=7). Propranolol also inhibited isoprenaline induced relaxation. L-NAME significantly decreased the pEC_{50} for K^+ and significantly increased the maximum response. This study provides evidence that inhibition of nitric oxide synthase attenuates the relaxation to isoprenaline. This suggests that in this preparation endothelial release of nitric oxide is a mechanism of β-adrenoceptor mediated vascular smooth relaxation; in addition NO release modifies contractions to K^+.

1.Gardiner SM, Kemp PA, Bennett T. (1991). Br J Pharmacol **103**,1725-1732
2.Mulvany MJ, Halpern W. (1977). Circ Res **41**,19-26

24P ENDOTHELIUM-DEPENDENT RELAXATIONS TO 5-HTR IN GUINEA-PIG ISOLATED JUGULAR VEIN ARE MEDIATED BY A RECEPTOR WHICH RESEMBLES THE 5-HT_{1D} SUBTYPE

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5-Hydroxytryptamine (5-HT) and sumatriptan have recently been shown to relax guinea-pig isolated jugular vein *in vitro* by the activation of a single population of endothelial 5-HT_1-like receptors (Gupta, 1992). The latter effects were competitively antagonised by both methiothepin and PAPP (1-[-2-[4-aminophenyl]ethyl]-4-[3-trifluoromethylphenyl]piperazine ; Schoeffter & Hoyer, 1990). The aim of the present study was to investigate further the pharmacological identity of this receptor.
Male Dunkin-Hartley guinea-pigs (300-500g) were killed by cervical dislocation. Isolated rings (2mm diameter) were suspended on parallel tungsten wires under a resting load of 0.2-0.5g, in Krebs solution gassed with 95% O_2 and 5% CO_2 at 37°C. 5-HT-evoked relaxant responses were measured isometrically in rings precontracted with U-46619 (30nM), in the presence of mesulergine (10μM) to antagonise respectively 5-HT_2 and 5-HT_1-like (sumatriptan-insensitive) receptors located on the smooth muscle. 5-HT (1.0nM-10.0μM) evoked concentration-dependent relaxations of guinea-pig isolated jugular vein which were not antagonised by high concentrations of the following ligands : (±) pindolol (1μM; 5-$HT_{1A/B}$), (-) propranolol (1μM; 5-$HT_{1A/B}$), spiperone (1μM; 5-$HT_{1A/2}$), and ICS 205,930 (10μM; 5-HT_3/putative 5-HT_4). However, the relaxations to 5-HT could be antagonised competitively by metergoline (0.1-1.0μM), and also by the α_2-adrenoceptor antagonists rauwolscine (0.3-10.0μM) and yohimbine (1.0-10.0μM; see below). The stereoisomer corynanthine (1.0-10.0μM) was without any antagonist effect.

Antagonist	pA_2[95% CL]	Schild slope[95% CL]	n
Metergoline	7.4[7.1-8.2]	1.36[0.76-1.96]	5
Rauwolscine	6.8[6.5-7.2]	1.11[0.81-1.42]	4
Yohimbine	7.1[6.8-7.7]	0.90[0.69-1.12]	4

Data are the mean pA_2 or Schild slope values [95% Confidence Limits; 95% CL] for n separate experiments.

Metergoline, rauwolscine and yohimbine have a high affinity towards the 5-HT_{1D} binding site (Waeber *et al.*, 1988) and have been used previously to demonstrate 5-HT_{1D}-like characteristics in rabbit saphenous vein, guinea-pig frontal cortex, and porcine coronary artery (Martin *et al.*, 1991; Middlemiss *et al.*, 1988; Schoeffter & Hoyer, 1990). These results are consistent with a 5-HT_{1D} receptor mediated relaxation in guinea-pig isolated jugular vein.

Gupta, P.(1992). *Br. J. Pharmacol.*, (in press).
Martin, G.R., Prentice,D.J. & Maclennan, S.J. (1991). *Fund. Clin. Pharmacol.*, **5** (5), 417.
Middlemiss, D.N., Bremer, M.E. & Smith, S.M. (1988). *Eur. J. Pharmacol.*, **157**, 101-107.
Schoeffter, P. & Hoyer, D. (1990). *J. Pharmacol. Exp. Ther.*, **252** , 387-395.
Waeber, C., Schoeffter, P., Palacios, J.M. & Hoyer, D. (1988). *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **337**, 595-601.

25P INVESTIGATION OF THE 5-HT RECEPTOR TYPE INVOLVED IN INHIBITION OF SPONTANEOUS ACTIVITY OF HUMAN COLONIC CIRCULAR MUSCLE

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There is evidence that 5-HT receptors designated as 5-HT_4, exist peripherally and centrally. Those identified in rat oesophagus mediate relaxant responses in precontracted tissues (Reeves et al., 1991; Baxter et al, 1991). We have investigated the action of 5-HT, other indoles and some substituted benzamides on the spontaneous contractions of the circular muscle of human colon. Strips of circular muscle from ascending or sigmoid colon were recorded isometrically in Krebs' gassed with 95% O_2, 5% CO_2 at 37°C and 15mN tension until regular spontaneous contractions were obtained. For each agonist results were obtained from 5-12 patients. 5-HT was tested on at least one strip from each patient. Agonists were added cumulatively (0.01-100μM), with a 2min contact time and produced inhibition of contractions. Results were expressed as a percentage reduction of amplitude measured before drug addition.

Compound	EC_{50}[1] (μM)	Equipotent molar ratio[2]	% max. inhibition of amplitude
5-HT	0.2(0.1-0.5)	1	85.2 ± 5.2
5-methoxytryptamine	0.7(0.2-3.2)	3	82.3 ± 6.2
α-methyl-5-HT	0.6(0.2-2.0)	3	80.3 ± 19.8
5-carboxamidotryptamine	3.9(0.8-18.4)	18	79.4 ± 6.0
2-methyl-5-HT	0.9[1](0.1-13.7)	30	37.8 ± 12.2
Renzapride	1.5(0.2-12.8)	7	73.4 ± 4.7
Zacopride	0.9[1](0.2-4.1)	13	68.3 ± 11.0
Metoclopramide	1.1[1](0.0-34.1)	37	46.4 ± 12.1

[1] Zacopride EC_{30}; 2-methyl-5-HT and metoclopramide EC_{20}.
[2] The mean EPMR is the ratio of the relevant mean EC values. 5-HT EC_{30} 0.07(0.04-0.12μM); EC_{20} 0.03 (0.02-0.05μM).

After a concentration response curve to 5-HT was obtained, antagonists were added for 30min before repeating the 5-HT. Inhibition by 5-HT was not significantly affected by the 5-HT_1 and 5-HT_2 antagonist, methysergide (10μM) or the 5-HT_3 antagonist, ondansetron (1μM) but a rightward shift of the concentration response curve was observed in the presence of ICS 205-930 (10μM) with an apparent pK_B value of 5.82 ± 0.33. ICS 205-930 (10μM) did not affect isoprenaline-induced inhibition of contractions.

It is concluded that the 5-HT receptors mediating the inhibitory response were distinct from 5-HT_1-like, 5-HT_2 and 5-HT_3 subtypes. Pharmacologically, the receptors are characteristic of the 5-HT_4 receptors identified by Reeves et al (1991).

Baxter, G.S., Craig, D.A. & Clark, D.E. (1991) Naunyn-Schmiedeberg's Arch. Pharmacol. 343, 439-446.
Reeves, J.J., Bunce, K.T. & Humphrey, P.A.A. (1991) Br. J. Pharmacol. 103, 1067-1072.

26P EVIDENCE TO SUGGEST THAT BUSPIRONE POTENTIATES THE VAGAL BRADYCARDIA INDUCED BY THE "DIVING RESPONSE" ELICITED BY SMOKE IN ANAESTHETIZED RABBITS

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Cardiac vagal motoneurones (CVMs) are activated by stimulation of arterial baroreceptors, carotid body afferents, cardiopulmonary receptors and receptors in the nasal cavity involved in the "diving response". Activation of CVMs by cardiopulmonary receptors is, in part, mediated by activation of central 5-HT_{1A} receptors (Bogle et al., 1990). The present experiments were carried out to investigate the effects of central application of buspirone, a partial agonist at 5-HT_{1A} receptors (Schoeffter & Hoyer, 1988), on the "diving response" in anaesthetized rabbits. This response can be elicited by stimulating nasopharyngeal receptors with cigarette smoke causing a bradycardia, a rise in BP and an apnoea (Peterson et al., 1983).

Rabbits were anaesthetized with urethane (1-1.5g kg^{-1}; i.v.; n=5). Arterial blood pressure (BP), heart rate(HR) and renal nerve activity (RNA) were recorded simultaneously. Bidirectional tracheal cannulation was performed which permitted both spontaneous respiration and the independent delivery of cigarette smoke to the nasal cavity. Smoke volume (10-50 ml) was chosen to produce a submaximal bradycardia. Atenolol (1mg kg^{-1}) was then administered i.v. and the smoke challenge repeated until a stable bradycardia was observed. Saline was then administered i.c. (20 μl over 20s) followed up to 20 min later by buspirone (200μg kg^{-1}; i.c.) the smoke challenge being repeated at regular intervals.

Atenolol caused falls in BP of 5±0.4 mmHg, HR of 44±9 bpm and RNA of 8±5% Saline (i.c.) had little effect on these variables while buspirone caused falls in BP of 5±1 mmHg, HR of 8±4 and RNA of 8±3%. Before saline smoke produced a bradycardia of 54±20 bpm, apnoea of 23±10s and an increase in RNA of 343±138%. Saline had little effect on these variables, a slight increase in the bradycardia by 5±4 bpm and apnoea by 3±1s. However by about 20 min buspirone had markedly potentiated these variables by 31±6 bpm and 10±3s respectively. The sympathoexcitation caused by smoke was inceased to 590±282% after buspirone.

These results suggest that 5-HT_{1A} receptors are involved in mediating the cardiovascular and respiratory effects caused by stimulation of nasopharyngeal receptors.

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Bogle,R.G., Pires,J.G.P. & Ramage,A.G. (1990). Br.J. Pharmacol., 100, 757-760
Peterson,F.D., Coote,J.H., Gilbey,M.P. & Futuro-Neto,H.A. (1983). Am.J.Physiol., 245, R433-R437
Schoeffter,P. & Hoyer,D. (1988). Br. J. Pharmacol., 95, 975-985

27P ROLE OF α_2 ADRENOCEPTORS AND Ca^{2+}-CHANNEL BLOCKERS ON INSULIN SECRETION RESULTING FROM GLUCOSE, α_1 AND β ADRENOCEPTOR STIMULATION IN CONSCIOUS RABBITS

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Though it is generally accepted that exogenous stimulation of α-2 adrenoceptors inhibits basal and stimulated insulin release (Reverte et al. 1991) the inhibitory role played by tonic sympathetic stimulation on insulin secretion it is a matter of controversy (John et al. 1990). It is also known than insulin secretion induced by glucose and several hormones seems to require extracellular calcium (Malaisse 1990) being calcium inflow mediated by voltage sensitive or voltage insensitive calcium channels. The present work was conducted to investigate, a)- the role of α-2 adrenoceptors on glucose evoked insulin secretion; b)- the effects of two calcium channel blockers on insulin secretion mediated by a glucose challenge, α-1 and β adrenoceptor stimulation.

Conscious, 24 h fasted rabbits were challenged with a glucose load (10mg/Kg/min, i-v for 30 min) in the absence and presence of yohimbine (YOH: 20μg/Kg/min, i-v for 30 min),idazoxan (10μg/Kg/min, i-v for 30 min), verapamil (0.17μg/Kg/min, i-v for 30 min) and elgodipine (a dihydropyridine derivate,1ng/Kg/min, i-v for 30 min) infused half an hour before glucose administration. Arterial immunoreactive insulin (IRI) plasma levels were significantly higher in animals pretreated with either idazoxan (Δ at the end of glucose infusion 1082±278 %, n=4, p<0.001) or YOH (Δ 655±238%, n=4 p<0.05) than rabbits receiving glucose alone (Δ=318±79%, n=6). Idazoxan also attenuated glucose induced hyperglycaemia (Δ at the end of glucose infusion in the presence and absence of the antagonist respectively was 2.9±0.35 mmol/l n=6, vs 4.1±0.75 mmol/l, n=6 p<0.005). Interestingly whereas verapamil did not alter glucose effects on both parameters, elgodipine enhanced glucose induced insulin secretion (Δ at 30 min in the presence of elgodipine: 597±84%, n=4, p<0.001) while simultaneously attenuating the blood glucose increase.

Verapamil suppressed the increase in IRI plasma levels evoked by amidephrine administration (AMID 10μg/Kg/min, i-v for 30 min; Δ at 45 min in the presence and absence of verapamil was 26.5±13.3%, n=6, p<0.001 vs 115.8±36.4%, n=6), but it did no modify the insulin secretory response mediated by isoprenaline (ISO 4.4μg/Kg/min, i-v for 30 min, Δ at 45 min 162.1±42.1%, n=9). Verapamil suppressed the insulin potentiated secretory rise produced by the concomitant administration of AMID+ISO (Δ at 15 min 655.5±150.4%, n=5 after combined agonist infusion vs: 10±13%, n=4, p<0.001 in rabbits pretreated with verapamil). Elgodipine reproduced the effects of verapamil on α-1 and α-1+β mediated insulin secretory responses. Our results add to the evidence for α-2 adrenoceptor involvement in the physiological regulation of insulin secretion in rabbits, and remark the importance of extracellular calcium to mediate the α-1 adrenoceptor excitatory response and to supporte insulin potentiation.

-Reverte, M., García-Barrado M.J., Moratinos, M. (1991) In Adrenoceptors: Structure, Mechanisms, Function. Ed.Bradshaw, C.M. pp 3369-370. Basel: B. Verlang.
-John, G., Doxey J., Walter, D. and Reid, J. (1990) Eur. J. Pharmacol. 187, pp 531-536.
-Malaisse W.J. (1990) In Insulin. Ed. Cuatrecasas, P. and Jacobs, S. pp 113-124. Berlin: Sprige-Verlang.

28P EFFECTS OF OVARIAN STEROIDS ON CONTRACTILITY OF HUMAN UMBILICAL ARTERY STRIPS

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During normal pregnancy, ovarian steroids contribute to the maintenance of low blood pressure by suppressing vascular tone. To gain further insight into the mechanisms of the vascular actions of 17-β-oestradiol and progesterone their effects have been studied on contractions of human umbilical artery strips evoked by 5-hydroxytryptamine (5-HT), noradrenaline (NA), potassium chloride (KCl) and oxytocin (OT).

Arteries were dissected from umbilical cords within 6h of normal delivery. They were cut open to form flat sheets, then incisions were made on opposite sides alternately to make "zig-zag" strips of mainly circular muscle. These strips were suspended for superfusion (10ml/min) with pre-gassed($95\%O_2+5\%CO_2$) Krebs' solution at 37°C (pH 7.4). Isometric contractions were recorded from a resting tension equivalent to 1g, set after an initial equilibration period during which the constancy of amplitude of responses to repeated 2min infusions of a submaximal concentration of 5-HT was confirmed. The following 2min infusions were then given in a randomised sequence: 10nM & 100nM 5-HT; 100nM & 1μM NA; 20mM & 40mM KCl and 10nM & 100nM OT. The infusions were repeated after 1hr superfusion with 50 or 500nM 17-β oestradiol or progesterone, or the ethanol vehicle in concentrations of 230μM or 2.3mM in the Krebs' solution (n=6-9 in each group). Amplitudes of contractions were calculated as %ages of those elicited by 40mM KCl in the initial phase of each experiment. Statistical comparisons utilised paired and unpaired 't' tests on $\log_{10}$ conversions of %age data.

Contractions evoked by 5-HT, but not by other agonists, increased in the presence of 230μM ethanol ($p<.05$, n=6 for 100nM 5-HT), but remained approximately constant with 2.3mM ethanol. 17-β-Oestradiol at 50nM enhanced contractions evoked by 100nM or 1μM NA ($p<.05$, n=6 for both) and at 500nM enhanced 100nM 5-HT-evoked contractions($p<.025$, n=6), but KCl- and OT-evoked contractions were unaffected by the oestrogen. In contrast, progesterone had no significant effect on 5-HT-induced contractions but at 50nM suppressed those evoked by OT ($p<.05$, n=6), and at 500nM suppressed those elicited by 100nM NA ($p<.05$, n=6) and 20mM KCl ($p<.005$, n=6).

These observations in human umbilical artery strips of oestrogen-mediated enhancement of amine-evoked contractions, and of progesterone imposed suppression of contractions elicited by small (20mM) but not larger (40mM) increments in extracellular K^+ concentration are entirely consistent with previously reported effects on other human vessel strips (Leathard & Eccles, 1991). From these findings it is easy to envisage how the gradual rise in oestrogen:progesterone ratio that occurs late in pregnancy could progressively diminish umbilical artery flow. This diminution would be exacerbated if local circulating amine levels were increased in stress. Furthermore, a premature increase in the oestrogen:progesterone ratio could, by these mechanisms, pre-dispose to maternal hypertension and reduced blood flow in the foeto-placental circulation, leading to inadequate oxygenation and nutrition of the developing foetus.

Leathard,HL & Eccles,NK (1991) In: New Advances in Headache Research 2, ed F Clifford Rose, pp187-192. London: Smith-Gordon.

29P TWENTY-ONE-DAY TREATMENT WITH THE GABA-T INHIBITORS ETHANOLAMINE-O-SULPHATE AND γ-VINYL GABA ELEVATES BASAL AND STIMULATED GABA RELEASE

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Gamma-vinyl GABA (GVG) and ethanolamine-O-sulphate (EOS) inhibit GABA-transaminase (GABA-T), preventing catabolism of the inhibitory amino acid GABA, thereby elevating tissue GABA levels, an approach useful in the treatment of epilepsy. GVG is used clinically for the treatment of refractory epilepsy. The purpose of the present study was to investigate the release of GABA following GABA-T inhibition.

18 Male Wistar rats (272 ± 6g) were divided into 3 groups receiving in their drinking water, 1g/l sucrose vehicle (controls), 3g/l GVG in vehicle or 3g/l EOS in vehicle. After 21 days the animals were sacrificed, the brains rapidly removed, the hippocampi dissected free, and the remaining tissue homogenised and frozen for analysis of GABA-T, GABA and protein content. Cross-chopped hippocampal slices were prepared as in Qume *et al.* (1992). Tissue was incubated for basal release in 500µl normal (2mM Ca^{++}) or low (0.1mM) Krebs for 5min, replaced with 500µl stimulation medium containing 20, 50, 100mM K^+ or 50mM K^+ with low Ca^{++}. The GABA content of the incubation medium and tissue was analysed by HPLC. The GVG treated animals received 251 ± 26mg/kg/day and EOS-treated 331 ± 23, assuming that all that was lost from the drinking bottle was consumed.

Animals treated with GVG or EOS showed classic signs of GABA-T inhibition: piloerection, hunched posture and akinesia, GVG treated animals did not gain weight. GABA-T activity was significantly depressed in treated tissues to 34-41% of controls. Tissue GABA levels were elevated significantly to GVG, 209% and EOS 177% of controls. Basal GABA release was significantly elevated from controls (mean ± sem): 56 ± 4 pm/500µl/5min/mg to GVG: 204 ± 38 and EOS: 150 ± 13. Potassium stimulated GABA release above basal levels were also elevated (Table 1). Basal GABA release was not Ca^{++} dependent. Low Ca^{++} medium significantly reduced stimulated GABA release to 45% in control animals, in contrast to treated animals where there was no evidence of Ca^{++} dependency.

Table 1: Stimulated GABA release above basal, (mean ± sem) units: nmoles/500µl/5min/mg.

mMK^{++}	CONTROL	GVG	EOS
20	59±10	143±34*	130±32*
50	179±29	299±56*	323±97
100	209±14	613±173*	757±150*
50/lowCa^{++}	95±16	313±91*	272±44*

*: $p<0.05$ Student's t-test, one tailed, n=6.

In conclusion, chronic (21 day) treatment with the GABA-T inhibitors GVG and EOS elevate both the basal and stimulated release of GABA, this stimulated release lacks Ca^{++} dependency.

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Qume, M., Davies, J. & Fowler, L. J. (1992). *Br. J. Pharmacol., In Press.*

30P INVOLVEMENT OF $GABA_B$ RECEPTORS IN EXPERIMENTAL ABSENCE SEIZURES

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Increasing GABA receptor function by $GABA_A$-(muscimol, THIP) and $GABA_B$-(R-baclofen) receptor agonists, by inhibitors of GABA reuptake (e.g. SKF 89976) or by vigabatrin, an inhibitor of GABA-transaminase, results in a dose-related increase in the number and duration of spike- and-wave discharges (SWD) in various animal models of petit-mal epilepsy and in humans (Vergnes et al., 1984), suggesting that absence epilepsy may be related to an excess of GABAergic transmission. As neither picrotoxin nor bicuculline alters SWD at non-convulsive doses (Vergnes et al., 1984) $GABA_A$-mediated mechanisms do not appear to be critical for the development of SWD in absence epilepsy; thus, the activity of $GABA_B$ receptors may be a more crucial factor for the generation and control of SWD. R-baclofen (2-4 mg/kg i.p.) not only increases SWD in a strain of rats (GAERS) with genetic absence epilepsy, but also induces SWD-like oscillations in the cortical EEG of non-epileptic control rats. In view of these results, we tested the centrally active $GABA_B$-receptor antagonist CGP 35348 (3-aminopropyl(diethoxymethyl)phosphinic acid) in GAERS. CGP 35348 (50-400 mg/kg) dose-dependently and progressively suppressed the spontaneous SWD in GAERS with a peak effect 60 min after injection. 50 ± 16% and 90 ± 3% suppression were obtained with 50 and 200 mg/kg, respectively. An increase in the cumulative duration of SWD by 150 to 300% was observed after systemic i.p. administration of 4 mg/kg R-baclofen, 8 mg/kg THIP, 1 mg/kg muscimol, 30 mg/kg SKF 89976, 600 mg/kg vigabatrin, 375 mg/kg γ-hydroxybutyric acid (GHB), 20 mg/kg carbamazepine and 50 mg/kg phenytoin (n = 6-8 rats in each case). CGP 35348 dose-dependently suppressed the SWD aggravated by any of the foregoing treatments as well as the spontaneously occurring SWD. The suppressant effect of CGP 35348 was significant at 200 mg/kg and complete at 400 mg/kg. CGP 35348 antagonized the increase in the duration of SWD induced by carbamazepine and phenytoin without significantly decreasing their anticonvulsant effects. CGP 35348 produced no obvious side-effects and all animals remained awake with a normal, desynchronized background EEG.

These results suggest that $GABA_B$-mediated neurotransmission plays a predominant role in the development of SWD in generalized non-convulsive epilepsy and that $GABA_B$ receptor antagonists may be considered as potential anti-absence drugs. This anti-absence effect is presumably mediated by a hitherto unknown mechanism, because the drugs commonly used in absence epilepsy, ethosuximide and valproate, do not interact with $GABA_B$-receptors.

Vergnes, M., Marescaux, C., Micheletti, G., Depaulis, A., Rumbach, L., and Warter, J.M. (1984) Neurosci. Lett. 44 91-94

31P EFFECT OF (-)-BACLOFEN ON cAMP FORMATION IN RAT SPINAL CORD SLICES

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Activation of $GABA_B$ receptors in the spinal cord may be responsible for the antinociceptive and muscle-relaxant properties of baclofen (Price et al.,1984; Schwartz et al.,1988). Since it has been shown that baclofen can modulate the accumulation of cAMP produced by a variety of stimuli in tissue prepared from rat brain higher centres e.g. cerebral cortex slices (Hill,1985; Karbon & Enna, 1985), we have compared the effect(s) of (-)-baclofen on stimulated cAMP accumulation in rat spinal cord slices with those reported in cerebral cortex slices. Whole spinal cords from male Wistar rats (250 g) were excised and sliced into 350 x 350 µm microprisms using a McIlwain tissue chopper. After several washes in Krebs-Ringer bicarbonate buffer, slices were preincubated with (-)-baclofen or GABA for 10 min and challenged for a further 10 min with either forskolin (Fk)(10 µM) or the adrenoreceptor agonists (-)-noradrenaline (NA) or (-)-isoprenaline (ISO) (100 µM). To prevent GABA uptake by the slices, the sodium concentration in the incubation buffer was reduced to 40 mM using Tris-base as a substitute (Hill, 1985).

(-)-Baclofen dose-dependently (10-100 µM) inhibited Fk-induced formation of cAMP (inhibition at 100 µM 58 ± 9.5%, n = 3 rats). By contrast it affected neither NA- nor ISO-induced accumulation of cAMP at any concentration tested (10-100 µM). GABA (10-300 µM) mimicked the effect of (-)-baclofen on Fk-induced accumulation of cAMP (inhibition at 300 µM 38.5 ± 4.97%, n = 8 rats). Surprisingly, the inhibition of Fk-induced accumulation of cAMP produced by (-)- baclofen and GABA was not prevented by the $GABA_B$ antagonist CGP 35348 (3-aminopropyl-diethoxy-methyl-phosphinic acid) (100 µM - 1.5 mM).

In conclusion, whilst the action of (-)-baclofen on Fk-induced cAMP formation observed previously in rat cerebral cortex slices could also be demonstrated in spinal cord slices, the enhancement of ß-adrenoreceptor activation could not. The lack of effect of CGP 35348 cannot be explained at present, but may support the concept of $GABA_B$ receptor heterogeneity.

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Hill, D.R. (1985) *Br. J. Pharmacol.* **84**, 249-257.
Karbon,E.W. & Enna,S.J. (1985) *Mol. Pharmacol.* **27**,53-59.
Price, G.W., Wilkin, G.P., Turnbull, M.J. & N.G. Bowery (1984) *Nature* **307**, 71-74.
Schwartz, M., Klockgether,T., Wullner,U., Turski,L. & Sontag K-H. (1988) *Exp. Brain Res.* **70**, 618-626.

32P REGIONAL SENSITIVITY OF $GABA_B$ BINDING SITES TO PERTUSSIS TOXIN CHANGES IN THE RAT BRAIN DURING ONTOGENY

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Pertussis toxin (PTX) produced from Bordatella pertussis, reduces ^{3}H-GABA binding to $GABA_B$ receptors in the cortex, hippocampus and cerebellum of adult rat brain. $GABA_B$ binding in the corpus striatum of adult animals appears to be insensitive to PTX (Bowery et al.,1990). The effect of PTX on $GABA_B$ binding during ontogeny has not hitherto been studied systematically. We have therefore compared the effects of PTX on $GABA_B$ binding in young (5-6wks;80-120g) and adult (10-12wks;270-300g) rat brain regions, and present our preliminary findings.

Crude synaptic membranes were prepared from cerebral cortices, corpus striata, hippocampi and cerebella pooled from young or adult Wistar rats, according to the method of Bowery et al (1983). Membrane pellets were halved and incubated in activated PTX (7-15µgPTX/mg protein) or in vehicle (equal volumes) for 30 minutes at 29°C. The reaction was stopped by the addition of ice-cold tris (50mM, pH 7.4) and centrifugation (10,000g for 10 minutes). Pellets were washed immediately prior to assay for $GABA_B$ binding. Briefly, membranes were incubated with ^{3}H-GABA (5nM; 91.5Ci/mmol) in tris-HCl (50mM, pH 7.4 containing $CaCl_2$ 2.5mM) at 23°C with isoguvacine (40µM) to saturate $GABA_A$ sites. Non-specific binding was determined by further addition of excess (-)baclofen (100µM). The reaction was terminated by centrifugation, the pellets washed superficially and radioactivity counted by scintillation spectroscopy. The mean (± sem) ratio of specific $GABA_B$ binding (fmol/mg protein) in the presence and absence of PTX is shown in the table below:

Ratio of Specific $GABA_B$ binding PTX:Control				
	Cortex	Striatum	Hippocampus	Cerebellum
Adult	0.664 ± 0.079	0.981 ± 0.105	0.641 ± 0.151	0.664 ± 0.118
Young	0.961 ± 0.140	0.594 ± 0.090**	0.536 ± 0.071	0.981 ± 0.160

** $p < 0.05$ Student's t-test
n = 4-6 experiments
10-15 animals pooled/experiment

The main finding of our study is that the sensitivity to PTX on $GABA_B$ binding in the corpus striatum declines with maturation. This did not occur in the other regions studied.

Bowery, N.G., Hill, D.R. and Hudson, A.L. (1983). Br. J. Pharmacol. **78:** 191-206.
Bowery, N.G., Knott, C., Moratalla, R. and Pratt, G.D. (1990). In: GABA and Benzodiazepine Receptor Subtypes (Eds Biggio, G. and Costa, E. Publ. Raven Press, New York. pp 127-139)

33P EVIDENCE THAT (±)-BACLOFEN-INDUCED HYPOACTIVITY IS NOT MEDIATED BY $GABA_B$ RECEPTORS

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Hypoactivity, a distinct form of sedation, has mainly been used to study presynaptic alpha2-adrenoceptor function (Heal et al., 1981). However, a similar syndrome can be produced by a range of other drugs including baclofen (Heal et al., 1981, 1989; Gray et al., 1987). Although Gray et al. (1987) implied that baclofen-hypoactivity may be a useful model for studying GABA-B function, insufficient pharmacological evidence exists to test this assertion. We have now extensively characterised this response using (±)-baclofen, its optical enantiomers and a wide range of receptor antagonists.

Hypoactivity was measured in adult C57/Bl/6Ola mice (15 - 30 g) as previously described (Heal et al., 1981). Four behavioural parameters (passivity, tactile responsiveness, posture and gait) were scored 0 - 3 every 10 min. for 60 min. (max. score = 72). Drugs were given i.p. dissolved in 0.9% saline. Antagonists (or vehicle) were given 30 min. prior to baclofen (or vehicle).

(±)-Baclofen dose-dependently induced hypoactivity (ED50 (95% confidence limits) = 6.8 (5.3 - 9.0) mg/kg, max. response at 20 mg/kg = 62±1, n = 8).The response to (±)-baclofen (5 mg/kg, 26.9±0.4, n = 75) was not altered by prazosin (1 mg/kg), idazoxan (1 mg/kg), (±)-propranolol (3, 10 mg/kg), quipazine (3 mg/kg), mepyramine (1, 10 mg/kg), ranitidine (3, 10 mg/kg), scopolamine (1 mg/kg), mecamylamine (3, 10 mg/kg), SCH 23390 (0.1 mg/kg) or BRL 34778 (0.05 mg/kg) (data not shown). However, ritanserin (0.1 - 1 mg/kg), a 5-HT2 receptor antagonist, potentiated the response to (±)-baclofen but had no effect when given alone (ritanserin 0.5 mg/kg + (±)-baclofen 5 mg/kg = 40.4±1.5, n = 10; saline + (±)-baclofen 5 mg/kg = 28.4±0.9, n = 10; $p<0.05$). However, inhibition of 5-hydroxytryptamine (5-HT) synthesis with p-chlorophenylalanine had no effect on (±)-baclofen-induced hypoactivity (data not shown). Surprisingly, the GABA-B antagonists, phaclofen (0.5 - 10 mg/kg) and saclofen (1 - 30 mg/kg) failed to attenuate the response to (±)-baclofen (5 mg/kg). In view of this, we determined the effects of the enantiomers of (±)-baclofen and found that the majority of the activity resided in the (+)-isomer which has low affinity for GABA-B receptors ((+)-baclofen ED50 = 3.7 (3.2 - 4.4) mg/kg, max. response at 20 mg/kg = 63.7±6.4, n = 10 ; (-)-baclofen ED50 = 10.1 (5.1 - 28.3) mg/kg, response at 20 mg/kg = 25.5±4.7, n = 4). A subsequent physical chemical study of the material used in these experiments confirmed its optical activity.

These data confirm that dose-dependent hypoactivity is induced in mice by (±)-baclofen. Involvement of alpha1, alpha2, beta, 5-HT1, H1, H2, D1, D2 and muscarinic receptors can be discounted, although the response was modulated by 5-HT2 receptors. While our data agree with those of Robinson et al. (1989) showing phaclofen and saclofen to be poor antagonists of (±)-baclofen-induced responses, the fact that this response was mediated by the (+)-isomer, which has 100-fold less affinity than the (-)-isomer for the GABA-B receptor (Robinson et al., 1989), leads us to conclude that this response is not mediated by GABA-B receptors.

Gray, J. A., Goodwin, G. M., Heal, D. J. & Green, A. R. (1987) Br. J. Pharmacol. 92, 863 - 870.
Heal, D. J., Akagi, H., Bowdler, J. M. & Green, A. R. (1981) Eur. J. Pharmacol. 75, 231 - 237.
Heal, D. J., Prow, M. R., Martin, K. F. & Buckett, W. R. (1989) Br. J. Pharmacol. 97, 406P.
Robinson, T. N., Cross, A. J., Green, A. R., Toczek, J. M. & Boar, B. R. (1989) Br. J. Pharmacol. 98, 833 - 840.

34P CHARACTERISATION OF HUMAN ILEUM NK_2 RECEPTOR STABLY EXPRESSED IN CHINESE HAMSTER OVARY CELLS USING NK AGONISTS

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The human NK_2 receptor gene from trachea and gastrointestinal tissue has recently been cloned (Gerard et al., 1990). We have established a stable cell line in chinese hamster ovary cells which have been transfected with the human ileum NK_2 receptor gene. The pharmacology of this transfected receptor has been investigated by measuring changes in cytosolic calcium in response to stimulation by neurokinin agonists, using fura-2.

Cells were pre-loaded with fura-2 (2μM, 30min at 37° C) in Krebs-Henseleit solution containing BSA (1mg/ml). Aliquots were spun (100g for 3min) and re-suspended in Krebs-Henseleit solution ($5x10^5$ cells/ml). Changes in fluorescence in response to neurokinin stimulation were measured using a Shimadzu RF5000 fluorimiter. Each response was determined using a single aliquot and experiments were designed to eliminate time dependent bias. Responses were expressed as % maximum fluorescence to the selective NK_2 receptor agonist GR64349 (Hagan et al., 1991).

GR64349 caused rapid, concentration-related increases in cytosolic calcium (EC_{50} - 7.9nM, 95% confidence limits 5-14nM, n=8). In marked contrast, the selective NK_1 receptor agonist GR73632 (Hagan et al., 1991) and the selective NK_3 receptor agonist, senktide caused only small effects which could not be quantified. The endogenous mammalian tachykinins, NKA, NKB and substance P also caused increases in calcium fluorescence with a rank order of potency NKA>NKB>>substance P. EC_{50} values and maximum responses are given below. The NK_2 receptor antagonist MEN 10207 (Maggi et al., 1990) was tested for its ability to antagonise responses to GR64349. However, MEN10207 produced agonist effects in its own right with an EC_{50} value and maximum response similar to that of GR64349 (see below).

Agonist	EC_{50} (nM)	Maximum Response (% GR64349 max)
GR64349	7.9 (5-14)	100
GR73632	>3000	18 ± 8
Senktide	>3000	8 ± 7
NKA	5.3 (3-10)	112 ± 20
Substance P	>3000	28 ± 4
NKB	61 (15-259)	88 ± 13
MEN10207	8.4 (3-26)	104 ± 5

Values are geometric means (95% confidence limits) or mean ± s.e.m. from 3-8 experiments.

These results suggest that chinese hamster overy cells, transfected with the human ileum NK_2 receptor gene, express functional receptors which display the expected pharmacological properties of NK_2 receptors. Further studies with antagonist ligands are in progress. These cells should provide a useful system for studying human NK_2 receptors in vitro.

Gerard, N.P., Eddy, R.L., Shows, T.B. and Gerard, C. (1990). J. Biol. Chem., **265**, 20455-20462.
Hagan, R.M., Ireland S.J., Jordan, C.C., Beresford, I.J.M., Deal, M.J. and Ward, P. (1991). Neuropeptides, **19**, 127-135.
Maggi, C.A., Pattachini, R., Guiliani, S., Rovero, P., Dion, S., Regoli, D., Giachetti, A and Meli, A. (1990). Br. J. Pharmacol., **100**, 588-592.

35P EVIDENCE FOR FUNCTIONAL NK_1 AND NK_3 NEUROKININ RECEPTORS IN THE MEDIAL HABENULA NUCLEUS OF THE RAT BRAIN *IN VITRO*

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Three pharmacologically distinct receptors (NK_1, NK_2 and NK_3) have been defined for the mammalian neurokinins. This classification has been based largely on the activities of selective agonists in various smooth muscle preparations. The endogenous neurokinins Substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) are not sufficiently selective to discriminate between neurokinin receptor types, but receptor selectivity has been achieved by modifications of SP, NKA and NKB. Hence, [Sar^9,$Met(O_2)^{11}$]-SP, [β-Ala^8]-NKA(4-10) and succinyl-[Asp^6,N-$MePhe^8$]-SP(6-11) (SENKTIDE) are three of the most potent and selective agonists currently available at the NK_1, NK_2 and NK_3 receptors, respectively (Guard and Watson, 1991). Little has been done to functionally characterize the neurokinin receptor types present in the CNS. Here, we describe NK_1- and NK_3-mediated electrophysiological responses in neurons of the rat medial habenula nucleus (MHb) *in vitro*.

Extracellular recordings were made from rat MHb in isolated coronal brain slices using conventional electrophysiological techniques. Serial coronal slices were cut using a Vibratome, and a single slice transferred to a perfusion bath and allowed to equilibrate in artificial cerebrospinal fluid (ACSF) at 37°C for 1 hour before recordings were commenced. Recordings were made from single spontaneously firing MHb neurons using microelectrodes filled with ACSF and with tip resistances of 25-40MΩ. Drugs were dissolved in ACSF and bath applied for 1 minute. Doses of drugs were applied in order of increasing concentration with at least 20 minutes between dose applications.

Recordings were made from 86 neurons in 69 brain slice preparations. No neuron was observed to respond to [β-Ala^8]-NKA(4-10) (0/15). However, neurons responded to [Sar^9,$Met(O_2)^{11}$]-SP and to SENKTIDE with a rapid and dose-dependent increase in firing rate, indicating the presence of NK_1 and NK_3 but not NK_2 receptors in the rat MHb. The mean EC_{50} for [Sar^9,$Met(O_2)^{11}$]-SP was 74nM (range of 2-209nM) (n=20), and the mean EC_{50} for SENKTIDE was 48 nM (range of 9-128nM) (n=14). Ninety percent of the neurons displayed a response to one or both of these neurokinin analogues. 34/72 neurons responded to both [Sar^9,$Met(O_2)^{11}$]-SP and SENKTIDE, 29/72 neurons responded to [Sar^9,$Met(O_2)^{11}$]-SP only, 2/72 neurons responded to SENKTIDE only and 7/72 neurons responded to neither agonist. These data suggest that NK_1 and NK_3 receptors are differentially expressed on subpopulations of neurons within the nucleus.

Our results are consistent with published autoradiographical studies that have demonstrated a high density of [^{3}H]-[Sar^9, $Met(O_2)^{11}$]-SP binding sites and a moderate density of [^{3}H]-SENKTIDE binding sites in the rat MHb, suggesting the presence of NK_1 and NK_3 receptors, respectively, in this nucleus (Dam *et al.*, 1990a and 1990b).

Dam, T.V., Escher, E., Quirion, R. (1990a). *Brain Res.* 506, 175-179.
Dam, T.V., Martinelli, B., Quirion, R. (1990b). *Brain Res.* 531, 333-337.
Guard S., Watson, S.P. (1991). *Neurochem Int.* 18(2), 149-165.

36P ACTIVATION OF NK_1 RECEPTORS STIMULATES PLASMA PROTEIN EXTRAVASATION IN GUINEA-PIG DURA MATER AND CONJUNCTIVA: TISSUES INNERVATED BY THE TRIGEMINAL NERVE

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Neurogenic inflammation (vasodilatation and plasma protein extravasation - PPE) within dura mater has been suggested to contribute to migraine headache (Moskowitz, 1984). The blood vessels within dura and extracranial tissues such as conjunctiva are innervated by trigeminal and upper cervical sensory nerves which contain substance P (SP) and calcitonin gene related peptide (CGRP). SP, but not CGRP, has been shown to stimulate PPE in all these tissues (Markowitz et al, 1987) but the neurokinin receptor type/s mediating this response have not been elucidated. Using selective neurokinin receptor compounds we have characterised this response in guinea-pigs.

Male Dunkin-Hartley guinea-pigs (200-250g) were anaesthetised with pentobarbitone (18mg/kg ip), ketamine (40mg/kg im), xylazine (8mg/kg im) and atropine (1mg/kg ip). Animals were artificially ventilated to maintain an arterial blood pH of about 7.4. The selective neurokinin agonists, GR73632 (NK_1), GR64349 (NK_2) and senktide (NK_3) (Hagan et al, 1991a) were injected intravenously 5 min after iv administration of [^{125}-I]human serum albumin (1.8MBq/kg). Animals were perfused via the left cardiac ventricle with 0.9% saline 10 min after injection of agonist to remove intravascular radioactivity. Tissues (dura and conjunctiva) were weighed and radioactivity counted. Results were expressed as a ratio of drug treated: vehicle treated animals. Results are shown in Table 1.

Table 1 Plasma protein extravasation in anaesthetised guinea-pigs, ratio of vehicle:drug treated animals

	Neurokinin agonist/antagonist (nmol/kg iv)				
	GR73632 (3nmol) (N=3)	GR73632 (10nmol) (N=7)	GR82334 (200nmol) (N=5)	GR73632(3nmol) +GR82334(200nmol) (N=3)	GR73632(10nmol) +GR82334(200nmol) (N=6)
DURA	1.0±0	1.4±0.1*	1.0±0.3	0.9±0.1	1.1±0.2
CONJUNCTIVA	13.3±0.5**	14.7±3.0**	2.2±0.9	1.2±0.3	9.1±2.3**

*P<.05 **P<.01 compared to vehicle, Students t-test (on cpm/mg)

The NK_1 agonist, GR73632 (3 and 10nmol/kg iv), produced a large increase in PPE in conjunctiva. A smaller (40%), but significant increase in PPE was also seen in dura with the higher dose. NK_2 and NK_3 receptor agonists were ineffective in all tissues (GR64349 at 10nmol/kg iv and senktide at 30nmol/kg iv, data not shown). The selective NK_1 antagonist, GR82334 (15min pretreatment; Hagan et al, 1991b) inhibited PPE induced by GR73632 in dura and conjunctiva (Table 1).

These studies suggest that NK_1 receptors mediate neurokinin-induced PPE in guinea-pig dura and extracranial tissue.

Hagan, R.M., Ireland, S.J., Jordan, C.C., Beresford, I.J.M., Deal, M.J. and Ward, P.(1991a) Neuropeptides, 19, 127-135.
Hagan, R.M., Ireland, S.J., Bailey, F., McBride, C., Jordan, C.C. and Ward, P.(1991b) Br. J. Pharmac., 102, 168P.
Moskowitz, M.A. (1984) Ann. Neurol., 16, 157-168.
Markowitz, S., Saito, K. and Moskowitz, M.A. (1987) J. Neurosci., 7, 4129-4136.

37P INTERACTION OF THE NON-PEPTIDE NK_1 TACHYKININ RECEPTOR ANTAGONIST (±)CP-96,345 WITH L-TYPE CALCIUM CHANNELS IN RAT CEREBRAL CORTEX

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We have previously reported that the non-peptide NK_1 tachykinin receptor antagonist CP-96,345 (Snider *et al.*, 1991), tested as the (2S,3S) and (2R,3R) racemic mixture, blocks contractile responses to the NK_1 receptor agonist [Sar^9,Met(O$_2$)^{11}]SP in the guinea pig ileum in a non-competitive manner (Boyle *et al.*, 1991). This appears to be a non-specific action since responses to histamine and carbachol are similarly affected. These results suggested a possible effect of (+)CP-96,345 on depolarization-contraction-coupling processes. In the present study, we have investigated whether this effect may be due to an interaction with voltage-sensitive calcium channels by examining the effects of (+)CP-96,345 on the binding of [^{3}H]-nimodipine and [^{3}H]-diltiazem to L-type calcium channels in rat cerebral cortex membranes.

Binding assays were performed according to the methods of Schoemaker & Langer (1989) and Bolger *et al.* (1983), for [^{3}H]-diltiazem and [^{3}H]-nimodipine binding, respectively, with minor modifications. Non-specific binding was defined using 10 µM diltiazem or 1µM nifedipine, respectively. Peptidase inhibitors (chymostatin, 2µg ml^{-1}, leupeptin, 4µg ml^{-1}, bacitracin, 40µg ml^{-1}, phosphoramidon, 2µM) and 0.1% BSA were included in the incubation buffer where appropriate.

Both unlabelled diltiazem and (+)CP-96,345 inhibited [^{3}H]-diltiazem (4nM) binding to rat cortex membranes with K_i values of 33.6nM (-4.1, +4.7) and 29.2nM (-2.3, +2.4), respectively (geometric mean, -sem, +sem; n=5-6). Nifedipine and nimodipine inhibited [^{3}H]-nimodipine (0.1nM) binding with K_i values of 1.03nM (-0.16, +0.17) and 0.33nM (-0.03, +0.03), respectively (n=3). In contrast, (+)CP-96,345 (1nM-10µM) produced a concentration-dependent potentiation of [^{3}H]-nimodipine binding with a maximal stimulation of 186 ± 29% above basal and an EC_{50} value of 83nM (-22.9, +31.6; n=4). In comparison, (+)CP-96,345 inhibited the binding of [^{125}I]-Bolton Hunter labelled substance P to NK_1 binding sites in rat cortex with a K_i value of 82nM (-9.4, +11.5; n=4). Scatchard analysis of [^{3}H]-nimodipine binding in the absence and presence of 100nM (+)CP-96,345 indicated that (+)CP-96,345 increased the affinity of [^{3}H]-nimodipine for its binding sites without affecting the B_{max} (control: K_D = 0.32nM, B_{max} = 511fmol/mg protein; in the presence of 100nM (+)CP-96,345: K_D = 0.074nM, B_{max} = 496fmol/mg protein; n=2). Substance P, and the NK_1 receptor-selective ligands, [Glp^6,L-Pro^9]SP(6-11), [Sar^9,Met(O$_2$)^{11}]SP, GR82334 and L-668,169, had no significant effect at 10µM on either [^{3}H]-diltiazem or [^{3}H]-nimodipine binding. Similarly, the NK_2 receptor-selective ligands [β-Ala^8]NKA(4-10), GR64349 and L-659,877, and the NK_3 receptor-selective agonist senktide, had no significant effect at 10µM on [^{3}H]-diltiazem or [^{3}H]-nimodipine binding.

In summary, these results indicate that in addition to interacting with NK_1 tachykinin receptors, (+)CP-96,345 possesses high affinity for the diltiazem site on L-type calcium channels. Accordingly, (+)CP-96,345 may mediate its biological effects via an interaction with both NK_1 receptors and L-type calcium channels.

Bolger, G.T., Gengo, P., Klockowski, R., Luchowski, H, Siegel. H., Janis, R.A., Triggle, A.M. & Triggle, D.J. (1983). *J. Pharm. Exp. Ther.*, 225, 291-309.
Boyle, S.J., Howson, W. & McKnight, A.T. (1991). *Br. J. Pharmacol.*, 104, (Suppl.), 146P.
Schoemaker, H. & Langer, S. (1989). *J. Pharm. Exp. Ther.*, 248, 710-715.
Snider, R.M., Constantine, J.W., Lowe III, J.A., Longo, K.P., Lebel, W.S., Woody, H.A., Drozda, S.E., Desai, M.C., Vinick, F.J., Spencer, R.W. & Hess, H-J. (1991) *Science* 251, 435-437.

38P THE NOVEL CALCIUM ANTAGONIST SR 33557 DISPLAYS HIGH AFFINITY FOR A DISTINCT BINDING SITE IN RAT BRAIN

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In skeletal muscle and cardiac sarcolemmal membranes, SR 33557 has been shown to interact with high affinity to a binding site which appears to be distinct, but allosterically coupled to sites for other calcium antagonists (Chatelain *et al.*, 1991). Indeed, in skeletal muscle a complicated picture is emerging with many separate, allosterically coupled binding sites being claimed (Janis & Triggle, 1991). However, in rat brain only sites for the prototypical calcium antagonists (labelled by [^{3}H] dihydropyridines, [^{3}H] diltiazem and [^{3}H] verapamil) have thus far been demonstrated. We now demonstrate a high affinity binding site for SR 33557 in rat brain, which appears to be distinct but allosterically coupled to the dihydropyridine site.

[^{3}H] Dihydropyridine binding assays were carried out as previously described (Kenny *et al.*, 1990). [^{3}H] SR 33557 binding to rat cerebral cortex membranes was carried out in 50 mM Tris-HCl, supplemented with 0.01% bovine serum albumin in a total volume of 1.0 ml. Assays were incubated for 120 min at 25^{0}C in the dark and filtered over Whatman GF/B filters pre-soaked with 0.05% polyethylenimine. Non-specific binding was determined in the presence of 1 µM SR 33557.

In rat cerebral cortex SR 33557 displaced [^{3}H] PN 200-110 binding with high affinity (pKi 9.54 ± 0.04, nH 1.01) which was decreased 5 fold in the presence of Ca^{2+} (5mM). Saturation experiments with [^{3}H] PN 200-110 in rat cortex carried out in the presence of SR 33557 (0.1-1.0 nM) indicated that the compound caused a decrease in Kd without any effect on Bmax (control: Bmax 186 ± 5 fmol/mg protein, Kd 0.04 ± 0.006 nM; 188 ± 12 fmol/mg protein, 0.24 ± 0.03 nM in the presence of 1 nM SR 33557, n=4). In kinetic experiments, SR 33357 did not change the rate of dissociation of [^{3}H] PN 200-110 induced by the addition of 1 µM nitrendipine (control: 0.007 ± 0.0009 min^{-1} and 0.006 ± 0.001 min^{-1} in the presence of 5 nM SR 33557, n=4). These data are indicative of an apparently competitive interaction of SR 33557 at the [^{3}H] PN 200-100 site. D-cis diltiazem fully reversed the inhibition of binding produced by all concentrations of SR 33557 examined (0.1-2.0 nM) whereas d-cis diltiazem did not reverse the effects of nitrendipine indicating that the site of action of SR 33557 was not at the dihydropyridine site.

Displacement studies were carried out with [^{3}H] SR 33557 in rat cerebral cortex and the rank order and affinities of a range of calcium antagonists (nitrendipine pKi 9.48 ± 0.24: nH 0.90, Bay K 8644 pKi 8.23 ± 0.17: nH 0.87, fluspirilene pKi 8.13 ± 0.17: nH 0.87, verapamil pKi 7.97 ± 0.03: nH 0.98, flunarizine 7.15 ± 0.14) was similar to that observed at the [^{3}H] PN 200-110 site in the same tissue. However, divalent cations (inactive at the [^{3}H] PN 200-110 site) displaced [^{3}H] SR 33557 from its site in rat cortex (IC50, mM: Mg^{2+}, 11.06 ± 1.1, Mn^{2+} 5.01 ± 0.7, Ca^{2+} 2.09 ± 0.48; Cd^{2+} 0.026 ± 0.015) with similar potency to that described at the [^{3}H] SR 33557 binding site in skeletal muscle membranes (Chatelain *et al.*, 1991).

These findings suggest that the binding site for SR 33557 in rat cortical membranes is distinct from, but tightly coupled to, the dihydropyridine site, and appears to be similar to the site characterised in skeletal muscle and cardiac sarcolemmal membranes.

Chatelain, P., Beaufort, P., Meysmans, L. & Clinet, M. (1991). *Mol. Pharmacol.*, **39**, 64-71.
Janis, R.A. & Triggle, D.J. (1991). In: *The Calcium Channel* (eds) Hurwitz, L., Partridge, L. & Keach. CRC press. London.
Kenny, B.A., Fraser, S., Kilpatrick, A.T. & Spedding, M. (1990). *Br. J. Pharmacol.*, **100**, 211-216.

39P CHRONIC HYOSCINE AND OXOTREMORINE TREATMENT ALTER THE SENSITIVITY OF RAT FRONTAL CORTEX PYRAMIDAL CELLS TO ACETYLCHOLINE AND CARBACHOL

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Chronic administration of hyoscine and oxotremorine to rats produced, up-regulation and down-regulation respectively, of cortical muscarinic receptors (Ben-Barak *et al.*, 1981). The aim of the present study was to investigate whether these changes in receptor density are paralleled by changes in neuronal sensitivity to acetylcholine (ACh) and carbachol applied iontophoretically.

Hyoscine (10 mg/kg s.c. in 0.9% w/v NaCl solution daily for 16 days) and oxotremorine (0.5 mg/kg s.c. in 0.9% NaCl solution twice daily for 11 days and 10 mg/kg in sesame oil once daily for 9 days) were administered to Sprague-Dawley rats (7 rats in each group) and control rats (3 in each group) received the vehicles alone. Twenty-four h after the last injection, the rats were anaesthetized with urethane (1.5 g/kg i.p.) and the sensitivities of pyramidal cells in the frontal cortex to iontophorized ACh and carbachol were determined. Each drug was applied with an ejection current of 30 nA for 20 s and the average responses of 3 applications (separated by 1 min recovery periods) were obtained. The control neuronal firing rate, monitored over 20 consecutive 1 s epochs was compared with the firing rate during drug administration and for 20 s after ceasing drug application by a Wilcoxon signed ranks test. Neurones were considered to be sensitive when their firing rate increased or decreased ($P < 0.05$) either during, or within 20 s of, drug application. The two control groups responded similarly to the two agonists and were therefore combined.

Chronic hyoscine treatment significantly increased the sensitivity of cortical neurones to ACh from 52.8% in the control group ($n = 161$) to 84.3% in the hyoscine-treated group ($n = 178$, $P < 0.0001$). There was a similar increase ($P < 0.3$) in sensitivity to carbachol, the proportion of sensitive neurones increasing from 55.3% in the control group to 78.7% in the hyoscine-treated group ($P < 0.0001$). Hyoscine treatment also increased the spontaneous firing rate of frontal cortex neurones ($P < 0.0001$), reduced the delay between drug application and onset of effect and prolonged the durations of action of ACh and carbachol.

In contrast, chronic oxotromorine treatment significantly decreased the sensitivity of neurones in the frontal cortex sensitivity to ACh from 52.8 % in the control group (treated with 0.9% sodium chloride daily for 11 days and sesame oil for 9 days) to 33.8% in the oxotremorine-treated group ($n = 172$, $P < 0.001$). There was a parallel reduction in the effect of carbachol, spontaneous firing rate decreasing from 55.3% in the control group to 38% in the oxotremorine-treated group ($P < 0.05$). The spontaneous firing rate of neurones ($P < 0.0001$) was also decreased by oxotremorine whereas the delays to onset of ACh's and carbachol's actions were increased and the durations of their effects were decreased. Atropine applied iontophoretically blocked the effects of ACh and carbachol in more than 90% of neurones studied.

The results show that the changes in cortical muscarinic receptor density which have been reported to occur in rats treated chronically with hyoscine and oxotremorine are paralleled by changes in their sensitivity to ACh and carbachol.

Ben-Barak, J., Gazait, Silman, I. & Dudai, Y. (1981) Eur. J. Pharmac. **74**, 73-81

40P "FourDAPines": A NEW CLASS OF ILEO-SELECTIVE ANTIMUSCARINIC DRUG

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4-Diphenylacetoxy-N-methylpiperidine (4DAMP) benzyl bromide, an intermediate in the synthesis of bis-4DAMP compounds (Barlow & Shepherd, 1985), has selectivity similar to that of 4DAMP methobromide. Experiments *in vitro* using tissues from guinea-pigs surprisingly showed the hydrobromide of the tertiary base, benzyl-4-DiphenylAcetoxyPiperidine ("Benzyl-FourDAPine") has even greater selectivity (Table 1), comparable with that of p-fluoro-hexahydro sila-diphenidol (Lambrecht et al., 1989).

	ATRIA	ILEUM		
	RATE	(30°) FORCE	30°	37°
4DAMP benzyl bromide	6.89 ±0.02 (4)	6.64 ±0.02 (4)	7.82 ±0.03 (5)	7.74 ±0.01 (5)
Benzyl-4DAPine HBr	6.00 ±0.21 (4)	5.65 ±0.18 (4)	7.65 ±0.07 (5)	7.41 ±0.04 (5)
(±)p-fluoro-hexahydro sila-diphenidol	5.91 ±0.10 (6)	5.56 ±0.19 (8)	7.53 ±0.05 (8)	7.53 ±0.05 (8)

TABLE 1
Mean log.K ± se in dose-ratio experiments with carbachol as agonist and 1.0 μM antagonist: results for (±)p-fluoro-hexahydro sila-diphenidol are those of Barlow et al. 1990.
(n) = number of experiments

The antagonism on ileum appears to be competitive with concentrations of antagonist < 10μM.
A search among analogues (Barlow & Veale, 1990, 1991) has shown that selectivity exceeding 2 log. units (100-fold) is found with other aralkyl groups, such as thiophenylmethyl, and with cyclohexylmethyl, and this selectivity extends to activity *in vivo*. In anaesthetised rats many substituted "Fourdapines" antagonise the effects of bethanechol on blood-pressure in doses which do not antagonise its effects on heart rate (Barlow, Bond & McQueen, unpublished observations).

An X-ray crystal structure determination of 4DAMP benzyl bromide and of benzyl Fourdapine HBr shows that the benzyl group lies axial to the piperidine ring on the same side of the molecule as one of the benzene rings in the diphenylacetic acid group. It is possible that this arrangement hinders binding to muscarinic receptors in atria.

Barlow R.B., & Shepherd M.K., (1985) Br. J. Pharmacol., 85, 427-435
Barlow R.B., Holdup D.W., Harris G., Veale M.A. & Williams A. (1990) Br. J. Pharmacol., 99, 622-626
Barlow R.B. & Veale M.A. (1990) UK Patent 9023023.6
Barlow R.B. & Veale M.A. (1991) UK Patent 9121631.7
Lambrecht G., Feifel R., Wagner-Roder M., Strohmann C., Zilch H., Tacke R., Waelbroeck M., Christophe J., Boddeke H. & Mutschler E. (1989) Eur. J. Pharmacol., 168, 71-80

41P STIMULATED PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP_2) HYDROLYSIS IN HUMAN AND BOVINE BRONCHIAL SMOOTH MUSCLE

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Muscarinic agonists evoke contractions of tracheal smooth muscle, which are associated with hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), leading to production of inositol (1,4,5) trisphosphate ($I(1,4,5)P_3$), e.g. in canine (Duncan *et al.*, 1987) or bovine, (Chilvers *et al.*, 1989) trachea. In bronchial smooth muscle however, evidence for this mechanism has relied upon measurement of accumulation of radiolabelled inositol phosphates- a method which does not allow for accurate assessment of the time course of inositide formation. In this present study, levels of $I(1,4,5)P_3$ have been measured directly using a specific $I(1,4,5)P_3$ binding assay.

Small pieces of human or bovine bronchial tissue were dissected free of connective tissue and fat in Krebs-Henseleit solution at 37°C, gassed with 5% O_2/ 95% CO_2. Tissues were stimulated with methacholine (MCh; 10^{-5}-10^{-3}M) and the reaction stopped after 5, 10, 20, 30, 60, 120 or 300s in a 20% perchloric acid solution at 4°C. Samples were then homogenised and a sample taken for estimation of protein using Peterson's modification of the micro-Lowry method. Further samples of the homogenate were assayed for accumulation of $I(1,4,5)P_3$ using a specific binding assay (Palmer & Wakelam, 1990).

In both human and bovine bronchial smooth muscle, methacholine evoked a rapid, transient increase in levels of $I(1,4,5)P_3$, returning to baseline by 30s. Maximally effective concentrations of methacholine (10^{-3}M) resulted in mean increases of 210% (n=5; $p<0.05$) in $I(1,4,5)P_3$ mass in human and 461% (n=6; $p<0.001$) in bovine bronchial smooth muscle each measured at 10s. The observed increases in levels of $I(1,4,5)P_3$ were concentration-dependent and in bovine tissue, a pD_2 value of 4.7 ± 0.6 was calculated. A second rise in $I(1,4,5)P_3$ levels was observed at 60s in both tissue types (mean increase in response to 10^{-3}M MCh: 167% ($p<0.05$) in human; 345% ($p<0.05$) in bovine tissue). Both the early and the later rises in $I(1,4,5)P_3$ were abolished by pre-incubation with atropine (10^{-5}M).

These results confirm that methacholine stimulates PIP_2 hydrolysis, leading to $I(1,4,5)P_3$ formation, in both human and bovine bronchial smooth muscle. The time course for production of $I(1,4,5)P_3$ - which releases Ca^{2+} from the endoplasmic reticulum - is in keeping with a receptor-activated response initiating smooth muscle contraction. The biphasic nature of the response is similar to that seen in canine trachea (Duncan *et al.*, 1987) where the secondary rise was suggested to result from inositol (1,3,4,5)-tetrakisphosphate formation - an effect not observed in bovine trachealis muscle (Chilvers *et al.*, 1989).

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Chilvers, E.R., Challiss, J., Barnes, P.J. & Nahorski, S.R. (1989). *Eur. J. Pharmacol.*, **164**, 587-590.
Duncan, R.A., Kranowski, J.J., Davis, J.S., Polson, J.B. *et al.*, (1987). *Biochem. Pharmacol.*, **36**, 307-310.
Palmer, S. & Wakelam, M.J.O. (1990). In *Methods in Inositide Research*. Ed. Irvine, F., pp 127-134. Raven Press Ltd. N.Y..

42P CONDITIONED TASTE AVERSIONS IN RATS AFTER ADMINISTRATION OF NICOTINE INTRACEREBRALLY

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Conditioned taste aversions (CTA) have been extensively studied and may be used to assess the aversive stimulus properties of nicotine (Kumar et al 1983). Pharmacological studies have shown this stimulus to be mediated centrally (Kumar et al 1983; Reavill et al 1986). The aim of the present study was to use microinjection techniques to identify neural substrates that mediate nicotine-induced CTA. Furthermore the relationship between the aversive and locomotor depressant effects of nicotine are examined within this study.

Male hooded rats were implanted with guide cannulae under stereotaxic control. Following recovery they were allowed to drink distinctively flavoured solutions (sodium chloride or saccharin) for 15 min; immediately afterward either nicotine or saline (0.5µl, isotonically balanced) was administered intracerebrally and the rats were placed back in their home cages. In experiments with intraventricular injections, rats were placed into photocell activity cages immediately following injection. Flavour-injection pairings were counterbalanced in each group of rats. CTA was assessed from two-stimulus tests during which both flavours were presented simultaneously, and a percentage consumption of drug-paired flavour significantly below 50% was indicative of CTA. Injection sites were confirmed by histology.

Following two conditioning trials with nicotine administered systemically, significant CTA was apparent (0.1 mg/kg 23.8±5.9%, $t(7)=4.08$, $P<0.01$; 0.4 mg/kg 12.4±3.1%, $t(7)=9.55$, $P<0.001$). Under these conditions, CTA was absent when nicotine was administered into the 3rd ventricle (4µg, 33.5±7.3%, 32µg, 47.4±9.2%) or the 4th ventricle (4µg, 60.4±8.8%). However decreases in locomotor activity were apparent in two groups (3rd ventricle 32µg, $t(7)=4.5, <0.01$; 4th ventricle 4µg, $t(7)=2.5$, $P<0.05$) during the conditioning phase as found previously (Shoaib & Stolerman 1992). Significant CTA was observed when nicotine (8µg) was administered bilaterally into the nucleus accumbens (28.2±4.2%, $t(17)=4.76$; $P<0.001$), but not in the striatum (47.4±7.1%), ventral tegmental area (50.4±6.5%), dorsal hippocampus (39.9±6.8%) or the mesopontine tegmentum (47.0±9.8%). CTA produced with intra-accumbens nicotine (8µg) was confirmed in a second experiment but not in rats pretreated with the nicotinic receptor antagonist mecamylamine (2.0 mg/kg SC).

The aversive effects produced by systemically administered nicotine may be mediated, in part through nicotinic receptors located in the nucleus accumbens. Results from intraventricular injections suggest that locomotor depression associated with nicotine administrations can be separated from the aversive stimulus effects as measured by the CTA procedure. Acknowledgement: we thank MRC for financial support.

Kumar, R., Pratt, J.A. & Stolerman, I.P. (1983) Br. J. Pharmac. 79, 245-253
Reavill, C., Stolerman, I.P., Kumar, R. & Garcha, H.S. (1986) Neuropharmacology 25, 1067-1069
Shoaib, M. & Stolerman, I.P. (1992) Br. J. Pharmac. 105, 167P

43P AMITRIPTYLINE BUT NOT PAROXETINE IMPAIRS COGNITIVE PERFORMANCE IN RATS

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The tricyclic antidepressant amitriptyline has been reported to cause memory impairment clinically (reviewed by Thompson 1991). The selective serotonin reuptake inhibitors (SSRIs) may represent a class of antidepressants lacking such detrimental effects on memory, although one recent report has indicated that fluoxetine may cause memory impairment (Mirrow, 1991). Since few studies have investigated the effects of antidepressants on memory in animals, the present series of experiments were carried out to compare the effects of acute administration of amitriptyline and the SSRI paroxetine on a delayed non match to sample task (DNMTS) in rats.

Male Lister Hooded rats (380-400 g), maintained on 80% of their free feeding body weight, were trained to a criteria of 95% correct responses at 0 sec delay on DNMTS (Saghal, 1987). All treatments were administered i.p. in a volume of 1 $ml.kg^{-1}$. In both experiments, each rat served as its own control, with drug treatments and vehicle being administered once in a balanced cross over design. A one week washout period was allocated between each treatment. Two separate experiments were carried out. The vehicle used was 1% methylcellulose in 0.9% saline. Before the beginning of each experiment rats were tested with no treatment to ensure a consistent baseline performance. In the first experiment, rats received vehicle or amitriptyline (3 or 10 $mg.kg^{-1}$; n=24). In a second experiment rats received vehicle or paroxetine (1 or 3 $mg.kg^{-1}$; n=12)). Amitriptyline (10 $mg.kg^{-1}$) produced a significant impairment of performance ($F = 7.46$; $P<0.001$) as shown in Table 1, although there was no significant interaction between treatment and delay.

Table 1: Percentage of correct responses with amitriptyline (10$mg.kg^{-1}$) and paroxetine (3 $mg.kg^{-1}$) (n=24)

	Delay (s) 0	2	4	8	12	16
V	93.9 ± 1.2	92.9 ± 1.7	84.6 ± 2.6	80.9 ± 2.5	73.7 ± 3.5	69.9 ± 4.3
amitriptyline	89.4 ± 2.6	83.1 ± 2.8	74.9 ± 2.8	72.9 ± 3.2	66.6 ± 3.1	66.9 ± 2.8
V	94.2 ± 2.1	85.7 ± 3.3	85.0 ± 3.1	76.8 ± 4.6	72.9 ± 5.0	65.7 ± 6.2
paroxetine	89.2 ± 2.8	84.8 ± 3.5	81.0 ± 3.6	72.7 ± 3.0	65.2 ± 3.6	62.0 ± 3.2

Neither dose of paroxetine was significantly different from control. In neither experiment was a significant number of missed trials observed with any treatment. In summary, acute treatment with amitriptyline produced an impairment in cognitive performance. The fact that this impairment with amitriptyline occurred across all delays suggests that the effects seen are not specific to working memory but rather may reflect an effect on some delay-independent process such as attention or retrieval. By contrast paroxetine did not impair performance in this model.

Mirrow S (1991) Am. J. Psychiatry 148 : 948-949
Saghal A (1987) Psychopharmacology 93 : 243-249
Thompson B J (1991) Human Psychopharm. 6 : 79-90

44P ANXIOLYTIC EFFECT OF PAROXETINE IN THE RAT SOCIAL INTERACTION MODEL OF ANXIETY

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Selective serotonin reuptake inhibitors (SSRIs) may have clinical anxiolytic properties following chronic treatment (Boyer, McFadden & Feighner, 1991). However there is little evidence in support of this from animal models. In the present study the effects of chronic administration of the SSRI paroxetine have been investigated in a validated rat social interaction (SI) test.

Male CD rats (12-14/group) were pre-dosed p.o., daily, for 20 days with paroxetine (1, 3 or 10 mg/kg) or vehicle (1% methyl cellulose). On day 17, the animals were housed singly and on day 21, sixty minutes after receiving a final dose p.o., rats were placed with an unfamiliar palrmate from the same treatment group into a novel white perspex test arena, under bright light (creating a high-anxiety state). Video monitoring was used to score locomotor activity and active SI (measured as time spent sniffing, grooming, following, mounting, biting, crawling over or under) by two remote, independent observers for 15 minutes. For comparison a group of animals that had been pre-dosed chronically with vehicle were tested on day 21, sixty mins after receiving 4 mg/kg chlordiazepoxide p.o..

Table 1: Effect of chronic paroxetine and acute chlordiazepoxide on rat social interaction

Pretreatment p.o. (once daily x 20 days)		Test day 21 treatment p.o.		Total SI (s)	Locomotion (lines crossed)
Vehicle		Vehicle		63 ± 11	294 ± 23
Paroxetine	1mg/kg	Paroxetine	1 mg/kg	109 ± 16	257 ± 27
"	3mg/kg	"	3 mg/kg	126 ± 18*	332 ± 35
"	10mg/kg	"	10 mg/kg	102 ± 13	276 ± 14
Vehicle		Chlordiazepoxide	4 mg/kg	125 ± 19*	296 ± 24

Significantly different from vehicle * $P<0.05$ by Dunnetts test following significant 1 way ANOVA. All values are means ± SEM.

Paroxetine, at 3 mg/kg, significantly increased SI, with no effect on locomotion, indicating an anxiolytic-like effect. This effect was comparable to that seen following acute chlordiazepoxide (4mg/kg). There was a suggestion of increased SI following paroxetine at 1 or 10 mg/kg, but statistical significance was not achieved.

This anxiolytic action of paroxetine in the rat SI model substantiates the clinical evidence for its therapeutic use in the treatment of anxiety in addition to its established role as an antidepressant.

Boyer, McFadden & Feighner (1991). In: *Selective Serotonin Reuptake Inhibitors*. Ed. Feighner, JP and Boyer, WP, p109-117. Wiley.

45P STRAIN DIFFERENCES IN BEHAVIOURAL RESPONSES IN MURINE MODELS OF ANXIETY

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Inhibition of marble burying by MF1 mice has been proposed as a "correlational" model of anxiety (Njung'e & Handley, 1991a). In the present study we have investigated this, together with other indices of anxiety in two different mouse strains.

Marble burying (Njung'e & Handley, 1991a); and exploration of the elevated plus maze (EPM) and light/dark box (Stanford et al, 1988) were measured in drug-free adult TO and C57BL/10 mice. In other experiments, the effects of three selective 5-HT uptake inhibitors (Njung'e & Handley, 1991b) on marble burying and EPM activity of male TO mice were assessed.

Table 1. Strain Differences in Anxiety

Subject	Sex	Marbles Buried	Light/Dark Transitions	Time in Light(s)	Open: Total Entries (%)	Open: Total Time (%)
TO	♂	17.1 ± 0.7	31.2 ± 2.4	116.6 ± 3.8	36.3 ± 3.5	17.9 ± 2.9
TO	♀	17.3 ± 0.7	32.0 ± 2.3	129.3 ± 2.3	40.1 ± 2.0	20.1 ± 4.1
C57BL/10	♂	8.7 ± 1.2	13.7 ± 1.2	119.6 ± 10.9	34.0 ± 4.2#	11.1 ± 2.9#
C57BL/10	♀	6.4 ± 1.6	16.7 ± 0.9	126.9 ± 7.1	24.1 ± 5.5#	9.2 ± 3.9#

Each value represents the mean ± s.e.m. of n=10 (except # n=9)

TO mice buried significantly ($P<0.05$; 2-way ANOVA) more marbles, made more light/dark transitions and open arm entries, and spent longer in the open arms than C57BL/10 mice (Table 1). Chlordiazepoxide (5mg/kg) and citalopram (20mg/kg) significantly ($P<0.05$; Dunnett test) inhibited marble burying and stimulated exploration of the EPM open arms in male TO mice. However, zimelidine (30mg/kg) reduced marble burying only, while fluvoxamine (20mg/kg) increased open arm exploration. These data show a strain, but not sex difference in marble burying, and suggest a relationship between this behaviour and that shown in the EPM.

T.A. is a B.Sc (Hons) Pharmacology Student.

Njung'e, K. & Handley, S.L. (1991a) Pharmac. Biochem. Behav. 38 63-67
Njung'e, K. & Handley, S.L. (1991b) Br. J. Pharmac. 104 105-112
Stanford, S.C., Parker, V. & Morinan A. (1988) J. Affect. Dis. 15 175-180

46P INHIBITION OF FORSKOLIN-STIMULATED CYCLIC AMP FORMATION BY t-ACPD IN GUINEA-PIG CEREBRAL CORTICAL SLICES

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Within the central nervous system the excitatory amino acid (EAA), glutamate exerts its effects via both ionotropic and G-protein coupled metabotropic receptors (Monaghan, *et al.*, 1989) which are linked to phospholipase C (Mayer and Miller,1990). In this study we have investigated the effects of a selective metabotropic receptor agonist, t-ACPD (1-aminocyclopentane-trans-1,3-dicarboxylic acid) and its stereoisomers, on another second messenger system, ie forskolin-stimulated cyclic AMP formation. [^{3}H]-cAMP formation was measured in cross-chopped slices of cerebral cortex slices from Dunkin-Hartley guinea-pigs (either sex) by the pre-labelling technique previously described by Donaldson *et al.*,(1990). Potential antagonists were added 10 mins before t-ACPD or other amino acids followed 5 mins later by forskolin. The incubation was stopped after a further 10 mins by the addition of 200 µl IM HCl.

In concentrations up to 300 µM, the racemic mixture of t-ACPD, consisting of 1S,3R and 1R,3S isomers, was without effect on basal [3H]-cyclic AMP formation but reduced the accumulation due to forskolin (30 µM) in a concentration-dependent manner with an IC50 value of 35 ± 4 µM (n=3) and a maximum inhibition of 77 ± 7%. Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), N-methyl-D-aspartate (NMDA) and quisqualate (all 300 µM) failed to mimic the inhibitory effect of t-ACPD. The ionotropic EAA receptor antagonists (6,7-dinitroquinoxaline-2,3-dione, 100 µM; MK-801, 100 µM; 7-chlorokynurenate, 100 µM) and the putative metabotropic EAA receptor antagonists (L-2-amino-3-phosphonopropionate, 3 mM; L-2-amino-4-phosphonobutyrate, 1 mM) failed to significantly ($p>0.05$) reduce the inhibitory effect of t-ACPD. In the presence (but not absence) of adenosine deaminase, glutamate (1 mM and 3 mM) also significantly ($p<0.05$) reduced the forskolin response although the magnitude of the reduction was smaller than that due to t-ACPD, with a maximum inhibition of approximately 30%. The inhibitory effect of t-ACPD was stereoselective, with the 1R,3S isomer being apparently as efficaceous as the 1S,3R, but nearly 400-fold less potent (IC50 values were 1.0 ± 0.5 mM and 2.6 ± 0.7 µM, respectively, n=3).

The data presented support the existence of a non-NMDA receptor, possibly coupled in a negative fashion to adenylate cyclase, in guinea-pig cerebral cortex. Whether this is the same as the recently reported $mGluR_2$ receptor is yet to be determined.

J.C. is an M.R.C. CASE student.

Donaldson, J., Kendall, D. A. and Hill, S. J. (1990). *J.Neurochem.*,54 (5),1484-91.
Mayer, M. L. and Miller, R. J. (1990). *TIPS*, 11, 254-260.
Monaghan, D. T., Bridges, R. J. and Cotman, C. W. (1989). *Annu. Rev. Pharmacol. Toxicol.*, 29, 365-402.

47P CHARACTERIZATION OF A NEW EXCITATORY AMINO ACID (EAA) RECEPTOR TYPE ACTIVATED BY (1S,3R)-ACPD AND SELECTIVELY BLOCKED BY (S)-4C3H-PG

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In motoneurones of the newborn rat spinal cord (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate ((1S,3R)-ACPD) produces a desensitizing depolarizing response (Sunter *et al*, 1991). This effect is not blocked by antagonists of either NMDA or AMPA/kainate receptors and was considered likely to be associated with activation of metabotropic EAA receptors. We have now used the hemisected isolated spinal cord of the newborn rat to identify (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3H-PG) as a selective antagonist of (1S,3R)-ACPD-induced depolarization and investigated the ability of this antagonist to block (1S,3R)-ACPD-stimulated phosphoinositide (PI) hydrolysis in rat cortical tissue.

Recordings of motoneuronal responses were made from ventral roots of the hemisected spinal cord of 1-5 day old rats. Depolarizations were induced by short (1 min) applications of (1S,3R)-ACPD (25-50 μM), NMDA (5-10 μM), AMPA (0.5-1.0 μM) and kainate (1-2 μM) added to the superfusion medium (nominally Mg^{2+}-free) in the absence and presence of (S)-4C3H-PG. (S)-4C3H-PG was tested also on the stimulation of PI hydrolysis by (1S,3R)-ACPD (20 and 100 μM) in 6-8 day old rat cortical slices (300 x 300 μm) labelled with [^{3}H]myoinositol.

(S)-4C3H-PG (300 μM) reduced 1S,3R-ACPD-induced depolarizations by 68$\pm$2% (n = 4), while having little or no effect at higher concentrations (500 μM - 1mM) on responses induced by NMDA, AMPA or kainate. (S)-4C3H-PG (500 μM) had no significant inhibitory effect on (1S,3R)-ACPD stimulated PI hydrolysis in cortical tissue, showing instead a moderate agonist activity.

These results reveal a dissociation between depolarizing and metabotropic effects of (1S,3R)-ACPD and suggest the existence in the newborn rat spinal cord of a new type of depolarizing EAA receptor for which (1S,3R)-ACPD and (S)-4C3H-PG act as a selective agonist and antagonist, respectively. The relationship between the antagonist effects of (S)-4C3H-PG reported here and the ability of the same substance to depress monosynaptic excitation in the spinal cord (Birse *et al*, 1992) remains to be determined.

Sunter, D.C., Edgar, G.E., Pook, P.C-K., Howard, J.A.K., Udvarhelyi, P.M. & Watkins, J.C. (1991) Br.J.Pharmac. **104**, 377P.
Birse, E.F., Mewett, K.N., Pook, P.C-K., Tyrell, K., Udvarhelyi, P.M., Wharton, B. & Watkins, J.C. (1992) J.Physiol. (in press)

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48P SELECTIVE ANTAGONISM OF (1S,3R)-1-AMINOCYCLOPENTANE-1,2-DICARBOXYLATE ((1S,3R)-ACPD) BY (S)-4C3H-PG IN THE RAT VENTROBASAL THALAMUS

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We have previously shown that the putative metabotropic glutamate receptor agonist *trans*-1-aminocyclopentane-1,3-dicarboxylate (*t*-ACPD) (Schoepp *et al.*, 1990) has an excitatory action on ventrobasal thalamus (VB) neurones which appears to be mediated by a receptor which is distinct from the known ionotropic excitatory amino acid receptors (Salt & Eaton, 1991). We have attempted to characterise this excitatory action by using the novel antagonist 4-carboxy-3-hydroxyphenylglycine (4C3H-PG) (Jones *et.al.*, 1992). Extracellular action potential recordings and iontophoretic drug applications were made with multibarrel glass electrodes in the VB of urethane-anaesthetised rats (Salt & Eaton, 1991).

The active form of t-ACPD, (1S,3R)-ACPD, was applied to VB neurones as were the agonists NMDA and kainate or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA). The racemic mixture of the antagonist isomers, (R,S)-4C3H-PG, was found to reduce responses to (1S,3R)-ACPD, as well as responses to NMDA. The S-isomer of the antagonist, (S)-4C3H-PG, was found to reduce selectively responses to (1S,3R)-ACPD (15±11.1% of control) while responses to other agonists were relatively unaffected (NMDA=84±12.3% of control, AMPA=99±33.5% of control, kainate=105±14.2% of control). In contrast, (R)-4C3H-PG was found to antagonise responses to NMDA (8±9.2% of control), whilst having less effect on responses to (1S,3R)-ACPD, AMPA or kainate. These results indicate that (S)-4C3H-PG is a selective antagonist of (1S,3R)-ACPD responses in the thalamus. However, in view of the finding that this antagonist does not antagonise (1S,3R)-ACPD-stimulated phosphoinositol hydrolysis (Jones *et.al.*, 1992), it seems unlikely that the excitatory action of (1S,3R)-ACPD is mediated via metabotropic glutamate receptors. It is possible that the excitatory action of (1S,3R)-ACPD is via a previously undefined receptor, for which (S)-4C3H-PG is a selective antagonist.

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Jones, P.L.S.J., Porter, R.H.P., Birse E.F., Pook, P.C-K., Sunter, D.C., Udvarhelyi, P.M., Wharton, B., Roberts P.J & Watkins, J.C. (1992) *Brit. Pharm. Soc. Proc.*, THIS MEETING.
Salt, T.E. & Eaton, S.A. (1991) *Eur. J. Neuroscience* **3**, 1104-1111.
Schoepp, D., Bockaert J. & Sladezek, F. (1990) *TiPS* **11**, 508-515.

49P GLUTAMATE INDUCES HYPERALGESIA IN THE RAT PAW

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Multiple injections of different hyperalgesic substances such as prostaglandin E_2 and substance P into the rat paw induce a state of sustained hyperalgesia We have suggested recently that sensory neuropeptides, particularly substance P, could be involved in the sustained hyperalgesic response (Nakamura-Craig et al., 1991). There is evidence of the coexistence of substance P and glutamate in some primary sensory neurons, suggesting that both agents may be released by terminals of primary afferents from the same dorsal root ganglia (Battaglia and Rustioni, 1988). We have now investigated the possibility that glutamate participates in the mechanisms underlying sustained hyperalgesia.

Male Wistar rats (150-170g, n=5 per group) were used. Hyperalgesia in the rat paw was measured by a modification of the Randall-Selitto test (Ferreira et al., 1978). The intensity of hyperalgesia (mean±s.e. mean) was quantified as the variation in reaction time (Δ reaction time, s) obtained by subtracting the value measured after administration of the hyperalgesic substances from the control reaction time.

L-glutamate caused hyperalgesia when injected into the rat paw, the ED_{50} and confidence limits being 0.7 (0.5-1.2)pg/paw. D-glutamate injected into the paw in doses up to 100 pg did not induce any hyperalgesic response. A single subplantar injection of glutamate (100pg) induced hyperalgesia (Δ reation time, 22.5±0.3s, 1 h after the injection) that was still present 8 days later (8.6±0.9s). Furthermore, 11 days after this single injection of glutamate, when the hyperalgesic response had subsided, a challenge of subthreshold doses of glutamate (0.1fg/paw) caused hyperalgesia (18.8±0.5s, at 1h) that lasted for at least 24h. Daily subplantar injection of subthreshold doses of glutamate (0.1 or 1.0 fg, twice daily) over 4 days led to the development on the fifth day of hyperalgesia (13.7±0.7s or 17.7±0.6s, respectively) that was still present 8 (6.2±0.9s) and 10 (6.4±1.3s) days, respectively, later. The glutamate-induced hyperalgesia (100pg, 23.6±0.5s) was inhibited by the NMDA antagonist (AP-7, 240mgkg^{-1}, s.c., 5.7±0.93s, $p<0.001$) but not by the non-NMDA antagonist (DNQX, 80 μgkg^{-1}, s.c., 23.2±0.8s).

Prostaglandin E_2-induced hyperalgesia (100ng/paw, 21.5±0.43s) was reduced by the NMDA antagonist (AP-7, 240mgkg^{-1}, s.c., 7.6±0.47s, $p<0.001$) and by the non-NMDA antagonist (DNQX, 80 μgkg^{-1}, s.c., 7.4±0.93s, $p<0.001$), suggesting that prostaglandin E_2-induced hyperalgesia probably involves the participation of glutamate and other excitatory amino acids.

The present findings suggest that glutamate may play an important role in the mechanisms of hyperalgesia, particularly in sustained hyperalgesia that could be associated with chronic pain.

Battaglia, G. and Rustioni, A. (1988) J. Compar. Neurol. 277, 302.
Ferreira, S.H., Lorenzetti, B.B. and Correa, F.M.A. (1978) Eur. J. Pharmacol. 53, 39.
Nakamura-Craig, M., Follenfant, R.L. and Gill, B.K. (1991) Br. J. Pharmac. 102, 380p.

50P ANALGESIC EFFECTS OF THE GLUTAMATE RELEASE INHIBITOR, LAMOTRIGINE, IN THE RAT

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There is evidence to suggest that glutamate is involved in the spinal mechanisms of pain (Woolf and Thompson, 1991). Furthermore, we have recently demonstrated that glutamate is a potent hyperalgesic agent suggesting its participation in some chronic pain conditions (Follenfant and Nakamura-Craig, at this meeting). The anticonvulsant effects of the antiepileptic drug lamotrigine (Lamictal®) have been ascribed to inhibition of the release of the excitatory amino acid glutamate (Leach et al., 1986). We have therefore investigated the effect of lamotrigine in models of hyperalgesia in rats.

Hyperalgesia was induced in the right hind paw of male Wistar-strain rats weighing 150-200g, by subplantar injection of prostaglandin E_2 (PGE_2; 100ng in 100μl) or glutamate (L-glutamic acid monosodium salt; 0.1ng). Δ Reaction times (s) to paw pressure (20mm Hg, constant) were calculated as the difference between initial values, measured before any treatment, and those obtained after the hyperalgesic stimulus at 2-3h after (acute) or at various times during and after the development of a sustained hyperalgesia to twice daily injection of PGE_2 (100ng for 4 days). In further studies, the responses of single motor units of the flexor digitorum longus muscle to noxious paw pinch (15s duration at 3min intervals) were measured in barbiturate-anaesthetised rats (Hartell & Headley, 1990). Pretreatment with lamotrigine (20-100mg/kg po, 1h) inhibited in a dose-related manner, the development of acute hyperalgesia induced by PGE_2, ED_{50}= 79 (72-88) mgkg^{-1}, but not glutamate (Δ reaction time 18.6±1.1s for 100 mgkg^{-1} c.f. 22.0±0.4s for controls, n=6). When given after the stimulus, lamotrigine (10-100mgkg^{-1}, p.o.) inhibited in a dose-related manner the hyperalgesia induced by PGE_2, ED_{50}= 114(75-206)mgkg^{-1} but was less effective against glutamate (Δ reaction time, 15.9±0.6s for 100mgkg^{-1} c.f. 21.3±0.7s for controls, n=6). A single oral dose of lamotrigine given 1h before the first of 8 injections of PGE_2, inhibited the development of sustained hyperalgesia measured on the fifth day, ED_{50}= 26.2 (21.6-30.5mgkg^{-1}). The twice daily injection of PGE_2 sensitises the paw to further, subthreshold, stimulus once the initial sustained hyperalgesia has subsided. Lamotrigine (10-40mgkg^{-1}, p.o.) given on day 22, 1h prior to PGE_2 (0.1ng subplantar), inhibited the sensitisation hyperalgesia in a dose-related manner, ED_{50}= 18.4(16.4-20.4) mgkg^{-1}. In addition, the responses of single motor units of the flexor digitorum longus muscle to noxious pinch (19.0±4.2 spikes/min) were attenuated significantly by lamotrigine (10mgkg^{-1}, i.v., 6.4±1.1 spikes/min, at 10 min, n=5, $p<0.001$).

These results suggest that glutamate release inhibitors may represent a new class of analgesics for the treatment of some chronic pain conditions in man where hypersensitivity exists and conventional analgesics are ineffective.

Leach, M.J. et al (1986). Epilepsia, 27 (5), 490-497.
Woolf, C.J.& Thompson, S.W.N.(1991). Pain, 44, 293-299.
Hartell, N.A.& Headley, P.M.(1990). Br. J. Pharmacol., 101, 563-568.

51P EXPRESSION OF PROTOONCOGENES IS INDUCED BY ISCHAEMIA IN THE MONGOLIAN GERBIL

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The protooncogenes c-*jun* and c-*fos* are members of the same family of early onset genes. Changes in c-*fos* message and Fos-like immunoreactivity have been recorded after cerebral ischaemia in both focal and global models (Uemura *et al.*, 1991; Dempsey *et al.*, 1991). The present study examines the expression of c-*jun* and c-*fos* following transient global ischaemia in the gerbil.

Female gerbils (60-80g) were anaesthetized with forane and ischaemia induced by occlusion of the common carotid arteries for 7min. The animals were allowed to recover and sacrificed at times varying from 30min to 72h, following the insult. Control animals were sham operated. Brains were excised and frozen on dry ice. Sections (10μm) were fixed in 4% paraformaldehyde and hybridised at 42°C overnight with oligonucleotide probes to c-*fos*, c-*jun* and *jun*-B. The probes were labelled with [^{35}S]- dATP at the 3'position by end labelling techniques. Following stringency washes (30min in 1xSSC, at room temperature and 30min at 55°C) slides were dried and apposed to XAR-5 film (Kodak) for 4 and 14 days. Radioactivity was measured in optical density units using a MCID Image Analyser. The ischaemic damage within the hippocampus was assessed in sections by staining with cresyl fast violet and measuring damaged areas (Hayward *et al.*, 1992).

Ischaemia caused a rapid rise in mRNA for c-*jun*, c-*fos* and *jun*-B. The time course of the induction was different for each protooncogene. Significant increases in c-*jun* expression occurred at 30min ,peaked at 1h but remained elevated for 24h. In contrast c-*fos* mRNA was significantly increased at 30min, peaked at 1h and returned to basal values after 4h. The ischaemia-induced increase in expression of c-*jun* and c-*fos* mRNA in the hippocampus was region specific. Expression was highest in the dentate gyrus (c-*fos* 276 ±7% control 1h: c-*jun* 196 ± control at 1h and 171 ± 6% at 4h; n=4), next highest was the CA3 region (c-*fos* 143 ± 1% at 1h; c-*jun* 116 ± 2% at 1h and 139 ± 4% above control at 4h.) The mRNA levels were unchanged in the CA1 region. The expression of *jun*-B, was similar to that of c-*fos* (204 ± 6% above control at 1h in the dentate gyrus and 103 ± 1% at 4h). The ischaemic damage was not observed in these animals until some 72h after the occlusion. After 7 days the degree of staining within the hippocampus was 108 ± 11μm² (25) in experimental animals compared with 374 ± 8μm² (25) in sham operated. No significant change was observed in any other region of the hippocampus. These results emphasise the involvement of the early onset genes in neuronal insult and possibly neuroprotection.

Dempsey, R.J., Carney, J.M. & Kindy, M.S. (1991). J. Cereb. Blood Flow Metab., 11,979-985.
Hayward, N.J.,McKnight, A.T. & Woodruff, G.N. (1992). Mol Neuropharmacol., in press.
Uemura, Y., Kowall, N.W. & Moskowitz, M. (1991). Brain Res., 552, 99-105.

52P NEUROTROPHIC EFFECTS OF SR 57746A IN A VARIETY OF NEURODEGENERATIVE MODELS *IN VIVO*

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Compounds possessing neurotrophic properties may represent a possible treatment for neurodegenerative disorders such as Alzheimer's disease. SR 57746A [1-(2βnaphthyl ethyl)-4-(3-trifluoromethyl phenyl)-1,2,5,6 tetrahydropyridine hydrochloride] is a new compound with neurotrophic activity in a number of in vitro preparations (Fournier *et al.*, 1992). The neurotrophic effects of this compound have been evaluated in vivo using three distinct rat models of neurodegeneration : a septo-hippocampal lesion produced by injection of vincristine sulphate (0.55 μg in 1 μl) into the medial septum; transient global ischaemia produced by a 4-vessel occlusion (Pulsinelli *et al.*, 1982); and sciatic nerve crushing as described by De Koning *et al.* (1986). Rats were administered vehicle or 2.5-10 mg/kg p.o. SR 57746A, after initiation of the degenerative process, then once daily for 11 days in the first two models, and 16 days in the third. Twelve days after vincristine administration, there was a marked loss of hippocampal cholinergic nerve terminals, as indicated by reduced choline acetyltransferase (ChAT) activity (sham = 242 ± 19 pmol/mg prot/min, lesion = 133 ± 25, $P<0.01$). SR 57746A dose-dependently reversed this reduction of ChAT (after 5 mg/kg = 204 ± 24, $P<0.05$; after 10 mg/kg = 243 ± 27, $P<0.01$ vs lesion + vehicle). These results were confirmed by immuno-histological evaluation of hippocampal acetylcholinesterase-containing terminals. The reversal by SR57746A of the loss of hippocampal ChAT induced by intraseptal vincristine has also been reproduced in marmosets (control hippocampal ChAT activity = 283 ± 5 pmol/mg prot/min; vincristine + vehicle = 143 ± 18, $P<0.01$ vs. controls; vincristine + 10 mg/kg/day po SR 57746A for 11 days = 238 ± 32, $P<0.05$ vs vincristine + vehicle). Ischaemia-induced neuronal damage was quantified using a scale from 0 (no damage) to 4 (all neurones damaged). Median scores for damage were reduced by 30-40% by SR 57746A treatment in hippocampal CA1 ($P<0.05$, chi-2 test), CA2 ($P<0.02$), and CA3 ($P<0.01$) regions, and in the striatum ($P<0.05$). Behavioural deficits in these models, (short-term social memory in the former, and exploratory behaviour in the latter) were also significantly reduced by SR 57746A treatment. In the peripheral neurodegenerative model, sensorimotor function, measured on a scale varying from 2 (no recovery) to 0 (total recovery), improved more rapidly in rats treated with 10 mg/kg SR 57746A (in a typical experiment, the median score in SR 57746A-treated rats was lower than that in vehicle-treated rats from the beginning of recovery, and this difference achieved statistical significance after day 14, $P<0.01$, Fisher test). In summary, SR 57746A possesses notable neurotrophic activity in a variety of neurodegenerative models in vivo. These results, taken together with those observed in vitro (Fournier *et al.*, 1992), suggest that SR 57746A may possess therapeutic potential for the treatment of neurodegenerative diseases.

De Koning P., Brakke J.H. & Gispen W.H. (1986). J. Neurol. Sci. 74, 237-246.
Fournier J., Gauthier T., Keane P.E.; Coudé F.X., Guzzi U., Soubrié P. & Le Fur G. (1992) This meeting.
Pulsinelli, W.A., Brierley, J.B. & Plum, F.(1982). Ann. Neurol. 11, 491-498.

53P SELECTIVE PROTECTION OF STRIATAL/EXTRASTRIATAL D_1/D_2 RECEPTORS FROM INACTIVATION BY N-ETHOXYCARBONYL-2-ETHOXY-1,2-DIHYDROQUINONOLINE: AN AUTORADIOGRAPHIC STUDY

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N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinonoline (EEDQ) is an irreversible striatal dopamine (DA) receptor antagonist, both *in vivo* and *in vitro* (Nowak et al., 1988). We report the effects of EEDQ on striatal/extrastriatal [^{3}H]-SCH 23390 and [^{3}H]-spiperone binding (D-1 and D-2 DA receptor sites respectively) with/without pretreatment with DA agonists and antagonists.

Male Wistar rats were pretreated with either saline (ip), SKF 38393 (5.1-411 μmole/kg; sc), SCH 23390 (0.006-6.2 μmole/kg; ip), quinpirole (0.27-27 μmole/kg; ip) or raclopride (0.02-20 μmole/kg; ip). 20 min later the animals were injected with EEDQ (7.5 mg/kg in 50% ethanol; ip; n=54) or its vehicle (controls; n=6). Twenty four hours later the animals were killed and the brains frozen in isopentane at -45^{0}C. Coronal sections (10 μm) were incubated with either 0.5 nM [^{3}H]-SCH 23390 or 0.5 nM [^{3}H]-spiperone. Specific binding was displaced with 10 μM sulpiride ([^{3}H]-spiperone) and 10 μM cis-flupentixol ([^{3}H]-SCH 23390). Densitometric analysis of the autoradiographs revealed that EEDQ treatment reduced [^{3}H]-SCH 23390 and [^{3}H]-spiperone binding in the striatum (D-1, 42%; D-2, 37% of control), substantia nigra pars compacta (SNc; D-2, 67% of control; p>0.05), substantia nigra pars reticulata (SNr; D-1, 23% of control) and the entopeduncular nucleus (EP; D-1, 20%) (Table 1). Pretreatment with either SKF 38393 or SCH 23390 (but not quinpirole or raclopride) dose dependently protected [^{3}H]-SCH 23390 binding in the striatum, SNr and EP. Pretreatment with quinpirole, raclopride or SKF 38393 (but not SCH 23390) protected [^{3}H]-spiperone binding in the striatum and SNc (Table 1). However, with SKF 38393, this effect was only observed at the highest dose used (411 μmole/kg).

Table 1: Effect of DA agonists/antagonists pretreatment on EEDQ induced DA receptor inactivation. Values are expressed as % control ± s.e.mean. *P < 0.01 compared to controls (ANOVA and Duncan's multiple range test).

Pretreatment (μmole/kg)	[^{3}H]-SCH 23390 binding			[^{3}H]-spiperone binding	
	Striatum	SNr	EP	Striatum	SNc
Saline	42 ± 4*	23 ± 5*	20 ± 5*	37 ± 5*	67 ± 9
SKF 38393 (411)	93 ± 2	86 ± 5	111 ± 3	82 ± 9	86 ± 9
SCH 23390 (6.2)	105 ± 6	122 ± 2	73 ± 8	47 ± 22*	63 ± 17
Quinpirole (2.73 SNc; 27.3 for rest)	57 ± 19*	36 ± 20*	40 ± 23*	111 ± 4	119 ± 11
Raclopride (20)	58 ± 12*	34 ± 12*	24 ± 5*	85 ± 9	93 ± 18

This study further demonstrates that SCH 23390/SKF 38393 and quinpirole/raclopride show a high degree of selectivity to striatal/extrastriatal D-1 and D-2 DA receptors *in vivo*, although at high doses this selectivity is not apparent with SKF 38393.

Nowak, G., Arnt, J. & Hyttel, J. (1988) Eur. J. Pharmacol. 153, 309-311.

54P CHLOROQUINE-INDUCED SEIZURES IN MICE: THE ROLE OF DOPAMINERGIC SYSTEM

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Chloroquine, an antimalarial agent, has been reported to cause convulsions both in standard doses and overdose (Fish & Espir, 1988). However, the mechanism of this seizure remains uncertain. The role of dopamine in seizures, even though conflicting, has been reported (Jones & Robert, 1964). In this report we describe the effects of some dopaminergic agents on chloroquine-induced seizures in mice.

Male albino mice (25-30 g) were used. The assessment of convulsant activity was performed as described previously (Vellucci & Webster, 1984). A predetermined dose of chloroquine (65 mg/kg, ip) produced convulsion in all animals used (n=8). Pretreatment times prior to injection of chloroquine (65 mg/kg, ip) were: L-dopa, 15 min; apomorphine, 5 min; benserazide, 30 min; and haloperidol, 1 h. The incidence and times of onset of tonic seizures were noted.

L-Dopa (12.5-50 mg/kg, sc), benserazide (5 mg/kg, ip) plus L-dopa (50 mg/kg, sc) and apomorphine (0.2-0.8 mg/kg, sc) profoundly decreased the latency of chloroquine (65 mg/kg, ip)-induced seizures (P<0.001, Student's t-test). Haloperidol (0.25-1.0 mg/kg, ip), on the other hand, significantly reduced the incidence (P<0.05, Chi-squared test) and prolonged the latency of the seizures (P<0.001, Student's t-test) as well as profoundly antagonising the effects of L-dopa (25-50 mg/kg, sc) and apomorphine (0.4 -0.8 mg/kg, sc) on chloroquine seizures.

It is significant that haloperidol, a potent dopamine receptor antagonist (Anden *et al* 1970), antagonised both chloroquine seizures and the effects of L-dopa, a precursor of dopamine (Friedman & Gershon, 1972), and apomorphine, a potent dopamine receptor agonist (Ernst, 1967) on chloroquine seizures. These data suggest the involvement of dopaminergic mechanism in chloroquine-induced seizures.

Anden, N.E., Butcher, S.G., Carrodi N., Fuxe, K. & Ungerstedt, U. (1970) Brain Res. 94(2) 279-296.
Ernst, A.M. (1967) Psychopharmacologia 10, 316-323.
Fish, D.R. & Espir, M.L. (1988) Br. Med. J. 297, 526-527.
Friedman, E. & Gershon, 5. (1972) Eur.J. Pharmacol. 18, 183-188.
Jones, B.J. & Robert, D.J. (1968) Br. J. Pharmacol.34 (1), 27-31.
Vellucci, S.V. & Webster, R.A. (1984) Eur. J. Pharmacol. 97, 289-293.

55P CHARACTERIZATION OF THE TWO FORMS OF THE D_2 DOPAMINE RECEPTOR EXPRESSED IN MAMMALIAN CELL LINES

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There are currently at least six subtypes of dopamine receptors that have been identified by gene cloning. Two forms of the D_2 dopamine receptor have been found; a long (D_{2L}) and a short (D_{2S}) form. We have expressed the rat D_{2L} dopamine receptor in Chinese Hamster Ovary cells (CHO6 cell line) and mouse fibroblast (Ltk-) cells (Ltk59 cell line). We also have an Ltk- cell line expressing the D_{2S} dopamine receptor (LZR1 cell line), supplied by Civelli and colleagues (Bunzow *et al.*, 1988). Saturation analysis using [^{3}H]spiperone binding on cell membranes has shown that these cell lines express the D_2 receptor at high levels (approximately 1 pmol/mg protein) and have the expected Kd values for rat D_2 dopamine receptors (approximately 0.05nM). Competition experiments versus [^{3}H]spiperone using D_2 dopamine receptor antagonists have been carried out on these cell lines (Table 1).

Table 1. Pharmacological profile of [^{3}H]spiperone binding to D_2 dopamine receptors in cell membranes.

Antagonist	CHO6	Ltk59	LZR1
(+)-Butaclamol	4.19 ± 0.75	4.09 ± 0.85	3.96 ± 0.28
(-)-Butaclamol	>10,000	>10,000	>10,000
Domperidone	0.95 ± 0.03	0.87 ± 0.2	1.22 ± 0.22
Haloperidol	0.94 ± 0.16	0.91 ± 0.16	0.64 ± 0.04
(-)-Sulpiride	16.16 ± 1.98	15.92 ± 1.68	6.03 ± 1.94(*)
Raclopride	3.74 ± 0.59	2.28 ± 0.26	0.87 ± 0.10(*)

Ki(nM) values, mean ± s.e.mean ($n \geq 3$),(*) $P < 0.05$, Student's t-test.

The two isoforms of the receptor appear to have similar pharmacological profiles for the "classical" antagonists and the data fit one binding site models. However, the substituted benzamides, (-)-sulpiride and raclopride, appear to have higher affinity for the D_{2S} dopamine receptor. These cell lines will therefore be useful in investigating differences between the two isoforms of the D_2 dopamine receptor.

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Bunzow, J.R., Van Tol, H.H.M., Grandy, D.K., Albert, P., Salon, J., Christie, M., Machida, C.A., Neve, K.A. and Civelli, O. (1988), Nature **336**, 783-787.

56P BIOCHEMICAL CHARACTERISATION OF A 5-HT_2 RECEPTOR IN THE HUMAN NEUROBLASTOMA CELL LINE IMR32

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The biochemical properties of the 5-HT_2 receptor are well characterised and the rat cDNA sequence has been cloned (Pritchett et al, 1988). Characterisation in human tissues has been limited to post-mortem brain studies (Pazos et al, 1985). We report here the biochemical and functional identification of a 5-HT_2 receptor in the human neuroblastoma cell line IMR32.

IMR32 cells (European Collection of Animal Cell Cultures) from passage 75-90 were grown in DMEM + 10% (v/v) foetal calf serum (FCS) until almost confluent. Cells were differentiated by incubation for a further 2-3 days in DMEM + 2% (v/v) FCS + 1mM dibutyryl cAMP. For mRNA analysis, total RNA was extracted with urea-lithium chloride. PolyA+ mRNA prepared using Dynabeads was electrophoresed in a 1.2% (w/v) agarose-glyoxal gel, transferred to a nylon membrane and probed with pBluescriptSK- containing the rat 5-HT_2 receptor cDNA labelled with [^{32}P]dCTP. The probe recognised a diffuse band with an apparent molecular size of 5.6Kb consistent with hybridisation to the 5-HT_2 receptor transcript.

For radioligand binding studies, cells were harvested then homogenised in 5mM Tris/EDTA. After washing, the membranes were resuspended in 50mM Tris, 5mM $MgCl_2$, 1mM EGTA, pH 7.4. Binding studies were carried out using [^{3}H]ketanserin with non-specific binding defined by 1μM methysergide. Binding was saturable within the range 0.1 - 6 nM [^{3}H]ketanserin indicating a single site of high affinity (Kd = 0.56 ± 0.10 nM) and capacity Bmax = 135 ± 27 fmol/mg protein (mean ± S.E. mean, n=3). Inhibition of [^{3}H]ketanserin binding revealed a typical 5-HT_2 receptor profile, with antagonist potency decreasing in the series spiroperidol > ketanserin > mianserin > fluphenazine. 5-HT was the most potent of the monoamine neurotransmitters (IC_{50} = 4.2 ± 0.1 μM, n_H=0.68 ± 0.04; n=3). Addition of the stable GTP analogue guanylylimidodiphosphate (10μM) reduced the affinity of 5-HT (IC_{50}=32μM) and increased the slope of the inhibition curve (n_H=1.07), suggesting interaction of the receptor with guanine nucleotide binding protein(s).

To investigate polyphosphatidylinositol (PI) stimulation, cells were grown on glass cover-slips in 24-well Costar plates and the differentiating medium was supplemented with [^{3}H]inositol (1μCi/ml). After 2-3 days in this medium the cells were washed with DMEM containing 20mM LiCl. Stimulation of the PI cycle was initiated by addition of agonist to each well, maintained for 30min then terminated by transfer of the cover-slips to methanol / chloroform / HCl (100 / 50 / 1). Tritiated inositol phosphates ([^{3}H]IP_x) were separated from [^{3}H]inositol on Dowex AG-1x columns. 5-HT stimulated [^{3}H]IP_x production in a concentration-dependent manner with EC_{50} = 1.9 ± 0.4 μM and maximal stimulation of 280 ± 5 % basal level (mean ± S.E. mean, n=3). This effect was inhibited by ketanserin or spiroperidol but not by prazosin or atropine, all at 1μM.

We conclude that IMR32 cells express a 5-HT_2 receptor whose biochemical properties correspond closely with those reported in animal tissues. This cell line may therefore prove a useful model to investigate the characteristics of the human 5-HT_2 receptor.

Pazos, A., Hoyer, D., & Palacios J.M. (1985) Eur. J. Pharmacol. 106, 531-538
Pritchett, D. B., Bach, A. W. J., Wozny, M., Taleb, O., Dal Toso, R., Shih, J. C. & Seeburg P. H. (1988) EMBO J. 7, 4135-4140

57P 5-HT_2 RECEPTORS MEDIATE AGONIST-EVOKED INCREASES IN INTRACELLULAR CALCIUM IN THE HUMAN NEUROBLASTOMA CELL LINE, IMR32

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Increases in intracellular calcium concentration ($[Ca^{2+}]_i$) mediated by 5-HT_2 receptors have been demonstrated previously in C6 glioma cells developed from rat (Reiser et al., 1989). In this study the human neuroblastoma cell line IMR32 has been characterised with respect to functional responses to 5-HT by monitoring $[Ca^{2+}]_i$ using fura-2 calcium imaging techniques.

IMR32 cells were obtained from the European Collection of Animal Cell Cultures and grown on 13mm diameter glass coverslips in DMEM + 10% FCS. For studies of calcium responses to 5-HT the cells were first differentiated by switching the medium to DMEM + 2% FCS with 1mM dibutyryl cyclic AMP for 3-7 days. Differentiated cells were then loaded with fura-2 by incubation in differentiation medium containing 10μM fura 2/AM for 30 minutes at 37°C. The coverslips were then transferred to a laminar flow chamber and continuously perfused at room temperature with Hank's BSS containing 10mM HEPES pH7.4 and 2mM $CaCl_2$. Cells were allowed to equilibrate for 10 minutes until stable fluorescence ratios were attained before beginning measurements. Fura-2 was excited alternately at 340nm and 380nm. The emitted light was monitored at 510nm and the cytosolic calcium concentration was calculated from the ratio of the fluorescent intensities at the two excitation wavelengths (Grynkiewicz et al., 1985).

Brief (10 second) applications of 5-HT were able to stimulate, in a dose-dependent manner, increases in $[Ca^{2+}]_i$, with an EC_{50} of 0.4μM. The neuropeptide bradykinin and carbacol were also able to stimulate increases in $[Ca^{2+}]_i$, with EC_{50} values of 66nM and 3μM respectively. 5-HT-mediated increases in $[Ca^{2+}]_i$ occurred in both calcium-containing and calcium-free media implying release of calcium from intracellular stores. Furthermore, in calcium-free medium this release of cytosolic calcium was blocked when the stores were first depleted with 1μM ionomycin. This 5-HT-mediated response was also blocked by 10nM concentrations of the 5-HT_2 antagonists ketanserin, spiperone, and 1μM concentrations of the 5-HT_2 partial agonist DOI, but was unaffected by 1μM pindolol or granisetron. 1μM 8-OH-DPAT, 5-CT, and 10μM 2-methyl-5-HT had no effect on $[Ca^{2+}]_i$ while 10μM DOI, 5-methoxy-N,N-dimethyl-tryptamine and 1μM quipazine were all able slightly to increase $[Ca^{2+}]_i$, consistent with reports that these compounds acting as 5-HT_2 partial agonists.

Taken together these data suggest the presence of functional 5-HT_2 receptors on IMR32 cells. This finding would make these neuroblastoma cells a good model for the study of intracellular calcium regulation by 5-HT_2 receptors in a human neural cell line.

Grynkiewicz, G., Poenie, M., & Tsien, R. (1985) J. Biol. Chem. 260, 3440-3445
Reiser, G., Donie, F., & Binmoller, F.-J. (1989) J. Cell Sci. 93, 545-555

58P THE METABOLISM OF 8-OH-DPAT IN RATS

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The serotonin system is involved in various psychiatric disorders. 8-OHDPAT, a potent and selective serotonergic agonist, is used as a ligand for the characterisation of the 5-HT_{1A} receptor subtype. Although active when administered intracerebrally or parenterally, 8-OHDPAT is inactive orally and metabolic studies have been initiated to account for this discrepancy.

Male Sprague-Dawley rats were given [2 -^{3}H]-8-OHDPAT (1mg/Kg; 40μCi/Kg) by i.p. injection and their urine and faeces collected over 24 hour periods for 3 days. Elimination of ^{3}H was monitored by scintillation counting and urinary metabolites separated using a reverse-phase-HPLC system and characterised by LC-MS.

The total elimination of ^{3}H over 3 days was 94.25 ± 3.35 % of dose with the bulk (>70 %) of the excreted material present in the 0-24 h urine. HPLC of the 0-24 h urine showed a major peak of R_t 4 min containing 99% of urinary ^{3}H, accompanied by a very minor peak at the same R_t as 8-OHDPAT (10.8 min). Incubation of the urine with ß-glucuronidase, but not sulphatase, caused the early eluting peak to disappear. The peak corresponding to 8-OHDPAT increased to 80% of urinary ^{3}H and a new peak appeared at R_t 7.5 min containing 20% of urinary ^{3}H. LC-MS showed this peak to be 8-hydroxy-2-(N-n-propylamino)tetralin, the product of N-despropylation and confirmed the peak at R_t 10.8 min as 8-OHDPAT. The *in vivo* metabolism of 8-OHDPAT therefore involves oxidative N-dealkylation and glucuronidation of the free hydroxyl group of both the parent drug and its major metabolite.

In vitro studies using rat liver microsomes have confirmed that the N-dealkylation of 8-OHDPAT is cytochrome P-450-mediated, on the basis of co-factor requirements and specific inhibitors. The substantial increase in N-despropylation in liver microsomes prepared from phenobarbitone-treated rats indicates the participation of phenobarbitone-inducible forms of Cyt.P450 in this reaction.

59P "REAL-TIME" VOLTAMMETRIC MEASUREMENT OF NORADRENALINE EFFLUX AND UPTAKE IN RAT BRAIN SLICES

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Fast cyclic voltammetry (FCV) at carbon fibre microelectrodes is a "real time" electrochemical method for the detection of evoked monoamine efflux *in vivo* and *in vitro*. Previous reports have demonstrated the suitability of FCV for the detection of dopamine (DA: Palij et al. 1990) and, more recently, 5-hydroxytryptamine (5-HT: O'Connor & Kruk, 1991) in brain slices. In this study we applied FCV to the detection of stimulated noradrenaline (NA) efflux in slices of rat nucleus interstitialis stria terminalis (NIST).

All experiments were conducted in 350 μm slices of NIST superfused with oxygenated artificial CSF at 32°C. The carbon fibre microelectrode was positioned in the ventral part of the NIST, immediately ventral to the anterior commissure. Local NA efflux was evoked by constant-current pulse trains (30 pulses, 0.2 ms, 100 Hz, 10 mA) applied at adjacent bipolar tungsten stimulating electrodes. NA efflux was monitored using FCV at the following parameters:- scan rate: 480 V/s, scan range: -1.0 to +1.4 V vs Ag/AgCl, interscan interval: 500 ms. All drugs were administered via the artificial CSF.

Stimulation trains every 5 minutes evoked amine efflux in NIST that was stable and reproducible over at least 2½ hours (longest period tested). NA was confirmed as the detected species in NIST by three distinct lines of evidence: Firstly, the voltammogram of the released species had a single redox couple and was indistinguishable from the signals of catecholamines NA and DA. 5-HT could be excluded by its twin reduction peaks. Secondly, stimulated efflux matched the known local NA innervation density: efflux was greater in ventral than dorsal NIST. Thirdly, amine efflux was modified predictably by the drugs tested: Pargyline (2 x 10^{-6}M) had no effect on efflux or uptake confirming that the released species was neither a deaminated metabolite nor removed from the extracellular space (ECS) by deamination. Tetrodotoxin (10^{-6}M) or omission of Ca^{2+} from the superfusate significantly ($P<0.001$) reduced amine efflux by 90.2 and 88.0% respectively. Ro 4-1284 (10^{-6}M) decreased ($P<0.001$) amine efflux by 75.8 %. Desipramine (5 x 10^{-8}M), the selective NA uptake blocker, significantly increased amine efflux (+114%, $P<0.05$) and uptake half-life +290%, $P<0.01$). Fluvoxamine (5 x 10^{-7}M) and GBR 12909 (3 x 10^{-7}M), had no effect on amine efflux although fluvoxamine caused a modest (+91.0%, $P<0.05$) increase in uptake half-life.

Taking the combined anatomical, electrochemical and pharmacological data together, the study demonstrates that the monoamine released in NIST by local electrical stimulation was NA and that its removal from the ECS was primarily by a NA uptake system. Peak NA efflux per train was 139 ± 22 nM and the half-life of uptake was 3.1 ± 0.3 s (mean ± s.e.m., n=31). The study confirms that, in addition to DA and 5-HT, FCV may also be used to monitor NA efflux and uptake from small nuclei in "real time".

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O'Connor, J.J. & Kruk, Z.L. (1991) *J. Neurosci. Meth.* **38**, 25-33.
Palij, P., Bull, D.R., Sheehan, M.J., Millar, J., Stamford, J.A., Kruk, Z.L. & Humphrey, P.P.A. (1990) *Brain Res.* **509**, 172-4.

60P PRE- AND POST-JUNCTIONAL ACTIONS OF NPY IN PRODUCING INHIBITION OF THE ELECTRICALLY-EVOKED NEUROGENIC TWITCHES OF RAT ISOLATED VAS DEFERENS

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Neuropeptide Y (NPY) is present in the nerve supply of rodent vas deferens and is known to inhibit the electrically-evoked neurogenic twitches of the vas (Lundberg *et al.* 1982). The inhibition is believed to be prejunctional and this model has been widely used to estimate the prejunctional inhibitory activity of NPY (Wahlestedt *et al.* 1986; Grundemar & Hakanson 1990). However since in the vas deferens the contractile responses to noradrenaline (NA) and α,β-methylene ATP, substances acting directly on smooth muscle receptors, are potentiated by NPY (Ellis & Burnstock 1990), the inhibition of neurogenic twitches by NPY is not an accurate index of its prejunctional inhibitory action, unless its postjunctional potentiating activity is also taken into account. In the present investigation, we have therefore determined the inhibition of electrical field stimulation (EFS)-evoked twitches and the potentiation of NA-evoked contraction by NPY 1-300 nM in order to assess its true prejunctional activity.

Male Wistar rats (200-250 g) were killed by decapitation; 1 cm long segments of vas deferens from the prostatic end were prepared and suspended between parallel platinum electrodes in a 1 ml organ bath containing Krebs-Henseleit solution aerated with 95% O_2 + 5% CO_2 at 37°C. Isometric contractions in response to EFS (0.08 ms pulse duration, supramaximal voltage, frequency = 0.016 Hz) and to NA 10 µM were obtained, first without and then in the presence of NPY, 1-300 nM. EFS-evoked twitches were tetrodotoxin (0.5µM)-sensitive and therefore neurogenic. NPY inhibited the EFS-evoked twitches in a concentration-dependent manner (% inhibition : mean ± s.e.mean = 11.5 ± 3, 18 ± 1, 32 ± 4, 43 ± 4, 58 ± 3 and 73 ± 4 by NPY 1, 3, 10, 30, 100 and 300 nM respectively; IC_{50} = 62 ± 10 nM; n = 6). Contractile responses to NA were potentiated by NPY concentration dependently (% potentiation : mean ± s.e.mean = 20 ± 2, 34 ± 3, 40 ± 1, 45 ± 2, 50 ± 2 and 62 ± 2 by NPY 1, 3, 10, 30, 100 and 300 nM respectively; n = 6). After allowing correction for the postjunctional potentiation, the prejunctional twitch inhibition by NPY was substantially greater (% inhibition : mean ± s.e.mean = 32.3 ± 3, 40 ± 1, 47.5 ± 4, 58 ± 3, 77 ± 2 and 86 ± 4 by NPY 1, 3, 10, 30, 100 and 300 nM respectively; IC_{50} = 12 ± 2.5 nM; n = 6).

In conclusion the results emphasise the necessity of introducing appropriate correction for postjunctional activity while evaluating the prejunctional inhibitory potency of NPY on neurogenic twitches of rat vas deferens.

Ellis, J.L. & Burnstock, G. (1990) *Br. J. Pharmacol.* **100**, 457-462.
Grundemar, L. & Hakanson, R. (1990) *Br. J. Pharmacol.* **100**, 190-192.
Lundberg, J.M., Terenius, L., Hokfelt, T., Martling, C.R., Tatemoto, K., Mutt, V., Polak, J., Bloom, S.R. & Goldstein, M. (1982) *Acta Physiol. Scand.*, **116**, 477-480
Wahlestedt, C., Yanaihara, N. & Hakanson, R. (1986) *Regul. Peptides.* **13**, 307-318.

61P PREJUNCTIONAL INHIBITORY ACTIVITY OF SOME C- AND N-TERMINAL FRAGMENTS OF NPY IN RAT ISOLATED VAS DEFERENS

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Inhibitory effect of neuropeptide Y (NPY) fragments on electrically-evoked neurogenic contractions of the rat vas deferens have been studied by several groups of researchers, and have led to the conclusion that the amidated C-terminal of NPY molecule is essential for the prejunctional inhibitory activity (Wahlested *et al.* 1986; Grundemar & Hakanson 1990). These studies, leading to the forementioned conclusion, were carried out under the tacit assumption that NPY fragments lack any postjunctional activity. Since in the vas deferens noradrenaline (NA)-evoked and ATP-evoked contractions are potentiated by NPY (Ellis & Burnstock 1990) and NPY 13-36 (Cook & Wickenden 1990), we have re-assessed the prejunctional inhibitory activity of some NPY fragments after taking into account their postjunctional potentiating activity.

Male Wistar rats (200-250g) were killed by decapitation; 1cm long segments of vas deferens from prostatic end were suspended between parallel platinum electrodes in a 1ml organ bath containing Krebs-Henseleit solution aerated with 95% O_2 + 5% CO_2 at 37°C. Isometric contractile responses to EFS (0.08ms pulse duration, supramaximal voltage, frequency = 0.016 Hz) and to NA 10μm were obtained first without and then in the presence of NPY and NPY fragments (NPY 11-36, 16-36, 18-26, 21-26, 1-25 and 1-35) at concentrations of 0.1-3000nM. Stimulation at 0.016 Hz was chosen because this stimulation frequency has been shown to minimise the interfering influence attributable to the local release of endogenous NPY (Khan & Zar 1992). NPY and all NPY fragments inhibited the EFS-evoked twitches and potentiated the postjunctional contractile response to NA concentration dependently. The pIC_{20} values (mean ± s.e.mean) of NPY and NPY fragments for the prejunctional inhibition of the twitch are given in Table 1, both before and after correction for the postjunctional potentiating activity.

Table 1 pIC_{20} values for inhibition of vas deferens twitches by NPY and its fragments (mean ± s.e.mean; n = 6)

Peptide	Apparent	Corrected
NPY 1-36	8.30 ± 0.02	9.18 ± 0.08
NPY 11-36	7.42 ± 0.04	8.00 ± 0.14
NPY 16-36	7.37 ± 0.15	8.04 ± 0.06
NPY 18-36	7.00 ± 0.08	7.25 ± 0.24
NPY 21-36	5.85 ± 0.17	6.40 ± 0.26
NPY 1-25	5.95 ± 0.18	6.25 ± 0.20
NPY 1-35	6.07 ± 0.12	6.76 ± 0.16

The results indicate that both C- and N-terminal NPY fragments possess pre- as well as postjunctional activities in the vas deferens but the possession of the C-terminal enables the NPY fragment to have a greater prejunctional potency.

Cook, M. & Wickenden, A.D. (1990) *Br. J. Pharmac.* **100**, 481P.
Ellis, J.L. & Burnstock, G. (1990) *Br. J. Pharmac.* **100**, 457-462.
Grundemar, L. & Hakanson, R. (1990) *Br. J. Pharmac.* **100**, 190-192.
Khan, R.M. & Zar, M.A. (1992) *Br. J. Pharmac.* in press
Wahlestedt, C., Yanaihara, N. & Hakanson, R. (1986) *Regul. Peptides* **13**, 307-318.

62P EFFECT OF PALYTOXIN ON RAT ISOLATED PROSTATIC VAS DEFERENS

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Palytoxin (PTX), a particular potent toxin isolated from marine coelenterates of the genus *Palythoa*, produces intense contractions of isolated smooth muscle preparations. The precise mechanism of action of PTX remains unclear (Habermann, 1989) and may vary between tissue preparations. Previous experiments on rat detrusor suggested that the primary mode of action was to initiate the release of transmitter from motor nerves. We now present some of our observations on the effects of PTX on the isolated vas deferens of the rat.

Male Wistar rats (200-250 g) were killed by decapitation. Segments of prostatic vas deferens (1-1.5 cm long) were suspended between parallel platinum electrodes in a 2 ml organ bath containing Krebs-Henseleit solution aerated with 95% O_2 + 5% CO_2 at 37°C. PTX (30-100 nM) initiated concentration dependent, biphasic contractions of the muscle. The first phase of the contraction peaked 0.5-1 min after exposure to toxin and the second phase peaked at 20-25 min. The maximum tension generated during the first phase was lower than that during the second (mean maximum tension ± s.e.mean = first phase 0.58 ± 0.03 g; second phase 0.75 ± 0.05 g, n=6). Occasionally, a third peak, lying between the first and second peaks could be distinguished. The contraction subsided almost completely in about 120-150 min despite the continued presence of PTX in the bath. A second exposure to PTX (100 nM), failed to evoke any visible response even after extensive washing. The first phase of the contraction was inhibited by 80% by phentolamine and by 90% by nifedipine, but was unaffected by tetrodotoxin, 6-OHDA, α,β-methylene ATP and low K^+. The second phase was inhibited by 70% by 6-OHDA and 45% by phentolamine and nifedipine. Neurogenic contractile responses elicited by electrical field stimulation (0.4 ms pulse duration, 0.017 Hz, supramaximal voltage) were at first augmented and then abolished by PTX (100 nM).

The results are compatible with the view that the contractile action of PTX in rat prostatic vas deferens is due (in part at least) to the release of endogenous motor transmitter(s) (Posangi et al., 1991).

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Habermann, E. (1989). *Toxicon* 27, 1171-1181.
Posangi, J., Zar M.A. & Harris J.B. (1991). *J.Physiol.* 434, 65P.

63P NEUROTOXIC AND MYOTOXIC ACTIVITY OF THE CRUDE VENOM OF THE TWO SUBSPECIES OF RUSSELL'S VIPER, *Daboia russelii*

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Daboia russelii is a venomous snake found throughout South-East Asia. Bites by the subspecies found in Myanmar (D. r. siamensis) cause oedema, haemorrhage and hypotension, with little evidence of neuromuscular damage. In contrast, bites in Sri Lanka by D. r. pulchella cause neuromuscular paralysis, haemolysis and rhabdomyolysis but little oedema (Warrell, 1986). We wish to determine whether the apparent differences reported by clinicians can be reproduced in the laboratory.

Both venoms blocked neuromuscular transmission in the indirectly stimulated mouse hemi-diaphragm preparation, but the venom of D. r. pulchella was slightly more potent than that of D. r. siamensis (ED_{50} 10.3 ± 2.6 µg ml^{-1} cf. 22.1 ± 5.0 µg ml^{-1} n = 4/5). Both venoms elicited a triphasic response in indirectly stimulated preparations partially blocked with high $[Mg^{2+}]_o$: depression of twitch tension; reversal; a progressive loss of twitch tension over 30 min. Identical results were seen with curarized preparations and when EPP's were recorded using intracellular electrodes in preparations incubated in low $[Ca^{2+}]_o$/high $[Mg^{2+}]_o$. The mechanical response of the field-stimulated vas deferens muscle of the guinea pig was also triphasic following exposure to the venoms.

Venom (10 µg in 0.1 ml 0.9% w/v NaCl) was inoculated into tibialis anterior muscles of mice. D. r. pulchella venom caused extensive necrosis of the muscle fibres but little or no oedema; D. r. siamensis caused much less necrosis, but muscle wet weight was increased by 21%. Cultured rat and human myotubes were exposed to the two venoms (80 µg ml^{-1}). D. r. pulchella caused the destruction of the myotubes by 6h, and by 24h the dishes contained little but debris. Cultures maintained in the presence of D. r. siamensis survived beyond 24h. The response of diaphragm muscle to direct stimulation was reduced by 30% following exposure to the venom of D. r. pulchella (30 µg ml^{-1}, 60 min) and by 11% following exposure to the venom of D. r. siamensis.

Our data show that some features of bites by these snakes can be reproduced under laboratory conditions, although the neurotoxic activity of the venom of D. r. siamensis is anomalous. The venom of all sub-species of D. r. russelii is rich in phospholipase activity and the results are consistent with the view that toxic phospholipases are responsible for the clinical pathology following a bite (see Harris, 1991).

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Harris, J.B. (1991) In Snake Toxins ed. Harvey, A.L. 91-129, Pergamon Press, New York.
Warrell, D.A. (1986) In Natural Toxins ed. Harris, J.B. 25-45, Clarendon Press, Oxford.

64P NOTECHIS 11'2: A ROUTE TO THE DETERMINATION OF TOXIC SITES ON A SERIES OF VENOM PHOSPHOLIPASES A_2

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Snake venoms are a rich source of phospholipase A_2 (PLA_2) [EC 3.1.1.4]. The molecules typically comprise 120-125 amino acid residues and are predominantly α-helical structures cross-linked by 7 or 8 disulphide bridges. Many venom PLA_2 proteins are highly toxic, possessing neurotoxic and/or myotoxic properties. Some experimental evidence suggests that within the molecule the sites responsible for myotoxicity and neurotoxicity are distinct from each other, and from the enzymatic site, although the latter seems to be an essential factor involved in the expression of toxicity (see Harris, 1991).

The venom of the Australian tiger snake, *Notechis scutatus scutatus*, contains a series of such proteins. The series includes two toxins, notexin and notechis II-v, which are enzymatically active neuro- and myotoxic compounds with very low LD_{50}'s (17 and 45 µg/kg i.p.), and the non-toxic compound notechis II-i, which has very low enzymatic activity, probably due to the substitution of the ordinarily conserved glycine 30 residue by serine; glycine 30 is implicated in the binding of the essential co-factor, Ca^{2+}.

Notechis 11'2 is a recently purified, non-lethal, PLA_2 homologue from the venom (Bouchier *et al*, 1991). The isoform contains all the residues purportedly required for enzymatic activity, including glycine-30. We have now shown that notechis 11'2 is a potent myotoxin. This allowed us to establish a hierarchy of myotoxic potency; notexin>notechisII-v>notechis11'2>>notechisII-i. The facts that (i) this order does not correlate with our observed maximal enzymatic rates; notechis II-v>notexin>notechis 11'2>>notechis II-i, and (ii) that notechis 11'2 has a comparable enzymatic activity to non-myotoxic PLA_2 homologues, support the postulated existence of regions upon PLA_2 associated with the expression of neuro- and myotoxicity; these regions are presumably binding regions that attach the molecule to specific target tissues.

Kini and Iwanaga (1986) have postulated that a cationic motif is associated with myotoxicity, but this motif is missing in the myotoxic compound notechis 11'2.

Site directed and cassette mutagenesis experiments are currently underway using a cDNA clone encoding notechis 11'2 (Hodgson *et al*, unpublished). The aim is to insert into 11'2 sequences from notexin that may be associated with neurotoxicity (and hence lethality).

Bouchier, C., Boyot, P., Tesson, F., Trémeau, Bouet, F., Hodgson, D., Boulain, J.-C. & Ménez, A. (1991) Eur. J. Biochem. 202, 493-500.
Harris, J.B. (1991) In Snake Toxins ed. Harvey, A.L. 91-129, Pergamon Press, New York.
Kini, R.M. & Iwanaga, S. (1986) Toxicon 24, 895-905.

65P EFFECT OF SR 58611A, A NOVEL ATYPICAL β-ADRENOCEPTOR (β3) AGONIST, ON BROWN ADIPOSE TISSUE LIPOGENESIS

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SR 58611A is a putative ß3-adrenoceptor agonist phenylethanolaminotetraline (PEAT) developed for treating conditions of abnormally enchanced gastrointestinal motility (Bianchetti & Manara, 1990). CBA/Ca obese-diabetic mice exhibit reduced lipogenesis compared to normal littermates (Al-Qatari et. al., 1991). Brown adipose tissue (BAT) possesses atypical ß adrenoceptors (ß3) which stimulate energy expenditure by increasing fatty acid synthesis (lipogenesis) and thermogenesis. The aim of this study was to investigate the effect of SR 58611A on lipogenesis in adipose tissue of lean and obese-diabetic mice.

In vivo lipogenesis rates were estimated in fed control (lean) and obese-diabetic male CBA/Ca mice by measuring the incorporation of ^3H into fatty acids extracted from adipose tissue following i.p. injection of ^3H_2O, using the methods described by Mercer & Trayhurn (1983). Propranolol (10mg/kg) was given as i.p. injection 90 minutes prior to ^3H_2O injection. SR 58611A (1 mg/kg i. p.) was administered acutely 1 hour and insulin (1 IU/kg i.p.) was injected 15 minutes prior to ^3H_2O.

SR 58611A (1mg/kg) lowered BAT lipogenic rate slightly from control values of113.81±8.99(11) to 87.8±14.1(16) (means± S.E.M.(n)µg atoms H incorporated/hr/g fat free tissue weight) in lean mice. However, in obese mice, lipogenic rates were increased significantly ($p<0.05$, t-test) from obese control values of 56.03 ±15.48(8) to 98.6 ±15.4 (8). Insulin administered prior to SR 58611A further increased lipogenic rates significantly ($p<0.002$,t-test) to 193.61±22.7(7) in lean mice but did not alter rates in obese mice. In obese mice, propranolol reduced basal lipogenic rates significantly ($p<0.001$,t-test) to 36.6±10.3(7) and when given prior to SR 58611A reduced lipogenic rates back to control values in obese mice. In white adipose tissue (WAT), SR 58611A increased lipogenesis significantly ($p<0.001$, t-test) in lean but not in obese mice.

Since SR 58611A acts to stimulate lipogenesis and thermogenesis selectively in obese mice, and the effect is blocked by propranolol, this suggests that SR 58611A may have anti-obesity properties by virtue of its selective ß3-agonist activity.

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Al-Qatari M. , Leighton I. A. and Taberner P. V. (1991) Br. J. Pharmacol. 104: 347P.
Bianchetti A.& Manara L. (1990) Br. J. Pharmacol. 100: 831-839.
Mercer S. W. & Trayhurn P. (1983) Biochem. J. 212: 393-398.

66P FURTHER CHARACTERIZATION OF THE ATYPICAL β-ADRENOCEPTORS IN GUINEA-PIG GASTRIC FUNDUS

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We have previously demonstrated that the β-adrenoceptors present in guinea-pig gastric fundus (GPGF) are "atypical", being particularly sensitive to the relaxant activity of the selective atypical β-adrenoceptor agonist, BRL35135, and insensitive to classical β-adrenoceptor antagonists (Coleman et al, 1987). In the present study, we have further evaluated the potencies of a range of β-adrenoceptor agonists and the effect of the putative atypical β-adrenoceptor antagonist, alprenolol (Blue et al, 1990) in this preparation.

Strips of GPGF were suspended in oxygenated (5% $C0_2$ in 0_2) Krebs solution containing atropine (0.4µM), phenoxybenzamine (1µM), indomethacin (2.8µM) and propranolol (1µM). The preparations were contracted by the addition of a submaximally-effective concentration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 0.3-3µM). The β-adrenoceptor agonists were added to the organ bath in a cumulative manner (0.01-30µM).

Isoprenaline (Iso) caused concentration-related relaxation of GPGF, the mean maximal inhibition of $PGF_{2\alpha}$-induced tone being 81% (95% CL, 70-85%, n=40) and the mean EC_{50} 1.8µM (1.4-2.5µM, n=40). Salbutamol (Salb), fenoterol (Fen), BRL35135, BRL37344, noradrenaline (Nor), adrenaline (Adr) and clenbuterol (Clen) also all caused concentration-related relaxation of GPGF, the concentration-effect curves appearing parallel and of similar maxima to those for Iso. The rank order of agonist potency (equipotent concentration, Iso=1) was BRL35135 (0.3) > BRL37344 (0.4) > Iso (1.0) > Nor (4.3) ~ Clen (6.3) ~ Adr (7.3) ~ Fen (8.4) ~ Salb (10.6). Data are derived from at least six individual experiments.

Alprenolol (0.1-10µM) caused a concentration-related rightward shift of the concentration-effect curves to Iso (pA_2 6.0, slope 0.85, n=5) and BRL35135 (pA_2 6.8, slope 0.72, n=5). None of the slopes of the Schild plots was significantly different from unity ($p>0.05$). These pA_2s for alprenolol differ from those previously reported for blockade of β_1- and β_2-adrenoceptors in other tissues (8.1 and 8.0 respectively, Hoefle et al., 1975).

These data further characterize the atypical β-adrenoceptors in GPGF, and show that clenbuterol, fenoterol and salbutamol possess only relatively weak agonist activity at these receptors.

Blue, D.R., Bond, R.A., Adham, N., Delmondo, R., Michel, A.D., Eglin, R.M., Whiting, R.L. & Clarke, D.E. (1990). J. Pharm. Exp. Ther., 252 1034-1042.
Coleman, R.A., Denyer, L.H. & Sheldrick, K.E. (1987). Br. J. Pharmac., 90, 40P.
Hoefle, M.L., Hastings, S.G., Meyer, R.F., Corey, R.M., Holmes, A. & Stratton, C.D. (1975), J. Med. Chem., 18, 148-152.

67P DRUG EFFECTS ON VASCULAR FLOW IN THE ISOLATED PERFUSED BOVINE EYE USING RADIO-LABELLED MICROSPHERES

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We have previously reported (Millar & Wilson, 1991) the lowering of intraocular pressure (IOP) by sodium nitroprusside (SNP) and now show how its vasodilator activity extends to all the main ocular vascular beds. Within 50 min of slaughter, bovine eyes were cannulated via a long posterior ciliary artery and perfused with an oxygenated Krebs solution at 2.25 $ml.min^{-1}$. IOP was measured by cannulation of the anterior chamber and a water manometer. At least 60 min was allowed for IOP to equilibrate; only eyes with a constant IOP in the range 6.5-12.5 mm Hg were used. Approximately 4,500 ^{141}Ce-labelled microspheres of 15 μm diameter were introduced into the arterial perfusate without disturbing the flow rate. After 5 min the eye was dissected and each region weighed and counted for gamma radioactivity.

Timolol (30 nmol) significantly reduced IOP (Table 1) but produced no change in perfusate flow in the iris or ciliary body, while a 300 nmol dose revealed an overall vasoconstrictor effect, though this was only significant in the iris. SNP significantly lowered IOP; while little vasodilatation was observed in the absence of noradrenaline (NA; data not shown), arterial perfusion of NA (10^{-5} M) produced vascular tone (Table 1), against which significant vasodilatation occurred in all regions examined.

Table 1.

	Dose (nmol)	Vascular flow ($\mu l.min^{-1}.g^{-1}$ tissue) n	iris	ciliary body	choroid	n	Δ IOP (mm Hg)
Control		4	234± 96	104±46	73± 6	9	+0.1±0.4
Timolol	30	4	269± 54	183±19	24± 6*	8	-2.2±0.3**
Timolol	300	4	37± 17*	89±37	52±10	11	-1.9±0.2**
Control+		5	30± 24[a]	18±12[a]	40±20		
SNP+	300	5	240±103[b]	117±67[b]	142± 7[b]	7	-2.0±0.3**

Means ± s.e.mean. + Vascular flow measured in eyes perfused with Krebs containing NA (10^{-5} M).
*P<0.05, Mann-Whitney test. **P<0.01, IOP at start vs end, Student's t-test.
[a]P<0.05, NA-perfused vs control; [b]P<0.05, SNP vs control, both NA-perfused; Mann-Whitney test.

The data indicate a complex effect of timolol on the ocular vasculature, in addition to its ability to lower IOP. SNP produces marked vasodilatation in all preconstricted vessels. Decreases in IOP following these drugs do not appear to be related to their vascular effects.

Millar, C. & Wilson, W.S. (1991) Br. J. Pharmacol. **104**, 55P.

68P EFFECTS OF THE ISOPROPYL ESTER OF PALMITOYL CARNITINE ON VASCULAR TISSUES OF THE RAT, AND THE GUINEA-PIG TAENIA-COLI

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Endogenous acyl carnitines accumulate in the ischaemic myocardium and are thought to contribute to myocardial damage (Corr *et al* 1984). Some synthetic acyl carnitine esters unlike their endogenous counterparts have coronary dilator effects in the rat, (Criddle *et al* 1990), the duration of which can be modified by altering fatty acid chain length (Criddle *et al* 1991). In this study we compare the actions of the isopropyl ester of palmitoyl carnitine (P1PI) on a number of smooth muscle preparations from the rat and guinea-pig.

Aortic rings and spontaneously contracting portal veins, together with constant flow perfused superior mesenteric vascular beds (5$ml.min^{-1}$), Langendorff perfused hearts (10$ml.min^{-1}$) and caudal artery vascular beds (1$ml.min^{-1}$) of male Wistar rats (250-300g) were studied. Taenia-coli preparations from male Dunkin-Hartley guinea-pigs (300-350g) were also used. All preparations (n≥4), were initially set up using Krebs-Henseleit solution containing 11.1mM glucose and either 3.2mM K^+ (hearts) or 5.9mM K^+ (other preparations) gassed with 95%O_2/5%$_2$. Potassium, phenylephrine or noradrenaline were used to contract some of the preparations, while calcium induced dose response curves were studied in potassium depolarized taenia-coli preparations and rat mesenteric vascular beds.

In perfused heart preparations bolus doses of P1PI (0.1-10nmoles) or constant infusions (1-1000nM) caused dose related coronary dilation. Vasodilator responses were also seen using similar doses in potassium depolarized mesenteric and tail artery preparations. In portal veins, P1PI (0.1-50μM) had no effect on the amplitude or frequency of the basal spontaneous contractions, or on the increased contraction amplitude induced by phenylephrine (0.1μM). In the potassium depolarized or phenylephrine preconstracted rat aorta, P1PI had no relaxant effect at concentrations upto 20μM, at higher concentrations there was a small relaxant effect which was associated with the development of spontaneous contractile activity . In taenia-coli preparations P1PI (0.1-10.0μM) had no significant effect on calcium induced contractions but it did inhibit calcium induced contractions in the perfused mesenteric vascular bed.

These result show that P1PI is a relatively selective dilator of small resistance vessels in the rat with little activity on large conductance vessels. Its inhibitory action on the mesenteric vascular bed appears to be the result of calcium channel blockade.

Corr,P.B.,Gross,R.W.,Sobel,B.E.(1984) Circ.Res.**55**,135-154
Criddle,D.N.,Dewar,G.H.,Wathey,W.B.,Woodward,B.(1990) Br.J.Pharmacol.**99**,477-480.
Criddle,D.N.,Dewar,G.H.,Radniknam,M.,Wathey,W.B.,Woodward,B. (1991) J.Pharm.Pharmacol.**43**,636-639.

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69P EVIDENCE FOR THE INVOLVEMENT OF INTERLEUKIN-5 IN ANTIGEN-INDUCED EOSINOPHIL ACCUMULATION IN GUINEA-PIG LUNG

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The guinea-pig has been used widely to evaluate the efficacy of anti-asthma drugs on granulocyte, particularly eosinophil, accumulation in the lung, but the mediators involved in this response are unknown. Interleukin-5 (IL5) has been proposed as a pivotal cytokine for eosinophilic responses (Sanderson, et al., 1988). This view has recently been reinforced since IL5 can induce eosinophilia (Fattah et al., 1990; Sanjar et al., 1991) and a neutralizing antibody to IL5, TRFK5, can inhibit eosinophilia in parasitized mice (Coffman et al., 1989).
Guinea-pigs sensitized and boosted with ovalbumin [OA (10 μg) + $AL(OH)_3$ (2 mg) + Pertussis vaccine (0.1 ml) injected i.p. on two occasions, two weeks apart] were exposed to increasing concentrations of OA (0.5-5000 μg/ml nebulizer solution) or phosphate buffered saline (PBS, control) for 30 minutes. At various times after challenge (0-72 hours), animals were killed with an over-dose of sodium pentobarbitone (200 mg/kg i.p.) and bronchoalveolar lavage (BAL) was performed with 3 x 10 ml of Ca^{2+}- and Mg^{2+}-free Hank's Balanced Salt Solution (HBSS) containing BSA (0.1%) and EDTA (10 mM). Total cells were counted in a Coulter counter (J series) and differential cell counts were made with a light microscope from Leishman stained cytospin preparations. Cell numbers are presented as mean±s.e.m. x 10^5/ml BAL fluid. After OA challenge (50 μg/ml, n=4 per group), eosinophil accumulation was maximal at 24 hours (5.7±0.7) and still present at 72 hours (4.8±0.7). The 24 hour time point was used for dose-response studies to OA, and the results of these studies are shown in the Table (*=P<0.01, **=P<0.0005).

				OA μg/ml			
	PBS	0.5	5.0	50	500	1000	5000
n	14	8	12	20	10	5	9
Eosinophil	1.0±0.2	1.2±0.2	4.5±0.6**	5.6±0.5**	4.7±0.5**	5.9±0.6**	4.5±0.3**
Neutrophil	0.2±0.2	0.03±0.01	1.0±0.3	0.6±0.2	1.8±0.5*	1.9±0.3*	4.2±0.6**

OA challenge caused a concentration-dependent granulocyte accumulation in the lungs of guinea-pigs, which was selective for eosinophils at concentrations below 500 μg/ml. TRFK5 (0.01-1.0 mg/kg i.v.) or rat IgG (1 mg/kg i.v., control for TRFK5) was injected 30-45 minutes prior to OA (50 μg/ml) challenge (n=10 animals per group). TRFK5 inhibited eosinophil accumulation 24 hours after challenge,in a dose-related manner (IgG+PBS 0.6±0.1; IgG+OA, 6.7±0.8; TRFK5 0.01+OA, 5.0±1.0, NS; TRFK5 0.1+OA, 2.4±0.7, P<0.005; TRFK5 1.0 + OA 1.5±0.4, P<0.0005). These studies with TRFK5 confirm a major role for IL5 in eosinophil recruitment in the guinea-pig after antigen challenge.

Coffman, R.L., Seymour, B.W.P., Hudak, S., Jackson, J. & Rennick, D. (1989), Science, **245**, 308-310
Fattah, D., Quint, D.J., Proudfoot, A., O'Malley, R., Zanders, E.D. & Champion, B.R. (1990), Cytokine, **2**, 112-121.
Sanderson, C.J., Campbell, H.D. & Young, I.G. (1988). Immunol. Rev., **102**, 29-50.
Sanjar, S., McCabe P.J. & Reynolds, L.H. (1991). Am. Rev. Respir. Dis. **143**, A755.

70P *IN VIVO* ADMINISTRATION OF AN ANTI-CD18 MONOCLONAL ANTIBODY INHIBITS LEUKOCYTE-DEPENDENT PLASMA EXUDATION IN RABBIT SKIN

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The adhesive interactions between leukocytes and endothelial cells are mediated by distinct families of surface determinants (Springer,1990). In particular, the family of hetero-dimeric leukocyte integrins (CD18/CD11a-c) are involved in the adhesion and migration of leukocytes from blood vessels. In this study, a murine monoclonal antibody (6.5E) to the common β chain (CD18) has been investigated for its effects on leukocyte-dependent and independent plasma exudation in rabbit skin.

Plasma exudation was induced by the intradermal injection of bradykinin (0.03-10ng) or the chemotactic peptide FMLP (0.1-30ng) and measured over a 30min period as the local accumulation of ^{125}I-HSA injected i.v. 5 min prior to the intradermal injections. Six doses of bradykinin and FMLP were injected in volumes of 0.1ml into the shaved dorsal skin of the same rabbit and each dose was given in replicates of six in a random grid. All intradermal injections were given in combination with the vasodilator prostaglandin E_2 (30ng). Intradermal injections of saline caused an average plasma leakage of 9.01 ± 0.71μl (mean ± s.e.m.) whereas a dose-dependent increase in plasma extravasation was seen after bradykinin (15.2 4 ± 2.14μl to 54.37 ± 8.33μl per site) and FMLP (16.38 ± 1.81μl to 59.8 ± 6.7μl per site). Pre-treatment of rabbits with colchicine (1mg.kg^{-1} i.v.), a drug which disrupts microtubules and restricts neutrophil movement, reduced plasma exudation induced by FMLP to 11.76 ± 1.04 to 17.39 ± 1.51μl but had no significant effect on plasma exudation induced by bradykinin. This supports the proposal that plasma leakage caused by FMLP is neutrophil-dependent whereas the response to bradykinin is neutrophil-independent (Hellewell et al.,1989). Pre-treatment of rabbits with 6.5E (0.0072-0.24mg.kg^{-1} i.v., 3-5 animals per dose) caused a dose-dependent inhibition of FMLP-induced plasma leakage (ED_{50}=0.06mg.kg^{-1}) but had no effect on responses to bradykinin. The higher dose of antibody (0.24mg.kg^{-1}) completely inhibited the response to all doses of FMLP. Administration of 6.5E did not change circulating leukocyte numbers and administration of an isotype-matched murine antibody (0.24mg.kg^{-1}) had no effect on FMLP-induced plasma exudation.

These results demonstrate that an antibody to CD18 can selectively suppress leukocyte-dependent plasma leakage in vivo. Further studies are in progress to determine which α-subunits of the integrin molecule (CD11a, b or c) are involved in this model of inflammation.

Hellewell, P.G., Yarwood, H. & Williams, T.J. (1989) Br. J. Pharmac. 97, 181-189.
Springer, T.A. (1990) Nature 346, 425-533

71P ICI D2148: A POTENT, ORALLY-ACTIVE NON-REDOX INHIBITOR OF 5-LIPOXYGENASE

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Inhibitors of 5-lipoxygenase have therapeutic potential in a variety of inflammatory diseases including asthma, rheumatoid arthritis, psoriasis and ulcerative colitis. Recently we reported a novel class of non-redox inhibitors which exhibit enantioselective inhibition of 5-lipoxygenase (McMillan et al, 1990). Further development of this series led to the discovery of ICI D2138 (6-([3-fluoro-5-(4-methoxy-3,4,5,6-tetrahydro-2H-pyran-4-yl)phenoxy]methyl)-1-methyl-2-quinolone). The pre-clinical pharmacology of ICI D2138 is described here.

The effects of ICI D2138 on leukotriene (LT) synthesis in vitro and in vivo were measured as described previously (Foster et al, 1990; McMillan et al, 1990). Leukotriene-dependent bronchospasm was induced by allergen challenge of sensitised guinea pigs pre-treated with indomethacin, propranolol and pyrilamine (Anderson et al, 1983). Leukotriene-dependent inflammation in mouse ear was induced by topical administration of arachidonic acid in animals pre-treated with indomethacin by modification of the method of Opas et al (1985).

ICI D2138 potently inhibited LTC_4 synthesis in murine macrophages (IC_{50} = 3 x 10^{-9}M). In human blood, ICI D2138 inhibited LTB_4 synthesis (IC_{50} = 2.4 x 10^{-8}M) but did not significantly inhibit thromboxane B_2 synthesis at 500μM, which indicates a selectivity ratio with respect to cyclo-oxygenase of at least 25,000. ICI D2138 inhibited rat blood LTB_4 synthesis ex vivo with oral ED_{50} values at 3 and 10 hours after dosing of 0.9 and 4.0mg/kg respectively. LTB_4 synthesis in inflamed rat air pouch was inhibited with oral ED_{50} values of 0.3 and 2.0mg/kg at 3 and 10 hours respectively. Pharmaco-dynamic studies in dogs demonstrated that a single oral dose of 5mg/kg ICI D2138 inhibited blood LTB_4 synthesis ex vivo for at least 32 hours. ICI D2138 inhibited LT-dependent inflammation in mouse ear (ED_{50} 1-3mg/kg p.o.) and inhibited LT-dependent allergic bronchospasm in guinea pig (ED_{50} = 50-100μg/kg i.v.).

ICI D2138 is the most potent and most selective inhibitor of 5-lipoxygenase yet reported. Based on these pre-clinical data, ICI D2138 was selected for clinical development and has been shown to be well tolerated and to inhibit LT synthesis ex vivo in healthy volunteers (Yates et al, 1992).

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Foster, S.J. et al (1990) Br. J. Pharmacol. 99, 113-118.
McMillan, R.M. et al (1990) Br. J. Pharmacol. 101, 501-503.
Opas, E.E. et al (1985) J. Invest. Dermatol. 84, 253-256.
Yates, R.R. et al (1992) Am. Rev. Respir. Dis. In press.

72P EFFECT OF DOXAPRAM ON TRITIUM OVERFLOW FROM THE NEONATAL RAT CAROTID BODY *IN VITRO*

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A fundamental step in the transduction of physiological chemostimuli involved in the control of respiration by the carotid body is a release of catecholamines (predominantly dopamine) from type I cells within the organ (Fidone & Gonzalez, 1986). However, the mechanism by which doxapram, a commonly used respiratory stimulant (Nishini *et al.*, 1982) excites the carotid body is unknown. Here, we have addressed the question of whether doxapram can induce catecholamine release from the carotid body, by examining its effects on [^{3}H] overflow from carotid bodies pre-incubated with [^{3}H]-tyrosine.

Carotid bodies were removed from halothane-anaesthetised rat pups (age 8 to 11 days, usually 3 rats per experiment) into ice-cold physiological saline (in mM: NaCl 111; KCl 4.7; $CaCl_2$ 2.2; $MgCl_2$ 1.1; $NaHCO_3$ 22.5; HEPES 5; Na glutamate 17; glucose 5.5; pH 7.4) and then incubated at 37°C in physiological saline containing [^{3}H]-tyrosine (6μM), DL-6-methyl-5,6,7,8-tetrahydropteridine (100μM) and ascorbic acid (142μM) for 3h. They were then rinsed and placed in a perfusion chamber (flow rate approximately 1ml min^{-1}, 37°C). For the first hour, the perfusate was discarded. Thereafter the experiment started and 1 min samples were collected and counted for [^{3}H] by liquid scintillation counting. Correction for quench utilized the spectral index of the external standard. At the end of the experiment the tissue was removed from the perfusion chamber, solubilized in OptiSolv and the [^{3}H] content determined. All values given are means $\pm$ s.e.mean.

The mean tissue [^{3}H] content at the start of the experiment was 3.71$\pm$1.21 x 10^6 d.p.m. (n=23), and resting release per 1 min collecting period was 2769$\pm$298 d.p.m. (n=23). In fractional terms this is 1.05$\pm$0.09 x 10^{-3}. [^{3}H] release evoked by carotid body stimuli was taken as that over and above release expected under resting conditions, and is expressed in fractional terms.

[^{3}H] overflow was consistently evoked (6.84$\pm$1.70 x 10^{-3}, n=12) by raising extracellular [K^+] to 60mM (isotonic substitution for Na^+), or by addition of 2mM NaCN to the perfusate (6.12$\pm$1.27 x 10^{-3}, n=4), in agreement with earlier studies on catecholamine release from the carotid body of other species (Fidone & Gonzalez, 1986). Doxapram, applied in the perfusate for 3 min, produced a concentration-dependent enhancement of ^{3}H overflow: 15μM, 2.25$\pm$0.65 x 10^{-3}; 50μM, 4.07$\pm$1.63 x 10^{-3}; 150μM, 6.84$\pm$3.21 x 10^{-3} (n=9).

These results suggest that doxapram can enhance presumed catecholamine release from the carotid body, and in so doing support the idea that the drug shares similarities with physiological stimuli in its mechanism of excitation of the carotid body.

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73P CAPSAICIN DESENSITISATION FOLLOWED BY ATROPINE ADMINISTRATION ABOLISHES AIRWAY HYPERREACTIVITY INDUCED BY OZONE IN GUINEA-PIGS

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Inhalation of ozone (O_3) by a variety of animals including cat, guinea-pig and man results in an increased responsiveness of the airways in vivo, but not in vitro, to a wide range of substances producing bronchoconstriction. The inability to demonstrate bronchial hyperreactivity (BHR) in vitro suggests that reflexes may be important in the mechanism of action of O_3. We have previously shown that in O_3 exposed guinea-pigs, BHR to inhaled substance P, which can be released from nerve endings in the lung by capsaicin and other irritants, is entirely attributable to a functional inhibition of neutral metalloendopeptidase (NEP), but this mechanism incompletely explains the non-specificity of BHR (Yeadon, et al, 1990). Accordingly, the effect of capsaicin desensitisation on O_3-induced BHR in guinea-pigs has been studied.

Airway responsiveness to histamine was assessed as previously described (Yeadon, et al, 1990). In brief, male Hartley guinea-pigs (400-600g) were exposed to O_3 (3ppm, 2h) during quiet tidal breathing, and under subsequent pentobarbitone anaesthesia, increases in pulmonary inflation pressure (PIP) resulting from inhalation of nebulised histamine (1.25-160μg ml^{-1},10s) were recorded. Concentration-effect curves were constructed from each animal using a 20min dose-cycle. The concentration of histamine producing a 20cmH_2O increase in PIP (PC_{20}) was found by interpolation, and this was used as an index of BHR. Capsaicin (CAPS) desensitisation consisted of administration of a total of 110mg kg^{-1} s.c. capsaicin or vehicle given as divided doses over 3d, under protection afforded by terbutaline and theophylline (0.1 and 10mg kg^{-1} i.p., respectively) and halothane/O_2 anaesthesia. 3w later, responses to inhaled capsaicin were characterised by a PC_{20} for capsaicin of 8.5$\pm$5ug ml^{-1} in vehicle-treated animals, but no responses were obtained in desensitised animals with inhaled capsaicin at concentrations of $\leq$300μg ml^{-1}. In guinea-pigs without capsaicin pretreatment, O_3 induced a fall in PC_{20} for histamine, and atropine administration (1mg kg^{-1} i.v.) significantly but incompletely reversed this BHR (Table 1). Atropine had no significant effect in non O_3-exposed animals. In CAPS animals, PC_{20} for histamine was slightly but significantly increased in air-exposed animals, but atropine still had no further effect. However, after O_3 exposure, the fall in PC_{20} was significantly less than in non-desensitised guinea-pigs (Table 1). In addition, when atropine was administered to CAPS animals, O_3 exposure did not result in BHR.

TABLE 1: Responsiveness to inhaled histamine (PC_{20}; ug ml^{-1}, 10s nebulisation. Mean$\pm$sem, n=4-6)

	Pre-capsaicin		Post-capsaicin	
	Air	Ozone	Air	Ozone
-Atropine	81$\pm$11	***4.7$\pm$1.	122$\pm$12	***11$\pm$1.7
+Atropine	161$\pm$40	**21.3$\pm$3.6	131$\pm$23	77$\pm$24

(**, ***; significantly different from non ozone-exposed group, $P<0.01$, $P<0.001$).

In conclusion, capsaicin desensitisation coupled with atropine, but neither alone, prevents BHR to inhaled histamine induced by O_3 exposure. Failure of capsaicin pretreatment alone to abolish BHR after O_3 may reflect the inability of this irritant to deplete fully the neuropeptides in the adult animal, but the data may be interpreted to suggest that during O_3 exposure, neuropeptides are released which augment the cholinergic, pressor component of bronchoconstriction to histamine.

Yeadon, M., Wilkinson, D. & Payne, A. N. (1990). Br. J. Pharmacol., 99, 191P.

74P EOSINOPHIL RECRUITMENT TO LUNG TISSUE INDUCED BY ALLERGEN CHALLENGE IN SENSITISED GUINEA-PIGS: INHIBITION BY BETAMETHASONE AND THE 5-LIPOXYGENASE INHIBITOR, BW B70C

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Both animals and man respond to inhalation of specific allergen with an immediate bronchoconstriction and a 'late-phase' response characterised by an influx of inflammatory cells, predominately eosinophils. To investigate the role of 5-lipoxygenase products in the accumulation of eosinophils in lung tissue after allergen we have explored the effect of BW B70C ((E)-N-(3-(3-(4-fluorophenoxy)phenyl)-1-(R,S)-methylprop-2-enyl)-N-hydroxyurea), a potent, selective, orally available and persistent 5-LO inhibitor (Salmon & Garland, 1991) in guinea-pigs actively sensitised with ovalbumin (1mg i.p. and s.c., used 3w later). Groups of 4-6 animals were pretreated with mepyramine (5mg kg^{-1} i.p.) 30min prior to exposure to nebulised allergen (estimated dose retained 50ug) and 4-48h later, blood and lung diaphragmatic lobe samples were taken for haematology, histology (wax sections, H&E stain) and for spectrophotometric determination of myeloperoxidase (MPO; U g^{-1} wet weight lung) content (Mullane, et al, 1985). In some experiments, animals were treated with BW B70C (10-50mg kg^{-1} p.o., -1h and +12h) or betamethasone (2mg kg^{-1} p.o., -1 and +6h) prior to challenge. Neither agent inhibited MPO directly, thus effects on lung MPO content were ascribed to inhibition of cell recruitment.

Allergen challenge produced moderate respiratory symptoms in most animals, but lung MPO (% of time-matched, unchallenged control) content was significantly increased by 4h (360$\pm$100; $P<0.05$), peaking at 24h (770$\pm$85; $P<0.05$), and incompletely resolved by 48h (240$\pm$49; $P<0.01$). Histology showed that the great majority of infiltrating cells were eosinophils, and 'blinded' assessments of tissues from several study groups supported the use of tissue MPO content as an index of cell infiltration. In blood, total leukocyte count was elevated between 4-16h (2-2.5 fold; $P<0.01$), but was not different from initial values by 24h. These changes were not attributable to haemoconcentration as evidenced by erythrocyte counts. The changes induced by allergen challenge were only seen in animals from whom food was withdrawn overnight prior to allergen: in food-replete animals, only the acute bronchoconstrictor response was observed. In food-withdrawn animals, BW B70C produced a dose-related reduction of the increase in lung MPO after challenge (+24h; 50mg kg^{-1} single dose; control challenge=570$\pm$71% of unchallenged; drug-treated=210$\pm$70%; 77% inhibition, $P<0.001$). A single 10mg kg^{-1} dose of BW B70C was ineffective (620$\pm$86% of control challenged), but 20mg kg^{-1} given in two divided doses resulted in 67% inhibition (250$\pm$40%; $P<0.001$). This suggests that the acute bronchospasm plays no part in the later eosinophil accumulation, since in this study, no BW B70C-treated animals demonstrated acute responses after allergen challenge. In a further study, betamethasone (2mg kg^{-1} p.o., -1 and +6h) completely prevented the allergen-induced increase in lung MPO (+24h; $P<0.001$). If LTB4 is the mediator of eosinophil recruitment, then its release must persist for > 12h, since at the lower dose given (10mg kg^{-1}, p.o.) BW B70C should reduce LTB_4 production in blood for this duration (Salmon & Garland, 1991).

In conclusion, since both a steroid and an inhibitor of 5-LO are able to reduce eosinophil recruitment in experimental allergen challenge, the effect of a 5-LO inhibitor on cell accumulation in late-phases responses to allergen challenge in asthmatics will be of interest.

Mullane, K. M., Kraemer, R., Smith, B. (1985). J. Pharmacol. Methods, 14, 157-67.
Salmon, J. A. & Garland, L. G. (1991). Prog. Drug Res., 37, 9-90.

75P EFFECT OF DEXAMETHASONE AND MOGUISTEINE ON ALLERGEN-INDUCED EARLY BRONCHOCONSTRICTION AND LATE-PHASE AIRWAY LEUCOCYTE RECRUITMENT IN SENSITIZED GUINEA-PIGS

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Moguisteine, a non narcotic antitussive drug, displays airway antiinflammatory properties inhibiting the hyperreactivity, the eosinophil infiltration and the plasma leakage induced by tobacco smoke exposure in anaesthetized guinea pigs (Gallico *et al.*, 1990). We have now extended our studies to an animal model of atopic asthma evaluating the allergen - induced early bronchoconstriction and the late-phase airway leucocyte recruitment in 1% ovalbumin (OA) sensitized (inhalation for 3 min on days 1 and 7) male barrier bred Dunkin Hartley guinea pigs. Seven days later a first group of anaesthetized (pentobarbital 55mg/kg i.p.) forced-ventilated (50 strokes/min; 7ml/kg air) guinea pigs were primed with propranolol (1mg/kg i.v.) and indomethacin (5mg/kg i.v.) 15 min before OA, and mepyramine maleate (0.5mg/kg i.v.) 10 min before antigen exposure (Kreutner *et al.*, 1989). The guinea pigs were then challenged with an aerosol of 1% OA for 5 sec, which provoked an early leukotriene-dependent bronchoconstriction measured as an increase in pulmonary inflation pressure (PIP). Dexamethasone given p.o. 7h before challenge, dose-dependently reduced the PIP increase, ED_{50} 12.2 (C.L. 95%: 4.7-19.2mg/kg) whereas moguisteine (150mg/kg p.o. 2h before challenge), proved inactive. A second group of conscious guinea pigs were primed with mepyramine maleate (10mg/kg i.p.) 30 min before challenge with a 2% OA aerosol for 3 min; bronchoalveolar lavage (BAL) was done 17h and 72h later (Hutson *et al.*, 1988). The OA challenge induced in sensitized guinea pigs a marked increase in the total cell number, neutrophils and eosinophils 17h and 72h after the challenge in comparison with the unchallenged sensitized animals. Moguisteine (150mg/kg p.o., 2h before challenge) and dexamethasone (30mg/kg p.o., 7h before challenge) prevented the cell accumulation in BAL at both times.

CELLS x 10^6 means ± S.E. n = 6	NO CHALLENGE	VEHICLE		DEXAMETHASONE 30mg/kg p.o.		MOGUISTEINE 150mg/kg p.o.	
		17h	72h	17h	72h	17h	72h
TOTAL CELLS	6.1 ± 0.3	13.2 ± 1.6	19.4 ± 1.0	5.7 ± 0.1	6.8 ± 0.4	6.6 ± 0.4	7.5 ± 0.6
NEUTROPHILS	0.05 ± 0.01	1.9 ± 0.3	0.5 ± 0.01	0.07 ± 0.03	0.06 ± 0.01	0.2 ± 0.04	0.2 ± 0.04
EOSINOPHILS	0.07 ± 0.08	3.0 ± 0.5	7.2 ± 0.6	0.5 ± 0.08	0.7 ± 0.08	0.9 ± 0.1	1.3 ± 0.1

These findings indicate that moguisteine is inactive on early-phase bronchoconstriction and therefore does not interfere with the arachidonic acid metabolism. Moguisteine is effective, though to a lesser extent that dexamethazone, in inhibiting allergen-induced leucocyte recruitment in the guinea pig lung, suggesting a specific effect on cell infiltration.

L. Gallico, A. Borghi, C. Dalla Rosa, B. Lumachi, O. Tofanetti and S. Tognella *An. Rev. Respir. Dis.* 141: A654 (1990)
Kreutner A., Sherwood J. and Rizzo C. *Agents and Actions* 28: 173 (1989)
Hutson P.A., Church M.K., Caly T.F., Miller O. and Holgate S.T. *An. Rev. Respir. Dis.* 137: 548 (1988)

76P EFFECT OF A NITRIC OXIDE SYNTHASE INHIBITOR ON BRONCHOCONSTRICTION AND BRONCHODILATATION INDUCED BY ELECTRICAL STIMULATION OF THE VAGI IN THE ANAESTHETISED CAT

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Evidence suggests that non-adrenergic, non-cholinergic (NANC) relaxation due to electrical field stimulation of guinea-pig trachea (Tucker et al., 1990) in vitro may be mediated, in part, by nitric oxide (NO). In addition, NO may also exert an inhibitory modulation of cholinergic contractions in the guinea-pig trachea in vitro (Belvisi et al., 1991). We have investigated the involvement of NO on electrically-evoked bronchoconstriction (BC) and bronchodilatation (BD), in vivo, in anaesthetised cats using the NO synthase inhibitor, L-N^G-nitro-arginine-methyl-ester (L-NAME) (Rees et al., 1990).

Male cats (2.6-3.5kg) were anaesthetised with chloralose (60-80mg kg^{-1} iv) after induction with 5% halothane, paralysed with dimethyl tubocurarine and artificially ventilated. Dynamic compliance (C_{Dyn}), lung resistance (R_L) and cardiovascular parameters were monitored by on-line computer. BC was evoked by bilateral electrical vagal stimulation (BVS) at 1,3,10 & 30Hz, (5ms, 15V for 5s) in cats infused with saline (0.1ml min^{-1} iv). BD_1 at 1,3,10 & 30Hz (1ms) was evoked by BVS in cats with airways constricted with 5-HT (10 or 20μg kg^{-1} min^{-1} iv) after atropine and propranolol (each at 0.5mg kg^{-1} iv). Control frequency-response curves were obtained. L-NAME was infused (3.3mg kg^{-1} min^{-1} iv) 30min before and throughout the second frequency-response curve. As appropriate, L-Arginine (L-Arg) or D-Arginine (D-Arg) were infused (33mg kg^{-1} min^{-1}) 10min before L-NAME.

Both BVS-induced BD and BC were frequency-related. L-NAME had no significant effect on BVS-induced BD ($p>0.05$; n=5). L-NAME infusion had no effect on baseline airway parameters per se, but significantly ($p<0.05$) increased BVS-evoked BC at the higher frequencies: R_L 30Hz, Δ change: pre-saline 0.024±0.007, post-saline 0.03±0.009cmH_2O l^{-1} s^{-1}; pre-L-NAME 0.034±0.015, post-L-NAME 0.105±0.035cmH_2O l^{-1} s^{-1}, n=5). L-NAME significantly increased ($p<0.005$) mean arterial blood pressure (BP) by 18±1.2mmHg. L-Arg had no significant effect infused alone on either R_L or C_D (n=5), but significantly ($p<0.01$) decreased BP by 25±4.8mmHg. In the presence of L-Arg, the effect of L-NAME was attenuated, since there was no longer a significant ($p>0.05$) increase in R_L (At 30Hz, Δ change: pre-L-NAME+L-Arg 0.02±0.004, post-L-NAME+L-Arg 0.04±0.02cmH_2O l^{-1} s^{-1}, n=5). However, the stereoisomer, D-Arg was without effect alone or when infused concomitantly with L-NAME (n=5). D-Arg had no significant effect on BP.

Although L-NAME significantly enhanced the bronchoconstrictor response to BVS, which was reversed by L-Arg but not by D-Arg, it had no significant effect on BVS-induced BD. These results demonstrate that NO may have a neuromodulatory role predominantly by limiting cholinergic bronchoconstrictor responses.

Belvisi,M.G., Stretton,D., Barnes,P.J. (1991) Br.J.Pharmacol., 102, 80P.
Rees,D.D., Palmer,R.M.J., Shulz,R., Hodson,H.F. & Moncada,S. (1990) Br.J.Pharmacol., 101, 746-752.
Tucker,J.F., Brave,S.R., Charalambous,L., Hobbs,A.J. & Gibson,A. (1990) Br.J.Pharmacol., 100, 663-664.

77P EXAMINATION OF THE ROLE OF CHARYBDOTOXIN-INACTIVATED POTASSIUM CHANNELS ON AIRWAY TONE IN THE ANAESTHETIZED GUINEA-PIG

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Recently, the role of the charybdotoxin (ChTX)-sensitive large conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) has become of great interest since it was shown that ChTX inhibits ß-adrenoceptor agonist-induced relaxation of guinea-pig and human airway smooth muscle preparations *in vitro* (Jones *et al*, 1990; Belvisi *et al*, 1991). This suggests that ß-adrenoceptor stimulation may result in the opening of a BK_{Ca} channel in airway smooth muscle. To investigate further this phenomenon we have examined the effects of ChTX on pulmonary inflation pressure (PIP) and on mean arterial blood pressure (BP) in the anaesthetized (pentobarbitone 60 mg/kg, i.p.), ventilated (66 strokes/min, 1 ml/100 g) guinea pig. Basal PIP, the effect of histamine or the reversal of histamine-induced increases in PIP by salbutamol were compared in vehicle (saline)- and ChTX (10 µg, i.t. bolus)-treated animals. The effects of ChTX (10 µg, i.v.) were examined on resting BP and on salbutamol (5 mg/kg, i.v.)-induced hypotension. ChTX or vehicle was administered 2 min before salbutamol and 3 min before histamine challenge.

TABLE 1 **Effects of ChTX on pulmonary inflation pressure in the anaesthetized, ventilated guinea pig**

	% Change in PIP			
	Vehicle	*n*	*ChTX*	*n*
Basal tone	+9.2 ± 0.2	5	+5.2 ± 0.6	5
Histamine(ED_{50})-induced tone	+13.2 ± 3.0	5	+3.4 ± 1.8	5
Histamine(ED_{50})+Salbutamol(5µg i.t.)	-75 ± 6	5	-80 ± 6	5

ChTX (10 µg, i.v., n = 3) increased resting BP by 38 ± 6 %. Salbutamol (n = 4) produced a 24 ± 5 % fall in BP which was completely abolished following ChTX treatment ($p < 0.01$, n = 4). The lack of effect of ChTX on airway tone *in vivo*, cannot be attributed to rapid inactivation of the peptide since it had a very marked effect on the cardiovasculature. Hence, in the anaesthetized guinea pig, BK_{Ca} channels seem not to be important for the regulation of basal or histamine-induced airway tone, nor do they appear to play a part in the action of salbutamol.

Jones, T.R., Charette, L., Garcia, M.L. & Kaczorowski, G.J. (1990) J. Pharmacol. Exp. Ther. 255, 697-706.
Belvisi, M.G., Miura, M., Stretton, D., Yacoub, M.H. & Barnes, P.J. (1991) Eur. Respir. J. 4 (suppl. 14), 306s.

78P EFFECT OF CROMAKALIM ON SYMPATHETIC-INDUCED RELAXATION IN GUINEA-PIG ISOLATED TRACHEA

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The K^+ -channel activator, cromakalim, attenuates vagal constriction in guinea-pig trachea (McCaig & De Jonckheere, 1989), an effect which arises prejunctionally and may involve inhibition of acetylcholine release (Wessler et al., 1992). We have now examined the effect of cromakalim on sympathetic nerve-mediated relaxation in guinea-pig isolated trachea.

Guinea-pig trachea, with right sympathetic supply and recurrent laryngeal nerve, was dissected and responses monitored as reductions in intraluminal pressure (ILP) in the closed, Krebs-filled trachea. Frequency response curves to stimulation of the stellate ganglion (40V, 1ms pulses at 1-50Hz every 2min) were generated in the absence then presence of cromakalim (10^{-7} to 10^{-5} M) and in time-matched controls. The effect of cromakalim on cumulative concentration-effect curves to applied noradrenaline were studied also for comparison.

Cromakalim attenuated sympathetic-induced decreases in ILP in a concentration-dependent manner, shifting the frequency response curve to the right and reducing the maximum response. Attenuation at 20Hz, for example , was 26±22%, 39 ± 12% and 54±14% in the presence of cromakalim 10^{-7}, 10^{-6} and 10^{-5}M, respectively. Cromakalim itself reduced ILP but similar results were obtained when control resting ILP was restored prior to sympathetic stimulation. Responses to applied noradrenaline (10^{-8} to 10^{-5}M) were attenuated to a similar degree by cromakalim.

These results indicate that cromakalim attenuates sympathetic relaxation in isolated guinea-pig trachea. The evidence suggests a postjunctional rather than prejunctional effect on sympathetic neurotransmission since responses to applied noradrenaline were reduced to a similar degree. Since noradrenaline acts in part through the activation of K^+- channels, attenuation may result from competition at this level.

McCaig, D.J. & De Jonckheere, B. (1989) Br. J. Pharmacol. 98, 662-668.
Wessler, I., Pohan, D., Maclagan, J. & Racke, K. (1992) Br. J. Pharmacol. in press

79P CROMAKALIM, BUT NOT MINOXIDIL SULPHATE, INHIBITS THE RELEASE OF INTRACELLULAR CALCIUM IN THE ISOLATED RABBIT AORTA

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We have previously reported that the potassium channel openers cromakalim (CROM) and minoxidil sulphate (MXS) exhibit markedly different profiles of activity in a range of vascular preparations (Wickenden et al., 1991). One possible explanation for these observations is that CROM may be able to inhibit both calcium entry through voltage operated calcium channels (VOC's) and intracellular calcium release whereas MXS may preferentially inhibit the former. In order to test this possibility the ability of CROM and MXS to inhibit responses dependent on either calcium entry through VOC's or the release of intracellular calcium was compared.

Rings of rabbit thoracic aorta were suspended in Kreb's buffer at 37°C under a resting tension of 1g. As a measure of the ability to inhibit responses dependent on the entry of calcium through VOC'S cumulative concentration response curves were determined for either CROM or MXS in KCl (20mM) precontracted preparations. The magnitude of the transient response obtained to noradrenaline (NA, 1uM) in calcium free Kreb's (supplemented with EGTA, 1mM and $MgCl_2.6H_2O$, 10mM) was determined in the presence of a single concentration of CROM, MXS or vehicle in order to assess the ability of these agents to inhibit intracellular calcium release.

CROM and MXS relaxed the KCl (20mM) precontracted aorta with mean (± sem) pD_2 values of 6.79 ± 0.06 (n=8) and 6.22 ± 0.04 (n=8) respectively. The maximum relaxation achieved by CROM (75.2 ± 8.9%) was significantly ($p<0.01$) greater than that achieved by MXS (41.8 ± 6.6%). The mean (± sem) magnitude of the transient response to NA was 54.1 ± 5.6%, 40.4 ± 5.6%, 28.9 ± 3.1% ($p<0.01$), 21.2 ± 4.1% ($p<0.01$) and 28.9 ± 4.8% ($p<0.01$) of the control response to NA in normal Kreb's buffer in the presence of vehicle, 0.3, 1, 3 and 10uM CROM respectively (n=4). In contrast MXS had no significant effect on the transient response to NA. The mean (± sem) magnitude of the transient response was 46.3 ± 2.5%, 49.2 ± 4.4%, 48.2 ± 3.7% and 53.4 ± 4.1% of the control response to NA in normal Kreb's in the presence of vehicle, 0.3, 3 and 30uM MXS respectively (n=4).

In summary, CROM but not MXS appears able to inhibit the release of calcium from NA sensitive intracellular stores in the rabbit aorta. Both agents apparently inhibit the entry of calcium through VOC's in the same preparation. Differences in the ability to inhibit the release of intracellular calcium may therefore underlie the different pharmacological profiles of these agents in different vascular preparations.

Wickenden, A.D., Grimwood, S., Grant, T.L. and Todd, M.H. (1991). Br.J.Pharmacol., 103, 1148-1152.

80P RELAXIN, A POTASSIUM CHANNEL OPENER IN THE ISOLATED RAT UTERUS?

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The uterine relaxant hormone relaxin is antagonized by glibenclamide *in vivo* in the rat suggesting a role for glibenclamide-sensitive potassium (K+) channels in relaxin's mechanism of action (Downing & Hollingsworth, 1991). Further investigation of relaxin as a K+-channel opener (KCO) was performed with the isolated rat uterus in comparison with BRL38227, a KCO (Clapham *et al.*, 1991), and salbutamol.

Uterine strips from non-pregnant rats pre-treated with oestradiol benzoate were mounted for isometric tension recording in the longitudinal plane. 3 cumulative concentration-effect (C-E) curves were constructed to KCl (10, 20, 40 and 80 mM). For 30 min prior to and during the 3rd C-E curve, tissues were incubated with BRL38227 (0.2 or 2.0 μM), salbutamol (1.5 or 15.0 nM), porcine relaxin (20 or 200 ng/ml) or vehicle. These concentrations are 1 and 10 times the IC_{50} for inhibition of oxytocin-induced spasm. The magnitude of the spasms induced by each of the four KCl concentrations in curve 3 were compared with those in curve 2 by paired Student's *t*-test. Only the spasms to KCl 20 and 40 mM were significantly reduced by BRL38227 whereas the spasms to all KCl concentrations were reduced by salbutamol and relaxin.

Single cumulative C-E curves were constructed to BRL38227, salbutamol or relaxin against oxytocin (0.2 nM)-induced spasms and the IC_{50} calculated. Strips were pre-incubated with tetraethylammonium (TEA, 10 mM), glibenclamide (10 μM) or vehicle for 30 min. For each relaxant, IC_{50} values in vehicle- and antagonist-exposed tissues were compared by paired Student's *t*-test and $\log_{10}$ concentration ratios (CR) calculated. The C-E curve to BRL38227 was shifted ($P<0.001$) to the right by both TEA and glibenclamide ($\log_{10}$ CR 2.09±0.14; 2.07±0.22). Comparatively, only a small reduction in sensitivity was induced by TEA and glibenclamide to salbutamol (0.73±0.10 [$P<0.01$]; -0.05±0.07 [N.S.] respectively) and relaxin (0.87±0.35; 0.28±0.08 [$P<0.05$] respectively).

Relaxin's profile in the isolated uterus does not reflect that of a KCO (Piper *et al.*, 1990). Relaxin did not selectively inhibit spasm to low concentrations of KCl and it was only slightly antagonized by TEA and glibenclamide. The profile of relaxin more closely resembles that of salbutamol than that of BRL38227. The discrepancy between this data and that *in vivo* may be that glibenclamide *in vivo*, as an antagonist of relaxin, was not acting as a K+-channel blocker or the action of relaxin *in vitro* differs from that *in vivo*.

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Clapham, J.C. *et al.* (1991) Arzneim. Forsch. **41**, 385-391.
Downing, S.J. & Hollingsworth, M. (1991) Br. J. Pharmacol. **104**, 71-76.
Piper, I. *et al.* (1990) Br. J. Pharmacol. **101**, 901-907.

81P CONTRIBUTION OF ACETYLCHOLINE AND ATP TO THE NEUROGENIC CONTRACTION OF ISOLATED URINARY BLADDER SMOOTH MUSCLE FROM ADULT AND NEONATAL RABBITS

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In rabbit urinary bladder smooth muscle the non-cholinergic portion of the neurogenic contraction can be blocked by arylazidoaminoproprionyl-ATP, ANAPP3 (Theobald & Hoffman, 1986), or by purinoceptor desensitisation using α,β,methylene-ATP (Kasakov & Burnstock, 1982) and is therefore thought to be due to ATP acting on P_{2x}-purinoceptors. We have recently shown that exogenous ATP is relatively more potent in producing contraction of bladder muscle from neonates than from adult rabbits (Sneddon & McLees, 1991). We have now compared the neurogenic contractions obtained in isolated urinary bladder smooth muscle to test the possibility that ATP makes a greater contribution to neurogenic contractions in the neonate.

Longitudinal strips of detrusor muscle 2 mm wide and 1-2 cm long were prerared from adult (>14weeks) or neonatal (<6 days) rabbits and maintained at 35°C in a physiological salt solution and bubbled with 95% O_2, 5% CO_2. Field stimulation was applied for 20s to produce neurogenic contractions using a pulse width of 0.2 ms at frequencies from 1 - 16 Hz. In each tissue a frequency response curve was obtained before and after the introduction of either atropine (10^{-6}M) or desensisitation of P_{2x}-purinoceptors by addition of several, cumulative doses of 10^{-6}M α,β,methylene-ATP (as described by Kasakov & Burnstock, 1982). Each of these procedures was shown in preliminary experiments to be selective for inhibiting responses mediated by acetylcholine and ATP respectively. In each tissue the contractile response to 100 mM KCl was obtained, and all responses to nerve stimulation were expressed as a pecentage of this response. Data is presented as mean ± s.e. mean and compared using Student's t-test for unpaired data.

In the adult tissues the control contractile responses to nerve stimulation were biphasic, with an initial, rapid phase reaching a peak within 4s, before declining to a lower level of tension. In the neonatal tissue the responses had a slower rise, reaching a maintained plateau in about 10s. The magnitude of contractile responses to field stimulation was greater in neonatal tissues than in tissue from adult rabbits, and this difference was statistically significant at stimulation frequencies of 1, 2 and 4Hz (e.g. at 4Hz in the adult the mean response was 42.0±5.6% and in the neonate 75.6±9.1%, $p<0.01$, n=11). In the adult tissues, introduction of 10^{-6}M atropine greatly reduced the maintained portion of the neurogenic contraction, but left a large, initial, phasic contraction unchanged (e.g. at 4Hz the mean control response was 51.1±7.8% and after atropine 58.6±12.3%, n=6). In the neonatal tissue, however, atropine reduced the magnitude of all parts of responses to all stimulation frequencies, and this reduction was statistically significant at 1, 2, 4, and 8Hz (e.g. at 4Hz the control response was reduced from 128±29% to 26.8±10.2%, $p<0.01$, n=6). In the adult tissues, α,β,methylene-ATP reduced particularly the initial phase of the response, and this reduction was statistically significant at 1, 2 and 8Hz (e.g. at 2Hz the control response was reduced from 43.6±12.7% to 12.4±4.3%, $p<0.05$, n=6). In the neonatal tissues α,β,methylene-ATP produced a profound reduction in all parts of the contractile response obtained at all frequencies, which was statistically significant at 1, 2, 4 and 8Hz (e.g. at 4Hz the control was reduced from 122±28% to 31.4±25.4%, $p<0.05$, n=5). We conclude that the contribution made by ATP and acetylcholine to neurogenic contractions of the urinary bladder changes during development.

Kasakov, L. & Burnstock, G. (1982) *Eur. J. Pharmacol.*, **86**, 291-293.
Sneddon, P & McLees, A. (1991) *Proc. British Pharmacological Society London Meeting*, P111.
Theobald, R.J. & Hoffman V. (1986) *Life Sci.*, **38**, 1591-1595.

82P RENAL OUTFLOW OF DOPAMINE: A COMPARATIVE STUDY WITH L-DOPA AND GAMMA-GLUTAMYL-L-DOPA

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There is evidence to suggest that endogenous dopamine may be important in the renal handling of sodium, through the activation of specific tubular D_1 receptors. Gamma-glutamyl-L-DOPA (GluDOPA) is a synthetic dipeptide which undergoes conversion into L-DOPA by the enzyme gamma-glutamyl transpeptidase in the brush border of renal tubular cells; this offers the possibility to selectively increase the formation of dopamine in renal tissues (Worth et al., 1985). However, in human kidney preparations it has been suggested that the dopamine with origin in GluDOPA, in comparison with that formed from L-DOPA, is less available to leave the compartment where the synthesis has occurred (Soares-da-Silva et al., 1992). In the present work the outflow of newly-formed dopamine in cortical fragments of the rat kidney using L-DOPA and GluDOPA as precursors for dopamine has been studied; the outflow of DOPAC, the deaminated metabolite of DA, was also measured. In some experiments, rats were given L-DOPA (10 and 30 mg/kg, i.p.) or GluDOPA (16.7 mg/kg, i.p.) and sacrificed 15 min later, the kidneys removed and cortical fragments prepared and placed in perifusion chambers. In another series of experiments, cortical fragments of rat kidney were incubated for 15 min with L-DOPA (50 and 100 μM) or GluDOPA (83.5 and 167 μM) and subsequently treated as described above. In both conditions, kidney fragments were perifused with warm (37°C) and gassed (95% O2 and 5% CO2) Krebs' solution containing 50 μM tropolone and allowed a 30 min stabilization period; thereafter five consecutive 10 min fractions of the perifusate were collected. The levels of dopamine and DOPAC (in nmol/g/10 min) in the perifusate were logaritmically transformed, plotted against time of perifusion and the constant rates of dopamine and DOPAC outflow calculated. The fractional outflow was also calculated. The outflow of dopamine and DOPAC was found to progressively decline, the constant rates of loss (DA, $k=0.021\pm0.002$; DOPAC, $k=0.019\pm0.002$) being similar using either L-DOPA or GluDOPA. However, with both precursors the fractional outflow for DOPAC (10.6±1.4%) was nearly twice that for dopamine (5.8±0.8%) With GluDOPA, both tissue and perifusate levels of dopamine were nearly twice those found with an equimolar concentration of L-DOPA. In addition, the DOPAC/dopamine ratios in tissues (0.36±0.04) and in the perifusate (0.85±0.09) of experiments performed with L-DOPA were significantly higher ($P<0.01$) than those found with GluDOPA (tissue, 0.14±0.01; perifusate, 0.35±0.05). These results show that the amount of newly-formed dopamine leaving tubular epithelial cells is a constant source for DOPAC. It is also suggested that dopamine with origin in GluDOPA is less deaminated than that resulting from L-DOPA; it appears that this different behavior might be related to differences in the availability of dopamine to leave the cell or to differences in the access to monoamine oxidase.

Worth, D.P., Harvey, J.N., Brown, J. & Lee, M. (1985). Clin. Sci., 69, 207-214.
Soares-da-Silva, P., Fernandes, M.H. & Pestana, M. (1992) Br.J.Clin.Pharmacol. "in press".

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83P INFLUENCE OF 3-0-METHYLDOPA ON THE SYNTHESIS OF DOPAMINE IN THE RAT KIDNEY

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Renal synthesis of dopamine, from decarboxylation of circulating dihydroxyphenylalanine (DOPA), may be physiologically important due to the natriuretic and diuretic effects of the amine (Siragy et al., 1989). Decarboxylation of L-DOPA is believed to represent the major pathway of its metabolism (Fernandes et al., 1991); however, other pathways may arise, namely, convertion to 3-O-methylDOPA (3OMD), by cathecol-O-methyltransferase. In addition, 3OMD has been shown to competitively inhibit the active transport of L-DOPA in some non-renal tissues; 3OMD has also been shown to be decarboxylated by the aromatic L-amino acid decarboxylase (Männistö & Kaakkola, 1989). The present work has examined the effect of 3OMD in the synthesis of dopamine from L-DOPA in the rat kidney. Renal cortical slices were incubated with 10μM L-DOPA for 5 to 30 min in the absence (control conditions) or in the presence of 10 and 50μM 3OMD. L-DOPA, dopamine and their methylated and deaminated metabolites, 3OMD, 3-methoxytyramine (3-MT), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were assayed by h.p.l.c. with electrochemical detection. The amount of dopamine and DOPAC formed in control kidney slices loaded with 10 μM L-DOPA, during a 15 min incubation period, was, respectively, 12.9$\pm$1.4 and 5.0$\pm$0.6 nmol/g. In the presence of 10μM 3OMD, tissue levels of dopamine and DOPAC were, respectively, 11.9$\pm$1.5 and 4.5$\pm$0.5 nmol/g; in the presence of 50μM 3OMD the tissues levels of dopamine and DOPAC were, respectively, 18.2$\pm$1.8 and 5.6$\pm$1.3 nmol/g. The amount of 3OMD accumulated in kidney tissues loaded with 10μM L-DOPA during 15 min was found to be very low (9.7$\pm$1.1 pmol/g). Also, very low amounts of other methylated compounds were found to occur (63.2$\pm$11.4 pmol/g and 33.1$\pm$3.4 pmol/g for 3-MT and HVA, respectively). Similar results were obtained while using other incubation periods (5, 10, 20 and 30 min). In the cortex, the external medulla and the internal medulla of rats injected with 30mg/kg L-DOPA the levels of dopamine were, respectively, 87.4$\pm$6.2, 93.5$\pm$12.1 and 169.8$\pm$23.9 nmol/g and for DOPAC these values were, respectively, 39.3$\pm$3.7, 36.1$\pm$3.3 and 110.1$\pm$22.5 nmol/g; in rats injected with L-DOPA (30mg/kg) plus 30mg/kg 3OMD no significant differences were observed in the accumulation of dopamine and DOPAC in the three renal areas studied. These results show that the renal synthesis of dopamine from L-DOPA, both under *in vitro* and *in vivo* experimental conditions, does not appear to be affected by 3OMD and methylation does not represent an important alternative mechanism of L-DOPA metabolism under *in vitro* experimental conditions.

Fernandes, M.H., Pestana, M. & Soares-da-Silva, P. (1991) Br. J. Pharmacol.,102, 778-782.
Siragy , H.M., Felder, R.A., Howell, N.L., Chevalier, R.L., Peach, M.J. & Carey, R.M. (1989) Am. J. Physiol., 257, F469-F477.
Männistö, P.T. & Kaakkola, S.(1989) TiPS, 10, 54-56.

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84P *IN VIVO* UROSELECTIVITY OF ALFUZOSIN COMPARED TO PRAZOSIN AND TERAZOSIN

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Alfuzosin, a selective α_1-adrenoceptor antagonist, shows therapeutic efficacy in the symptomatic treatment of benign prostatic hypertrophy (BPH), at doses which do not produce hypotensive effects (Jardin et al., 1991). In animals, i.v. administration of alfuzosin preferentially blocks α_1-adrenoceptors of lower urinary tract rather than those of vascular smooth muscle (Lefèvre-Borg et al., 1990). The aim of this study is to compare, by i.v./i.a. injection and by oral administration, the tissue selectivities of alfuzosin, prazosin and terazosin, three α_1-antagonists used in the treatment of BPH.

Male cats (3.5 - 4.5 kg) were anaesthetized with pentobarbitone (42 mg/kg i.p.). A catheter was introduced into the urethra and secured by a ligature around the bladder neck. Animals were pretreated with i.v. propranolol (0.75 mg/kg) + atropine (0.5 mg/kg) + chlorisondamine (0.5 mg/kg). Increases in urethral pressure (UP) were evoked by electrical stimulation of sympathetic fibers of the hypogastric nerve (5 V, 20 Hz, 2 ms over 15 sec) before and after i.v. or intraduodenal administration of alfuzosin, prazosin or terazosin. To measure effects on blood pressure, unrestrained conscious spontaneously hypertensive rats (SHR: 10 - 12 months old), prepared for continuous arterial blood pressure recording, were dosed i.a. or p.o. with alfuzosin, prazosin or terazosin. The doses of each compound producing a 50% reduction in UP (UP_{50}) and a 20% decrease in arterial blood pressure (AP_{20}) in SHR were established and are shown in the table.

	UP_{50} mg/kg iv	AP_{20} mg/kg ia	Ratio AP/UP	UP_{50} mg/kg id	AP_{20} mg/kg po	Ratio AP/UP
ALFUZOSIN	0.08	0.26	3.2	0.36	4.0	11
PRAZOSIN	0.016	0.016	1	0.12	0.13	1
TERAZOSIN	0.07	0.07	1	0.12	0.42	3.5

This study shows that alfuzosin is able to decrease the urethral resistance at doses lower than those which produce effects on blood pressure. In this respect, alfuzosin is more uroselective than prazosin and terazosin, regardless of route of administration. The observed uroselectivity of alfuzosin is consistent with the clinical results and differentiates this α_1-adrenoceptor antagonist from prazosin and terazosin.

Lefèvre-Borg F. et al. (1990) Prostate and α-Blockers, Ed. Adis, Chester, 73-86.
Jardin A. et al. (1991) Lancet 337, 1457-1461.

85P ENHANCEMENT OF α_1-ADRENOCEPTOR MEDIATED CONTRACTIONS BY PARTIAL DEPOLARISATION OF RAT AORTIC SMOOTH MUSCLE

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α-agonists contract the rat aorta by activating α_1-adrenoceptors (Beckeringh et al 1984). These promote extracellular Ca influx for contractions through plasmalemmal Ca channels which may be activated by agonist-induced membrane depolarisation, and they also cause intracellular Ca release following agonist-stimulated hydrolysis of membrane phosphatidylinositol (Morel & Godfraind 1991; Wilson et al 1991). Subthreshold KCl concentrations which partially depolarise smooth muscle without themselves producing contractions are reported to enhance α_1-vasoconstrictor responses in some blood vessels (e.g. Nelson et al 1988). Here, we have investigated if similar effects are demonstrable in the rat aorta.

Thoracic aortic rings (male Sprague-Dawley rats, 300-500g) were suspended for isometric recording of contractions under 1g resting tension in organ baths with Krebs buffer, normally containing 4.7 mM KCl. The buffer also contained 1μM propranolol (to block β receptors) and 0.1mM ascorbic acid (to inhibit noradrenaline oxidation). Rings were 'rubbed' to remove endothelium before use, and thus was confirmed by the absence of relaxations to 1μM acetylcholine in pre-contracted (35mM KCl) preparations. Contractions to noradrenaline (NA), phenylephrine (PE), methoxamine (MA) and clonidine (CL) were studied by cumulative dosing schedules. Dose response curves (DRCs) for each agonist were obtained initially in Krebs with 4.7mM KCl and subsequently in higher (14.7mM) KCl Krebs. Although not producing contractions per se, the higher KCl concentration enhanced responses to NA, PE and MA, characterised by leftward parallel shifts of DRCs indicating around 2 - 2.5-fold increases in sensitivity to agonists without changes in maximal responses (n = 8 tissues). However, with CL, maximal responses were additionally increased by around 3-fold. The influence of the Krebs KCl concentration upon contractile effects of submaximal doses of NA (10^{-8}M) and CL (10^{-7}M), induced by adding $CaCl_2$ (2.5mM) to tissues in Ca-free Krebs, was also studied. These contractions were significantly greater in 14.7mM than in 4.7mM KCl, by 50 ± 12% for NA and 98 ± 16% for CL (n = 8).

Partial depolarisation of rat aortic smooth muscle enhances α_1-mediated contractions to an extent which may depend upon the nature of the agonist used. Contractions resulting from Ca addition to tissues in Ca-free medium depend upon α_1-agonist induced extracellular Ca influx. Partial depolarisation could enhance these contractions, at least in part, by reducing the degree to which α_1-agonists must depolarise the muscle membrane before voltage-dependent Ca channels open.

Beckeringh, J.J., Thoolen, M.J.M.C., de Jonge, A., Wilffert, B., Timmermans, P.B.M.W.M. & van Zwieten, P.A. (1984) J. Pharmacol. Exp. Ther. 229, 515-521.
Morel, N. & Godfraind, T. (1991) Br. J. Pharmacol. 102, 467-477.
Nelson, M.T., Standen, N.B., Brayden, J.E. & Worley, J.F. (1988) Nature, 336, 382-385.
Wilson, V.G., Brown, C.M. & McGrath, J.C. (1991) Exp. Physiol. 76, 317-346.

86P DOSE-RELATED EFFECTS OF DOBUTAMINE ON DIASTOLIC FUNCTION IN THE MINI-PIG

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(±)Dobutamine is a synthetic catecholamine which possesses predominantly β_1 and β_2-adrenoceptor activity (Hayes et al., 1985). While its positive inotropic actions are well established, its effects on diastolic function remain to be clarified. We investigated the effects of dobutamine on diastolic function by analysis of left ventricular volume curves, together with indices of systolic function. Mini-pigs (10 - 13 kg) were anaesthetised (pentobarbital 25 mg/ kg i.m. and 0.15 mg/ kg/ min i.v.) and ventilated. A Swan-Ganz thermodilution catheter was placed in the right heart for cardiac output determination. A pressure-sensitive tip catheter was placed in the left ventricle, together with an 8-electrode impedance catheter (connected to a Leycom Sigma 5 processing unit) for continuous measurement of ventricular volume. Dobutamine HCl was infused (2- 16 nmol/kg/min i.v.) and the following parameters determined in real time; cardiac output (CO), heart rate (HR), stroke volume (SV), end-diastolic volume (EDV), ejection fraction (EF), mean arterial blood pressure (MABP) left ventricular dp/dt_{max}, time-constant of left ventricular isovolumic pressure decay (τ_L), diastolic filling time (DFT), early peak filling rate (PFR) and time to early peak filling rate (TPFR).

Dose	CO	HR	SV	EDV	EF	MABP	dp/dt_{max}	$-dp/dt_{max}$	τ_L	DFT	PFR	TPFR
nmol/kg/min	l/min	bpm	ml	ml	%	mm Hg	mm Hg/s	mm Hg/s	ms	ms	ml/s	ms
control	1.12±.02	102±2	11.0±.1	31.5±.8	35.3±0.7	100±2	1474±30	2041±34	32.6±.3	202±6	88±2	82±3
2	1.30±.03	113±2*	11.5±.2	29.7±.9*	39.6±1.1*	109±3	1979±60**	2333±38**	31.3±.3**	156±5*	104±5	76±4
4	1.39±.04*	119±2	11.6±.2*	27.6±.9**	43.3±1.4*	111±4	2544±112**	2487±62*	29.6±.3**	155±9*	114±4**	71±4
8	1.56±.04**	134±2**	11.6±.2	24.5±1***	49.2±1.6**	112±3*	3679±160**	2761±78**	27.4±.3**	132±6*	119±3***	67±5*
16	1.73±.05**	148±3**	11.6±.2*	22.4±1***	54.7±2.3**	114±3*	4695±245**	2917±110*	25.4±.4**	112±8*	126±4***	61±5*

Data are shown as the mean ±s.e.mean of 6 animals, with * P<0.05; ** P<0.01: *** P<0.001

Dobutamine, over the dose range 2- 16 nmol/kg/min, elicited an increase in cardiac output by increasing heart rate with a minor increase in stroke volume. End-diastolic volume was reduced due to increased ejection fraction and contractility, while isovolumic left ventricular pressure decline was accelerated. Stroke volume was maintained at high doses despite the reduction in diastolic filling time, by means of an earlier and faster peak filling rate. This study demonstrates that dobutamine has significant and dose-related effects on diastolic as well as systolic function in the mini-pig.

Hayes J.S.,Bowling, N. & Pollock,G.D. (1985) *J. Pharmacol. Exp. Ther.* **235, 58 - 65**

87P DO SALMETEROL AND FORMOTEROL POSSESS AGONIST ACTIVITY AT ATYPICAL β-ADRENOCEPTORS IN GUINEA-PIG GASTRIC FUNDUS?

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We have previously demonstrated that the β-adrenoceptors mediating relaxation of guinea-pig gastric fundus (GPGF) are 'atypical' in nature (Coleman et al, 1987; Nials et al. this meeting). In the present series of experiments, we have compared the relaxant activities of two newly developed β-adrenoceptor agonists, formoterol (Form) and the long-acting β-adrenoceptor agonist, salmeterol (Salm) on this preparation.

Strips of GPGF were prepared as described by Nials et al, (1992), and contracted by the addition of a submaximally-effective concentration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 0.3-3.0μM).

In the presence of propranolol (1μM), isoprenaline (Iso, 0.1-30μM), Form (0.1-30μM) and Salm (0.3-30μM) all caused concentration-related relaxations of $PGF_{2\alpha}$-induced tone, the mean EC_{50} values being 1.8μM (1.4-2.5μM, n=40), 1.2μM (0.5-3.1μM, n=7) and 6.2μM (2.0-18.8μM, n=6) respectively. No differences were observed in the maximum responses achieved by the three agonists. The putative 'atypical' β-adrenoceptor blocking drug, alprenolol (0.1-10μM; Blue et al, 1990) caused concentration-related rightward shifts of the concentration-effect curves to Iso (pA_2 6.0, slope 0.85, n=5) and Form (pA_2 6.1, slope 0.97, n=4). However, even at a concentration of 10μM, alprenolol failed to significantly antagonize responses to salmeterol. We went on to investigate the effect of prolonged exposure to the selective 'atypical' β-adrenoceptor agonist, BRL35135, on subsequent responses to Iso, Form and Salm. Pretreatment of GPGF with BRL35135 (30μM) for 45 min, followed by repeated washing over a further 30 min, resulted in a subsequent substantial loss of potency in both Iso and Form, seen as a rightward shift in the concentration-effect curves, with variable degrees of reduction in response maxima. In contrast, similar curves to Salm were unaffected.

These data suggest that like Iso, Form relaxes GPGF by an agonist action at 'atypical' β-adrenoceptors, and is at least as potent as Iso in this regard. In contrast, the rather weaker activity of Salm is not mediated by 'atypical' β-adrenoceptors. Salm has previously been reported to exhibit non-β-adrenoceptor mediated inhibitory effects on human lung macrophages (Baker & Fuller, 1990), but as in GPGF, this is only observed at concentrations far in excess of those required to exhibit specific β_2-adrenoceptor agonist activity.

Baker, A.J. & Fuller, R.W. (1990). Am. Rev. Resp. Dis., 141, A394.
Blue, D.R., Bond, R.A., Adham, N., Delmendo, R., Michel, A.D., Eglen, R.M., Whiting, R.L. & Clarke, D.E. (1990). J. Pharmac. Exp. Ther., 252, 1034-1042.
Coleman, R.A., Denyer, L.H. & Sheldrick, K.E. (1987). Br. J. Pharmacol., 90, 40P.
Nials A.T., Barker, R.C. Coleman, R.A. & Vardey, C.J. (1992, this meeting).

88P ANGIOTENSIN II DECREASES, WHILE CAPTOPRIL INCREASES, MYOCARDIAL NORADRENALINE UPTAKE: A MECHANISM FOR ACE INHIBITORS REDUCING CARDIAC DEATH?

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Previous in vitro studies have suggested that angiotensin II (AII) inhibits myocardial noradrenaline uptake (Peach et al 1969). In 1986 Sumners and Raizada showed that AII had a biphasic effect on NA uptake, reporting a stimulatory effect in the short term and an inhibitory one 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 has been to develop an animal model which allows us to examine the effect of AII on myocardial NA uptake in vivo. We have previously shown that with short term infusions (10 mins) of AII had no apparent effect on myocardial NA uptake (Arnott and Struthers 1990); but we have now studied a longer term infusion and studied the effect of captopril.

Male Sprague-Dawley rats (250-400g) were anaesthetised with Urethane (25% w/v, 6ml/kg) and jugular, femoral and carotid catheters inserted. The carotid catheter was connected via transducer to a chart recorder to allow monitoring of both heart rate and blood pressure. After anaesthesia the animals were left undisturbed for forty minutes before an infusion of AII (20ng/kg/min or 80ng/kg/min) was begun through the femoral line. Thirty minutes later the animals received a bolus dose of [3H]-NA (10μCi/kg) through the jugular line. After a further 3.5 minutes the rats were sacrificed by anaesthetic overdose. The hearts were then removed, blotted, weighed and homogenised in ice-cold 0.4N perchloric acid before counting in the scintillation counter. We also investigated the effect of the angiotensin converting enzyme, Captopril on [3H]-NA uptake by pre-dosing animals with a 2mg/ml solution of captopril in their drinking water for the 4 days prior to cannulation. This solution was sweetened on the final day to encourage drinking.

The sub-pressor dose of AII (20ng/kg/min) caused a significant reduction ($p < 0.05$) in the amount of [3H]-NA taken up by the myocardium. This dose reduced myocardial uptake from 198002 $\pm$6760 dpm/g/wet weight tissue to 151602 $\pm$5987 (mean $\pm$ s.e. mean). Conversely when a higher (pressor) dose of AII (80ng/kg/min) was infused for thirty minutes no significant change in the uptake of [3H]-NA was observed when compared to the control situation (saline only infused). In the experiments in which the effect of captopril on myocardial NA uptake was examined, it was found that this angiotensin converting enzyme inhibitor caused a significant increase ($p < 0.05$) in the amount of [3H]-NA taken up into the myocardium, the levels rising from 150178 $\pm$22595 dpm/g/wet weight tissue in the control to 203228 $\pm$15231 dpm/g/wet weight tissue in the treated animals.

We have therefore found that AII decreases while captopril increases myocardial uptake of NA in vivo. This animal model is akin to ischaemia and infarction where NA floods into the myocardial cavity. AII therefore is liable to lead to the prolongation and potentiation of the released NA whereas captopril encourages its uptake and disposal. This captopril induced reuptake of myocardial NA may be one mechanism whereby ACE inhibitors reduce mortality in chronic heart failure.

Arnott, M.R. & Struthers, A.D. (1990) Br. J. Pharmacol., **101**, 542P
Cleland, J.G.F., Dargie, H.J., Ball, S.G., Gillen, G., Hodsman, G.P.P., Morton, J.J., East, B.W., Robertson, I., Ford, I & Robertson, J.I.S. (1984) Br. Heart J. **54**, 305-312
Peach, M.J., Bumpus, F.M. & Khairallah, P.A. (1969) J. Pharmac. exp. Ther. **167**, 291-299
Sumners, C. & Raizada, M.K. (1986) Am. J. Physiol. **250**, (Cell Physiol. 19), C236-C244

89P EFFECT OF THE DEOXYRIBONUCLEIC ACID DERIVATIVE DEFIBROTIDE ON PLASMA FIBRINOLYTIC ACTIVITY IN THE RAT

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It is well established that compromised fibrinolytic activity, resulting from changes in plasma levels of tissue plasminogen activator (tPA) or plasminogen activator inhibitor (PAI-1), is asso ciated with the development of thrombotic disorders. Defibrotide (Crinos, Italy) is a deoxyribonucleic acid derivative of bovine lung that has been shown to exhibit anti-thrombotic activity attributed to a pro-fibrinolytic action via modulation of endothelial cell function and enhanced prostacyclin formation.

Following treatment of rats with Defibrotide we have investigated changes in the fibrinolytic response of plasma using a functional chromogenic assay for tPA (IU/ml) and PAI activity (AU/ml, one AU quenching one IU of tPA activity) and the euglobulin clot lysis time assay (ECLT). Anti-thrombotic activity was also investigated in anaesthetised rats measuring pulmonary deposition of ^{125}I-fibrin(ogen) in response to intravenous thrombin infusion.

Acute administration of Defibrotide resulted in increased plasma fibrinolytic activity, evidenced by a marked decrease in ECLT times, a pronounced reduction in plasma PAI activity accompanied by a net increase in tPA activity, and did not involve stimulation of prostacyclin synthesis (Table 1).

Table 1. Effect of Defibrotide on plasma fibrinolytic activity Values Mean ± S.E.M n=6 (*p<0.05)

	tPA (IU/ml)	PAI (AU/ml)	50% ECLT (mins)
Control	9.7 ±1.8	14.5 ±1.7	131.0 ±6.5
Defibrotide 25mg/kg	14.6 ±1.7*	8.9 ±3.0	50.2 ±2.5*
Defibrotide 50mg/kg	19.7 ±2.3*	6.3 ±1.8*	32.5 ±3.5*
Defibrotide 100mg/kg	16.6 ±2.0*	4.1 ±1.6*	41.2 ±2.6*
Defibrotide 50mg/kg + Indomethacin 25mg/kg (i.p. one hour prior to defibrotide)	-		30.5 ±2.4*

Intravenous infusion of thrombin (2000 U/Kg/hr; 15 mins) to animals pretreated with ^{125}I fibrinogen (100ug i.v.) resulted in pulmonary accumulation of labelled fibrin(ogen). Treatment with Defibrotide (20mg/kg/min, i.v infusion for the duration of experiment) attenuated this response.

These data suggest significant increased fibrinolytic and anti-thrombotic activity can result from reduced plasma PAI activity following treatment of rats with the deoxyribonucleic acid derivative Defibrotide.

90P EFFECT OF PLATELET ACTIVATING FACTOR ON CIRCULATING WHITE BLOOD CELL COUNT, PLATELET COUNT AND ON PLASMA tPA AND PAI-1 ACTIVITY IN THE RAT

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Platelet activating factor (PAF) is a potent agonist on white blood cells (WBC) and platelets (PL) in guinea pig, rabbit and human. However rodent PL have been reported as unresponsive to PAF *in vitro* whilst WBC sensitivity to PAF is unclear. The aim of the present study was to establish the effect of PAF on rat PL and WBC *in vivo* by monitoring circulating blood cell counts alongside estimation of plasma tPA and PAI-1 activity since levels of these agents have been claimed to be influenced by the activation state of WBC or PL *in vivo*.

PL and WBC counts were monitored using the on line continuous cell counting techniques (Smith and Freuler 1973). Activity of tPA and PAI-1 was estimated 2, 30, 60 and 180 mins after PAF dosing using a commercially available amidolytic assay with chromogenic substrate. Doses of PAF were administered to rats by intravenous injection. Results were subjected to unpaired Student's t-test.

PAF (0.3-3μg/kg) had no significant effect on circulating PL counts. In these same animals however a subsequent 40μg/kg collagen i.v. bolus injection induced a substantial (32.7± 2.2%) fall in PL count confirming sensititvity to a recognised agonist.

PAF did however cause a profound and dose-related fall in circulating WBC count (Table 1) accompanied by a dose-dependent delay in time to establishing a new baseline count. These responses were also accompanied at the higher PAF dosed by a significant rise in tPA activity but no change in PAI-1 activity.

Table 1 Acute effect of PAF on circulating WBC, plasma tPA and plasma PAI-1

PAF Dose (μg/kg)	Fall in WBC Count (Mean % ± sem)	Plasma tPA activity (Mean IU/ml ± sem)	Plasma PAI-1 activity (Mean AU/ml ± sem)	
Vehicle	-	8.3 ± 1.7	31.4 ± 3.2	
0.01	7.8 ± 2.6	n.d.	n.d.	
0.03	15.1 ± 3.9	n.d.	n.d.	
0.10	26.2 ± 2.6	n.d.	n.d.	
0.30	29.4 ± 4.0	10.6 ± 2.3	29.3 ± 2.9	
1.00	46.9 ± 7.8	16.8 ± 2.5	26.8 ± 5.2	
3.00	63.4 ± 6.7	29.6 ± 3.3	27.1 ± 2.9	n.d. = not determined

At longer time points (30-180 mins) there was no effect of PAF (3.0μg/kg) on plasma tPA or PAI-1 activity.

We conclude that in the rat PAF induces a dose dependent fall in WBC but not PL accompanied by increases in plasma tPA activity at higher doses but little effect on PAI-1. It remains to be established whether the observed tPA increase is via an effect on WBC or through a direct action at the vascular endothelium.

91P HETEROGENEITY OF NK_2 TACHYKININ RECEPTORS IN HAMSTER, RAT AND RABBIT SMOOTH MUSCLES

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The pharmacology of tachykinin receptors has progressed with the discovery of selective NK_2 receptor antagonists such as MEN 10,207, R 396 and L 659,877 which have enabled the identification of distinct NK_2 receptor subtypes in the endothelium-deprived rabbit pulmonary artery (RPA) and hamster trachea (HT) (Maggi et al., 1990, 1991). MEN 10,207 and R 396 show an opposite pattern of high affinity interaction with the above preparations, the former being more potent (about 100 fold) on the RPA while R 396 exhibits the converse picture (Maggi et al., 1990). The cyclic hexapeptide L 659,877 resembles R 396 (which is a linear derivative of L 659,877) in its interaction with the NK_2 subtypes, although it is much less discriminating (Patacchini et al., 1991). In this study, we have extended the observations on the antagonism of R 396 and L 659,877 to a number of NK_2-bearing tissues from different species. The isolated organs used were: hamster and rat urinary bladder (longitudinal strips), hamster colon (longitudinal segments), hamster stomach (zig-zag strips from the pyloric region), rat stomach (longitudinal strips from the fundus) rat and rabbit vas deferens (pars prostatica) and rabbit bronchus (deprived of epithelium). Antagonism was studied against [βAla^8]-NKA(4-10), a selective NK_2 receptor agonist, in the presence of peptidase inhibitors (thiorphan, captopril and bestatin 1 μM each). Results, expressed as $pK_B \pm$ S.E. of the mean (n= 6-8), are reported below.

Table 1

	Hamster			Rat			Rabbit	
	bladder	colon	stomach	bladder	stomach	vas deferens	bronchus	vas deferens
R 396	7.5 ± 0.2	7.5 ± 0.2	7.5 ± 0.1	6.4 ±0.2	6.5 ± 0.2	6.3 ±0.1	5.6 ±0.2	5.5 ± 0.2
L 659,877	8.0 ± 0.1	7.6 ± 0.2	8.1 ±0.1	7.7 ±0.2	7.7 ± 0.2	8.0 ± 0.2	7.0 ± 0.1	6.9 ± 0.1

As shown, L 659,877 possesses a rather uniform pattern of high affinity for the rat and hamster and lower affinity for the rabbit tissues. On the contrary R 396 shows three distinct clusters of affinity for the three species, exhibiting the highest affinity for the hamster tissues and the lowest for the rabbit tissues. These results rise the question whether they reflect the existence of multiple subtypes or species-related variations of the NK_2 receptor.

Maggi, C.A., Patacchini, R., Astolfi, M., Rovero, P., Giuliani, S. & Giachetti, A. (1991). *Ann. N. Y. Acad. Sci.* **632**,184-191
Maggi, C.A., Patacchini, R., Giuliani, S., Rovero, P., Dion, S., Regoli, D., Giachetti, A. & Meli, A. (1990). *Br. J. Pharmacol.* **100**, 588-592.
Patacchini, R., Astolfi, M., Quartara, L., Rovero, P., Giachetti, A. & Maggi, C.A. (1991). *Br. J. Pharmacol.* **104**, 91-96.

92P INVESTIGATION INTO DIFFERENCES IN TACHYKININ NK_1 RECEPTORS BETWEEN AND WITHIN SPECIES USING A PEPTIDE AND A NON-PEPTIDE NK_1 RECEPTOR ANTAGONIST

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The non-peptide NK_1 antagonist CP-96,345 is substantially less potent at blocking NK_1 receptors in rat and mouse compared to other species (Beresford et al., 1991). To investigate the possibility of subdivision of the NK_1 receptor within species, we have measured the potencies of (±)CP-96,345 and the peptide NK_1 antagonist GR82334 for NK_1 receptors in functional preparations from guinea-pig, rabbit and rat.

Both (±)CP-96,345 (3nM-3μM) and GR82334 (0.1-30μM) behaved as competitive antagonists of contractions induced by the selective NK_1 agonist substance P methylester (SPOMe) in guinea-pig ileum (GPI), guinea-pig bladder (GPB), guinea-pig trachea (GPT; Ireland et al., 1991), rabbit iris sphincter muscle (RISM; Hall et al., 1991, with modifications) and rat bladder (RB), SPOMe-induced relaxation of phenylephrine-contracted rabbit thoracic aorta (RTA; Beresford et al., 1991) and SPOMe-induced depolarization of neonatal rat hemisected spinal cord (RSC; Beresford et al., 1991). There were small (3-5-fold) differences in the affinity estimates of GR82334 and (±)CP-96,345 in different guinea-pig tissues (Table 1). Activities in rabbit aorta were similar to those obtained in guinea-pig, while affinity estimates in rabbit iris were 17-26-fold lower than those in aorta. Both GR82334 and (±)CP-96,345 were substantially less potent in rat tissues compared to guinea-pig preparations and rabbit aorta. GR82334 was equiactive in rat bladder and spinal cord, while (±)CP-96,345 was 7-fold less active in bladder compared to spinal cord.

Table 1

	Affinity (pK_B) estimate						
	GPI	GPB	GPT	RTA	RISM	RB	RSC
(±)CP-96,345	8.89±0.02*	8.29±0.11	8.17±0.10	8.81±0.06*	7.39±0.12	6.30±0.11	7.13±0.10*
Schild slope	0.9 (0.8-1.1)	0.6 (0.2-1.1)	0.9 (0.6-1.3)	0.9 (0.7-1.1)	0.8 (0.3-1.3)	n.d.	0.8 (0.4-1.2)
GR82334	7.64±0.01	7.06±0.04	7.23±0.10	7.37±0.08	6.11±0.09	6.53±0.06	6.38±0.09
Schild slope	1.1 (0.9-1.2)	0.8 (0.7-1.0)	1.0 (0.5-1.4)	0.9 (0.5-1.3)	1.3 (0.8-1.9)	1.3 (1.0-1.5)	0.8 (0.3-1.3)

Results are mean ± s.e. mean of 3-20 experiments. 95% confidence limits of Schild slopes are indicated in parentheses.
Antagonist contact time was 30min. *Data from Beresford et al., 1991.

The present results further suggest the existence of species variants in tachykinin NK_1 receptors. Further work is required to determine if the apparent differences in the potencies of GR82334 and (±)CP-96,345 observed between different NK_1 receptor-containing preparations from the same species are indicative of the existence of NK_1 receptor subtypes.

Beresford, I.J.M., Birch, P.J., Hagan, R.M. and Ireland, S.J. (1991) Br. J. Pharmacol., **104**, 292-293.
Hall, J.M., Mitchell, D. and Morton, I.K.M. (1991) Eur. J. Pharmacol., **199**, 9-14.
Ireland, S.J., Bailey, F., Cook, A., Hagan, R.M., Jordan, C.C. and Stephens-Smith, M.L. (1991) Br. J. Pharmacol., **103**, 1463-1469.

93P COMPARISON OF THE APPARENT AFFINITY OF GR82334 AT NK_1 RECEPTORS IN VARIOUS TISSUE PREPARATIONS

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The NK_1 antagonist GR82334 (Hagan et al., 1991a) appears to have different affinities at NK_1 receptors in isolated functional preparations of peripheral tissues obtained from rabbit, rat or guinea-pig (Beresford et al., 1992). Since NK_1 receptors in the brains of rat and rabbit may differ (Beresford et al., 1991), it was of interest to evaluate the affinity of GR82334 at NK_1 receptors in brain. In addition, in preparations known to contain NK_1 receptors, the guinea-pig ileum longitudinal smooth muscle myenteric plexus (GPI) and human astrocytoma cells (U-373 MG; Lee et al., 1989), comparison has been made of estimates of affinity of GR82334 obtained using radioligand binding (pK_i) and functional assays (pK_B).

Membranes from rat or rabbit cerebral cortex or GPI were incubated with [^{3}H]-substance P ([^{3}H]-SP, 0.5-1.0 nM) at 22°C for 40 min. Bound radioactivity was separated by rapid filtration through glassfibre filters (Whatman GF/B). Whole U-373 MG cells attached to 24-well plates (2.5 x 10^5 per well, Falcon) were incubated with [^{3}H]-SP (1-2nM) at 22°C for 1 hr after which unbound radioactivity was removed by washing. Measurement of increases in intracellular calcium ion concentration induced by the NK_1 agonist GR73632 (Hagan et al., 1991b) were made in suspensions of U-373 MG cells (1-2.5 x 10^5 per ml) pre-loaded with fura2.

Table 1

	Apparent Affinity				
Rat cortex	Rabbit cortex	GPI	GPI*	U-373 MG	U-373 MG
pK_i	pK_i	pK_i	pK_B	pK_i	pK_B
6.13±0.23	5.90±0.13	5.58±0.20	7.64±0.01	7.70±0.10	7.73±0.04

Values are means ± s.e. mean of 3-4 determinations (pK_i) or 15-18 determinations (pK_B). pK_B values were estimated using the equation pK_B = log(concentration ratio - 1) - log(concentration of antagonist). Slopes of inhibition curves and Schild plots were not significantly different from unity. * Data from Beresford et al., 1992.

In U-373 MG cells, GR82334 behaved as a reversible competitive antagonist of GR73632: affinity was similar to that observed against [^{3}H]-SP binding in these cells and to that exhibited in functional assays in the GPI. In contrast, GR82334 was 30-100 fold less active against binding of [^{3}H]-SP to membranes prepared from GPI, rat cortex or rabbit cortex. It is not clear whether these differences indicate heterogeneity of NK_1 receptors or are related to the technique employed and perhaps artefactual.

Beresford, I.J.M., Birch, P.J., Hagan, R.M. & Ireland, S.J. (1991) *Br. J. Pharmacol.*, **104**, 292-293.
Beresford, I.J.M., Ireland, S.J., Stables, J.M., Stubbs, C., Ball, D., Hagan R.M. & Birch, P.J. (1992) *This meeting.*
Lee, C.M., Kum, W., Cockram, C.S., Teoh, R. & Young, J.D. (1989) *Brain Res.*, **488**, 328-331.
Hagan, R.M., Ireland, S.J., Bailey, F., McBride, C., Jordan, C.C. & Ward, P. (1991a) *Br. J. Pharmacol.*, **102**, 168P.
Hagan, R.M., Ireland, S.J., Jordan, C.C., Beresford, I.J.M., Deal, M.J. & Ward, P. (1991b) *Neuropeptides*, **19**, 127-135.

94P ANTIVASOCONSTRICTOR EFFECTS MEDIATED BY ADENOSINE A_1 RECEPTOR AGONISTS IN THE PITHED RAT

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Relaxation of precontracted isolated vessels by adenosine receptor agonists is generally attributed to activation of A_2 receptors. In contrast, hypotensive or antihypertensive responses in vivo often manifest a complex pharmacology which, in the rat at least, is not consistent with a predominantly A_2 receptor mediated event (Trivedi et al., 1991). We chose to investigate further the contribution of A_1 receptors to vasodepression in vivo by using a simplified model (the pithed rat) and focussing deliberately on adenosine agonists with high A_1 selectivity.

Male normotensive Sprague-Dawley rats (260-430 g) were anaesthetized, pithed and prepared for recording blood pressure (BP) and heart rate (HR) (Fozard et al., 1987). BP was maintained at 100-120 mm Hg by infusion of angiotensin II, 0.2-0.5 µg/kg/min. Compounds were injected intravenously (i.v.) and dosing was cumulative. Affinities for A_1 and A_2 receptors were measured in radioligand binding assays (Bruns et al., 1986).

Table 1: Adenosine receptor selectivity and cardiovascular effects of adenosine receptor agonists

	A_1 (K_i, nM)[a]	A_2	A_1 Selectivity	BP, ED_{30}[b] (nmol/kg)	HR, ED_{30}[b] (nmol/kg)
N^6-cyclopentyladenosine	0.7 ± 0.1	930 ± 110	1257	8	3
R-N^6-phenylisopropyladenosine	1.0 ± 0.1	457 ± 59	448	21	20
N^6-cyclopentyl-2-chloroadenosine	1.0 ± 0.2	1057 ± 82	1090	21	11
N^6-cyclohexyladenosine	1.2 ± 0.1	1260 ± 110	1024	26	12
N^6-[(1S,trans)-2-hydroxycyclopentyl]adenosine	3.5 ± 0.1	4965 ± 628	1430	22	11
N^6-[2-hydroxy-3-(naphthyl-1-oxy)propyl]adenosine	4.6 ± 0.6	6224 ± 992	1365	88	33
S-N^6-phenylisopropyladenosine	20 ± 3	2690 ± 350	132	208	333

[a] mean values from 4-8 experiments; [b] dose to lower BP and HR by 30 %; mean values from 4-6 experiments

All the compounds decreased BP and HR both potently and dose-dependently. Significantly, only the bradycardia but not the fall in BP could be immediately and completely reversed by 8-p-sulphophenyltheophylline (8-SPT), a non-selective A_1/A_2 receptor antagonist, 20 mg/kg, injected i.v. at the end of the agonist injection sequence. Thus, selective adenosine A_1 receptor agonists induce antivasoconstrictor effects in the angiotensin II-supported circulation of the pithed rat independently of the fall in HR. Despite an excellent correlation between the doses required to reduce BP by 30 % and the A_1 receptor affinities (r = 0.95) it may be premature to implicate exclusively these sites in the functional response until an explanation for the discriminatory blockade of HR and BP by 8-SPT is forthcoming.

Bruns, R.F., Lu, G.H. & Pugsley, T.A. (1986). Mol. Pharmacol. 29, 331-346.
Fozard, J.R., Mir, A.K. & Middlemiss, D.N. (1987). J. Cardiovasc. Pharmacol. 9, 328-347.
Trivedi B.K., Blankley, C.J., Bristol, J.A., Hamilton, H.W., Patt, W.C., Kramer, W.J., Johnson, S.A., Bruns, R.F., Cohen, D.M. & Ryan M.J. (1991). J. Med. Chem. 34, 1043-1049.

95P ENDOTHELIUM-DERIVED NITRIC OXIDE (NO) IN THE RAT THORACIC AORTA DOES NOT MEDIATE THE SUPPRESSION OF CLONIDINE-INDUCED TONUS

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The induction of VSM tone with clonidine, and a variety of other agonists, is suppressed by the presence of the endothelium (Egleme et al, 1984), and the mediator of this inhibitory effect is thought to be spontaneously released EDRF (Martin et al, 1986). Assuming that NO is the principal EDRF, the inhibition of its synthesis by L-arginine analogues, such as nitro-arginine methyl ester (L-NAME) and methyl-arginine (L-NMMA), should mimic the effects of endothelial denudation. This hypothesis has been tested by studying the effects of clonidine and other related α-adrenoceptor agonists on isolated ring preparations (3-5 mm) of rat thoracic aorta, suspended under 2 g resting tension in Krebs solution at 37°C and gassed with 95% O_2/5% CO_2 mixture.

Clonidine elicited a biphasic contractile effect in aortic rings, and both a high affinity (10^{-9}-10^{-5}M) and a low affinity (10^{-4}-10^{-3}M) phase could be detected. Endothelial denudation significantly potentiated ($p < 0.05$) both the "high affinity"-mediated contractions (0.20 ± 0.06 vs 1.99 ± 0.14 g tension) and the "low affinity"-mediated contractions (1.51 ± 0.16 vs 3.11 ± 0.18 g tension). However, the effects of low (10^{-9}-10^{-5}M) concentrations of clonidine were not affected by 10 μM L-NAME, 100 μM L-NMMA nor by 10 μM glyburide, 5 mM tetraethylammonium, Ba^{2+} or ouabain, but were significantly ($p < 0.05$) enhanced by modification of $[K^+]_{ex}$ (0 or 15 mM K^+). Conversely, the effects of high (10^{-4}-10^{-3}M) concentrations of clonidine were significantly enhanced by L-NAME or L-NMMA in endothelium-intact but not in denuded rings.

The imidazoline derivatives, cirazoline (α_1-selective) and tramazoline (dual α_1/α_2 agonist) initiated aortic contractions at concentrations comparable with the effects of clonidine at the putative high affinity site, and such effects were potentiated by endothelial denudation and also by L-NAME. These data suggest that the inability of the NO synthase inhibitors to potentiate the effects of low concentrations of clonidine cannot clearly be related to differences in structure-activity relationships. B-HT 933 (an α_2-selective, non-imidazoline) contracted aortic rings at concentrations suggestive of a low affinity interaction and these contractions were potentiated by endothelial denudation and by L-NAME. Our data thus provide evidence dissociating the withdrawal of endothelial NO synthesis from the marked contractile effect of clonidine unmasked by endothelial denudation. It remains undetermined whether clonidine action at the putative low affinity site is mediated by an effect on an α_2-adrenoceptor.

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Egleme, C., Godfraind, T. & Miller, R.C. (1984) Br. J. Pharmac. **81**, 16-18.
Martin, W., Furchgott, R.F., Villani, G.M. & Jothianandan, D. (1986) J. Pharmac. Exp. Ther. **237**, 529-538.

96P EFFECTS OF PHYTATE ON SMOOTH MUSCLE PREPARATIONS *IN VITRO*

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Phytate (IP_6, inositol hexakisphosphate) is a neuroactive sugar for which a central transmitter function has been proposed (Vallejo et al., 1987; Thiebert et al., 1991). The work described here was carried out to investigate whether, in addition to these central effects, IP_6 has an action on peripheral tissues.

Contraction of each of the following preparations was measured isometrically: (i). longitudinal segments of male Purbright guinea-pig ileum (GPI) maintained at 32°C in aerated Tyrode solution; (ii). uterine horns taken from pro-oestrous CFLP mice and maintained at 37°C in carbogen (5% CO_2, 95% O_2)-aerated de Jalon's solution; and (iii). field-stimulated (80V pulses applied for 2 msec at 0.1 Hz) Wistar rat vasa deferentia maintained at 37°C in carbogen-aerated Kreb's solution.

Whereas up to 3 mM-IP_6 had no effect on basal tone or electrically-stimulated contractions of vasa deferentia (n=3), it caused dose-dependent contractions of uteri and GPI (EC_{50} = 376 ± 42µM (4) and 290 ± 47µM (12) respectively) (mean ± S.E. mean (n)). Maximal responses to IP_6 were 90 ± 4% (4) and 68 ± 14% (8) of the maximal responses to carbechol in uteri and GPI respectively. In both preparations heparin (50µg.ml^{-1}) had no effect on 300µM or 1mM-IP_6-stimulated responses (n=4). Atropine (100nM and 1µM), hexamethonium (5µM), mepyramine (20nM), methysergide (1µM), phenoxybenzamine (1µM), phentolamine (100nM) and 5 minute preincubation in Ca^{2+}-deficient Tyrode solution were similarly without effect on 300µM or 1mM-IP_6-stimulated contractions of GPI (n=4).

These data show that IP_6 stimulates dose-dependent contractions of mouse uteri and GPI but not of rat vasa deferentia. In GPI this effect is via a mechanism that is not cholinergic, histaminergic, α-adrenergic or serotinergic. Although greater than the K_d value for binding of IP_6 to central sites, the EC_{50} for this sugar's effects on uteri and GPI is similar to concentrations reported to modify neutrophil activity (Eggleton et al., 1991). The actions of IP_6 reported here also differ from its central effects in that they are insensitive to heparin and to calcium withdrawal (Smith et al., 1991; Thiebert et al., 1991).

Eggleton, P., Penhallow, J. & Crawford, N. (1991). Biochim. Biophys. Acta **1094**, 309-316.
Smith, S.E. & Durmuller, N. (1990). E.W.J. Pharmacol. **191**, 337-343.
Thiebert, A., Ferry, C., Danoff, S., Supattapone, S., Barrow, R. & Snyder, S. (1991) Neurbiol. **88**, 3165-3169.
Vallejo, M., Jackson, T., Lightman, S. & Hanley, M.R. (1987). Nature **330**, 656-658.

97P HISTAMINE H_1-RECEPTOR-MEDIATED ACCUMULATION OF TOTAL [^{3}H]-INOSITOL PHOSPHATES IN DDT$_1$MF-2 CELLS

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Radioligand binding studies using the histamine H_1-receptor antagonist ^{3}H-mepyramine have suggested that DDT$_1$MF-2 cells, a smooth muscle cell line derived from hamster vas deferens, possess a homogenous population of specific binding sites which have a low affinity for mepyramine (K_d = 219 nM; Mitsuhashi & Payan, 1988). In this study we have examined the pharmacological characteristics of histamine-stimulated ^{3}H-inositol phosphate accumulation in this cell line.

DDT$_1$MF-2 cells were grown in Dulbecco's modified Eagles medium (DMEM) containing 10% foetal calf serum (FCS) and 2 mM glutamine in humidified air/CO_2 (90:10) at 37°C (Dickenson & Hill, 1991). Monolayer cell cultures were loaded for 24 hr with ^{3}H-myo-inositol (37KBq/well) in 24 well cluster dishes in inositol-free DMEM containing 2 mM glutamine. Cells were then washed twice and incubated in Hanks/HEPES buffer (1ml/well), pH 7.4, for 30 min in the presence of 20 mM LiCl and antagonist drugs. Agonists were added in 10 µl of medium and the incubation continued for 10 min (unless otherwise indicated). Incubations were stopped with ice-cold methanol/0.12M HCl (1:1 v/v) and total ^{3}H-inositol phosphates isolated by anion exchange chromatography as described previously (Hawley *et al.*, 1991).

Histamine (0.1 mM, EC_{50} 1.9 ± 0.8 x 10^{-5} M, n=10, 10 min incubation) caused a rapid and significant increase in total ^{3}H-inositol phosphates in DDT$_1$MF-2 cells (3.3 ± 0.3 fold, n=10, $p<0.05$). At longer incubation times (> 10 min), the response to histamine (0.1mM) began to decline such that after 40 min incubation it was only 59 ± 3.8 % of that measured at 10 min (n = 11). The H_1-receptor agonists 2-thiazolylethylamine (EC_{50} 6.6 ± 1.9 x 10^{-5} M, n=4), N-α-methylhistamine (EC_{50} 9.0 ± 3.3 x 10^{-5} M, n=4), and 2-pyridylethylamine (EC_{50} 2.1 ± 1.2 x 10^{-4} M, n=4) produced a maximal increase in ^{3}H-inositol phosphate accumulation of 88.1 ± 7.8, 63.9 ± 17.4 and 62.2 ± 15.2 (%) respectively, when compared to 0.1 mM HA (100%). The response to 0.1 mM HA was antagonised by the H_1-antagonists mepyramine (IC_{50} 2.4 ± 0.9 x 10^{-9} M, K_d 0.4 nM, n=4), (+)-chlorpheniramine (IC_{50} 9.1 ± 1.3 x 10^{-9} M, K_d 1.3 nM, n=4) and (-)-chlorpheniramine (IC_{50} > 10 µM, n=4).

These results suggest that the histamine mediates an increase in total ^{3}H-inositol phosphate accumulation in DDT$_1$MF-2 cells via a "classical" histamine H_1-receptor with high affinity for mepyramine and (+)-chlorpheniramine.

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Dickenson, J.M. & Hill, S.J. (1991) Biochem. Pharmacol. **42**, 1545-1550
Hawley, J., Rubin, P.C. & Hill, S.J. (1991) Biochem.Soc.Trans. in press
Mitsuhashi, M. & Payan, D.G. (1988) J.Cell.Physiol. **134**, 367-375

98P COMPARISON OF ANTAGONIST pK_B ESTIMATES IN LUMEN-PERFUSED STOMACH ASSAYS FROM GUINEA-PIG, RAT AND MOUSE

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We have previously reported the underestimation of pK_B values for histamine H_2- and acetylcholine M- receptor antagonists on the isolated, lumen-perfused, mouse stomach assay (Shankley *et al.*, 1988). The extent of pK_B underestimation was positively correlated with the lipophilicity of the antagonists (log P oct/H_2O) and we suggested that the phenomenon was due to the loss of the antagonists from the receptor compartment into the stomach lumen.

The analysis has been extended to include similar lumen-perfused assays prepared from the immature guinea pig and rat (Welsh, 1992) and the following pK_B (s.e.) values were estimated:

	log P	guinea-pig right atrium	mouse stomach	rat stomach	guinea-pig stomach
famotidine	- 0.67	7.74(0.07)	7.50(0.11)	7.96(0.06)	7.60(0.09)
tiotidine	0.47	7.57(0.07)	6.96(0.11)*	7.40(0.05)	b=.54(0.05)
metiamide	0.61	6.06(0.13)	4.90(0.09)*	—	b=.54(0.08)
atropine	1.83	8.93(0.16)	7.78(0.11)*	8.89(0.08)	—

* - significantly different from the guinea-pig right atrium

On the guinea-pig stomach assay although the hydrophilic antagonist, famotidine, behaved in a simple manner, low Schild analysis slope parameters (b) were obtained with the two more lipophilic compounds, tiotidine and metiamide. The latter data might be expected if the loss of antagonist into the stomach lumen was sufficient to cause significant depletion of the antagonist from the organ bath. This hypothesis was tested by repeating the analysis using tiotidine on a guinea-pig mucosal sheet assay in which the tissue volume was greatly reduced compared to the whole stomach assay with respect to the organ bath volume. In this case, tiotidine behaved as a simple competitive antagonist although the pK_B value of 6.77(0.07) was still underestimated. However, on the rat assay, famotidine, tiotidine and atropine behaved as simple competitive antagonists and the pK_B values were not underestimated. These results will be discussed in terms of the compartmental model already presented (Shankley, 1985).

Shankley, N.P., Black, J.W., Ganellin, C.R. & Mitchell R.C. (1988) Br. J. Pharmac. 94, 264-274.
Welsh, N.J. (1992) PhD thesis University of London.
Shankley N.P. (1985) PhD thesis University of London.

99P ACTIONS OF DAU 6285 AT PUTATIVE 5-HT_4 RECEPTORS IN GUINEA-PIG COLON AND RAT OESOPHAGUS

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DAU 6285 (1H-benzimidazole-1-carboxylic acid, 2, 3-dihydro-6-methoxy-2-oxo 8-methyl-8-azabicyclo (3,2,1) oct-3-yl ester) is a novel benzimidazolone derivative which is reported to act as a silent antagonist of 5-HT at 5-HT_4 receptors in mouse embryo colliculi neurones, guinea pig ileum and human atrium. (Turconi *et al.*, 1991). In the present study we have examined the actions of DAU 6285 in the tunica muscularis mucosae of the rat oesophagus (Baxter *et al.*, 1991) and in the longitudinal muscle-myenteric plexus preparation of guinea pig distal colon (Wardle & Sanger 1991). Both show a high sensitivity to 5-HT, and may represent the most efficiently coupled 5-HT_4 receptor preparations described to date.

Oesophageal tunica muscularis mucosae and guinea pig distal colon were set up as described previously (see Baxter *et al.*, 1991; Wardle & Sanger 1991)

Both 5-HT and high concentrations of DAU 6285 caused a concentration-dependent relaxation of carbachol pre-contracted rat oesophagus (pEC_{50} [95% CL] estimates of 8.0 [7.8-8.3], n=8, and 4.8 [4.5-4.9] n=4, respectively). Relaxant effects evoked by DAU 6285 appear to be be mediated by muscarinic cholinoceptor blockade as DAU 6285 (30μM) caused a 2 to 3-fold rightward displacement of concentration-effect curves to carbachol, but did not affect contractile responses elicited by Substance P. Furthermore, full responsiveness to DAU 6285 remained after desensitisation of 5-HT_4 receptors with 5-methoxytryptamine (10 μM for 90 minutes). At lower concentrations (0.3-3.0 μM), DAU 6285 did not relax the oesophagus in its own right, but antagonised relaxant responses mediated by 5-HT (pA_2 = 7.22, slope = 1.01). In guinea pig distal colon, 5-HT but *not* DAU 6285 (≤ 3.0 μM), evoked a concentration-dependent contractile response (pEC_{50} = 10.2 [9.9-10.5], n=4). DAU 6285 (0.1-1 μM, n=4) caused a concentration-dependent and surmountable antagonism of responses to 5-HT (pA_2 estimate of 7.25, slope = 1.35).

The present study supports the finding in guinea pig ileum, mouse colliculi neurones, and human atria and demonstrates that DAU 6285 also acts as a silent antagonist of 5-HT at 5-HT_4 receptors in rat oesophagus and guinea pig colon.

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Baxter, G.S., Craig, D.A. & Clarke, D.E. (1991) *Naunyn-Schmiedeberg's Arch. Pharmacol.* 343, 439-446
Wardle, K.A. & Sanger, K.A. (1991) *Br. J. Pharmacol.* in press
Turconi, M., Schiantarelli, P., Borsini, F., Rizzi, C.A., Ladinsky, H. & Donneti, A. (1991) *Drugs of the Future* 16, 1011-1026

100P A COMPARISON OF THE EFFECTS OF SUMATRIPTAN AND 5-CARBOXAMIDOTRYPTAMINE ON BLOOD PRESSURE IN THE ANAESTHETISED AND ANGIOTENSIN II-SUPPORTED PITHED RAT

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The hypotensive effects of 5-carboxamidotryptamine (5-CT) in the anaesthetised rat have been suggested to be due to activation of 5-HT_1-like receptors mediating endothelium independent relaxation (Martin *et al.*, 1987). The present study shows that sumatriptan produces a transient hypotensive response in the anaesthetised rat and compares the mechanism of this response with that of 5-CT.

Male rats were anaesthetised with pentobarbitone sodium (70 mg/kg i.p.) and prepared with cannulae in the trachea, jugular vein and carotid artery. Agonists were administered as cumulative bolus doses with a 5 min dose interval, 30 min after receiving antagonist or vehicle control. The minimum mean arterial blood pressure (MABP) after each dose was recorded and compared with the value immediately before the first agonist dose. Sumatriptan (0.1-10 mg/kg i.v.) produced a dose-related, transient fall in MABP with a maximum of 58.5 ± 2.9 mmHg at 10 mg/kg i.v. which was abolished at all doses tested following pretreatment with methiothepin (1 mg/kg i.v.) whereas neither mesulergine (1 mg/kg i.v.) nor WAY100135 (3 mg/kg i.v.) a selective 5-HT_{1A} antagonist (Fletcher *et al.*, 1991) significantly altered the response to sumatriptan. 5-CT (0.01-3 μg/kg i.v.) also produced dose-related falls in MABP with a maximum of 77.0 ± 6.4 mmHg at 3 μg/kg i.v.which showed a slight shift to the right after pretreatment with mesulergine (1 mg/kg i.v.), 50 fold by methiothepin (1 mg/kg i.v.) and not significantly altered by WAY100135 (3 mg/kg i.v.).

In a second series of experiments rats were anaesthetised with halothane (5% in O_2), the trachea cannulated and the animal pithed through the orbit of the eye, ventilated with room air, (10 ml /kg) and cannulated as above but with a second jugular venous cannula through which a continuous infusion of angiotensin II (0.2-0.6 μg/kg/min) was given to maintain DBP above 100 mmHg. The animals were bilaterally vagotomised and the contralateral carotid ligated. In this preparation saline control injections (1 ml/kg + 0.5 ml flush) at 5 min intervals produced an increase in DBP of 33 ± 3 mmHg after 5 doses. Sumatriptan (0.1-10 mg/kg) produced no significant change in DBP from pre-dose values whereas 5-CT (0.03 - 3 μg/kg) produced dose dependent falls in DBP (measured 5 min after dosing) of up to 73.0 ± 3.7 mmHg which were abolished by pretreatment (10 min) with methiothepin (1 mg/kg i.v.) and shifted to the right approximately 5 fold by mesulergine (1 mg/kg i.v.).

These results suggest that 5-CT and sumatriptan reduce the MABP in the anaesthetised rat by different mechanisms. Both may be a result of 5-HT_1-like receptor activation since they are both blocked by methiothepin although methiothepin was more effective against the response to sumatriptan than that to 5-CT in the anaesthetised rat. Neither the response to sumatriptan nor that to 5-CT in the anaesthetised rat were mediated by activation of 5-HT_{1A} receptors since they were not affected by WAY100135. Mesulergine, however attenuated the response to 5-CT but had no effect on that to sumatriptan. Moreover, 5-CT but not sumatriptan, reduced DBP in the angiotensin II-supported pithed rat, suggesting that the response to sumatriptan in the anaesthetised rat may involve a neurogenic mechanism. This is unlikely to be a central effect since sumatriptan does not cross the blood brain barrier in the rat (Humphrey *et al.*, 1991) but may involve a requirement for a functional autonomic nervous supply.

Fletcher, A., Bill, D.J., Bill, S.J., Brammer, N., Cliffe, I.A., Forster, E.A., Reilly,Y. & Lloyd, G.K. (1991) *Soc. Neurosci. Abstr.* **17**, 92
Humphrey, P.P.A., Feniuk, W., Marriott , A.S., Tanner, R.J.N., Jackson, M.R. & Tucker, M.L. (1991) *Eur. Neurol.* **31** 282-290.
Martin, G.R., Leff, P., Cambridge, D. & Barrett, V.J. (1987) *Naunyn-Schmiedeberg's Arch Pharmacol.* **336**, 365-373.

101P THE EFFECT OF 8-OH-DPAT ON DRUG-INDUCED EMESIS IN THE FERRET

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Emesis can be induced in both animals and man by a wide variety of stimuli and may be the result of activation of different neuronal pathways relaying information to the 'vomiting centre'. Circumstances in which agents evoke emesis by more than one input may require the use of a combined antiemetic regime. Recently, Lucot & Crampton (1989) using buspirone and 8-OH-DPAT (8-hydroxy-2(di-n-propylamino)-tetralin) suggested that the 5-HT_{1A} receptor may play a pivotal role to mediate a 'universal antiemetic' protection in the cat. In the present studies we investigate the ability of 8-OH-DPAT to inhibit apomorphine-, morphine-, copper sulphate- and cisplatin-induced emesis in the ferret.

Ferrets of either sex (0.7-1.4 kg) were administered 8-OH-DPAT (0.25-0.75 mg kg^{-1}) or vehicle (saline 0.9 % w/v; 0.5 ml kg^{-1}) subcutaneously as a 15 min pretreatment prior to the administration of apomorphine (0.25 mg kg^{-1} s.c.), morphine (0.5 mg kg^{-1} s.c.) or $CuSO_4.5H_2O$ (100 mg kg^{-1} intragastric), or 45 min post injection of cisplatin (10 mg kg^{-1} i.v.). Animals treated with apomorphine, morphine or copper sulphate were observed for 30 min and animals treated with cisplatin were observed for 240 min The numbers of retches and vomits were recorded. If an animal failed to either retch or vomit then a value of 'onset' was taken as the observation time.

Table 1. The effect of 8-OH-DPAT on drug induced emesis in the ferret.

Treatment	mg/kg	Onset (min)	Retches	Vomits	Treatment	mg/kg	Onset (min)	Retches	Vomits
Apomorphine	0.25	2.8±0.7	25.7±5.5	2.7±0.8	Morphine	0.5	2.2±0.3	30.8±4.9	4.3±1.2
+ 8-OH-DPAT	0.25	10.1±6.6	8.8±4.2	1.8±0.9	+ 8-OH-DPAT	0.25	1.5±0.3	33.8±12.7	6.0±1.8
	0.5	14.7±6.4	6.2±4.3*	0.6±0.6		0.5	18.2±7.0*	9.3±8.6	1.3±1.3
	0.75	30.0±0.0*	0.0±0.0*	0.0±0.0*		0.75	11.8±6.3	8.8±4.2*	3.0±1.5
$CuSO_4.5H_2O$	100.0	3.3±0.5	80.6±9.4	8.0±0.3	Cisplatin	10.0	79.9±6.1	72.3±11.9	5.0±0.7
+ 8-OH-DPAT	0.25	4.3±0.7	42.0±8.4*	5.5±0.7*	+ 8-OH-DPAT	0.25	54.1±4.6*	153.5±35.1	15.0±3.9*
	0.5	6.1±2.1	26.0±4.9*	4.0±0.9*		0.5	104.1±24.4	54.0±21.0	5.8±1.6
	0.75	6.0±2.1	16.0±5.9*	3.0±1.1*		0.75	111.4±43.4	23.0±9.0*	2.8±1.0

Results represent the means±s.e.m.s of 4-6 determinations. Significant differences between respective controls and 8-OH-DPAT treated animals are indicated as *P<0.05 (Mann-Whitney U test).

8-OH-DPAT had a differential capacity to antagonise the emesis induced by all four emetogens and indeed at 0.25 mg kg^{-1} s.c. potentiated cisplatin induced emesis. In addition, during the pretreatment time some animals (16 %) exhibited transient emesis to 8-OH-DPAT (onset of approximately 5 min, comprising 1-7 episodes of 0-4 vomits and 2-16 retches) lasting for approximately 2-4 min. The intensity of the emetic response induced by 8-OH-DPAT was not dose related and did not correlate to the antiemetic efficacy.

In conclusion, 8-OH-DPAT has the capacity to both induce and inhibit emesis in the ferret. The mechanism of action of 8-OH-DPAT to induce emesis remains to be elucidated; the ability to antagonise emesis may be related to a 5-HT_{1A} receptor interaction.

Lucot, J.B. & Crampton, G.H. (1989) Pharmacol. Biochem. Behav. **33**, 627-631.

102P PARALLEL STUDIES OF ^{133}Xe CLEARANCE AND ^{113}Sn MICROSPHERE ACCUMULATION DURING SPONGE-INDUCED ANGIOGENESIS AND THE EFFECTS OF INTERLEUKIN-1α

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In 1987, we described quantitative studies of angiogenesis in sponge implants in rats in which a simple ^{133}Xe wash-out technique was used for repeated quantitation of relative blood flow changes through the sponges over a period of weeks (Andrade *et al.*, 1987). Since the sponges originally contained no blood vessels, the development of blood flow was considered to represent a neovascularisation. This assumption has been supported by histological observations that the sponges were gradually infiltrated by host blood vessels. We have now undertaken two additional approaches to validate this technique.

Firstly, we measured absolute blood flow in the sponges using the tracer microsphere technique as described by Hiley *et al*, (1980). In this method, increases in flow to a vascular region will lead to the trapping of more of the microspheres which have been injected into the left ventricle. Since the diameter of capillaries is less than that of the microspheres (15±5 μm, labelled with ^{113}Sn and obtained from NEN, Dreieich, F.R.G.), the latter are trapped in the tissues and sponge implants and the fraction of injected radioactivity in a particular tissue or sponge represents the amount of cardiac output which it has received. Quantitation of blood flow is achieved by the use of the reference organ technique in which blood is removed from a femoral artery by a syringe pump at 0.5 ml/min.

On Days 3, 5, 7, 10 and 14 after sponge implantation, the blood flows were 0.146±0.023 (n = 8), 0.161±0.030 (n = 4), 0.232±0.034 (n = 8), 0.340±0.025 (n = 14), and 0.335±0.033 (n = 4) ml/min/g respectively. There was a linear correlation with changes in ^{133}Xe clearance (r = 0.957). Sponge implants given 3 pmol interleukin-1α (IL-1α) daily showed significantly higher ^{133}Xe clearance values than the control group, e.g. the control 6 min ^{133}Xe clearances on Days 7 and 10 were 16.8±1.7 (n = 8) and 24.5±0.4% (n = 12) while after IL-1α clearances were 26.6±1.2 (n = 8) and 35.3±1.9% (n = 16, $P < 0.01$). With IL-1α, consistently higher absolute blood flows were detected in the test sponges; on Days 7 and 10 control flows were 0.232±0.034 and 0.340±0.025 as compared to IL-1α values of 0.517±0.081 and 0.545±0.100, respectively ($P < 0.05$, n = 12-14).

Secondly, we carried out comparative studies on ^{133}Xe clearance and the amount of neovasculature in the sponges as determined by the carmine dye method of Kimura *et al.* (1991). In these experiments, 5 ml 10% carmine solution containing 5% gelatin were injected into the penile veins of rats after ^{133}Xe measurements. A good correlation between these two parameters was established (r = 0.974). Again, sponge implants given 3 pmol IL-1α daily showed significantly higher clearance values than the controls, e.g. the control 6 min ^{133}Xe clearances on Days 6 and 10 were 14.5±1.1 and 29.1±1.3% (n = 12) while IL-1α increased them to 25.3±0.7 and 37.9±1.6% (n = 12, $P < 0.01$). Consistently higher levels of carmine were detected in the test sponges; carmine contents (mg) on Days 6 and 10 were 3.67±0.10 and 6.11±0.37 as against IL-1α values of 4.86±0.35 and 9.67±0.38, respectively ($P < 0.05$, n = 4 for all).

Altogether, these data show that measurements of relative blood flow changes in sponge implants by the ^{133}Xe clearance method provide an accurate means to assess new blood vessel formation, and illustrate the general applicability of this model in angiogenesis research.

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Andrade, S.P., Fan, T.-P.D. & Lewis, G.P. (1987) Br. J. Exp. Path. **68**, 755-766.
Hiley, C.R., Yates, M.S., Roberts, P.J., & Bloom, A.E., (1980) Nephron, **26**:244-248
Kimura, M., Kobayashi, S., Luo, B. & Kimura, I. (1990) Int. Arch. Allergy Appl. Immunol. **93**, 365-370

103P COMPARATIVE STUDIES ON THE ANGIOGENIC ACTIVITY OF IL-1 AND IL-8 IN A RAT SPONGE MODEL

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Angiogenesis is thought to be involved in the progression of rheumatoid arthritis and several cytokines such as interleukin (IL)-1, IL-6, IL-8 and tumour necrosis factor (TNF) were found in synovial fluid from rheumatoid joints. It has been reported that IL-8 can induce neutrophil and lymphocyte infiltration and destroy synovial membrane (Oppenheim *et al.*, 1991). Here we use a rat sponge model to test the possibility that IL-8 may contribute to the aberrant neovascularisation in chronic inflammatory diseases.

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (180-200g) and neovascularisation was assessed as a function of blood flow through the implants every two days over a period of 14 days, using a ^{133}Xe clearance technique (Andrade *et al.*, 1987).

Daily administration of 3 pmol (50 ng) IL-1α, 3 pmol (50 ng) TNF-α or 6 pmol (100 ng) basic fibroblast growth factor (bFGF) caused an intense neovascularisation. Lower doses of IL-1α (0.3 pmol), TNF-α (0.3 pmol) or bFGF (0.6 pmol) produced no apparent effect on sponge-induced neovascularisation. However, these doses of IL-1α, but not TNF-α, acted in synergy with subthreshold doses of the neuropeptide substance P (SP, 10 pmol), or the inflammatory autacoid bradykinin (BK, 10 pmol) to produce an angiogenic response similar to that seen with higher doses of the cytokines. In contrast, 3 pmol (75 ng) or 0.3 pmol (7.5 ng) of IL-6 did not affect angiogenesis and no synergism between subthreshold dose of SP (10 pmol) and IL-6 (0.3 pmol) was observed in this model.

Daily doses of 3 pmol (25 ng) of IL-8 induced significantly higher clearance values than the control group, e.g. the 6 min ^{133}Xe clearance value of the control group on Day 6, 8, 10,12 were 14.5±1.1, 23.1±0.9, 29.1±1.8, 34.0±2.0% (n=12) while IL-8 increased it to 24.3±0.9, 31.9±0.8, 38.0±1.7, 41.6±2.6%, respectively (n=12, $P<0.05$). Histological studies of sponge sections stained with haematoxylin & eosin or an endothelial cell marker *Bandeirea simplicifolia* lectin I, isolectin B_4 showed increased cellularity and vascularity in the test sponges as compared to controls. Lower doses of IL-8 (0.3 pmol) did not induce a significant angiogenic response. Furthermore subthreshold doses of SP (10 pmol) or BK (10 pmol) failed to act synergistically with IL-8 (0.3 pmol) to produce an enhancement of angiogenesis.

Thus, IL-8 may be involved in the modulation of angiogenesis commonly associated with chronic inflammatory diseases but its mechanism of action appears to be different from that of IL-1.

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Andrade, S.P., Fan, T.-P.D.& Lewis, G.P. (1987) Br J Exp Path **68**, 755-766
Oppenheim, J.J., Zachariae, C.O.C., Mukaida, N. & Matsushima, K. (1991) Annu Rev Immunol **9**, 617-48

104P NEUTROPHIL RECRUITMENT INTO MOUSE AIR-POUCH BY INTERLEUKIN-1β IS MEDIATED BY TYPE 1 RECEPTORS

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Two receptors for the pro-inflammatory cytokine interleukin-1 (IL-1) have been so far characterized: type I receptor ($IL\text{-}1_{RI}$) present on T cells and endothelial cells, and type II receptor ($IL\text{-}1_{RII}$) present on B cells and PMN (Dinarello, 1991). The antagonist ($IL\text{-}1_{RA}$) binds to both receptors with similar affinity (McMahan *et al.*, 1991). These reagents were used to characterize IL-1-induced PMN recruitment *in vivo*.

IL-1β (I.R.I.S., Siena, Italy) was injected in 0.5 ml of carboxymethylcellulose (0.5% in phosphate buffered saline, PBS) into 6-day-old pouches formed on back of male albino mice (28-30 g). Pouches were washed 4h later with 2 ml of PBS (containing heparin 50 units ml^{-1}). Lavage fluids were centrifuged (240 g x 10 min at 4°C) and cells suspended in 2 ml of heparin-PBS, stained with Turk's solution (1:10 dilution) and counted. Either $IL\text{-}1_{RA}$ (Upjohn, Kalamazoo, MI) or a monoclonal antibody (mAb) prepared against $IL\text{-}1_{RI}$ (Genzyme, Maidstone, Kent, UK) or a mAb anti-$IL\text{-}1_{RII}$ (I.R.I.S., Siena, Italy; Ghiara *et al.*, 1991) were injected concomitantly with the cytokine. Results are expressed as 10^6 PMN recovered from the pouch per mouse (mean ± s.e.).

IL-1β caused a dose-dependent PMN infiltration into the mouse air-pouch with 20ng producing a maximal response at the 4h time point. $IL\text{-}1_{RA}$ dose-dependently inhibited this migration with a maximal effect at a molar ratio of $1:10^4$. Of the two mAbs, anti-$IL\text{-}1_{RI}$ almost abolished the migration to 5ng IL-1β ($\approx ED_{50}$), whereas anti-$IL\text{-}1_{RII}$ was ineffective as reported in the following table:

Treatment		Migration (10^6 PMN)	No. of Animals	% Inhibition	Mann-Whitney U-test
IL-1β	20ng	6.80 ± 0.64	15	0	---
+$IL\text{-}1_{RA}$	2μg	5.48 ± 0.77	7	19	not significant
	20μg	2.72 ± 0.58	7	60	$p<0.01$
	200μg	1.61 ± 0.23	7	77	$p<0.01$
IL-1β	5ng	2.55 ± 0.18	7	0	---
+anti-$IL\text{-}1_{RI}$	10μg	0.33 ± 0.18	7	87	$p<0.01$
+anti-$IL\text{-}1_{RII}$	10μg	3.55 ± 0.77	7	0	not significant

In conclusion, IL-1β-induced PMN recruitment *in vivo* is a receptor-mediated event blocked by $IL\text{-}1_{RA}$. Furthermore, $IL\text{-}1_{RI}$ activation appears to be implicated in this phenomenon, suggesting endothelial cell activation by the cytokine prior to PMN infiltration. These data contribute to an understanding of the molecular mechanism activated by IL-1β during the inflammatory process.

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Dinarello, C.A. (1991) Blood., 77, 1627-1652.
Ghiara P. *et al.*, (1991) Cytokine., 3, 473.
McMahan C.J. *et al.*, (1991) EMBO. J., 10, 2821-2832

105P DRUG EFFECTS ON ADJUVANT DISEASE IN THE MF1 MOUSE

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Adjuvant arthritis (AA), which in the rat is an accepted model of rheumatoid arthritis widely used in the screening of anti-rheumatic agents, has been reported previously not to occur in mice (Kerwar *et al*, 1988). The recent greater availability of genetically defined mouse strains led us to explore adjuvant disease induction in the mouse.

Mice (aged 8 - 12 weeks) received a subplantar injection of 50 µl of *Mycobacterium tuberculosis* in liquid paraffin. Hind paw volume was then determined by water displacement plethysmography at intervals throughout the experiment. A sustained (over 75 days) biphasic increase in paw volume occured in MF1 and C57BL mice, whilst C3H, CBA and NZB showed a less severe inflammation which declined more rapidly. The uninjected paw volumes of the MF1 mice also increased over the time course, suggesting a systemic response in this strain.

Female MF1 mice were dosed prior to adjuvant injection and daily throughout the experiments with indomethacin (0.5, 1.0 & 2.0 mg/kg *po*), dexamethasone (0.01, 0.1, 1.0 mg/kg *sc*) or azathioprine (2.0, 20 & 100 mg/kg *po*). Control groups were dosed with the relevant vehicle, and inhibitory effects were determined by comparison of the area under the curves of the mean paw volume against time graphs. Indomethacin inhibited the paw oedema at all the doses tested, whilst dexamethasone inhibited the condition only at 1 mg/kg/day. The inhibition induced by azathioprine exhibited a good dose response relationship, with significant inhibition occurring at the highest concentration tested.

The effectiveness of indomethacin and dexamethasone suggest the presence of an inflammatory component in this condition, whilst the inhibition caused by the immunosuppressant azathioprine points to the involvement of immune mechanisms. Adjuvant disease in the mouse appears to be a chronic inflammation with some of the characteristic systemic effects associated with the rat adjuvant arthritis model. A recent report by Isenberg *et al* (1990) also suggests that a chronic inflammation with some systemic characteristics can be induced in the mouse following an *id* injection of Freund's complete adjuvant. After further characterisation, the murine model reported here may provide an alternative to rat AA for the investigation of anti-rheumatic drugs.

Isenberg, D., Le Page, S., Katz, D., Tucker, L., Hutchins, P., Knight, B., Bakimer, R., Cooke, A. (1990) *Br. J. Rheum.* **29** (Supp 1), 15.
Kerwar, S.S., Ridge, S.C. & Oronsky, A.L. (1988) in *Handbook of animal models for the rheumatic diseases* Vol 1. Ed. Greenwald, R.A. & Diamond, H.S. USA: CRC Press, Inc.

106P THE ACTIONS OF MECLOFENAMIC ACID AT PROSTANOID RECEPTORS

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Meclofenamic acid (MA) is a non-steroidal anti-inflammatory drug that is thought to act mainly by inhibiting prostaglandin synthesis. Data from binding and functional studies (Rees *et al.*, 1988; McLean & Gluckman, 1983) now provide evidence that MA also has affinity for various prostanoid receptors. Prostanoid receptors have been classified, and preparations containing each receptor type have been identified (Coleman *et al.*, 1990). In the present study, we have tested MA for its ability to interact with a range of prostanoid receptors on smooth muscle preparations *in vitro*.

Guinea-pig stomach fundus (GPF), cat trachea (CT), guinea-pig vas deferens (GPVD), dog iris (DI) and rat aorta (RA) were isolated and mounted in organ baths containing modified Krebs solution with indomethacin 2.8µM, maintained at 37°C and gassed with 5% CO_2 in O_2 as described by Coleman *et al.*, (1987). Cumulative concentration-effect curves to the standard agonists were repeated on each preparation until constant responses were obtained. MA (30µM) was then added to the bathing solution and left in contact with the tissues for 30 minutes and a further curve to the standard agonist was constructed. Concentration-ratios were determined from which pK_B values were derived (Table 1).

Table 1.

Tissue	Receptor	Standard Agonist	Response	Antagonist Potency pK_B (± s.e.mean)	n
GPF	EP_1	PGE_2	Contraction	5.8 (±0.1)	9
CT	EP_2	PGE_2	Relaxation	<4.5	6
GPVD	EP_3	PGE_2	Twitch Inhibition	<4.5	6
DI	FP	$PGF_{2\alpha}$	Contraction	<4.5	4
RA	TP	U-46619	Contraction	6.6 (±0.1)	5

MA had no agonist activity on GPF, DI or RA, but had weak relaxant activity (equieffective concentration ratio > 700 (PGE_2 = 1)) on 3/6 preparations of CT, and produced a 24 (±4)% inhibition of electrically-induced contractions of GPVD (n=6). The mechanism of these weak and variable inhibitory actions on CT and GPVD is not clear. MA had weak antagonist activity on GPF and RA, but not on CT, GPVD or DI (Table 1). As MA had no effect on contractions of GPF and RA to carbachol (n=4) and potassium chloride (n=7) respectively, this antagonism appears to result from specific blocking activity at prostanoid EP_1- and TP-receptors.

Coleman, R.A., Kennedy, I., Humphrey, P.P.A., Bunce, K. and Lumley, P.(1990). In Comprehensive Medicinal Chemistry, vol 3, Ed. J.C. Emmett, Pergamon Press, Oxford. pp643-714.
Coleman, R.A.(1987). In Prostaglandins and Related Substances. A Practical Approach. Ed. C. Benedetto, R.G. McDonald-Gibson, S. Nigam, T.F. Slater. IRL Press, Oxford, Washington. pp267-303.
McLean, J.R. and Gluckman, M.I. (1983). Drug Res., 33, 627-631.
Rees, M.C.P., Canete-Soler, R., Lopez Bernal, A. and Turnbull, A.C.(1988). Lancet, II, 541-542.

107P INDOMETHACIN ACCUMULATION BY MURINE CANCER CELLS IN CULTURE

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A method for the determination of indomethacin (INDO) accumulation was developed to investigate drug entry into NC carcinoma cells *in vitro*. Previous studies of this type ignored non-specific binding of INDO to cells (Blanchard *et al.*, 1979) and assay vessels (Beaven & Bayer, 1980). Assays were performed in 1.5 ml Eppendorf microcentrifuge tubes containing 2 x 10^6 cells, 1.8 μM [^{14}C]-INDO (0.21 x10^6 dpm) with (blank) or without (total) added non-radiolabelled INDO (0.5 mM) in a total volume of 1.1 ml serum-free medium (Minimum Essential Medium). Samples were incubated at 37°C for 30 min and the reaction stopped by centrifugation (11600 *g*) followed by superficial washing of the pellet with ice-cold phosphate buffered saline pH 7.4 (PBS).

Specific association of INDO with the cells was rapid, being maximal at 5 min. Dissociation was also rapid: almost all (about 98%) of the cell-associated label was removed by washing the cell pellet twice in 1 ml aliquots of PBS by resuspension followed by recentrifugation as above. INDO accumulation was dependent on pH, and increased as the pH was lowered. However, at pH 6.8 this increase was only about 35% of the total specific accumulation expected from pKa calculations if INDO entered the cell by simple diffusion. The mean specific accumulation of label (total minus blank) was less in the presence of the sulphydryl group derivatisers p-hydroxy-mercuribenzoic acid and N-ethylmaleimide, by 53 ± 11% (P=0.02, n=5) and 51 ± 11% (P=0.05, n=5) respectively (means ± s.e.). The reducing agent dithioerythritol or the lectin concanavalin A had little or no effect on mean accumulation (100 ± 11%, P=0.66; and 94 ± 3.5%, P=0.43 of control respectively, both n=5). In comparison with whole cells, membrane preparations showed only 9.6 ± 0.3% of mean specific uptake (P=0.04, n=4). The above results suggest that INDO accumulation is rapid and dependent on the integrity of membrane proteins.

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Beaven M. A. & Bayer B. M. (1980) Biochem. Pharm. **29**: 2055-2061.
Blanchard J. C., Scheider Y. J., Tulkens P. & Trout A. (1979) Arch. Int. Pharmacodyn. **240**: 4-16.

108P THE EFFECTS OF INTRAVENOUS CHOLECYSTOKININ (CCK) ON PROLACTIN AND GROWTH HORMONE RELEASE IN THE PREPUBERTAL PIG

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Pekas and Trout (1990) have recently demonstrated that immunization of growing pigs against endogenous CCK increases their growth rate. While this observation may be causally related to the putative inhibitory effects of endogenous CCK on feeding (see Parrott *et al.*, 1991), it is also conceivable that endogenous CCK may regulate the release of metabolic hormones such as growth hormone (GH) (Ganong, 1991) or prolactin (see Ebenezer & Parrott, 1991). To test this hypothesis, the present study was carried out to determine whether i.v. administration of CCK would alter plasma concentrations of GH and prolactin in growing pigs. Prepubertal Large White pigs (n=7, 5 male, 2 female, b.wt. approx. 30kg) were surgically prepared under halothane anaesthesia with chronic venous catheters in the external jugular vein. The animals were injected i.v. with either physiological saline (control) or CCK (0.3, 0.7 and 1.3 μgkg), and blood samples were collected 10 min before and 2,5,10,20 and 30 min after injection for determination of plasma prolactin and GH by radioimmunoassay. All doses of CCK produced rapid, dose-related increases in plasma prolactin and GH concentrations. The 0.3, 0.7 and 1.3 μgkg^{-1} doses increased plasma prolactin from 0.66 ± 0.06 $ngml^{-1}$ to a peak of 1.32 ± 0.24, 1.59 ± 0.18 and 2.03 ± 0.32 $ngml^{-1}$ ($p<0.01$ in each case), respectively, approximately 5-10 min after injection and also raised plasma GH from 1.35 ± 0.31 $ngml^{-1}$ to 3.38 ± 1.16, 3.34 ± 1.04 and 3.69 ± 1.14 $ngml^{-1}$ ($p<0.01$, 0.05, 0.01), respectively, after 20-30 min; the 0.7 μgkg^{-1} dose also produced a GH peak after 5 min ($p<0.01$). Although these stimulatory effects of i.v. CCK on GH are inconsistent with the increased growth rate reported in pigs immunized against CCK (Pekas & Trout, 1990), it is possible that different results may be produced under chronic conditions. One explanation for the present findings may be that CCK induces a stress response in this species (Parrott *et al.* 1991) as both prolactin and GH are released during stress (Ganong, 1991). However, it is of note that CCK stimulated prolactin release at an iv dose (0.3 μgkg^{-1}) that does not induce cortisol secretion in pigs (Parrott *et al.* 1991).

Ebenezer, I.S. & Parrott, R.F. (1991) *Gen. Pharmacol.* **22**, 811-814.
Ganong, W.F. (1991) *Review of Medical Physiology*. pp. 389.
Parrott, R.F., Ebenezer, I.S., Baldwin, B.A. & Forsling, M.L. (1991) *Expt. Physiol.* **76**, 525-531.
Pekas, J.C. & Trout, W.E. (1990) *Growth Dev. Aging* **54**, 51-56.

109P MODULATION OF THE HYPOTHALAMO-PITUITARY-GONADAL AXIS BY LIPOCORTIN 1

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Within the neuroendocrine system, lipocortin 1 (LC1) is particularly concentrated in the median eminence area of the hypothalamus (Smith et al., 1990), where its distribution closely parallels that of the gonadotrophin releasing hormone (GnRH) nerve fibres (Strijbos et al., 1991), and in the anterior pituitary gland (Smith et al., 1989) suggesting that the protein may contribute to the mechanisms controlling hypothalamo-pituitary gonadotrophic activity. We have therefore examined the effects of human recombinant LC1 (hu-r-LC1, Biogen Inc, USA), an N-terminal LC1 fragment ($LC1_{1-188}$, ICI plc, UK) and two neutralising LC1 antibodies on the secretion of immunoreactive luteinising hormone (ir-LH) in young adult (≈ 200g) male CFY rats (i) in vivo in animals bearing indwelling intracerebroventricular (i.c.v.) cannulae (n=5-6/group) and (ii) in vitro by segments of anterior pituitary tissue (n=6/group) removed post-mortem and incubated in conditions described elsewhere (Hadley et al., 1991).
In vivo, central administration of hu-r-LC1 (0.3-1.2μg,i.c.v.) had no discernable effects on the serum ir-LH concentration. Similarly, an intraperitoneal injection of histamine (0.6mg/100g), which produced a significant ($P<0.01$) increase in the hypothalamic LC1 content (29.6±7.0 vs 79.1±17.0 pg/ml extract), failed to influence ir-LH release. By contrast, in both control and histamine treated rats, a polyclonal LC1 antibody (3μl i.c.v., diluted 1:200, ICI plc) produced significant ($P<0.01$) increases in the serum ir-LH concentration compared to corresponding controls treated with non-immune rabbit serum (3μl i.c.v., diluted 1:200). In vitro, hypothalamic extract (0.05 - 0.2 HE/ml), which is rich in GnRH, initiated concentration dependent increases in ir-LH release from the pituitary segments. The significant ($P<0.01$) responses to a submaximal concentration of the extract (0.1 HE/ml) were reduced by ≈ 50% ($P<0.01$) by pretreatment of the tissue with dexamethasone (0.1μM) which alone did not affect ir-LH release. The inhibitory effects of the steroid were neutralised by simultaneous inclusion in the incubation medium of a monoclonal LC1 antibody (diluted 1:15000, Zymed) but not by a correspondingly diluted control antibody (anti myosin light chain kinase, Sigma Chemical Co., UK). Neither hu-r-LC1 (10fg/ml - 100ng/ml) nor $LC1_{1-188}$ (10pg/ml - 1μg/ml) influenced the spontaneous release of ir-LH in vitro. By contrast, hu-r-LC1 (10pg/ml & 100ng/ml) reduced by ≈ 60% the significant ($P<0.01$) increases in hypothalamic extract stimulated ir-LH release (0.1HE /ml) although in other concentrations it was without effect. Similarly, $LC1_{1-188}$ (100pg/ml) reduced the secretory response to the tissue extract ($P<0.01$) but higher concentrations were ineffective in this regard.
The results suggest that lipocortin 1 may act at the level of both the hypothalamus and the anterior pituitary gland to modulate the neuroendocrine control of luteinising hormone release.

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Hadley, A.J., Flack, J.D. & Buckingham, J.C. (1991) Br. J. Pharmacol. **100**, 337P.
Smith, T. Flower, R.J. & Buckingham, J.C. (1989) J. Endocr. **123**, 46P.
Smith, T., Flower, R.J. & Buckingham, J.C. (1990) J. Endocr. **127**, (suppl.) 49.
Strijbos, P.J.L.M., Tilders, F.J.H., Carey, F., Forder, R. & Rothwell, N.J. (1991) Brain Res., **553**, 249-260.

110P CLASS III CALCIUM ANTAGONISTS DISPLAY HIGH AFFINITY FOR THE [^{3}H]-FLUSPIRILENE BINDING SITE IN SKELETAL MUSCLE

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At least four sites of action have been proposed for agents acting at L type voltage dependent calcium channels on the basis of ligand binding interactions. Thus far only sites for agents acting at the dihydropyridine (DHP), phenylalkylamine and benzothiazepine sites have been demonstrated in brain tissue. Sites for other prototypical agents such as fluspirilene (a diphenylbutylpiperidine) and SR 33557 (an indolizinsulfone) have been demonstrated in skeletal muscle or cardiac sarcolemmal membranes (Galizzi *et al.*, 1986; Qar *et al.*, 1987). Recently, we have been unable to demonstrate [^{3}H] fluspirilene binding in brain, whereas at the DHP site it acts in a similar manner to other class III calcium antagonists, although agents acting at the fluspirilene site appear to have selective effects against calcium channel activators in functional experiments (Kenny *et al.*, 1990). We have now examined the interaction of this group of agents with the [^{3}H] DHP and [^{3}H] fluspirilene site in skeletal muscle.

Dihydropyridine binding assays were carried out as described (Kenny *et al.*, 1990) with the selective ligand [^{3}H] PN 200-110 at a concentration of 0.03 nM in cerebral cortex and 0.2 nM in skeletal muscle membranes. [^{3}H] fluspirilene binding in skeletal muscle (0.1nM) was carried out essentially as described (Qar *et al.*, 1987).

Displacement of [^{3}H] calcium antagonists from rat cerebral cortex and rabbit skeletal muscle membranes.

	rat cerebral cortex		rabbit skeletal muscle			
	[^{3}H] (+) PN 200-110		[^{3}H] (+) PN 200-110		[^{3}H] fluspirilene	
Competing drug	pIC50	nH	pIC50	nH	pIC50	nH
NITRENDIPINE	9.36 ± 0.15	0.94	8.28 ± 0.13	1.02	6.53 ± 0.26	0.49
FLUSPIRILENE	7.69 ± 0.01	0.66	8.29 ± 0.10	1.09	9.04 ± 0.19	1.12
FLUNARIZINE	6.10 ± 0.20	1.28 (*98)	6.97 ± 0.05	0.84	7.39 ± 0.19	0.99
LIDOFLAZINE	6.44 ± 0.06	0.84 (*90)	6.99 ± 0.07	0.71	7.92 ± 0.21	1.14
BEPRIDIL	5.53 ± 0.10	1.00 (*68)	6.92 ± 0.24	0.69	7.42 ± 0.08	0.96
SR 33557	9.83 ± 0.09	1.01	9.31 ± 0.06	1.05	8.40 ± 0.20	0.92

(* % maximal inhibition at 10μM)

These results confirm that the affinity of flunarizine, lidoflazine and bepridil in displacing [^{3}H] DHP binding is increased in skeletal muscle, but these agents displace binding with Hill slopes consistantly less than unity. However, these agents displace [^{3}H] fluspirilene from its site of action in skeletal muscle membranes with higher affinity, indicating that fluspirilene may be a prototypical agent for this class of drug.

Galizzi, J.P., Fosset, M., Romey, G., Laduron, P. & Lazdunski, M. (1986) *Proc. Natl. Acad. Sci.* **83**, 7513-7517.
Kenny, B.A., Fraser, F., Kilpatrick, A.T. & Spedding, M. (1990) *Br. J. Pharmacol.*, **100**, 211-216.
Qar, J., Galizzi, J.P., Fosset, M. & Lazdunski, M. (1987) *Eur. J. Pharmacol.* **14**, 261-268.

111P EFFECTS OF ALINIDINE AND FALIPAMIL ON PN200-110 AND DESMETHOXYVERAPAMIL BINDING IN GUINEA-PIG VENTRICULAR MICROSOMES

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Alinidine and falipamil are specific bradycardic agents with an uncertain mechanism of action although their activity has been associated with modification of calcium currents (Kobinger & Lillie, 1988). Since there is little published information on the binding properties of these agents to the L-type calcium channel, we have investigated the binding of these compounds to the PN200-110 and desmethoxyverapamil binding sites in guinea-pig ventricular microsomes and related this to their negative chronotropic activity in vitro.

Crude microsomes were prepared from guinea-pig ventricle using Polytron homogenisation followed by centrifugation at 35,000xg for 1 hour. Binding to the dihydropyridine binding site on the L-type calcium channel was evaluated using the ligand [^{3}H]-PN200-110 (Gould et al., 1984) and binding to the phenylalkylamine binding site using the ligand [^{3}H]-desmethoxyverapamil (Garcia et al., 1984). The IC50 values for negative chronotropic activity were derived by conducting cumulative dose response curves in isolated spontaneously beating paired guinea-pig atria, preparations were suspended in Krebs solution at 37°C and gassed with 5% carbon dioxide in oxygen.

Falipamil and alinidine were found to completely displace desmethoxyverapamil binding with an IC50 of 8.5 ± 1.2 µM and 17.2 ± 2.1 µM respectively (n = 6). Alinidine was completely inactive at doses up to 100 µM against PN200-110 binding whereas falipamil caused a partial inhibition of binding (20%) at the highest concentration used (100 µM). The IC50 values for the negative chronotropic activity for falipamil and alinidine were 41.8 ± 5.3 µM (n = 11) and 156.5 ± 39.1 µM (n = 8) respectively.

Both falipamil and alinidine specifically displaced desmethoxyverapamil binding at concentrations lower than those observed for negative chronotropic activity. This observation for compounds of diverse chemical structure suggests that selective binding to the phenylalkylamine binding site on the L-type calcium channel may be involved in the mechanism of action of these compounds.

Garcia, M.L., Trumble, M.J., Reuben, J.P. & Kaczorowski, G.J. (1984) J. Biol. Chem. 259, 15013-15016.
Gould, R.J., Murphy, K.M.M. & Snyder, S.H. (1984) Mol. Pharmacol. 25, 235-241.
Kobinger, W. & Lillie, C. (1988) Cardiovasc. Drug. Revs. 6, 35-53.

112P COMPARISON OF RAT AORTA AND PORCINE CORONARY ARTERY RESPONSE TO BIG ENDOTHELIN-1 AND ENDOTHELIN-1

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There is now considerable evidence for the specific conversion of big endothelin-1 (big ET-1) to endothelin-1 (ET-1) by a phosphoramidon-sensitive endothelin converting enzyme (Yanagisawa et al. 1988). This conversion may occur in isolated tissues such as the porcine coronary artery (Fukuroda et al., 1990). In the present study, we have compared the effect of phosphoramidon and the endothelin antagonist BQ123 (Ishikawa et al., 1991) on the response of rat aorta (RAO) and porcine coronary artery (PCA) to these endothelins. Ring segments from RAO and PCA with or without endothelium (assessed by acetylcholine or ATP), were suspended in Kreb's solution, bubbled with 95% O_2/5% CO_2 at 37°C, under resting tensions of 1g and 2g respectively. Changes in tone were measured isometrically. The contractile activity of the endothelins was expressed as a % of the maximum response to 70mM KCl.

Table 1 EC50 values (nM) and [maximum response (% KCl maximum)]

	Rat aorta		Porcine coronary artery	
	ET-1	Big ET-1	ET-1	Big ET-1
+ endoth.	20 [155]	156 [123]	7 [89.5]	85 [88.4]
- endoth.	14 [155]	170 [137]	6 [90.1]	111 [93.6]

In RAO, phosphoramidon had little effect on the big ET-1 concentration response curve (ratios : 3.8, 3.6, 3.2, at 0.1, 0.3, 1 mM). In contrast, in the PCA, phosphoramidon, 0.1mM gave a ratio of 8.7. Greater shifts were obtained with 0.3mM and 1mM, but maximum responses to big ET-1 were not obtained. Phosphoramidon did not significantly affect the contraction to ET-1 in either RAO or PCA. BQ123 (0.3µM) relaxed the sustained maximum contraction to big ET-1 (1µM) in both RAO and PCA.
These results confirm the findings of Fukurada et al., (1990) that the endothelium does not modulate the responses to big ET-1 and ET-1 in the tissues studied. In addition, the data suggest that unlike the PCA, the contraction evoked by big ET-1 in RAO, is not a result of its conversion to ET-1 by a phosphoramidon-sensitive enzyme but, as evidenced by the effect of BQ123, may be a direct effect on an endothelin receptor.

Fukuroda, T., Noguchi, K., Tsuchida, S., Nishikibe, M., Ikemoto, F., Okada, K. & Yano, M. (1990). Biochem. Biophys. Res. Comm. 172, 390-395.
Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. & Masaki, T. (1988). Nature 332, 411-415.
Ishikawa, K., Fukami, T., Hayama, T., Niiyama, K., Nagase, T., Mase, T., Fujita, K., Kumagai, K., Urakawa, U., Ihara, M., Kimura, S. & Yano, M. (1991). Proc. 12th Amer. Peptide Symp., Abstract P506.

113P EFFECT OF A MAGNESIUM DEFICIENT DIET ON CYCLOSPORIN A-INDUCED CARDIAC CALCIFICATION

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Severe myocardial calcification accompanied by hypocalcaemia and hypomagnesaemia has been observed in CBA/Ca mice treated with cyclosporin A (CsA) (Borland et al, 1985a & b). The work here investigates the effects of CsA on animals rendered magnesium deficient by dietary manipulation.

CBA/Ca mice (♂ & ♀ 20-25g) were obtained from Bantin and Kingman Ltd., Humberside. Mice were fed a magnesium deficient diet (Special Diet Services, Witham, Essex). This was a semi-synthetic diet in powder form. It was made to a paste immediately before use with either distilled water (for Mg deficient diet), or MgSO4 water (for Mg sufficient diet). Mice were pair fed and were given distilled water ad lib.CsA (a gift from Sandoz) 80mg/kg was dissolved in olive oil. Magnesium sulphate was dissolved in distilled water and, where indicated, was used to make the food powder into paste such that the diet was then magnesium sufficient. Mice were allowed to acclimatize to the diet for a few days, then they were treated orally with either CsA or olive oil (0.1ml) daily for 12-14 days.

Mice were killed 24 hours after completion of treatment. The hearts were removed for measurement of tissue calcium content and were dried to constant weight followed by digestion in nitric acid. Tissue calcium and plasma calcium and magnesium content were determined by atomic absorption spectroscopy and were compared using students t-test.

		OLIVE OIL		CsA	
		Mg deficient	Mg sufficient	Mg deficient	Mg sufficient
Cardiac Ca^{++}	♀	8.2 ± 1.0	8.6 ± 0.7	858.1 ± 234.0**††	70.2 ± 16.8††
umol/g	♂	11.5 ± 2.0	7.7 ± 0.3	506.4 ± 106.3**†††	147.0 ± 43.1††
Plasma Mg^{++}	♀	0.86 ± 0.06***	1.64 ± 0.04	0.51 ± 0.10**††	1.03 ± 0.12†††
mmol/l	♂	0.82 ± 0.05***	1.09 ± 0.03	0.43 ± 0.03***†††	0.92 ± 0.04††
Plasma Ca^{++}	♀	2.24 ± 0.05	2.34 ± 0.04	2.07 ± 0.04*†	2.27 ± 0.08
mmol/l	♂	2.24 ± 0.08	2.43 ± 0.06	2.14 ± 0.09	2.01 ± 0.07†††

Values are mean ±S.E. n=5-10

*Significance of difference from Mg sufficient (*)p<0.05 (**)p<0.01 (***)p<0.001
(†)Significance of difference from olive oil (†)p<0.05 (††)p<0.01 (†††)p<0.001)

In all groups, the plasma magnesium concentration was significantly reduced by the magnesium deficient diet compared with the magnesium sufficient diet. Plasma magnesium concentration was significantly reduced in all of the groups treated with CsA, compared with their olive oil controls. Plasma calcium concentration was significantly reduced in ♀ mice treated with CsA on a magnesium deficient diet compared with both its magnesium sufficient group and its olive oil control. Plasma calcium was significantly reduced in the CsA Mg sufficient group compared with its olive oil control. There was no obvious correlation between plasma calcium concentration and changes in cardiac calcification. Heart calcium content was significantly increased in the CsA magnesium sufficient group compared with olive oil controls, and the magnesium deficient diet significantly increased the effect of CsA, but had no significant effect on the olive oil group.

In conclusion dietary deficiency of magnesium greatly magnifies the cardiac calcification caused by CsA in CBA/Ca mice.

Borland I.A., Sells R.A., Gosney J.R. & Benyon R.J.(1985a) Transplant proc, 17 1345
Borland I.A., Gosney J.R., Hillis A.N., Williamson E.P.M. & Sells R.A.(1985b) Lancet,1 103

114P PROTECTION OF HUMAN ERYTHROCYTES BY LAZAROIDS

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Lazaroids are 21-aminosteroids which have been shown to promote functional recovery of central nervous system from trauma and ischaemia (Anderson et al., 1988) and also to inhibit lipid peroxidation of brain tissues (McCall et al., 1987). It is however not known if they have plasma membrane stabilizing effect. In the present study we have investigated if lazaroids can protect human erythrocytes against haemolytic injury. Two lazaroids (U 74500A and U 74389F) were selected. Samples of antecubital vein blood were collected from 14 healthy male volunteers (age 21 to 28 years) and erythrocyte suspensions were prepared as described earlier (Sharma & Gulati, 1988). Erythrocytic Dye Decolorization Time (Sigma Chemicals) was measured to determine if any of the subjects was suffering from Glucose 6 phosphate dehydrogenase (G6PD) deficiency. Cell suspensions were incubated with drugs for periods ranging from 5 to 60 minutes. Erythrolysis was produced by 5 minute incubation with hypotonic saline (68.5mM sodium chloride in 10mM sodium phosphate) or by four hour incubation with hydrogen peroxide (to give a final concentration of 0.75%). The ability of lazaroids to protect haemolysis was also compared with chlorpromazine and cromolyn sodium both of which are known plasma membrane stabilizers. Stock solutions of lazaroids were prepared in 95% ethanol and were added in 25 µl volumes directly to erythrocyte suspensions. Final concentration of ethanol in experimental and control tubes was kept at 1.75%. Solutions of chlorpromazine and cromolyn sodium were prepared in 0.9% NaCl. Mean (± SD) IC_{50} (concentrations which produced 50% inhibition of erythrolysis) are shown in the table.

	Saline	Hydrogen peroxide
Chlorpromazine	4.3 ± 1.03 x 10^{-6}	3.8 ± 0.93 x 10^{-6}
Cromolyn sodium	4.6 ± 1.19 x 10^{-3}	3.7 ± 0.54 x 10^{-3}
U 74500A	2.3 ± 0.52 x 10^{-6}	1.6 ± 0.42 x 10^{-6}
U 74389F	2.9 ± 0.57 x 10^{-4}	4.8 ± 1.61 x 10^{-5}

The results show that lazaroids have plasma membrane stabilizing property. Out of the two compounds studied U 74500A was several fold more potent than U 74389F and also when compared with the two known stabilizers of plasma membrane. Further work will elucidate if lazaroids can protect G6PD deficient erythrocytes from drug induced haemolysis since all the subjects in the present study had normal levels of this enzyme.

Anderson, D.K., Braughler, J.M., Hall, E.D., Waters, T.R., McCall, J.M. and Means, E.D. (1988) J. Neurosurg. 69, 562.
McCall, J.M., Braughler, J.M. and Hall, E.D. (1987) Acta Anaesthesiol. Belgica 38(4) 417.
Sharma, S.C. & Gulati, O.P. (1988) Brit. J. Pharmacol. 93, 209p.

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115P MODIFICATIONS TO THE RATE OF WOUND CONTRACTION IN THE RAT INDUCED BY ALLOPURINOL

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Wound contraction is produced by the action of myofibroblasts within the granulation tissue formed at the sites of tissue damage. The myofibroblast, alternatively called the contractile fibroblast, can exert sufficient tension so as to compromise the functioning of an organ, such as the liver in alcohol induced cirrhosis or induce tissue alterations which can reduce the mobility of joints and cause strictures in body orifices. A desirable pharmacological objective would be the development of an agent which selectively inhibited myofibroblast contractility in such conditions. One such agent is allopurinol which has been suggested as alleviating the activity of myofibroblasts in Dupuytren's contracture (Murrell *et al.*, 1987); another is papaverine which 'in vitro' relaxes myofibroblasts (Gabbiani *et al.*, 1973). Neither of these agents have ever been experimentally investigated in a rodent wound healing model.

Full thickness wounds, 15 x 15mm, were made on the lower dorsum of adult female, hooded Lister rats. All the wounds were immediately covered with a Tegaderm(R) dressing and the animals then treated with a) control solvents - either 2% carboxymethylcellulose or saline, b) allopurinol - suspended in carboxymethylcellulose 50mg/kg p.o. p.d. for 7 days or c) papaverine - dissolved in saline, 5mg/kg p.o. p.d. for 4, 8 or 12 days. All the wounds were traced onto plastic sheets, digitised and the coefficient of wound contraction calculated as described previously (Cross & Naylor, 1990).

All the wounds healed by granulation and none were infected. The coefficients for wound contraction were:

Treatment	n	Coefficient of wound contraction (2-7 days)	P	Treatment	n	Coefficient of wound contraction (2-7 days)	P
Control	18	-0.075		Control	6	-0.073	
Allopurinol	14	-0.057	<0.05	Papaverine 4 days	6	-0.060	<0.05
				8 days	5	-0.085	-
				12 days	5	-0.069	<0.05

The histology of the wounds will be shown using photomicrographs. The results for the coefficients of wound contraction indicate that both allopurinol and papaverine decrease the rate of wound healing. However, for allopurinol, the wound defect was found to fill with more granulation tissue than the solvent treated animal. With papaverine a similar finding was made. These results indicate that inhibition of normal repair may facilitate a greater deposition of collagen and so aid the repair process.

Cross, S.E. & Naylor, I.L. (1990) Eur. J. Pharmacol. 183, 2271-2272.
Gabbiani, G. et al. (1973) J. Exp. Med. 135, 719-734.
Murrell, G.A.C. et al. (1987) Speculation in Science & Technology 10, 107-112.

116P NICKEL CYTOTOXICITY IN MAMMALIAN FIBROBLASTS DOES NOT INVOLVE DEPLETION OF GLUTATHIONE

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Nickel is a suspected human carcinogen, and has cytotoxic effects on the growth, viability and proteoglycan synthesis of connective tissues. One hypothesis for the mechanism of nickel-induced toxicity and carcinogenicity is that the metal indirectly damages cell macromolecules by producing active oxygen species as a result of nickel catalyzed redox reactions. The resulting oxidative stress placed on the cell would cause oxidation and depletion of essential cofactors such as reduced glutathione (GSH). We have tested this hypothesis using cultured 3T3 mouse fibroblasts treated with nickel chloride (20-500μM) for up to 7 days, by investigating the effect of the metal on cell growth (measured by total cell protein), viability (leakage of lactate dehydrogenase) and GSH content. Cells were grown in Dulbecco's medium containing 10% foetal calf serum.

Nickel caused a concentration dependent inhibition of fibroblast growth at concentrations of 100μM and above, but had no significant effect on cell viability, even after exposure of the cells to 500μM for 7 days. Table 1 shows that, when added during the log phase of growth (48h after passaging the cells), 500μM nickel was markedly cytostatic, but had no significant effect on cellular GSH levels.

Table 1 The effect of 500μM Nickel Chloride on 3T3 cell growth and GSH content

Treatment	Protein (mg/flask)		GSH(nmol/mg protein)	
Time	Control	Nickel	Control	Nickel
1h	0.29±0.05	0.23±0.02	22.01±3.10	29.28±2.28
2h	0.25±0.01	0.28±0.02	27.51±2.12	29.48±0.90
3h	0.31±0.01	0.24±0.03	28.18±2.56	29.80±3.02
4h	0.29±0.01	0.22±0.01*	32.84±1.63	34.42±1.75
6h	0.34±0.02	0.23±0.01*	28.18±1.28	27.82±3.89
8h	0.36±0.01	0.17±0.01*	27.67±2.80	25.01±1.80

Results are means ± SEM of 4 experiments, *P<0.05, by non-paired Student's t test. Nickel chloride was added 48h after passaging the cells during the log phase of cell growth.

These results indicate that oxidative stress is not likely to be involved in the cytostatic effect of nickel chloride on fibroblasts as intracellular GSH levels were not depleted.

117P BILIRUBIN UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES IN SV40-IMMORTALISED RAT HEPATOCYTES

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Immortalised hepatocyte cell lines produced by transfecting primary cells with DNA containing viral oncogenes are a potential alternative to primary rat hepatocytes for studying xenobiotic metabolism and toxicity in vitro. We have demonstrated that one such cell line, SV40RH1, retains many aspects of differentiated cell function at least in early culture (Nairn et al, 1990, Campbell et al, 1991). As a further index of differentiated function, the activity of bilirubin UDP-glucuronosyltransferase (GT) and its regulation by intracellular haem levels has been measured. GT activities were compared in freshly isolated Sprague-Dawley rat hepatocytes, the rat hepatoma cell line HTC and in the SV40 immortalised rat hepatocyte cell lines SV40RH1 and P9. SV40RH1 cells were grown on hydrated collagen gels, and both immortalised cell lines were grown in a hormonally-defined medium based on Ham's F12/Williams' E media. Where indicated dimethylsulfoxide (DMSO, 2%(v/v)) and/or 10^{-4}M 5-aminolaevulunic acid (ALA) were added to increase intracellular haem levels and these experiments were carried out in Dulbecco's medium containing 10% foetal calf serum (FCS) and the effect on GT activity measured 7 days later.

Table 1 shows that P9 cells retained GT activity at levels similar to those in freshly isolated hepatocytes, whereas the activity had declined in SV40RH1 cells. In contrast, the de-differentiated HTC cells contained very low levels of GT activity. GT activity in P9 cells was higher when the cells were grown in hormonally - defined medium than in medium containing 10% FCS (7.72 ± 0.67 (n=4) compared with 1.40 ±0.25 (n=4) pmol/min/mg protein).

DMSO and ALA increased GT activity in P9 cells grown in Dulbecco's medium containing 10% FCS to 2.36 ± 0.46 (n=4) and 3.27 ±0.60, (n=6) pmol/min/mg protein respectively, and when both agents were present together the effect was additive (6.84 ±1.09, (n=7) pmol/min/mg protein).

Table 1. GT activities in hepatocytes, HTC cells and in the immortalised liver cell lines (pmol/min/mg protein)

	hepatocytes	P9	SV40RH1	HTC
GT activity	8.37±0.57(4)	7.72±0.67(4)	2.86±0.08(3)	0.31±0.25(3)

Values are expressed as mean ± SE, with the number of experiments in parentheses.

These results indicate that GT activity is retained in immortalised hepatocytes at levels similar to those in freshly isolated hepatocytes, and furthermore the GT activity can be regulated by agents which increase intracellular haem content.

Campbell D. et al (1991) Brit. J. Pharmacol. 104 199P.
Nairn A. et al (1990) Biochem Soc. Trarns. 18 1201-1202.

118P GLUTATHIONE LEVELS IN BRAIN NEURODEGENERATIVE DISEASES AFFECTING BASAL GANGLIA

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Previously, we confirmed that levels of reduced glutathione (GSH) in the substantia nigra (SN) were decreased in Parkinson's disease (PD) as reported by others (Perry et al, 1986; Riederer et al, 1989). We also showed that the reduction of reduced glutathione levels was present in the early presymptomatic stages of the disease process (Sian et al, 1991). We have now examined the specificity of this change in other degenerative disorders that affect the SN, namely multiple system atrophy (MSA), and progressive supranuclear palsy (PSP).

Brain tissue was obtained after autopsy from 3 groups of control patients matched for age and post-mortem delay with PD, MSA and PSP patients. Brain samples were homogenised in 0.4M perchloric acid and centrifuged, the supernatant was analysed for oxidised glutathione (GSSG) by an enzymatic recycling procedure using a double-beam spectrophotometer and GSH using high pressure liquid chromatography-ultraviolet detection (see Sian et al, 1991).

There was a marked reduction of GSH in SN from the PD patients compared to controls (Table 1). However there was no change in the GSSG content in this area. There were no changes in GSH or GSSG levels in any other areas in PD. These results are in agreement with our earlier findings (Sian et al, 1991). In both MSA and PSP, there was a trend for a decrease in the GSH content of SN, but these effects were not statistically significant (Table 1). In the MSA patients there was an increase of GSH coupled with a reduction of GSSG levels in the lateral globus pallidus. In the PSP subjects there was a decrease of GSH in the caudate nucleus. There were no changes in GSH or GSSG levels in other brain regions in MSA or PSP.

Table 1 Glutathione levels in the substantia nigra from controls, MSA, PSP and PD patients.

GROUP	GLUTATHIONE CONTENT (μmoles/g wet weight)	
	GSH (reduced)	GSSG (oxidised)
Controls	0.80 ± 0.03 (3)	0.0027 ± 0.0004 (8)
MSA patients	0.61 ± 0.07(4)	0.0039 ± 0.0005 (4)
Controls	0.73 ± 0.08 (10)	0.0021 ± 0.0003 (10)
PSP patients	0.56 ± 0.20 (3)	ND
Controls	0.75 ± 0.06 (13)	0.0024 ± 0.0004 (12)
PD patients	0.45 ± 0.03**(16)	0.0031 ± 0.0004 (13)

Values are mean ± SEM. Number of patients in parentheses. **p< 0.01 as compared to control subjects (Student's t-test).
ND = Not determined due to insufficient tissue available.

The results suggest a selective reduction of GSH in SN of Parkinson's disease. The changes of glutathione levels in other brain areas from MSA and PSP patients probably reflect the different pathological processes underlying these disorders.

Perry, T.L., Yong, V.W. (1986). Neurosci. Lett. **67**, 269-274.
Riederer, P., Emin, S., Rausch, W-D., Schmidt, B., Reynolds, G.P., Jellinger, K., Youdim, M.B.H. (1989). J. Neurochem. **52**,515-520.
Sian, J., Dexter, D.T., Jenner, P., Marsden, C.D. (1991). Brit. J. Pharmacol. **104**, 281P.

119P HYPOTHERMIA AND THE NEUROPROTECTIVE ACTION OF ENADOLINE AND DIZOCILPINE

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Kappa opioid agonists and non-competitive NMDA antagonists are effective anti-ischaemic agents in the gerbil (Tang, 1985; Hayward *et al.*, 1992). There has been some debate concerning the mechanism of the neuroprotective effect of the NMDA antagonist dizocilpine (MK-801) in global models of ischaemia, particularly with regard to the possible contribution of drug-induced hypothermia to this action (Buchan & Pulsinelli, 1990; Gill & Woodruff, 1990). We have investigated this further by continuously monitoring brain and rectal temperature of gerbils subjected to ischaemia given either dizocilpine or enadoline (CI-977) at neuroprotective doses of 3 and 1 mg kg^{-1} s.c. respectively (Hayward *et al.*, 1992).

Under equithesin anaesthesia a guide cannula was implanted such that a thermocouple microprobe (Sensortek MT29/2) protruded 1 mm from the tip of the guide cannula, into the CA1 subfield of the right hippocampus of mature female gerbils. Two days later animals were subjected to 7 min forebrain ischaemia by occluding both carotid arteries. Enadoline was administered immediately before and at 4 h after ischaemic insult, whilst dizocilpine was administered only once, immediately before the ischaemic insult. Saline-injected animals were used as controls. Hippocampal temperature was recorded continuously, and rectal temperatures every 30 min, 1 h before surgery and for 7 h post ischaemia. Seven days later animals were sacrificed and measurements of neuronal damage made for both hippocampi (CA1/CA2) in cresyl fast violet stained coronal sections (20 μm) corresponding to 1.6 mm caudal to bregma using an image analyser.

Table 1: Effect of Enadoline and Dizocilpine on brain and rectal temperature, pre and post ischaemia.

	Temperature (°C)					
Time post	Saline		Enadoline		Dizocilpine	
ischaemia (min)	Brain	Rectal	Brain	Rectal	Brain	Rectal
-30	36.64±0.27	38.00±0.18	36.76±0.22	37.52±0.03	36.48±0.35	37.80±0.17
0	33.34±0.40	37.13±0.20	32.79±0.36	36.87±0.22	32.21±0.18	36.92±0.18
30	36.72±0.25	37.38±0.22	35.90±0.42	36.50±0.32	35.65±0.55	36.17±0.54
90	36.34±0.24	37.38±0.19	36.54±0.28	36.92±0.29	36.41±0.47	36.50±0.38

In control animals, marked degeneration of hippocampal CA1/CA2 pyramidal neurones was seen; Enadoline and dizocilpine significantly decreased the area of neuronal damage. The results show that brain temperature drops rapidly during ischaemia, due to the removal of the warming effect of cerebral blood flow, whilst body temperature is maintained normothermic. Enadoline and dizocilpine afforded marked neuroprotection without altering brain temperature during surgery or recirculation, compared to ischaemic (control) animals. Therefore the neuroprotective effect of dizolcilpine does not appear to be soley the consequence of hypothermia as suggested by Buchan & Pulsinelli (1990). The findings also suggest that enadoline does not produce its neuroprotective effect by lowering brain temperature in the gerbil.

Tang, A.H. (1985). Life Sci., 37, 1475-1482.
Hayward, N.J., McKnight, A.T. & Woodruff, G.N. (1992). Mol. Neuropharmacol., in press.
Buchan, A. & Pulsinelli, W.A. (1990). J. Neurosci., 10, 311-316.
Gill, R. & Woodruff, G.N. (1990). Eur. J. Pharmacol., 176, 143-149.

120P NEUROTROPHIC EFFECTS OF SR 57746A, A NEW NON-PEPTIDE COMPOUND: *IN VITRO* STUDIES

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A possible strategy for treating neurodegenerative disorders (e.g. Alzheimer's disease) is the search for compounds which mimic or increase the effects of nerve growth factor (NGF) (see Hefti & Schneider, 1991). SR 57746A [1-(2βnaphthyl ethyl)-4-(3-trifluoromethyl phenyl)-1,2,5,6 tetrahydropyridine hydrochloride] is a new compound active in a number of cell culture systems in which NGF is involved. When neuroblasts from 17-18 day-old rat foetal septa were incubated in a modified serum-free medium (Muller & Seifert, 1982) for 18 h with SR 57746A or its vehicle (DMSO), SR 57746A, like NGF, increased the number of cells bearing neurites. In a typical experiment, there were 29.4 ± 1.6 % (mean ± S.E.M., n = 10) of neurite-bearing cells in the presence of vehicle, and 40.1 ± 2.4 % (n = 10; P<0.01) in the presence of 24 nM SR 57746A. When the neuroblasts were incubated in a minimal serum-free medium for 4 days, SR 57746A, again like NGF, increased neuronal survival, as measured by the formazan blue method (Manthorpe *et al*, 1988). Optical density was 0.084 + 0.01 units in presence of vehicle, 0.12 + 0.01 (P<0.05) with 100 nM SR 57746A, and 0.14 + 0.01 (P<0.01) with 1.5 μM SR 57746A (n = 7-20). However, SR 57746A did not modify survival of these cells incubated in optimal N_2 medium (Bottenstein, 1985) or selenium-free medium. As subsequent screening studies had shown that SR 57746A possessed high affinity for 5-HT1A receptors (IC_{50} = 4 nM), buspirone and 8OHDPAT were also evaluated, but had no consistent effect on neuronal differentiation or survival. In another cell system in which NGF is active, PC12 cells maintained in the N_1 medium (Bottenstein, 1985), SR 57746A (25 nM to 2.5 μM) did not alone modify cell differentiation or survival, but increased the differentiating effect of NGF (0 % cells with neurites in the presence of vehicle or SR 57746A, 11.3 ± 1.5 % with 1 nM NGF, 18.9 ± 2.7 % when 1 nM NGF was associated with 250 nM SR 57746A, n = 10, P<0.05 vs. NGF alone). The effect of SR 57746A was also evaluated on the expression of the NGF gene in C6-2B glioma cells, cultivated in 10 % foetal calf serum, then stimulated for 3 h with SR 57746A or vehicle in serum-free medium. SR 57746A (100 nM) increased NGF mRNA (measured by Northern blot) by 46.2 ± 11.8 % (n = 4; P<0.05); 8OHDPAT (1-10,000 nM) was inactive. This effect was confirmed in two subsequent experiments. In conclusion, SR 57746A has direct or indirect neurotrophic effects in a number of cell systems. In NGF-sensitive cells, SR 57746A either produced a direct, NGF-like effect, or acted synergistically with NGF. In gliomal cells, SR 57746A increased NGF mRNA levels. Since 5-HT1A receptors do not seem to be involved in the neurotrophic effect of SR 57746A, further studies are required to determine the mechanisms underlying the neuroprotective activity of the compound.

Bottenstein J.E. (1985). In "Cell culture in the Neurosciences", J.E. Bottenstein, G. Sato, eds., 3-43.
Hefti F. & Schneider L.S. (1991). Clin. Neuropharmacol. 14, Suppl. 1, S62-S76.
Manthorpe G., Fagnani R., Skaper S.D. & Varon S. (1988). Dev. Brain Res. 25, 191-198.
Muller H.W. & Seifert N. (1982). J. Neurosci. Res. 8, 195-204.

121P CHARACTERIZATION OF A NOVEL KAINATE-BINDING PROTEIN FROM GOLDFISH CNS

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The retino-tectal system of the goldfish is a widely used model for the study of neuronal regeneration and for the mechanisms underlying synaptic formation and activity-dependent stabilization. The latter processes are thought to rely heavily on the involvement of excitatory amino acid (EAA) receptors. In order to begin to investigate the role of EAA receptor subtypes in these neuronal mechanisms we have determined the pharmacological properties of kainate binding in membrane fragments from goldfish CNS.

Goldfish membranes were prepared as described previously (Henley and Oswald, 1988) in Tris buffer (50mM Tris, 1.0mM EDTA, 1.0mM EGTA, pH 7.4 with citric acid). To initiate binding, goldfish homogenate (50µl) was added to test-tubes, in triplicate, containing 10µl of competing compound or vehicle and 10µl [^{3}H]Kainate (58Ci/mmol). The final assay volume was adjusted to 100µl with Tris buffer. Binding was allowed to proceed at 0°C for 90 min. The reaction was terminated by rapid filtration through GF/B filters pre-soaked in 0.05% polyethylenimine. The filters were washed with three 3 ml aliquots of ice-cold Tris buffer (total wash time ~9 sec.). Radioactivity remaining on the filters was assayed by liquid scintillation spectroscopy at an efficiency of approximately 48%.

In good agreement with previous reports (Henley and Oswald, 1988) the K_D value was 244 ± 23 nM and the B_{max} value 155 ± 43 pmol/mg protein. The rank order of potency for displacing ligands was domoate > kainate> CNQX > L-glutamate. It was notable, however, that a proportion of the [^{3}H]kainate binding was not displaced by L-glutamate even at mM concentrations. In general, these findings are consistent with the binding sites being ionotropic non-NMDA receptors as documented in other vertebrate systems (for review see Barnes and Henley 1992). However, the major proportion of [^{3}H]kainate binding in the goldfish CNS has recently been reported to be to a class of G-protein coupled receptors (Willard et al., 1991). Preliminary experiments in our laboratory have confirmed that [^{3}H]kainate binding is sensitive to modulation by certain GTP-analogues, suggesting these sites may represent a novel form of metabotropic EAA receptor.

We have performed radiation inactivation studies (in collaboration with Dr Mogens Nielsen, Sct Hans Hospital, Denmark) to determine the molecular target size of the kainate-binding site in goldfish CNS. A linear inactivation profile was obtained revealing an apparent molecular mass of ~31 kilodaltons. This value is considerably smaller than the reported molecular weights of rat brain EAA receptor subunits. Both the ionotropic and metabotropic classes of receptors cloned and expressed from rat brain have a deduced molecular weight of ~100 kilodaltons (see Barnes and Henley 1992). On the other hand, biochemical purification of kainate-binding proteins from several lower vertebrates have indicated that the subunit sizes are in the 40 - 50 kilodalton range. In an attempt to address this anomaly we have solubilized the goldfish CNS kainate-binding activity using the non-ionic detergent octylglucoside and we are currently attempting to determine the size of the soluble binding site by various physical and chemical fractionation methods.

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References

Barnes, J. M. and Henley, J. M. (1992) Prog. Neurobiol. in press.
Henley, J. M. and Oswald, R.E. (1988) Biochem. Biophy. Acta. 937, 102-111.
Willard, J.M., Ziegra, C.J. and Oswald, R.E. (1991) J. Biol. Chem. 266, 10196-10200.

122P DOPAMINE RELEASING ACTIONS OF NMDA IN RAT CAUDATE PUTAMEN STUDIED BY FAST CYCLIC VOLTAMMETRY

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Investigations in vitro (Clow & Jhamandas, 1989; Krebs et al., 1990) have shown that glutamic acid, acting at NMDA receptors in the absence of Mg^{2+} can stimulate the release of dopamine (DA) from nerve terminals in the rat caudate putamen (CPu). We have investigated the actions of NMDA on DA release and on DA overflow evoked by local electrical stimulation in caudate putamen slices in presence or absence of Mg^{2+}. Experiments were made in 350 µm coronal slices of rat CPu (0.7-1.7mm anterior to bregma;Paxinos & Watson, 1982). DA overflow following single pulse electrical stimulation (0.1 ms; 20 v) or addition of drugs to the perfusion medium was monitored by fast cyclic voltammetry at a carbon microelectrode, implanted 60-80 µm inside the slice using methods described by (Palij et al.1990).

In the presence of 2 mM Mg^{2+}, 20µM NMDA produced a transient rise in the voltammetric signal (peak equivalent to 19 ± 4 nM DA, n=3); electrically evoked DA overflow was not significantly affected by addition of 20µM NMDA.

In the absence of Mg^{2+} there was a sustained rise in the background voltammetric signal (equivalent to 50 ± 10 nM DA; n=21) and at the same time a significant potentiation of the electrically stimulated DA overflow (from 45 ± 6 nM in the presence of Mg^{2}+ to 90 ± 11 nM in the absence of Mg^{2+}; n=21, $p<0.001$). 1µM CPP had no effect on electrically stimulated DA overflow (n=9). 20µM NMDA evoked a transient rise in the background signal, equivalent to 57 ± 7 nM DA, n=17 . This effect was augmented by 20µM glycine (90 ± 16 nM DA, n=3) but depressed by 1µM CPP (33 ± 28 nM DA, n=3). 20 µM NMDA reduced DA overflow due to single pulse electrical stimulation (80 ± 12 nM before NMDA; 18 ± 5 nM after NMDA; n=10; $P<0.001$). This effect was fully blocked by 1µM CPP. At 4 sites (out of 21 examined) 20 µM NMDA produced a large transient rise in the background signal (equivalent to 670 ± 85 nM DA n=4) ; DA overflow following single pulse electrical stimulation was attenuated (13 ± 6 nM, n=4).

In the absence of Mg^{2+}, NMDA has two effects on DA overflow: a transient release of DA in the absence of electrical stimulation, followed by an attenuation of electrically stimulated DA overflow. The link (if any) between these effects requires further investigations. The presence of sites where very large DA overflow occurs suggests that the CPu is not the neurochemically homogenous structure which it is sometimes assumed to be.

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Clow D.W. & Jhamandas K. (1989). J. Pharmacol. Exp. Ther. 248, 722-28.
Krebs M.O., Desce J.M., Kemel M.L., Gauchy C., Godeheu G., Cheramy A, & Glowinski J. (1990). J. Neurochem. 56, 81-85.
Palij P, Bull D.R.,Sheehan M.J., Millar J., Stamford J., Kruk Z.L. & Humphrey P.P.A. (1990). Brain Res. 509, 172-17.
Paxinos,G & Watson,C.The rat brain in stereotaxic co ordinates. Academic.London. 1982.

123P EFFECTS OF DAILY FOCAL NMDA PRETREATMENT ON THE PARAMETERS OF AMYGDALOID ELECTRICAL KINDLING

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The brain mechanisms involved in the generation and spread of epileptic seizure activity are at present undefined. Electrical kindling is a model of epilepsy ideally suited to studying these processes. We have previously demonstrated that competitive antagonists acting at the NMDA sub-type of excitatory amino acid (EAA) receptors both retard the rate of electrical kindling and raise generalized seizure thresholds (GSTs) in fully kindled animals (Croucher et al, 1988; Croucher et al 1992). In addition, daily intra-amygdaloid microinjections of glutamate and/or aspartate causes kindling of full limbic seizures which are blocked by co-administration of an NMDA antagonist (Croucher and Bradford, 1990). In the present study we further examine the role of NMDA receptors in seizure processes.

Details of the surgical and kindling procedures are given elsewhere (Croucher et al, 1992). Briefly, male Sprague-Dawley rats were implanted with a guide cannula/bipolar electrode assembly into the basolateral amygdala. Electrical afterdischarge thresholds (ADTs) were estimated in each animal before daily focal intra-amygdaloid microinjections (0.5µl) of vehicle (phosphate buffer) or NMDA were given, followed 15min later by the electrical kindling stimulus. The effects of NMDA pretreatment on ADT and on the development of electrical kindling (electroencephalographic (EEG) and motor seizure (graded 0-5) responses) were determined. In fully kindled animals, the influence of NMDA pretreatment on GSTs was examined.

Initial ADT estimates (mean ± s.e.mean): control group 115.0 ± 29.2µA, n=5; NMDA group, 90.0 ± 17.0µA, n=5. A range of doses (1-5nmol) of NMDA was tested to determine the threshold dose for inducing minimal seizure activity. No seizure activity was evident up to 30 min following NMDA, 1-2nmol. After NMDA, 5nmol, 2/5 animals showed clear EEG and/or motor responses. NMDA, 2nmol (subthreshold dose) was thus selected for further study. Pretreatment with NMDA, 2nmol, increased the mean ADT to 160.0 ± 10.0µA ($P<0.05$). Buffer injection produced no significant change. Daily pretreatment with NMDA, 2nmol, significantly enhance the rate of electrical kindling with marked reductions in the number of stimulations required to reach each stage of kindling development. The number of daily stimulations required to attain full kindling were: control group, 11.6 ± 1.4; NMDA group, 6.8 ± 1.6 ($P<0.05$). The mean durations of evoked ADs were significantly increased after some, but not all, stimulations in the NMDA pretreated animals. Importantly, fully kindled animals showed greatly enhanced sensitivity to NMDA administration. Whilst NMDA, 0.5nmol was inactive, NMDA 1nmol reduced the GST (-53.3%, 2/5 animals) or induced spontaneous seizure activity in all animals tested. These results provide further evidence for the important involvement of central NMDA receptor-mediated events in seizure generation and expression.

Croucher, M.J. & Bradford, H.F. (1990) Brain Res. 506, 349-352.
Croucher, M.J., Bradford, H.F., Sunter, D.C. & Watkins, J.C. (1988) Eur.J.Pharmacol. 152, 29-38.
Croucher, M.J., Cotterell, K.L. & Bradford, H.F. (1992) Neurochem. Res. 17, 409-413.

124P ETHANOL WITHDRAWAL HYPEREXCITABILITY IN ISOLATED HIPPOCAMPAL SLICES IS PREVENTED BY THE COMPETITIVE NMDA ANTAGONIST, CGP39551

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The physiological basis of the hyperexcitability seen on cessation of long-term ethanol intake is not yet understood. We have described patterns of hyperexcitability in isolated hippocampal slices, after chronic ethanol treatment in vivo, with decreases in the thresholds for elicitation of single and multiple population spikes (Whittington and Little, 1991). We now investigate the effects of CGP39551, a competitive NMDA antagonist, on these threshold changes. (CGP39551 is 2-amino-4-methyl-5-phosphono-3-pentanoate-1-ethyl ester).

Male mice, C57 strain, 25 - 35g, were given ethanol, 24%, as sole drinking fluid for 15 weeks. Hippocampal slices were prepared immediately on withdrawal from ethanol, as previously described (Whittington and Little, 1991). They were perfused with Kreb's solution, in the absence of ethanol. Extracellular recordings were made from the pyramidal cell layer in area CA1, with stimulation of the Schaffer collateral/commissural pathway. Thresholds for elicitation of population spikes were established every 15 minutes. All times are from the beginning of slice preparation (ie. from ethanol withdrawal). The effects of CGP39551 were determined by addition of this compound to the perfusion medium, at 20µM, throughout the recording period. Comparisons were made by analysis of variance; n = 6 - 8 slices per group, each from a different mouse.

	Single spike thresholds (µA)				Multiple spike thresholds (µA)		
Time into W/D:	2h	3.5h	5.5h	7h	3.5h	5.5h	7h
Control	180±11	157±9	153±10	153±10	>1000	>1000	>1000
Ethanol W/D*	133±12	125±15	125±15	128±15	>1000	983±17	942±37
Ethanol W/D + CGP†	165±11	161±10	159±11	158±11	>1000	>1000	>1000
Control + CGP	175±19	168±20	167±17	162±15	>1000	>1000	>1000

Single spike thresholds: between 1.75h and 7h into W/D : *Con v Eth W/D $p<0.05$; †Eth W/D v Eth W/D + CGP $p<0.05$
Multiple spike thresholds: between 4.75h and 7h into W/D : *Con v Eth W/D $p<0.05$; †Eth W/D v Eth W/D + CGP $p<0.05$
Con = control; Eth = ethanol; W/D = withdrawal; CGP = CGP39551 20 µM (Maximum possible stimulation was 1000µA)

The results indicate that CGP39551 completely prevented the decreases in thresholds that follow withdrawal from chronic ethanol treatment, with no effect on control thresholds. These results are compatible with our intracellular recordings showing that NMDA-mediated slow EPSPs in area CA1 of the hippocampus were increased during ethanol withdrawal (Whittington et al., 1992) and our demonstration that CGP39551 prevented the behavioural manifestations of ethanol withdrawal (Ripley et al., 1991).

Ripley, T. L., Rabbani, M. and Little, H. J. (1991) Eur. J. Neurosci., Suppl. 4, 322
Whittington, M. A. and Little, H. J. (1991) Br. J. Pharmacol., 103, 1313-1320
Whittington, M. A., Lambert, J. D. C. and Little, H. J. (1992) J. Physiol., In Press

125P THE EFFECT OF THE NMDA ANTAGONIST, DIZOCILPINE, ON BOTH ACQUISITION AND REVERSAL OF SPATIAL AND VISUAL DISCRIMINATION LEARNING TASKS IN RATS

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N-methyl-D-aspartate (NMDA) receptor antagonists impair learning processes in rats (Morris et al, 1986) but also produce motor impairments (Tonkiss & Rawlins, 1991) which may impair performance of learning and memory tasks involving motor skills. Reversal learning may obviate this difficulty since learning is measured by choice accuracy rather than response latency, and can be compared to performance in acquisition. We examined the NMDA antagonist dizocilpine on acquisition and reversal of a spatial and a visual discrimination task.

Two groups of twenty male Lister hooded rats (Harlan Olac) were trained in a Y-maze on either an appetitively motivated spatial or visual task. For each group, rats were randomly assigned to one of two conditions, saline or dizocilpine (0.075 $mgkg^{-1}$ s.c.), administered twenty minutes prior to each test session. Rats were trained for fifteen trials per day, to a criterion of five consecutive correct responses on all tasks. In the spatial task rats were trained to respond to a fixed position, in the visual task, to one of two coloured inserts. Following the completion of acquisition, reward contingencies were reversed and the rats re-retrained. Trials to criterion, mean errors per block of five trials, mean % correct and mean choice latency were also recorded.

Table Effects of dizocilpine on Y-maze performance

	Spatial Task		Visual Task	
Treatment	Acquisition	Reversal	Acquisition	Reversal
Saline	10.1 ± 2.4	13.1 ± 1.3	37.1 ± 7.8	107.2 ± 8.7
Dizocilpine	12.7 ± 3.3	40.9 ± 7.0**	84.0 ± 20.6*	172.6 ± 17**

Values are trials to criterion, mean number of trials ± s.e.mean (n=10); *p<0.05, **p<0.01; (t-test).

Dizocilpine had no effect on acquisition of the spatial task, but increased trials to criterion ($p<0.01$) on reversal (Table). On the visual task, dizocilpine affected both acquisition ($p<0.05$) and reversal ($p<0.01$). Analysis of the other variables showed similar effects.

We conclude that dizocilpine does not produce a global performance deficit since it did not affect acquisition of the spatial task. Thus the dizocilpine induced impairment of reversal learning is not dependent on gross motor or sensory disturbance. Effects of dizocilpine on the acquisition and reversal of the visual task may relate to an effect on sensory systems, or to the difficulty of the task. The latter suggestion is prompted by the fact that control animals found the visual task more difficult than the spatial task. Thus, we suggest that a low dose of dizocilpine produces a specific learning impairment during the reversal of a spatial discrimination task.

Morris, R.G.M., Anderson, E., Lynch, G. & Baudry, M. (1986) Nature **319**, 774-776.
Tonkiss, J & Rawlins, J.N.P. (1991) Psychopharmacology **85**, 349-358.

126P CONTRADICTORY ACTIONS OF THE CALCIUM CHANNEL ANTAGONIST, DILTIAZEM, ON CHEMICALLY-INDUCED SEIZURES

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The dihydropyridine class of calcium channel blocking drugs has been shown to be effective in several animal models of convulsions (Dolin *et al.*, 1988). Flunarizine, a piperazine-derivative calcium channel blocker, is also anticonvulsant in several models. (Desmedt *et al.*, 1975). In the present study we examined the effects of diltiazem, a calcium channel blocker of the benzothiazepine class, on seizures induced by a variety of chemical agents.

Male TO mice (25-35g) were pretreated with diltiazem, i.p., at a range of doses, before i.p. administration of various convulsant drugs: bicuculline, N-methyl-DL-aspartate (NMDLA), 4-aminopyridine (4-AP) and the calcium channel agonist, Bay K 8644 given i.c.v. The incidence of seizures (defined as myoclonic jerking with loss of posture) was noted and the time (latency) to the first seizure was recorded. For NMDLA, the time to hyperactive behaviour was also recorded. In the case of Bay K 8644, only handling-induced convulsive behaviour was rated, with diltiazem given 30 min prior to Bay K 8644. Convulsion incidences compared using Fisher's exact test, latencies by Student's t-test.

Diltiazem 15 min before bicuculline 3mg/kg

Drug / dose	Incidence	Latency (s)
Saline	1/8	60
Diltiazem 10 mg/kg	6/8*	118 ± 41
Diltiazem 50 mg/kg	8/8**	176 ± 30
Diltiazem100 mg/kg	8/8**	199 ± 18

Diltiazem 30 min before 4-AP 8 mg/kg

Drug / dose	Incidence	Latency (s)
Saline	1/8	1048
Diltiazem 10 mg/kg	3/8	866 ± 119
Diltiazem 50 mg/kg	5/8†	1115 ± 321
Diltiazem 100 mg/kg	7/8*	1581 ± 156¶

Diltiazem 15 min before NMDLA 200 mg/kg

Drug/dose	Incidence	Latency (s)	Hyperactivity Latency (s)
Saline	2/8	462, 480	450 ± 132
Diltiazem 10 mg/kg	3/8	948 ± 180	630 ± 132
Diltiazem 50 mg/kg	6/8	1326 ± 90**	1284 ± 240*
Diltiazem100 mg/kg	4/8	1698 ± 90**	1782 ± 264**

KEY
* $p < 0.05$ c.f. control,
** $p < 0.01$ c.f. control
† $p = 0.06$ c.f. control
¶ $p < 0.05$ c.f. diltiazem 10mg/kg

The results showed that diltiazem increased seizure incidence, for bicuculline, NMDLA and 4-AP, but also may have increased the latencies to the first seizure. It also increased the time to hyperactivity for NMDLA. Diltiazem, 100 mg/kg, increased the handling induced convulsions measured 1h ($p < 0.01$) and 2h ($p < 0.01$) after i.c.v Bay K 8644 injection. It is often stated that calcium channel blocking drugs may be useful in the clinical treatment of epilepsy; with the class of calcium channel blocking drugs to which diltiazem belongs, this statement may not be true.

Dolin, S.J., Hunter, A.B., Halsey, M.J. and Little, H.J. (1988) Br. J. Pharmacol., 94, 413-422
Desmedt, L.K.C., Niemegeers, C.J.E. and Janssen, P.A.J. (1975) Arzneim.-Forsch. (Drug-Res.), 25, 1408-1413

127P A COMPARISON OF THE EFFECTS OF REPEATED DESIPRAMINE AND ECS TREATMENT ON RAT CORTICAL AND HIPPOCAMPAL 5-HT_{1A} RECEPTOR BINDING PARAMETERS

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It has been claimed that repeated antidepressant drug treatment increases rat frontal cortical 5-HT1A receptor number (Nagayama, 1990). This finding is in accord with results of electrophysiological (de Montigny, 1984), but not biochemical (Newman & Lerer, 1988; Sleight et al., 1988), studies. We have now compared the effect of repeated desipramine (DMI) and electroconvulsive shock (ECS) on 5-HT1A receptor binding in rat frontal cortex and hippocampus.

Male CD rats (100 - 125 g, Charles-River) received either 1 or 14 daily i.p. injections of 0.9% saline or DMI (20 mg/kg). In a second experiment, animals were given either a single ECS (200 V, 2 s) or 5 ECS spread over 10 days under halothane anaesthesia. Control animals received halothane. Frontal cortices and hippocampi were removed 24 h after the final treatments. 5-HT1A binding parameters were determined using [3H]8-OH-DPAT (8-hydroxy-2(di-n-propylamino)tetralin, 0.1 - 5 nM), with specific binding defined by 1 µM 5-hydroxytryptamine (5-HT).

Following repeated DMI administration, the number (Bmax) of 5-HT1A binding sites increased by 13% in the hippocampus (Table 1) and the Kd for [3H]8-OH-DPAT binding decreased ($P<0.05$) from 0.74 ± 0.02 nM (n=9) to 0.67 ± 0.02 nM (n=10). There were no changes in Bmax (Table 1) or Kd in the frontal cortex. A single dose of DMI had no effect on Bmax or Kd in either brain region studied; 5-HT1A receptor number was not altered by either repeated (Table 1) or a single ECS (data not shown). Similarly, neither regimen altered Kd values.

Table 1 The effect of repeated DMI (20 mg/kg) or ECS on [3H]8-OH-DPAT binding in rat brain membranes

Region	Control	DMI	Control	ECS
Frontal Cortex	152 ± 7 (9)	156 ± 7 (10)	129 ± 4 (10)	125 ± 4 (10)
Hippocampus	304 ± 14 (9)	342 ± 5*(10)	331 ± 21 (10)	366 ± 18(10)

Values are mean Bmax (fmol/mg protein) ± s.e.mean (n). *$P < 0.05$ Student's t-test versus control.

The increased 5-HT1A receptor number and affinity following repeated DMI administration confirms earlier binding (Nagayama, 1990) and electrophysiological (de Montigny, 1984) studies. However, the absence of changes following repeated ECS, which extend previous receptor binding studies using [3H]5-HT to label 5-HT1-like sites (Atterwill, 1980), suggests that the changes in functional responses to 5-HT1A receptor stimulation produced by ECS (de Montigny, 1984; Newman & Lerer, 1988) are not due to altered receptor number or affinity, but rather, are the consequence of modifications in post-receptor mechanisms.

Atterwill, C. K. (1980) J. Neurochem. 35, 729 - 734.
de Montigny, C. (1984) J. Pharmacol. Exp. Ther. 228, 230 - 234.
Nagayama, H. (1990) Proc. 17th C. I. N. P. 11-1-1, 13.
Newman, M. E. & Lerer, B. (1988) Eur. J. Pharmacol. 148, 257 - 260.
Sleight, A. J., Marsden, C. A., Palfreyman, M. G. Mir, A. K & Lovenberg, W. (1988) Eur. J. Pharmacol. 154, 255 - 261.

128P EVIDENCE FOR THE INVOLVEMENT OF 5-HT_{1C} RECEPTORS IN THE ANXIOGENIC-LIKE EFFECTS OF FENFLURAMINE IN A MODIFIED VOGEL CONFLICT TEST

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The phenylpiperazine 5-$HT_{1B/1C}$ receptor agonist, m-chlorophenylpiperazine (mCPP), has been reported to induce panic attacks in humans (Charney et al., 1987). Similarly, mCPP and another 5-$HT_{1B/1C}$ agonist, trifluoromethylphenylpiperazine (TFMPP), elicit anxiogenic-like effects in several animal models of anxiety, an action which appears to be mediated by activation of 5-HT_{1C} receptors (Kennett et al., 1989). Here we report that the 5-HT releasing agent, fenfluramine, induces an anxiogenic-like response in a modified Vogel water-lick conflict test. The pharmacology of this response is compared to that described for mCPP in previous studies.

Male Sprague-Dawley rats (170-240g; n=10/treatment) were water-deprived overnight (24h). Between 10.00 and 12.00h on the morning of the experiment the animals were placed individually in Plexiglass test chambers (43x21x21cm) and allowed to drink freely for a 5 min adaptation period from a metal drinking spout. Animals which made less than 700 licks within the 5 min period were excluded from further use. Approximately 3-4h later the test compounds or vehicle were administered i.p. or s.c. After a further 30 min the rats were replaced in the test chambers. On this occasion drinking from the sipper spout was punished with a mild electric shock (50µA), delivered through the sipper spout after every 20 licks. The number of licks made and shocks received over a 5 min test period (initiated by the first lick) were recorded. Under these test conditions vehicle-treated rats displayed a relatively high level of punished drinking (mean shocks received ± s.e.mean per 5 min ranged from 27.9±5.0 to 52.8±7.3). Fenfluramine (2.5-5.0 mg/kg s.c.) and mCPP (1.3-2.0 mg/kg s.c.) markedly and significantly ($P<0.05$; ANOVA/t-test) reduced (by 60-85%) punished drinking. Fenfluramine (2.5 mg/kg s.c.) did not significantly modify water consumption in water-deprived animals over a 5 min period when this parameter was measured in unshocked animals in their home cages. Pretreatment with the 5-HT_3 receptor antagonist WAY100289 (Rhodes et al.,1989; 0.03-3.0 mg/kg s.c.) or the 5-HT_2 antagonist ketanserin (0.3-3.0 mg/kg s.c.), had no effect on the anxiogenic-like response to fenfluramine (2.5 mg/kg), whereas mianserin (0.125 & 2.0 mg/kg s.c.) markedly ($P<0.05$) attenuated it. This higher dose of mianserin has previously been shown to block the anxiogenic-like effects of mCPP in rodent models (Kennett et al., 1989). The benzodiazepine lorazepam (0.3 mg/kg i.p.) and the 5-HT_{1A} partial agonists, ipsapirone (3 & 10 mg/kg i.p.) and zalospirone (Wy47,846; Haskins et al., 1989; 2.5 & 5.0 mg/kg i.p.), also reversed the anxiogenic-like response to fenfluramine. The effects of the 5-HT_{1A} receptor partial agonists may be mediated via activation of somatodendritic 5-HT_{1A} autoreceptors, thereby counteracting the increase in 5-HT release evoked by fenfluramine.

These data suggest that fenfluramine elicits an anxiogenic-like response in a modified Vogel conflict test, presumably by increasing 5-HT release on to postsynaptic 5-HT receptors. A preliminary examination of the pharmacology of this response indicates that it may be mediated via activation of 5-HT_{1C} receptors.

Charney, D.S., Woods, S.W., Goodman, W.K. & Heninger, G.R. (1987). Psychopharmacology, **92**, 14-24.
Haskins, J.T., Moyer, J.A., Andree, T.H., Muth, E.A. & Abou-Gharbia, M. (1989). Drug Dev. Res., **18**, 29-46.
Kennett, G.A., Whitton, P.A., Shah, K. & Curzon, G. (1989). Eur J. Pharmacol., **164**, 445-454.
Rhodes, K.F., Coleman, J., Dover, G., Lattimer, N., Saville, V.A. & Taylor, A. (1991). Fundamental Clin. Pharmacol., **5**, 462.

129P THE EFFECT OF ONDANSETRON ON THE ACQUISITION OF A VISUOSPATIAL LEARNING TASK IN THE COMMON MARMOSET (*Callithrix jacchus*)

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Previous studies using the marmoset have shown that the 5-HT_3 receptor antagonist ondansetron improves performance in a simple object discrimination reversal paradigm (Domeney *et al.*, 1991). Object discrimination is believed to involve amygdal function, since lesions of this area have been shown to impair stimulus-reward association in monkeys (Spielger & Mishkin, 1981). In contrast, lesions of the hippocampus in the marmoset are reported to impair the performance of rule-based behaviours, such as visuospatial discrimination learning (Ridley *et al.*, 1988). Since 5-HT_3 receptors are located in both amygdala and hippocampus (Kilpatrick *et al.*, 1987), the present studies were designed to investigate the effects of ondansetron on hippocampal function using a visuospatial discrimination learning task.

The studies used five adult marmosets (330-430g) of both sexes, experienced in visuospatial learning tasks using a Wisconsin General Test Apparatus. In this test paradigm the animal is presented with two sets of identical objects on successive trials. The reward strategy is such that on presentation of pair one the animals response must be to go left and on presentation of pair two, to go right, according to the pseudorandom schedule of Gellerman (1938). In order to make a correct choice the animal must decide, on the basis of which pair of objects is presented, where the reward is positioned. All animals completed 30 trials each day, criterion being set at 27 correct choices out of 30 trials in one test session. Drug treatment comprised a blind crossover design using ondansetron (1ng-100μg/kg s.c. b.i.d.) and vehicle.

Ondansetron (1-100ng/kg s.c. b.i.d.) failed to significantly ($P<0.05$) modify trials to criterion from 126.0±16.0 to 120.0±18.9, mean total errors per 5 trials from 5.3±0.5 to 5.6±0.2, errors to criterion from 30.3±7.2 to 31.4±10.8 or choice latency from 12.6±1.11 to 19.1±3.5 shown for vehicle and ondansetron (100ng/kg) respectively. Higher doses of ondansetron (10μg-100μg/kg s.c. bid) also failed to modify cognitive performance. On the basis of performance of the animals in this study it is suggested that the ability of ondansetron to improve the performance of marmosets in an object discrimination reversal paradigm may not be linked to effects on hippocampal function.

Domeney, A.M., Costall, B., Gerrard, P.A., Jones, D.N.C., Naylor, R.J. & Tyers, M.B. (1991) Pharmacol. Biochem. Behav. **38**, 169-175
Kilpatrick, G.J., Jones, B.J. & Tyers, M.B. (1987) Nature **330**, 746-748
Ridley, R.M., Samson, N.A., Baker, H.F. & Johnson, J.A. (1988) Brain Res. **456**, 71-87
Spielger, B.J. & Mishkin, M. (1981) Behav. Brain Res. 3, 303-317

130P THE 5-HT_{1A} LIGAND BP 554 MIMICS THE ANXIOLYTIC ACTIVITY OF BUSPIRONE, GEPIRONE AND IPSAPIRONE IN THE ELEVATED PLUS-MAZE IN RATS

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The response of rats to 5-HT1A ligands in the elevated plus-maze model of anxiety is controversial, for example, the clinically efficacious anxiolytic buspirone has been reported as anxiolytic (Söderpalm et al., 1989), inactive (Pellow et al., 1987) or anxiogenic (Moser, 1989) in this model. We have, therefore, evaluated buspirone, gepirone, ipsapirone, 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin) and a range of other ligands with high affinity for the 5-HT1A receptor in the elevated plus-maze.

Male CD rats (160-200g; Charles River) were orally administered either vehicle or a range of doses of the test drug 1h prior to placing in the centre of the elevated plus-maze (Luscombe et al., 1990). The number of entries into the open and closed arms was recorded during the following 10 min. BP 554 is 1-[3-(3,4-methylenedioxyphenoxy)propyl]-4-phenylpiperazine maleate; NAN 190 is 1-(2-methoxyphenyl)-4-(4-phthalimidobutyl)piperazine HBr; LY 165,163 is 1-[2-(4-aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine.

All the 5-HT1A ligands listed in table 1 induced marked anxiolytic effects after oral administration over a wide dose range. In contrast, 8-OH-DPAT (0.03-3.0 mg/kg) evoked only very weak, non dose-dependent anxiolytic effects after oral administration and an anxiolytic response at only a single dose (0.003 mg/kg) after subcutaneous (0.0001-0.1 mg/kg) treatment. The anxiolytic effect of all the 5-HT1A ligands in table 1, except spiroxatrine, decreased at higher doses to give an overall bell-shaped response; at these doses total arm entries were not reduced below those observed in control vehicle-treated rats. The increase in total arm entries at anxiolytic doses of the 5-HT1A ligands (table 1) was almost invariably due to an increase in open arm entries.

Table 1 Effect of 5-HT1A ligands on the performance of rats in the elevated plus-maze

	Range of oral	Range of	Most active	Open:closed arm entries ratio		Total arm entries	
Drug	doses tested	active doses	oral dose	Most active dose	Vehicle	Most active dose	Vehicle
Buspirone	0.003 - 3.0	0.01 - 3.0	0.01 mg/kg	0.33 ± 0.02**	0.06 ± 0.02	20.2 ± 1.4**	14.0 ± 1.1
Gepirone	0.0003 - 3.0	0.001 - 3.0	0.1 mg/kg	0.40 ± 0.02**	0.04 ± 0.02	23.3 ± 1.9*	16.0 ± 1.3
Ipsapirone	0.0003 - 3.0	0.001 - 1.0	0.003 mg/kg	0.43 ± 0.02**	0.03 ± 0.01	19.8 ± 1.0*	16.3 ± 0.8
Spiroxatrine	0.1 - 3.0	0.3 - 3.0	3.0 mg/kg	0.24 ± 0.03**	0.04 ± 0.01	18.8 ± 0.4*	16.8 ± 0.6
LY 165,163	0.003 - 30	0.03 - 3.0	1.0 mg/kg	0.31 ± 0.03**	0.03 ± 0.01	20.2 ± 0.8**	16.5 ± 0.8
NAN 190	0.001 - 10	0.003 - 3.0	0.03 mg/kg	0.36 ± 0.02**	0.03 ± 0.01	23.0 ± 0.3**	16.2 ± 0.4
BP 554	0.003 - 30	0.1 - 3.0	0.3 mg/kg	0.41 ± 0.04**	0.02 ± 0.01	17.8 ± 0.6*	16.0 ± 0.3

Values are mean ± SEM (n=6). ** $p < 0.01$, *$p < 0.05$ versus vehicle control (Dunnett's test, following 1-way ANOVA).

The data provide evidence that a range of ligands with high affinity for 5-HT1A receptors, particularly those which are not full agonists, mimic the ability of benzodiazepines to evoke a marked anxiolytic response in rats on the elevated plus-maze.

Luscombe, G. P., Mazurkiewicz, S. E. & Buckett, W.R. (1990) Br. J. Pharmacol. **100**, 356P.
Moser, P. C. (1989) Psychopharmacology **99**, 48-53.
Pellow, S., Johnston, A.L. & File, S.E. (1987) J Pharm Pharmacol **39**, 917-928.
Söderpalm, B., Hjorth, S. & Engel, J.A. (1989) Pharmacol. Biochem. Behav. **32**, 259-265.

131P THE DISCRIMINATORY STIMULUS (CUE) TO CLONIDINE APPEARS TO BE MEDIATED BY ITS α_2-ADRENOCEPTOR AGONIST PROPERTIES

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Clonidine produces a well established interoceptive discriminative stimulus or 'cue' in rat drug discrimination (D.D) studies (Lal & Yaden, 1985), which could be mediated by its α-2 adrenoceptor agonist properties, by its interaction with the catecholamine-insensitive imidazoline-preferring receptor (I.P.R; see Michel & Insel, 1989), or by a combination of these. In this study male Lister Hooded rats (n = 6) were trained to discriminate clonidine (0.02mgkg-1, IP) from saline in a fixed ratio (FR=10) D.D schedule, and a series of α-2 adrenoceptor agents of both imidazoline (I) and non-imidazoline (NI) structure were tested for their ability to generalise to (i.e. mimic) or antagonise the clonidine cue.

Table 1. The generalisation and antagonism of a clonidine-induced cue

GENERALISATION				ANTAGONISM			
Test drug		Dose (mgkg-1, i.p)	% total responses on clonidine lever	Test drug		Dose (mgkg-1, i.p)	% total responses on clonidine lever
0.9% saline		-	2.8±2.5*	L659,066	(NI)	5	100
Clonidine	(I)	0.02	100	RX811059	(I)	2.5	3.0±1.8*
Guanabenz	(NI)	0.32	100	Fluparoxan	(NI)	3	7.7±6.3*
UK14,304	(I)	0.16	91.9±3.7				

Results are means ± s.e.mean. *P<0.01 vs. clonidine (paired t-test). Antagonists given 30 min before clonidine.

Dose dependent generalisations to the clonidine cue were achieved with clonidine itself, and with the α-2 adrenoceptor agonists UK14,304 and guanabenz up to the doses shown in Table 1. Since the clonidine cue was mimicked by guanabenz, which does not act at the I.P.R (Ernsberger et al, 1990), and was antagonised by the selective α-2 adrenoceptor antagonists RX811059 (2-ethoxy-idazoxan) and the non-imidazolinic fluparoxan (Dickinson, 1991) this suggests it to be mediated by α-2 adrenoceptors. Furthermore, the fact that the clonidine cue was not antagonised by the peripherally acting α-2 adrenoceptor antagonist L659,066 (Clineschmidt et al, 1988) suggests it involves central as opposed to peripheral sites.

Clineschmidt, B.V., Pettibone, D.J., Lotti, V.J. & Hucker, H.B. (1988) J. Pharmacol. Exp. Ther. **102**, 32-40.
Dickinson, S.L. (1991) Drug News & Perspectives. **4**, 197-203.
Ernsberger, P., Giuliano, R., Willette, R.N. & Reis, D.J. (1990) J. Pharmacol. Exp. Ther. **253**, 408-418.
Lal, H. & Yaden, S. (1985) J. Pharmacol. Exp. Ther. **232**, 33-39.
Michel, M.C. & Insel, P.A. (1989) Trends in Pharmacol. Sci. **10**, 342-344.

132P AN ETHOLOGICAL STUDY OF THE EFFECTS OF THE D_2 DOPAMINE AGONIST, QUINPIROLE, ON BEHAVIOUR IN MICE

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The brain dopaminergic systems are highly responsive to stress and play an important role in the expression of submissive behaviour in rodents (Dixon et al. 1990; Upchurch & Schalert, 1982). Agonists at D_2 dopamine receptors induce hyperdefensiveness in mice resembling that which occurs after prolonged defeat experience (Belzung et al. 1991), but which is not abolished by administration of chlordiazepoxide. Ethopharmacological procedures have been employed in the present experiments to quantify dose-related changes to behaviour in mice treated with the selective D_2 dopamine receptor agonist, quinpirole.

Quinpirole hydrochloride, dissolved in physiological saline, was given by i.p. injection to adult male DBA/2 mice (0.25 mg/kg = LD, 0.5 mg/kg = MD and 1.0 mg/kg = HD, n = 14/group). Physiological saline was injected i.p. to the controls (n = 16). At 35 min. after injection, behaviour shown by each of the mice during social interactions with an untreated group-housed mouse in an unfamiliar neutral cage was recorded for a 5 min. period, using the ethological procedures described by Dixon et al. (1990). Behaviour of each mouse was also monitored for 5 min. in a two-compartment light-dark box. Results have been expressed as the mean duration (s.) ± s.e. mean.

Quinpirole dose-dependently increased the duration of flight behaviour (Control 9.9±5.3, LD 21.1±13.0, MD 41.7±15.7, HD 46.4± 15.6, P<0.05), with effects being most marked for the elements "retreat" and the rigid stationary posture "freeze". Quinpirole also increased immobility (Control 7.6±2.3, LD 203.1±10.9, MD 224.3± 11.0, HD 198.0±14.7, P<0.01), in which mice showed a flaccid sitting posture, often accompanied by outward splaying of the hindlimbs and a fine tremor of the head. Quinpirole also increased sniffing of the sawdust substrate (Control 1.6±1.0, LD 8.6±1.8, MD 9.8±2.0, HD 6.2±1.1, P<0.05). Quinpirole induced a dose-dependent reduction of non-social activity (Control 152.2±6.8, LD 68.7±9.7, MD 48.6±10.0, HD 53.7±9.1, P<0.01) and of social investigation (Control 137.2±7.6, LD 25.5±4.3, MD 20.4±3.6,HD 18.9±4.8, P<0.01). In the light-dark box, quinpirole reduced thenumber of transitions between light and dark compartments (Control 6.0±5.3, LD 1.1±1.8, MD 0.6±1.2, HD 0.6±1.9, P<0.05), but increased time spent in the light compartment into which mice had been originally placed (Control 204±80, LD 259±65, MD 284±34, HD 280±58, P<0.05). Significance of differences have been estimated by Mann Whitney-U and Kruskal Wallis tests.

The enhancement of flight and immobility by quinpirole, coupled with reductions of social investigation and non-social exploratory activity represent an anxiogenic effect differing in several respects from that mediated by benzodiazepine inverse agonists.

Belzung C. et al. (1991) Psychopharmacology 103, 449-454.
Dixon A.K. et al. (1990) Adv. Stud. Behav. 19, 171-204.
Upchurch M. & Schallert T. (1982) Behav. Neural. Biol. 35, 308-314.

133P EVIDENCE FOR DOPAMINERGIC INVOLVEMENT IN THE DEVELOPMENT OF TOLERANCE TO THE COCAINE DISCRIMINATIVE STIMULUS

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Dopamine (DA) uptake inhibition at the DA transporter is believed to be the main process involved in many behavioural effects of cocaine, including its ability to maintain self-administration (Ritz et al., 1987; Spealman et al., 1989; Howell & Byrd, 1991). Tolerance to cocaine's discriminative stimulus properties can be induced by frequently (t.i.d.) administering cocaine while drug discrimination training is discontinued (Wood et al., 1987). This tolerance involves DA systems (Wood & Emmett-Oglesby, 1987) and is centrally mediated (Wood et al., 1987). We therefore investigated whether tolerance to the cocaine stimulus can be induced by administering the specific DA uptake inhibitor GBR 12909 (Andersen, 1989). Rats were trained to discriminate cocaine (10 mg/kg, i.p.) in an operant Fixed Ratio 10 drug discrimination quantal assay (e.g. Goudie et al., 1986). GBR 12909 (20 mg/kg. p.o.) generalised fully to cocaine, as previously reported (Broadbent et al., 1991). The time/effect curve for *p.o.* administered GBR 12909 (20 mg/kg) was then determined in cocaine trained rats. GBR 12909 was a long acting agent after p.o. administration (20 mg/kg), full generalization to cocaine was seen in tests up to 8 h, but not 16 h post-administration. Subsequently, the i.p. cocaine generalization dose/effect curve was assessed in tests before and during chronic GBR 12909 treatment (20 mg/kg/day, p.o., for either 5, 10, or 20 days). During GBR 12909 treatment, cocaine discrimination training ceased. Tolerance to cocaine was seen, as the generalization dose/effect curve shifted significantly (alpha = 0.05) circa two fold to the right after 10 or 20 days treatment, although less tolerance was seen after only 5 days of GBR 12909 treatment. Thus the degree of tolerance was dependent upon the duration of treatment with GBR 12909. These data support studies implicating DA reuptake in cocaine's actions. They also support suggestions that DA systems may be implicated in tolerance to the cocaine discriminative stimulus (Wood et al., 1987). Since it is possible to induce tolerance to the cocaine stimulus by single daily injections of GBR 12909, rather than by multiple (3 x daily) injections of cocaine, which is a short acting drug (Wood and Emmett-Oglesby, 1987; Wood et al., 1987), GBR 12909 may be a very useful agent for analyzing the role of DA in tolerance to the discriminative ("subjective") effects of cocaine believed to be implicated in cocaine self-administration. Recently (Baldo & Kelley, 1991) it was reported that GBR 12909 can induce sensitization to the motor stimulant actions of cocaine. Thus the same drug can induce cross-tolerance or cross-sensitization to cocaine, depending upon the behavioural assay used to assess cocaine's effects.

Andersen, P.H. (1989) Eur. J. Pharmacol. 166,493-504.
Baldo, B.A. & Kelley, A.E. (1991) Pharmacol. Biochem. Behav. 27,105-108.
Broadbent, J., Michael, E.K., Riddle, E.E. & Appel, J.B. (1991) Behav. Pharmacol. 2,187-197.
Goudie, A.J., Atkinson, J. & West, C.R. (1986) Neuropharmacol. 25,85-94.
Howell, L.L. & Byrd, L.D. (1991) J. Pharmacol. Exp. Therap. 258,178-185.
Ritz, M.C., Lamb, R.J., Goldberg, S.R. & Kuhar, M.J. (1987) Science 237,1219-1223.
Spealman, R.D., Madras, B.K. & Bergman, J. (1989) J. Pharmacol. Exp. Therap. 251,142-149.
Wood, D.W. & Emmett-Oglesby, M.W. (1987) Eur. J. Pharmacol. 138,155-157.
Wood, D.W., Retz K. & Emmett-Oglesby, M.W. (1987) Pharmacol. Biochem. Behav. 28,401-406.

134P ALPHA$_2$ ADRENOCEPTOR ANTAGONISTS PREVENT A 5-HT$_{1A}$/5-HT$_2$ RECEPTOR INTERACTION

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5-HT$_{1A}$ and 5-HT$_2$ receptors appear to modulate each others effects but the mechanism of this interaction is unknown (eg. Backus et al., 1990). 5-HT$_{1A}$ agonist reduction of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) head-shakes appears to be presynaptic, being prevented by 5-HT depletion with pCPA (Handley & Dursun, 1991), while the 5-HT$_2$ receptor causing head-shakes is postsynaptic (Heal et al., 1986). In view of the ability of alpha-2 agonists to inhibit head-shaking (Handley & Brown, 1982) it is possible that the two sites could be linked by a noradrenergic mechanism. We have therefore investigated the effects of a range of alpha-2 antagonists on the ability of 8-OH-DPAT to inhibit head-shakes induced by DOI.

Male MF1 mice (20-30g, N=10-12) received a submaximal dose of DOI (1.0 mg/kg, i.p) 10 min after 8-OH-DPAT (0.1 mg/kg, i.p) or saline (SAL) and 30 min after i.p pretreatment with the alpha-2 antagonists or SAL. Head-shakes were counted from videotapes for 6 min starting at 5 min after DOI injection.

TABLE 1. ALPHA-2 ANTAGONIST REVERSAL OF THE INHIBITORY ACTION OF 8-OH-DPAT ON DOI HEAD-SHAKES

AGENTS	NUMBER OF HEAD-SHAKES (Means ± s.e. Mean) SAL+SAL+DOI	AGENT+SAL+DOI	SAL+8-OHDPAT+DOI	AGENT+8-OHDPAT+DOI	2-WAY ANOVA INTERACTION TERM
RX811059 (1.0 mg/kg)	17.4±1.4	22.8±1.9*	4.3±0.7	19.8±0.4**	[$F_{(1,44)} = 13.51$; $p<0.01$]
IDAZOXAN (0.5 mg/kg)	17.2±1.8	21.2±2.0	4.5±1.9	15.1±1.7**	[$F_{(1,36)} = 4.25$; $p<0.05$]
1-PP (2.0 mg/kg)	18.3±1.6	21.5±2.1	5.4±0.7	16.6±0.9**	[$F_{(1,44)} = 6.98$; $P<0.05$]
YOHIMBINE (1.0 mg/kg)	17.7±1.4	21.3±0.4	4.9±0.8	16.5±1.2**	[$F_{(1,44)} = 9.30$; $p<0.01$]

*Significantly different from SAL+SAL+DOI ($p<0.05$). **Significantly different from SAL+8-OHDPAT+DOI ($p<0.0001$).

Table 1 shows that 2-ethoxy-idazoxan (RX811059), idazoxan, 1-(2-pyrimidinyl)-piperazine (1-PP) and yohimbine all significantly reduced the inhibitory action of 8-OH-DPAT on DOI head-shakes. These results suggest that this 5-HT$_{1A}$ – 5-HT$_2$ interaction is under the modulatory control of alpha-2 adrenoceptors.

Backus, L.I., Sharp, T., Grahame-Smith, D.G. (1990) Br. J. Pharmacol. 100, 793-799.
Handley, S.L. & Brown, J. (1982) Neuropharmacol. 21, 507-510.
Handley, S.L. & Dursun, S.M. (1991) Br. J. Pharmacol. 104, 363P.
Heal, D.J., Philpot, J., O'Shaughnessy, K.M., Davies, C.L. (1986) Psychopharmacol. 89, 414-420.

135P CHARACTERISATION OF ANGIOTENSIN II RECEPTOR SUBTYPES IN HUMAN CEREBELLAR MEMBRANES

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Previous studies have identified angiotensin II receptor subtypes in central tissues originating from the rat (for review see Timmermans et al., 1991). However, with respect to the potential therapeutic indications of compounds that interact with central angiotensin II receptor subtypes (Barnes et al., 1990; 1991b), it is important to assess the presence and distribution of these receptors within the human CNS. Following our initial report identifying angiotensin II receptor subtypes in the human CNS using autoradiography (Barnes et al., 1991a) we presently report the pharmacological characterisation of these [^{125}I]angiotensin II labelled receptor recognition sites in human cerebellar membranes using subtype selective and non-selective ligands.

Brain tissues were obtained at autopsy within 24 hrs of death from male and female patients (aged between 64-80 yrs) who had died from a non-neurological non-psychiatric disorder. Tissue was homogenised (Polytron setting 7, 10 sec) in incubation buffer (mM; sodium chloride, 150 ; sodium dihydrogen phosphate, 50; magnesium chloride, 10; EGTA, 5 and 0.4 % w/v bovine serum albumin, pH 7.4) and centrifuged (48000 x g, 4°C, 10 min). The pellet was gently resuspended in incubation buffer and recentrifuged. The binding homogenate was formed by gentle resuspension of the pellet in incubation buffer (approximately 25 mg original wet weight/ml). For [^{125}I]angiotensin II binding, test-tubes in duplicate contained 150 µl competing compound (or vehicle; incubation buffer) and 100 µl [^{125}I]angiotensin II (final concentration 80 - 120 pM; 2200 Ci/mmol; NEN). 250 µl brain homogenate was added to initiate binding which was allowed to proceed for 60 min at 25°C before termination by filtration through GF/B filters followed by washing (16 sec, ice-cold buffer). Radioactivity was assessed using a gamma-counter. [^{125}I]Angiotensin II labelled a heterogeneous population of specific binding sites (defined by the inclusion of unlabelled angiotensin II, 1.0 µM). Thus the angiotensin II-1 receptor selective ligand, losartan, and the angiotensin II-2 receptor selective ligand PD123177 (1-(4-amino-3-methylphenyl)-methyl-5-diphenyl-acetyl-4, 5, 6, 7-tetrahydro-1H-imidazo [4, 5-C] pyridine-6-carboxylic acid; 1.0 µM) competed for the [^{125}I]angiotensin II binding site in a biphasic manner. In the presence of losartan (1.0 µM), angiotensin II, saralasin, losartan and PD123177 competed for the [^{125}I]angiotensin II binding site in human cerebellar membranes in a monophasic manner (pIC50; 9.77 ± 0.17, 9.61 ± 0.05, 4.23 ± 0.13 and 7.07 ± 0.22, respectively, mean ± SEM, n = 3). In the presence of PD123177 (1.0 µM), angiotensin II, saralasin, losartan and PD123177 also competed for the [^{125}I]angiotensin II binding site in a monophasic manner but the latter two compounds competed with differing affinities (pIC50; 9.97 ± 0.08, 9.75 ± 0.08, 7.91 ± 0.09 and 4.38 ± 0.13, respectively, mean ± SEM, n = 3).

The present study has confirmed the presence of angiotensin II-1 and II-2 receptor subtypes within the human cerebellum and demonstrated that their pharmacological profile is comparable to those previously identified in rat tissues. Such sites may provide viable therapeutic targets at which to direct compounds to influence the functioning of the human CNS.

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Barnes, J.M., Barber, P.C., Barnes, N.M. (1991a) Neuroreport 2, 605-608.
Barnes, N.M., Costall, B., Kelly, M.E., Murphy, D.A., Naylor, R.J. (1990) Neuroreport 1, 20-21.
Barnes, N.M., Costall, B., Kelly, M.E., Murphy, D.A., Naylor, R.J. (1991b) Neuroreport 2, 351-353.
Timmermans, P.B.M.W.M., Wong, P.C., Chiu, A.T., Herblin, W.F. (1991) Trends Pharmacol. Sci. 12, 55-62.

136P CENTRAL PENETRATION OF ANGIOTENSIN II ANTAGONISTS IN THE RAT USING THE TECHNIQUE OF *EX VIVO* BINDING

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Brain angiotensin II has been shown to play a role in the regulation of blood pressure, water intake, reproductive hormone levels and has been shown to modulate the levels of neurotransmitters (see Phillips, 1987). More recently the AT_1 receptor selective antagonist, losartan, has been found to improve cognitive performance in the rat (Dennes et al, 1991). Song et al (1991) have recently used ex vivo autoradiography to demonstrate the ability of losartan (Dup 753) to displace [^{125}I]-Sar^{1}Ile^8-AII binding from CNS tissue, indicating the ability to penetrate the blood brain barrier. In this study we have used the technique of ex vivo membrane binding to further characterize the brain penetration of the AT_1 selective antagonists GR117289 (Robertson et al, 1991) and losartan.

GR117289 (0.1-30mgkg^{-1}) and losartan (1-30mgkg^{-1}) were administered (s.c.) to male Lister Hooded rats. At various times later (0.5-72h) the rats were anaesthetised with pentobarbital and perfused transcardially with 50ml saline at 4°C. The livers and brains from individual animals were removed and the cortex/hippocampus (Cx/H) dissected out from the brain. The tissue was homogenised in Tris buffer (Tris 50mM, pH7.4) at a concentration of 12mgml^{-1} (liver) and 200mgml^{-1} (Cx/H). Specific [^{125}I]-Sar^{1}Ile^8-AII binding was measured as previously described (Michel and Barnes, 1991). Results are expressed as a percentage of binding (mean±s.e.mean) in vehicle treated control rats.

Table 1 Effect of 2h Pretreatment with GR117289

DOSE mgkg^{-1}	Liver binding (% control)	Cx/H binding (% control)	n
0.1	39±8 **	91±6	3
1.0	7±1 **	65±6	6
10.0	3±1 **	30±5 *	4
30.0	2±1 **	44±9 *	4

*p<0.05, **p<0.01 Student's t test performed on raw data (ie dpms)

The specific binding in the liver and Cx/H of control rats was 12765±316 and 1823±108 dpm respectively. GR117289 caused a dose-dependent inhibition of binding in the liver and Cx/H (see Table 1). The peak inhibition of binding in the Cx/H occurred after 2h (1mgkg^{-1} at 0.5h,1h,2h and 4h reduced binding to 83±5, 77±7, 65±6 and 73±4% respectively). The effect of GR117289 was long lasting. Binding in the Cx/H was still reduced 48h after drug treatment (57±3% after 10mgkg^{-1}), but had returned to control levels (97±8%) 72h after drug treatment.

Losartan also produced a dose-dependent reduction in liver and Cx/H binding. A 2h pretreatment with losartan (10mgkg^{-1}) reduced liver and Cx/H binding to 2±0.5 and 62±10% respectively.

These results show that GR117289 and losartan can penetrate the CNS and will be useful tools for investigating the central actions of angiotensin II.

Dennes R.P., Barnes J.C., Michel A.D., & Tyers M.B. (1991). Br.J.Pharmacol. In Press.
Michel, A.D. and Barnes, J.C. (1991). Br.J.Pharmacol., **96**, 132P.
Phillips M.I. (1987). Ann.Rev.Physiol. **49**, 413-435.
Robertson M.J.,Middlemiss, D., Ross, B.C., Drew, G.M., Scopes, D.I.C., & Dowle, M.D. (1991) Br.J.Pharmacol. **104**, 300P.
Song,K., Zhou,J.& Mendelsohn,A.O. (1991). Br.J.Pharmacol.**104**, 771-772.

137P ACUTE COCAINE ALTERS CEREBROVASCULAR AUTOREGULATION IN THE RAT NEOCORTEX

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The resistance vessels of the cerebrovascular bed are endowed with the ability to alter their calibre in response to fluctuations in perfusion pressure thereby maintaining a constant cerebral blood flow over a wide range (60-150mmHg) of arterial blood pressures. However, recent studies suggest that dopamine may alter the characteristics of this autoregulatory phenomenon, lowering the upper limit of autoregulation (Tuor and McCulloch, 1986). We have previously reported that cocaine has similar effects to dopamine in the cerebral circulation (Sharkey et al., 1991). If cocaine similarly alters autoregulation, this could provide a mechanism to account for cocaine-induced stroke. The purpose of the present study was to investigate whether cocaine could alter the cerebrovascular response to experimentally-induced hypertension.

Conscious rats were infused i.v. with angiotensin-II (5μg/ml; at a rate of 0.5-2.5ml/hr) or saline at the same rate. Once the desired mean arterial blood pressure (MABP) had been acheived, the rats were injected iv on a single occasion with either saline (2ml/kg; n = 30), or cocaine-HCl (5mg/kg; n = 30), and measurements of local cerebral blood flow (LCBF) (n = 30) and local cerebral glucose utilisation LCGU (n = 30) in 6 neocortical areas (piriform, somatosensory, anterior cingulate, entorhinal, primary auditory and primary visual) were performed using [^{14}C]iodoantipyrine or [^{14}C]2-deoxyglucose quantitative autoradiography respectively (Sharkey et al., 1991).

In saline-infused animals cocaine produced a transient increase (18±2mmHg mean±s.e.mean; n=10) in MABP and the typical stereotyped behavioural syndrome reported previously (Sharkey et al., 1991). The infusion of angiotensin increased MABP from 118±2 to between 145 and 160mmHg. At these levels the subsequent injection of cocaine had no further effect upon MABP.

Angiotensin-induced hypertension (MABP; 146-150) did not significantly alter blood flow or glucose use in any of the cortical areas examined in saline-treated rats. However, at higher pressures (between 151 and 160mmHg), LCBF was significant increased (from 189±10ml/100g/min to 690±54ml/100g/min) within parietal cortex. In contrast no significant alterations in LCGU were observed in animals at either of these levels of hypertension. Cocaine did not significantly alter LCBF or LCGU in normotensive animals. However at MABP > 145mmHg, all cocaine treated rats showed focal increases (2-4 fold) in LCBF in parietal cortex while LCGU remained relatively unaffected.

The cortical hyperaemia evident in cocaine-treated individuals at MABPs at which control animals are apparently able to autoregulate LCBF, suggests, that in the presence of cocaine, the upper limit of autoregulation is shifted to lower levels of MABP.

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Sharkey J., McBean DE., Kelly PAT (1991) Brain Res. 548, 310-314.
Tuor UI and McCulloch J. (1986) Neurosci. Lett. 63, 253-258.

138P CHARACTERIZATION OF FUNCTIONAL CALCITONIN GENE-RELATED PEPTIDE RECEPTORS IN A HUMAN NEUROBLASTOMA CELL LINE

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Calcitonin gene-related peptide (CGRP) and its receptors are widely distributed throughout the CNS and have been implicated in the mediation of nociception and neurogenic inflammation (Pedersen-Bjergaard *et al.*, 1991). This study has characterized functional CGRP receptors in a human neuroblastoma cell line, SK-N-MC (Van Valen *et al.*, 1990), in comparison to those in rat whole brain.

Radioligand binding studies were carried out essentially as described by Dennis *et al.* (1990) at room temperature for 1 h. Scatchard analysis of [^{125}I]-hCGRPα binding to SK-N-MC cell membranes generated linear plots, indicating a homogeneous receptor population with a K_d of 48 (35, 64) pM and a B_{max} of 35 (± 5.5) fmol/mg protein (all values quoted are means ± s.e. mean, except K_d values which are geometric means (- s.e. mean., + s.e. mean.), n = 3-4). The K_d was increased to 103 (93, 113) pM in the presence of 100μM GTP-γ-S while the B_{max} remained constant, suggesting possible G protein linkage. 2 nM hCGRP(8-37) had a similar effect (K_d = 114 (84, 155) pM), indicating competitive inhibition of binding. Binding of [^{125}I]-hCGRPα (40pM) was highly specific for hCGRP, as demonstrated by the large decrease in potency of other related peptides (Table 1). All binding characteristics were very similar in both SK-N-MC cell and rat brain membranes, suggesting that they possess the same CGRP receptor (Table 1).

Table 1 : pK_i (concentration to inhibit specific binding by 50%)

Compound :	hCGRPα	hCGRPβ	hCGRP(8-37)	salmon calcitonin	amylin
SK-N-MC cells :	10.2 ± 0.17	10.2 ± 0.18	8.96 ± 0.01	5.91 ± 0.15	5.56 ± 0.07
Rat brain membranes :	10.2 ± 0.10	10.4 ± 0.19	9.11 ± 0.07	5.86 ± 0.05	5.69 ± 0.06

Levels of cAMP in SK-N-MC cell membranes were measured essentially as described by Edwards *et al.* (1991) using Amersham's [^{125}I] scintillation proximity assay. hCGRPα was found to stimulate production of cAMP with a pEC_{50} of 9.80 (± 0.11), confirming linkage of the receptor to adenylate cyclase via a stimulatory G protein. hCGRP(8-37) alone had no effect on cAMP but caused a parallel rightward shift in the hCGRPα dose response curve (pA_2 = 9.20 ± 0.15, slope of Schild plot = 1.07). Thus, the receptor binding and functional studies showed that hCGRP(8-37) was a potent competitive antagonist at this hCGRP receptor, which belonged to the $CGRP_1$ subtype as defined by Dennis *et al.* (1990).

Dennis, T., Fournier, A., Cadieux, A., Pomerleau, F., Jolicoeur, F.B., St. Pierre, S. & Quirion, R. (1990) J. Pharmacol. Exp. Ther. 254, 123.
Edwards, R.M., Stack, E.J. & Trizna, W. (1991) J. Pharmacol. Exp. Ther. 257, 1020.
Pedersen-Bjergaard, U., Nielsen, L.B., Jensen, K., Edvinsson, L., Jansen, I. & Olesen, J. (1991) Peptides 12, 333.
Van Valen, F., Piechot, G. & Jürgens, H. (1990) Neurosci. Lett. 119, 195.

139P MODULATION OF ANTINOCICEPTION IN MICE BY HISTAMINE H_3-RECEPTOR LIGANDS

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Compounds which act at histamine H_1- and H_2-receptors in the central nervous system have been shown to modulate morphine-induced antinociception in mice (Freeman & Sturman, 1989; 1990) and may produce antinociception by themselves. In this study the effects of two compounds which act selectively at the presynaptic H_3-autoreceptor to modulate neurotransmitter release (Arrang *et al.*, 1987), the agonist (R)α-methylhistamine (RAMH) and the antagonist thioperamide (THIO), were examined for effects on nociception in mice.

Male BK:TO mice (30-50g) were pre-treated with RAMH, THIO or vehicle (saline) s.c.. The latency to paw-licking in the hot-plate test (49±1°C) at 30 and 45 mins after pre-treatment, alone and in combination with morphine (dosed 10 mins after pre-treatment), were then measured.

At both 30 and 45 mins after administration, RAMH (3 and 10 mg kg^{-1}) or THIO (3 and 10 mg kg^{-1}) alone had no effect on hot-plate latencies, compared with controls. However, when administered in combination with morphine (5 mg kg^{-1}), RAMH (3 mg kg^{-1}) increased latencies to paw-lick at 30 mins ($p<0.01$, n=9; Mann-Whitney) but not at 45 mins, while RAMH (10 mg kg^{-1}) had no effect at either 30 or 45 mins after pre-treatment, compared with controls. In contrast, THIO in combination with morphine (10 mg kg^{-1}) decreased latencies at both 3mg kg^{-1} ($p<0.05$, n=23) and 10 mg kg^{-1} ($p<0.05$, n=20) at 30 mins. No effect was seen with THIO 45 mins after pre-treatment.

The property of RAMH (which reduces histamine synthesis and release) to increase morphine-induced antinociception is in accordance with the effects of H_1-antagonists. However, THIO, which is reported to increase both histamine release and synthesis, significantly reduced morphine-induced antinociception.

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Arrang, J.-M., Garbarg, M., Lancelot, J.-C., Lecomte, J.-M., Pollard, H., Robba, M., Schunack, W. & Schwartz, J.-C. (1987) Nature, 327, 117-123.
Freeman, P. & Sturman, G. (1989) Br.J.Pharmacol., 97, 32P.
Freeman, P. & Sturman, G. (1990) Br.J.Pharmacol., 101, 556P.

140P A NOVEL DESIGN FOR AN INEXPENSIVE MICRODIALYSIS PROBE FOR DIRECT STEREOTAXIC IMPLANTATION INTO THE STRIATUM OF THE RAT

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Acute implantation of a microdialysis probe, or the introduction of the commercially available CMA/10 probe (CMA Microdialysis, Sweden) via a previously implanted guide cannula into the striatum, results in striatal DA release which is not completely sensitive to calcium depletion or to tetrodotoxin (Santiago and Westerink, 1990). In contrast, neuronal release of DA is measured after sub-chronic (24 h) implantation of a variety of probes (Westerink and De Vries, 1988; Santiago and Westerink, 1990). This, however, necessitates single useage of probes, making the commercially available microdialysis probes financially prohibitive. We therefore present a novel design for an inexpensive probe which can be stereotaxically implanted into the striatum of the rat at a fraction of the cost.

Thin-walled stainless steel tubing (23G) and two sizes of vitreous silica glass (VSG) tubing (inlet i.d. 40μm, o.d. 140μm; oulet i.d. 120μm, o.d. 170μm) were used in the construction of the probe. A 2mm section was removed from the body of the probe to allow entry of the inlet VSG tubing. The probe was equipped with Hospal AN 69 dialysis membrane (3mm) and had a dead volume of 0.63 ± 0.04μl, n=9. The relative *in vitro* recovery of the probe at 2.0μl/min with respect to DA was 26.6 ± 0.7%, n=9. Figure 1b shows the effect of 5mg/kg amphetamine i.p. on the basal efflux of DA from the striatum of freely moving rats, collected at 2.0μl/min.

Stereotaxic implantation of the probe was achieved through integration of the probe with a David Kopf stereotaxic frame by use of a probe clip (CMA Microdialysis). The probe was held above the side-arm, and thereby the depth of the clip (3mm) need not be included into the length of the of the probe below this point. This has facilitated the manufacture of a small probe which can be reliably implanted stereotaxically and function after the 24 hour post-operation recovery time required in this technique.

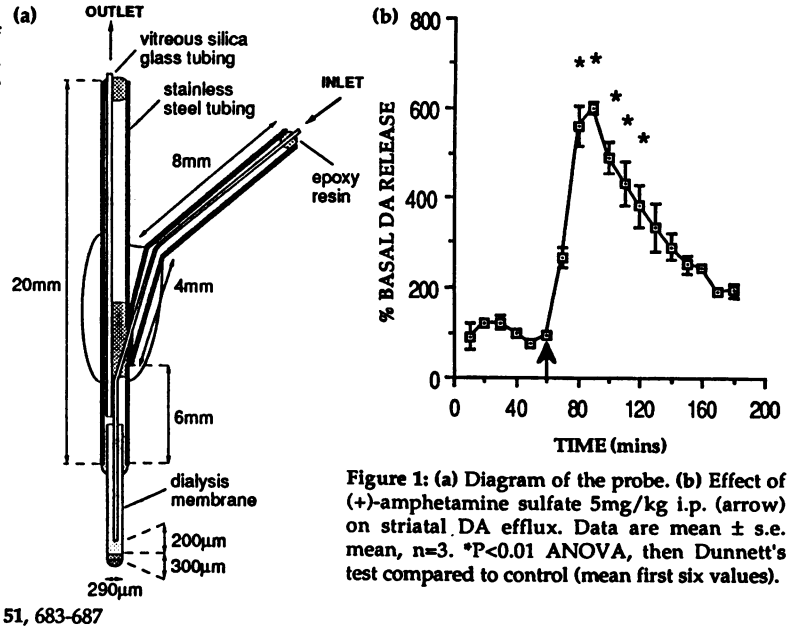

Figure 1: (a) Diagram of the probe. (b) Effect of (+)-amphetamine sulfate 5mg/kg i.p. (arrow) on striatal DA efflux. Data are mean ± s.e. mean, n=3. *P<0.01 ANOVA, then Dunnett's test compared to control (mean first six values).

Westerink, B. H. C. & De Vries, J. B. (1988) J. Neurochem. 51, 683-687
Santiago, M & Westerink, B. H. C. (1990) Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 407-414

141P MUSCARINIC SUPPRESSION OF EXCITATORY NEUROTRANSMISSION IN GUINEA-PIG OLFACTORY CORTEX SLICES IS UNLIKELY TO INVOLVE AN M_4-MUSCARINIC RECEPTOR SUBTYPE

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Five distinct muscarinic acetylcholine receptor subtypes have been identified by molecular cloning studies in mammalian brain and peripheral tissue, although only four have so far been defined pharmacologically (M_1-M_4) (see Hulme et al., 1990). M_4 receptors recently characterized in rabbit lung and chicken heart by Lazareno et al., (1990) show a moderate affinity for pirenzepine (M_1 selective antagonist) but a relatively high affinity for the 'cardioselective' antagonist himbacine. We have previously suggested that excitatory neurotransmission in the guinea-pig olfactory cortex slice is depressed by muscarinic agonists acting on presynaptic M_1-type receptors located on the lateral olfactory tract (LOT) and/or long association fibre nerve terminals (Williams & Constanti, 1988). In the present study, we have investigated the possibility that this response might involve an M_4 receptor subtype by testing the effectiveness of himbacine, on guinea-pig olfactory cortex slices maintained *in vitro*, using an extracellular recording method (Williams & Constanti, 1988). Two-minute applications of carbachol (CCh; 25-150 μM) reversibly depressed the surface N-wave potential evoked by stimulating the LOT, as previously described. Bath-application of himbacine (100 nM-1 μM; 45 min, n=7 slices) had no effect on the N-wave amplitude, but reduced subsequent responses to CCh. This effect persisted even after 1-3 hr washing in normal Krebs solution. No further suppression of the CCh response occurred when himbacine was pre-applied for 1 hr. Log dose-response lines for CCh were displaced in a parallel, competitive-type manner by increasing doses of himbacine. Schild plot analysis (Schild slope constrained to unity) of pooled data yielded a pA_2 value of 7.2 for this antagonist (n=7 slices). This value accords more with that expected for the interaction of himbacine with M_1 receptors (~7.2) than with functionally expressed M_4 receptors (~8.5-8.8) (Mathie et al., 1991; Caulfield & Brown, 1991).

We therefore suggest that M_4-type receptors are unlikely to be involved in mediating this presynaptic muscarinic response.

Himbacine was a kind gift from Prof. W.C. Taylor (University of Sydney, Australia).

Caulfield, M.P. & Brown, D.A. (1991). *Br. J. Pharmacol.* **104**, 39-44.
Hulme, E.C., Birdsall, N.M.J. & Buckley, N.J. (1990). *Annu. Rev. Pharmacol. Toxicol.* **30**, 633-673.
Lazareno, S., Buckley, N.J. & Roberts, F.F. (1990). *Mol. Pharmacol.* **38**, 805-815.
Mathie, A., Bernheim, L. & Hille, B. (1992). *J. Physiol.* **446**, Proc. suppl., 548P.
Williams S.H. & Constanti, A. (1988). *Br. J. Pharmacol.* **93**, 846-854.

142P THE EFFECTS OF NICOTINE ON LEARNING AND MEMORY: A STUDY USING AN 8-ARM RADIAL MAZE

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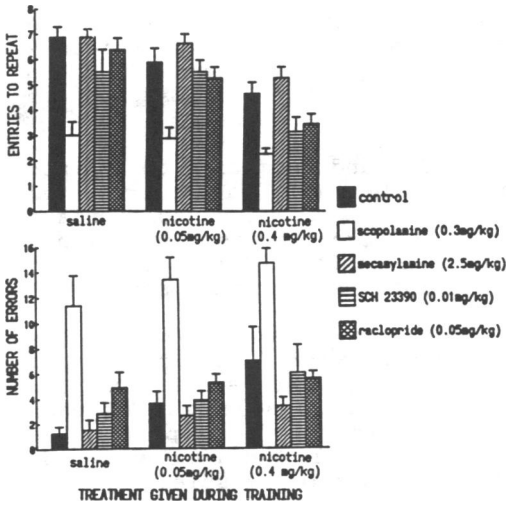
Previous experiments on the effects of nicotine on learning and memory, in rats, have yielded inconsistent results (Levin et al 1988; Mundy & Iwamoto 1987). In this study the effects of nicotine administration during training of an 8-arm radial maze task were examined.



Groups (n=8) of male Sprague-Dawley rats, treated with saline or nicotine (0.05 or 0.4 mg/kg) were trained to perform an 8-arm radial maze task in which all arms were baited with sweetened milk, using the procedure of McGurk et al (1989). When the performance was asymptotic the effects of scopolamine (0.3 mg/kg), mecamylamine (2.5 mg/kg), the specific dopamine receptor antagonists SCH 23390 (0.01 mg/kg) and raclopride (0.05 mg/kg) on maze performance were examined. The administration of nicotine during training dose-dependently impaired acquisition of the task and asymptotic levels of performance were lower than for the control group ($p < 0.05$) (Figure 1). Scopolamine evoked the expected disruption of performance (increased errors, $p < 0.001$; decreased number of entries before the first repeat, $p < 0.05$). SCH 23390 and raclopride both decreased entries to repeat ($p < 0.01$) but did not effect the number of errors made. The effects of all three drugs were independent of the treatment given during training. Mecamylamine was without effect on maze performance. The results indicate that both the cholinergic and dopaminergic systems may be involved in the processes of learning and memory and suggest that chronic stimulation of central nicotinic receptors disrupts acquisition of this learning and memory task.

Figure 1: Data are expressed as mean ± s.e. mean.

Levin, E.D., Lee, C., Rose, J.E., Reyes, A., Ellison, G., Jarvik, M. & Gritz, E. (1990) Behav. Neural Biol. **53**, 269-276.
McGurk, S.R., Levin, E.D. & Butcher, L.L. (1988) Behav. Neural Biol. **52**, 78-86.
Mundy, W.R. & Iwamoto, E.T. (1987) Pharmacol. Biochem. Behav. **30**, 119-122.

143P A COMPUTER SIMULATION TO TEACH THE ACTION OF DRUGS ON INTESTINAL (COLONIC) MOTILITY

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This program aims to teach, by investigation, the basic pharmacology of intestinal motility and is based on experiments that might be performed on isolated, perfused rat colon. The preparation allows longitudinal muscle activity to be recorded isometrically and propulsion of fluid to be recorded from an electronic drop counter.

The program is written in Turbo C++ (Borland) for IBM compatible microcomputers and will run under a range of graphics cards (Hercules, EGA, VGA). It makes use of an easy-to-use, windows-like menu display from which several options may be selected. INTRODUCTION and METHODS present essential information about the preparation and the apparatus used as a combination of text and high-resolution colour graphics. A DEMONSTRATION section also includes an animated sequence depicting how the colon responds to a bolus injection of saline and a test substance. The animation is based on a program written for Apple II microcomputer and has previously demonstrated to the Society (Leathard and Higman, 1990).

The main section, EXPERIMENTS allows the user to demonstrate the actions of several test substances either alone or in combination on the preparation (e.g. saline, acetylcholine, neostigmine, atropine, adrenaline and phenolphthalein). In each case the action of the test substance is demonstrated on basal activity of the colon and reflexly-evoked activity in response to raised intraluminal pressure. Simulated responses are displayed on the monitor, in high-resolution graphics and show the isometric (longitudinal muscle) tension and fluid flow on a simulated chart-recorder.

This work was supported by The Lord Dowding Fund (NAVS, UK).

Leathard, H. & Higman, M. (1990) Br. J. Pharmac. 99, 230P.

144P A COMPUTER-BASED, CASE-STUDY APPROACH TO TEACHING THE ANAEMIAS TO UNDERGRADUATE STUDENTS

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This program adopts a case-centred approach to teaching the anaemias to undergraduate students and extends a program written for the BBC microcomputer and previously demonstrated to the Society (Dewhurst & Williams, 1990). The program is written in Turbo C++ (Borland) for IBM-compatible computers and will run under both EGA and VGA graphics cards.

Briefly the program uses text, high-resolution colour graphics and animation to explain the underlying principles of the determination of haematological values (haematocrit, red cell count, haemoglobin concentration and blood group) and allows the user to simulate performing these determinations for normal subjects. The values obtained may then be input into a program to calculate MCV, MCH and MCHC and the full haematological profile may be output to an Epson-compatible printer (using the full IBM graphics character set).

In teaching the anaemias clinical data is given for ten sufferers to cover iron-deficiency (blood loss and dietary deficiency), megaloblastic (folate and vitamin B_{12} deficiencies), haemolytic (hereditary spherocytosis, glucose-6-phosphate dehydrogenase deficiency, sickle-cell, drug-induced autoimmune and thalassaemia) and aplastic anaemia. For each patient the program presents a brief medical history, a haematological profile, a peripheral blood film and the opportunity to access the results of relevant further tests. The task for the user is to make a correct diagnosis of the condition by selecting from a list of possible diagnoses. A HELP facility is available throughout this section of the program.

Dewhurst, D.G. & Williams, A.D. (1990) Br. J. Pharmac., 98, 940P.

145P ANTAGONISM OF THE TRACHEAL RELAXANT ACTIVITY OF POTASSIUM CHANNEL OPENERS BY GUANETHIDINE AND BRETYLIUM

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146P PHARMACOLOGICAL COMPARISON OF [^{3}H]HALOPERIDOL LABELLED SIGMA RECOGNITION SITES IN HUMAN AND RAT CEREBELLAR MEMBRANES

C. D. Hornsby[1] J. M. Barnes[2], N. M. Barnes[2], P. C. Barber[3], S. Champaneria[1], B. Costall[1], J. W. Ironside[4], R. J. Naylor[1], [1]Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP UK,[2]Department of Pharmacology and [3]Department of Pathology, The Medical School, University of Birmingham, Birmingham B15 2TT UK, [4]The Neuropathology Laboratory, Department of Pathology, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU UK.

147P ANXIOLYTIC-LIKE EFFECTS IN MICE INDUCED BY THE 5-HT_3 RECEPTOR ANTAGONIST, BRL 46470A

B. Gao and M.G. Cutler, Department of Biological Sciences, Glasgow Polytechnic, Cowcaddens Road, Glasgow G4 OBA.

ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work.

1P **Hall IP & Widdop S** Control of tissue cyclic AMP content in bovine tracheal submucosal glands

2P **De Jonckheere S & McCaig DJ** Comparison of the effects of two calcium modulators in trachea isolated from normal and albumin-sensitized guinea-pigs

3P **Foster A, Chapman ID, Mazzoni L & Morley J** Resolution of airway obstruction by reduction of airway hyperreactivity

4P **Taylor AD, Antoni F, Croxtall JD, Flower RJ & Buckingham JC** Modulation of corticotrophin (ACTH) release *in vitro* by lipocortin 1 (LC-1) and dexamethasone

5P **Ahluwalia A & Flower RJ** Topical steroids and ACE inhibitors: a common link?

6P **Mackay D** A method for estimating pA_2 values of competitive antagonists against neuronally- or ionophoretically-released agonists

7P **Welsh NJ, Shankley NP & Black JW** Evidence that pentagastrin acts both directly and indirectly by releasing histamine to stimulate gastric acid secretion in the rat

8P **Maubach KA, Patel M, Spraggs CF & Jordan CC** Multiple CCK receptor subtypes mediate contraction in guinea-pig gall-bladder

9P **Boyle SJ, Tang K-W, McKnight AT & Woodruff GN** Characterisation of CCK receptors in the circular muscle of the guinea-pig stomach corpus

10P **Griesbacher T & Lembeck F** Treatment of experimental acute pancreatitis by the bradykinin antagonist HOE 140

11P **Morita T, Edwards G, Englert H, Lang HJ & Weston AH** Comparison of the effects of HOE 511 and ciclazindol on responses to BRL 38227 in a variety of smooth muscles

12P **Cook SJ & Small RC** Role of K^+-channel opening in salmeterol-induced relaxation of trachealis muscle

13P **Sellers AJ & Ashford MLJ** The effect of lemakalim, a potassium channel opener, on the ATP-modulated potassium channels in rat isolated hypothalamic neurones

14P **Walland A & Weihs H** Alinidine prevents hypoperfusion-induced redistribution of myocardial flow in isolated rat heart as assessed by fluorescent microsphere technique

15P **Collis CS, Cave AC & Hearse DJ** The adenosine antagonist 8-(p-sulfophenyl)theophylline fails to abolish ischaemic preconditioning in isolated rat hearts

16P **Holden K, Sneddon JM & Munby J** KCl-induced augmentation of α_1-adrenoceptor pressor responses in the rat mesenteric vascular bed (MVB) is thromboxane-dependent

17P **Millican SA, Webster J & Wallace HM** ATP depletion after oxidant exposure in human vascular endothelial cells

18P **Knight RJ, Bowmer CJ & Yates MS** The effect of 8-cyclopentyl-1, 3-dipropylxanthine on *E. coli* endotoxin-induced renal dysfunction

19P **Clark KL, Robertson MJ & Drew GM** Effects of angiotensin AT_1 or AT_2 receptor blockade on basal renal function and on the renal effects of angiotensin II (Ang II) in the anaesthetised dog

20P **Soares-da-Silva P** Renal Na^+-H^+ exchanger activity, alpha -adrenoceptors and the control of dopamine outflow

21P **Adeagbo ASO, Triggle CR** Varying $[K^+]_{ext}$ reveals two components to acetylcholine (ACh)- and histamine (H)-induced vasodilatation in the perfused rat mesenteric arterial bed (MAB)

22P **Graham AM & Sneddon P** Evidence for nitric oxide as the inhibitory neurotransmitter in isolated rabbit anococcygeus

23P **Graves J & Poston L** Effect of inhibiting nitric oxide synthase on relaxations to isoprenaline in isolated rat mesenteric resistance arteries

24P **Gupta P** Endothelium-dependent relaxations to 5-HTR in guinea-pig isolated jugular vein are mediated by a receptor which resembles the 5-HT_{1D} subtype

25P **Tam FS-F, Hillier K & Bunce K** Investigation of the 5-HT receptor type involved in inhibition of spontaneous activity of human colonic circular muscle

26P **Ramage AG, Pires JGP, Gilbey MP & Futuro-Neto HA** Evidence to suggest that buspirone potentiates the vagal bradycardia induced by the "Diving Response" elicited by smoke in anaesthetized rabbits

27P **García-Barrado MJ, Moratinos J & Reverte M** Role of α_2 adrenoceptors and Ca^{2+}-channel blockers on insulin secretion resulting from glucose, α_1 and β adrenoceptor stimulation in conscious rabbits

28P **Leathard HL, Belcher E, Howarth SR, Richardson HL, Slater D, Arduino L & Wilson CA** Effects of ovarian steroids on contractility of human umbilical artery strips

29P **Qume M & Fowler LJ** Twenty-one-day treatment with the GABA-T inhibitors ethanolamine-O-sulphate and γ-vinyl GABA elevates basal and stimulated GABA release

30P **Bernasconi R, Marescaux C, Vergnes M & Bittiger H** Involvement of $GABA_B$ receptors in experimental absence seizures

31P **Malcangio M & Bowery NG** Effect of (-)-baclofen on cAMP formation in rat spinal cord slices

32P **Maguire JJ, Knott C & Bowery NG** Regional sensitivity of $GABA_B$ binding sites to pertussis toxin changes during ontogeny in the rat brain

33P **Martin KF, Hearson M & Heal DJ** Evidence that (±)-baclofen-induced hypoactivity is not mediated by $GABA_B$ receptors

34P **Stubbs CM, Dupere JRB, Birch PJ, Hagan RM, Chollet A & Kawashima E** Characterisation of human ileum NK_2 receptor stably expressed in Chinese hamster ovary cells using NK agonists

35P **Norris SK, Woodruff GN & Boden PR** Evidence for functional NK_1 and NK_3 neurokinin receptors in the medial habenula nucleus of the rat brain *in vitro*

36P **O'Shaughnessy CT, Connor HE & Feniuk W** Activation of NK_1 receptors stimulates plasma protein extravasation in guinea-pig dura mater and conjunctiva: tissues innervated by the trigeminal nerve

37P **Guard S & Watling KJ** Interaction of the non-peptide NK_1 tachykinin receptor antagonist (±)CP-96,345 with L-type calcium channels in rat cerebral cortex

38P **Kenny BA Spedding M** The novel calcium antagonist SR 33557 displays high affinity for a distinct binding site in rat brain

39P **Abdulla FA, Calaminici M, Gray JA, Sinden JD & Stephenson JD** Chronic hyoscine and oxotremorine treatment alter the sensitivity of rat frontal cortex pyramidal cells to acetylcholine and carbachol

40P **Barlow RB, Bond S, Holdup DW, Howard JA, McQueen DS, Veale MA, Smith TW, Stephenson GF & Batsanov AS** "FourDAPines": a new class of ileo-selective antimuscarinic drug

41P **Nally JE, McCall R, Young LC, McGrath JC, Wakelam MJO & Thomson NC** Stimulated phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis in human and bovine bronchial smooth muscle

42P **Shoaib M & Stolerman IP** Conditioned taste aversions in rats after administration of nicotine intracerebrally

43P **Bailey SJ, Rogers DC, Tulloch I, Fears R & Hunter AJ** Amitriptyline, but not paroxetine, impairs cognitive performance in rats

44P **Lightowler S, Williamson IJR, Hegarty J, Kennett GA, Fears RB & Tulloch IF** Anxiolytic effect of paroxetine in the rat social interaction model of anxiety

45P **Alder T & Morinan A** Strain differences in behavioural responses in murine models of anxiety

46P **Cartmell J, Kemp JA, Alexander SPH, Hill SJ & Kendall DA** Inhibition of forskolin-stimulated cyclic AMP formation by t-ACPD in guinea-pig cerebral cortical slices

47P **Jones PLStJ, Porter RHP, Birse EF, Pook PC-K, Sunter DC, Udvarhelyi PM, Wharton B, Roberts PJ & Watkins JC** Characterization of a new excitatory amino acid (EAA) receptor type activated by (1S,3R)-ACPD and selectively blocked by (s)-4C3H-PG

48P **Eaton SA, Salt TE, Udvarhelyi PM, Wharton B & Watkins JC** Selective antagonism of (1S,3R)-1-aminocyclopentane-1,2-dicarboxylate ((1S,3R)-ACPD) by (S)-4C3H-PG in the rat ventrobasal thalamus

49P **Follenfant RL & Nakamura-Craig M** Glutamate induces hyperalgesia in the rat paw

50P **Nakamura-Craig M & Follenfant RL** Analgesic effects of the glutamate release inhibitor, lamotrigine, in the rat

51P **McKibbon V, Hayward NJ, Poat JA, Woodruff GN & Hughes J** Expression of protooncogenes is induced by ischaemia in the Mongolian gerbil

52P **Keane PE, Fournier J, Steinberg R, Gauthier T, Coudé FX, Guzzi U, Soubrié P & Le Fur G** Neurotrophic effects of SR 57746A in a variety of neurodegenerative models *in vivo*

53P **Gnanalingam KK, Hunter AJ, Jenner P & Marsden CD** Selective protection of striatal/extrastriatal D_1/D_2 receptors from inactivation by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinonoline: an autoradiographic study

54P **Amabeoku GJ & Chikuni O** Chloroquine-induced seizures in mice: the role of dopaminergic system

55P **Castro S & Strange PG** Characterization of the two forms of the D_2 dopamine receptor expressed in mammalian cell lines

56P **Elliott JM, Phipps SL, Newton RA, Flanigan TP, Reavley AC, Cholewinski AJ, Leslie RA & Grahame-Smith DG** Biochemical characterisation of a 5-HT_2 receptor in the human neuroblastoma cell line IMR32

57P **Cholewinski AJ, Leslie RA & Grahame-Smith DG** 5-HT_2 receptors mediate agonist-evoked increases in intracellular calcium in the human neuroblastoma cell line, IMR 32

58P **Mason JP, Dring LG & Caldwell J** The metabolism of 8-OH-DPAT in rats

59P **Stamford JA & Palij P** "Real-time" voltammetric measurement of noradrenaline efflux and uptake in rat brain slices

60P **Khan RM & Zar MA** Pre- and post-junctional actions of NPY in producing inhibition of the electrically-evoked neurogenic twitches of rat isolated vas deferens

61P **Khan RM, Boublik JH, McDermott JR & Zar MA** Prejunctional inhibitory activity of some C- and N-terminal fragments of NPY in rat isolated vas deferens

62P **Posangi J, Zar MA & Harris JB** Effect of palytoxin on rat isolated prostatic vas deferens

63P **Faiz A, Harris JB & Zar MA** Neurotoxic and myotoxic activity of the crude venom of the two subspecies of Russell's viper, *Daboia russelii*

64P **Hodgson DR, Gasparini S, Ducancel F, Boulain J-C & Harris JB** Notechis 11'2: a route to the determination of toxic sites on a series of venom phospholipases A_2

65P **Al-Qatari M & Taberner PV** Effect of SR 58611A, a novel atypical β-adrenoceptor (β_3) agonist, on brown adipose tissue lipogenesis

66P **Nials AT, Barker RC, Coleman RA & Vardey CJ** Further characterization of the atypical β-adrenoceptors in guinea-pig gastric fundus

67P **Millar C, Carr RD, Humphries RG & Wilson WS** Drug effects on vascular flow in the isolated perfused bovine eye using radio-labelled microspheres

68P **Criddle DN, Reeves KA & Woodward B** Effects of the isopropyl ester of palmitoyl carnitine on vascular tissues of the rat, and the guinea-pig taenia-coli

69P **Sanjar S, McCabe PJ, Fattah D, Humbles AA & Pole SM** Evidence for the involvement of interleukin-5 in antigen-induced eosinophil accumulation in guinea-pig lung

70P **Rutter JA, Minion T, Howat D, Robinson M, Higgs D, Andrew D, Clements G & Hughes B** *In vivo* administration of an anti-CD18 monoclonal antibody inhibits leukocyte-dependent plasma exudation in rabbit skin

71P **Foster SJ, Crawley GC, Walker ERH & McMillan RM** ICI D2138: a potent, orally-active non-redox inhibitor of 5-lipoxygenase

POSTER COMMUNICATIONS

72P **Anderson-Beck R, Wilson L, Pickles M, Hughes IE & Peers C** Effect of doxapram on tritium overflow from the neonatal rat carotid body *in vitro*

73P **Yeadon M** Capsaicin desensitisation followed by atropine administration abolishes airway hyperreactivity induced by ozone in guinea-pigs

74P **Yeadon M, Dougan FL, Petrovic A & Payne AN** Eosinophil recruitment to lung tissue induced by allergen challenge in sensitised guinea-pigs: inhibition by betamethasone and the 5-lipoxygenase inhibitor, BW B70C

75P **Gallico L, Borghi A, Dalla Rosa C, Ceserani R & Tognella S** Effect of dexamethasone and moguisteine on allergen-induced early bronchoconstriction and late-phase airway leucocyte recruitment in sensitized guinea-pigs

76P **Shankley CE, Buchan P & Adcock JJ** Effect of a nitric oxide synthase inhibitor on bronchoconstriction and bronchodilatation induced by electrical stimulation of the vagi in the anaesthetised cat

77P **Sharma S, Lewis S & Raeburn D** Examination of the role of charybdotoxin-inactivated potassium channels on airway tone in the anaesthetized guinea-pig

78P **De Jonckheere B, De Jonckheere S & McCaig DJ** Effect of cromakalim on sympathetic-induced relaxation in guinea-pig isolated trachea

79P **Wickenden AD** Cromakalim, but not minoxidil sulphate, inhibits the release of intracellular calcium in the isolated rabbit aorta

80P **Hughes SJ, Downing SJ & Hollingsworth M** Relaxin, a potassium channel opener in the isolated rat uterus?

81P **Sneddon P & McLees A** Contribution of acetylcholine and ATP to the neurogenic contraction of isolated urinary bladder smooth muscle from adult and neonatal rabbits

82P **Pestana M & Soares-da-Silva P** Renal outflow of dopamine: a comparative study with L-DOPA and gamma-glutamyl-L-DOPA

83P **Fernandes MH, Vieira-Coelho MA & Soares-da-Silva P** Influence of 3-0-methylDOPA on the synthesis of dopamine in the kidney

84P **Lefèvre-Borg F, Lechaire J & O'Connor S** *In vivo* uroselectivity of alfuzosin compared to prazosin and terazosin

85P **Lyles GA, Chalmers J & Farrell RE** Enhancement of α_1-adrenoceptor mediated contractions by partial depolarisation of rat aortic smooth muscle

86P **Morris SJ, Roth B, Braun E & Ball HA** Dose-related effects of dobutamine on diastolic function in the mini-pig

87P **Barker RC, Coleman RA, Dahl MR, Nials AT & Vardey CJ** Do salmeterol and formoterol possess agonist activity at atypical β-adrenoceptors in guinea-pig gastric fundus?

88P **Arnott MR & Struthers AD** Angiotensin II decreases, while captopril increases, myocardial noradrenaline uptake: a mechanism for ACE inhibitors reducing cardiac death?

89P **Jamieson A, Alcock P, Wood L & Tuffin DP** Effect of the deoxyribonucleic acid derivative defibrotide on plasma fibrinolytic activity in the rat

90P **Fundafunda B, Smith GM, Jamieson A & Tuffin DP** Effect of platelet activating factor on circulating white blood cell count, platelet count and on plasma tPA and PAI-1 activity in the rat

91P **Patacchini R, Maggi CA & Giachetti A** Heterogeneity of NK_2 tachykinin receptors in hamster, rat and rabbit smooth muscles

92P **Beresford IJM, Ireland SJ, Stables JM, Stubbs CM, Ball D, Hagan RM & Birch PJ** Investigation into differences in tachykinin NK_1 receptors between and within species using a peptide and a non-peptide NK_1 receptor antagonist

93P **Ireland SJ, Beresford IJM & Stables J** Comparison of the apparent affinity of GR82334 at NK_1 receptors in various tissue preparations

94P **Carruthers AM, Milavec M, Gadient F & Fozard JR** Antivasoconstrictor effects mediated by adenosine A_1 receptor agonists in the pithed rat

95P **Adeagbo ASO & Triggle CR** Endothelium-derived nitric oxide (NO) in the rat thoracic aorta does not mediate the suppression of clonidine-induced tonus

96P **Arkle S, Groome RD & Sturge J** Effects of phytate on smooth muscle preparations *in vitro*

97P **White TE, Dickenson JM & Hill SJ** Histamine H_1-receptor-mediated accumulation of total [^{3}H]-inositol phosphates in DDT$_1$MF-2 cells

98P **Welsh NJ, Shankley NP & Black JW** Comparison of antagonist pK_B estimates in lumen-perfused stomach assays from guinea-pig, rat and mouse

99P **Baxter GS, Blackburn TP, Sanger GJ & Wardle KA** Actions of DAU 6285 at putative 5-HT_4 receptors in guinea-pig colon and rat oesophagus

100P **Spokes RA, Liddle CW & Middlefell VC** A comparison of the effects of sumatriptan and 5-carboxamidotryptamine on blood pressure in the anaesthetised and angiotensin II-supported pithed rat

101P **Rudd JA, Bunce KT & Naylor RJ** The effect of 8-OH-DPAT on drug-induced emesis in the ferret

102P **Hu DE, Riley CR & Fan T-PD** Parallel studies of ^{133}Xe clearance and ^{113}Sn microsphere accumulation during sponge-induced angiogenesis and the effects of interleukin-1α

103P **Hu DE, Hori Y & Fan T-PD** Comparative studies on the angiogenic activity of IL-1 and IL-8 in a rat sponge model

104P **Perretti M, Appleton I, Ghiara P, Parente L, Willoughby D & Flower RJ** Neutrophil recruitment into mouse air-pouch by interleukin-1_β is mediated by Type 1 receptors

105P **Hutchings A, Mulqueen M, Birchall A & Bradshaw D** Drug effects on adjuvant disease in the MF1 mouse

106P **Head SA, Louttit JB & Coleman RA** The actions of meclofenamic acid at prostanoid receptors

107P **Anderson EM, Kuonen DR & Bennett A** Indomethacin accumulation by murine cancer cells in culture

108P **Ebenezer IS, Parrott RF, Baldwin BA & Buttle HL** The effects of intravenous cholecystokinin (CCK) on prolactin and growth hormone release in the prepubertal pig

109P **Loxley HD, Taylor AD, Flower RJ & Buckingham JC** Modulation of the hypothalamo-pituitary-gonadal axis by lipocortin 1.

110P **Kenny BA & Spedding M** Class III calcium antagonists display high affinity for the [^{3}H]-fluspirilene binding site in skeletal muscle

111P **Dickinson K & Jones RB** Effects of alinidine and falipamil on PN200-110 and desmethoxyverapamil binding in guinea-pig ventricular microsomes

112P **Stoggall SM, Gregory L, Baker C & Wilson C** Comparison of rat aorta and porcine coronary artery response to big endothelin-1 and endothelin-1

113P **Borland IA, Bates RFL & Buckley GA** Effect of a magnesium deficient diet on cyclosporin A-induced cardiac calcification

114P **Sharma SC** Protection of human erythrocytes by lazaroids

115P **Naylor IL, Osman IA & Teo TC** Modifications to the rate of wound contraction in the rat induced by allopurinol

116P **Nugent C, Bertrand R & Grant MH** Nickel cytotoxicity in mammalian fibroblasts does not involve depletion of glutathione

117P **Grant MH, Watson J, Vass M, Willett B, Scott A & MacDonald C** Bilirubin UDP-glucuronosyltransferase activities in SV40-immortalised rat hepatocytes

118P **Sian J, Dexter DT, Marsden CD & Jenner P** Glutathione levels in brain neurodegenerative diseases affecting basal ganglia

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