British Journal of Pharmacology

April 1992

Volume 105 Proceedings Supplement 1P–314P

1P ROLE OF ACTIN CYTOSKELETON IN THE REGULATION OF THE RENAL SYNTHESIS OF DOPAMINE

P.Soares-da-Silva, Dept. of Pharmacology and Therapeutics, Faculty of Medicine, 4200 Porto Portugal

Renal tubular epithelial cells are endowed with an enormous capacity to decarboxylate L-DOPA into dopamine; Na^+ and mechanisms involved in the tubular transport of Na^+ have been shown to be determinant factors in the control of tubular synthesis of dopamine (Soares-da-Silva & Fernandes, 1990, 1991). The ultimate mechanism intervening in the process of Na^+ transport across epithelial cells of proximal tubules is that governed by Na^+ -K^+ ATPase. This enzyme is localized at the basolateral membrane of tubular epithelial cells and the association between the actin cytoskeleton and Na^+ -K^+ ATPase is determinant for the vectorial transport of Na (Molitoris, 1991). The aim of the present study is to determine the effects of inhibition of Na^+ -K^+ ATPase activity by ouabain and inhibition of actin cytoskeleton by cytochalasin B on the synthesis of dopamine in rat kidney slices loaded with L-DOPA. The deamination of newly-formed dopamine into 3,4-dihydroxyphenylacetic acid (DOPAC) was also examined; tissue levels of dopamine and DOPAC were determined by high pressure liquid chromatography with electrochemical detection. Slices of rat renal cortex were preincubated for 30 min in warmed (37°C) and gassed (95% O_2 and 5% CO_2) and incubated for further 15 min with increasing concentrations of L-DOPA (10-100 µM). The formation of dopamine from L-DOPA in the presence of 120 mM NaCl in the medium was found to be twice that in the presence of 20 mM NaCl; the osmolarity of the medium was kept constant by the addition of choline chloride. In experiments performed in the presence of 120 mM NaCl, but not in conditions of low Na^+ (20 mM NaCl in the medium), ouabain (100 and 500 µM) was found to inhibit the accumulation of newly-formed dopamine and DOPAC (14-57% reduction); this effect was more marked at 50 and 100 µM L-DOPA. Petreatment of rat renal slices with cytochalasin B (5, 10 and 50 µM) resulted in a concentration-dependent reduction in the accumulation of dopamine and DOPAC (10-49% reduction). The inhibitory effect of cytochalasin B on the formation of dopamine was no longer observed in kidney slices incubated simultaneously with ouabain (100 µM) or in conditions of low Na^+ (20 mM NaCl in the medium). These results show that the renal formation of dopamine is dependent on the concentration of Na in the medium and on the integrity of a tubular transport system for Na^+ , namely on the association between actin cytoskeleton and Na^+ -K^+ ATPase.

Molitoris, B.A. (1991). Am.J.Physiol., 260,F769-F778.
Soares-da-Silva, P. & Fernandes, M.H. (1990) Am.J.Hypertens., 3,7S-10S.
Soares-da-Silva, P. & Fernandes, M.H. (1990) Br.J.Pharmacol., 103,1923-1927.

Supported by a grant from the INIC (FmP1).

2P COMPARATIVE EFFECTS OF NEUROLEPTICS AND APOMORPHINE ON DOPMAINE RELEASE AND METABOLISM IN THE RAT CAUDATE NUCLEUS AND MEDIAL PREFRONTAL CORTEX

J.W. Dalley & R.A. Webster, Department of Pharmacology, University College London, Gower St, London WC1E 6BT.

Neuroleptic (NL) drugs probably act mainly by antagonising dopamine (DA). It is believed that atypical NLs, such as clozapine (CLZ), which give rise to fewer adverse motor disorders, may have less influence on the nigrostriatal DA system compared to typical NLs such as haloperidol (HAL) whilst remaining effective on mesolimbic and/or mesocortical DA systems.We have therefore compared the effects of CLZ and HAL alone both on the spontaneous efflux of DA and its metabolites (DOPAC and HVA) in the caudate nucleus (CP) and medial prefrontal cortex (MPFC) as well as on the effects of the DA agonist apomorphine(APO) in both areas.

Spague-Dawley rats (250-300g) were anaesthetised with halothane and intracerebral dialysis probes inserted into either the rostral CP (A+1.5-2.0, L2.5-3.0, V7 mm relative to bregma) or MPFC (A+2.5-3.0, L0.4-0.9, V5.5) and perfused with artificial CSF (mM)(Na^+ 147, Ca^{2+} 2.30, K^+ 4.00, Cl^- 155.6, pH 6.5) at 5µl/min. Perfusates were collected every 20 mins and analysed for DA, DOPAC and HVA using HPLC with electrochemical detection. The effects of clinically equivalent doses of CLZ (20 mg/kg) or HAL (0.5 mg/kg) were monitored for 4 hours after their i.p. administration. APO was administered in increasing doses (0.025, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10 mg/kg i.v.) at 40 min time intervals either alone or after NL pretreatment. The maximum percentage changes in basal efflux (mean ± s.e. mean n=5) to APO (1mg/kg i.v.) before and after each NL and to each NL alone are given in Table 1.

Table 1

	CP			MPFC		
	DA	DOPAC	HVA	DA	DOPAC	HVA
CLZ 20mg/kg	+4.8±1.2	+59.0±11.2*	+115.2±21.2*	+23.3±6.2*	+156.0±16.9*	+116.3±7.8*
HAL 0.5mg/kg	+3.9±1.9	+50.8±12.7*	+90.2±7.3*	+4.5±3.2	+142.2±30.7*	+174.1±23.5*
APO 1mg/kg	-33.7±5.2*	-72.7±13.1*	-59.5±12.8*	-23.2±2.7*	-65.5±7.1*	-53.5±5.0*
CLZ & APO	-12.5±8.1#	-55.8±2.1#	-13.1±2.4†	-30.1±6.3	+8.7±6.9†	+32.5±11.1†
HAL & APO	-0.9±4.8†	+21.4±6.1†	+41.8±5.3†	-11.4±4.4#	+72.2±8.2†	+120.5±16.9†

* $p<0.01$ versus basal efflux, # $p<0.05$, † $p<0.01$ versus apo 1mg/kg alone (Student's non-paired t-test). Basal efflux (pmol/20min (mean±s.e.mean n=5) CP:DA 0.071±0.012, DOPAC 18.1±1.4, HVA 11.7±2.0, MPFC:DA 0.052±0.010, DOPAC 1.14±0.22, HVA 2.34±0.42.

Both NLs enhanced DOPAC and HVA efflux in the two areas although only CLZ significantly facilitated DA efflux and this was restricted to the MPFC. Thus under these conditions the levels of DA metabolites may reflect the intraneuronal metabolism of DA rather than its release. The ability of CLZ to release DA in the MPFC may account for its effectiveness in alleviating the negative symptoms of schizophrenia. The reduction in DA efflux and metabolism induced by APO in both brain areas was reversed by HAL but only partially reduced by CLZ. This weak DA (APO) antagonism shown by CLZ in the CP would account for the low incidence of extrapyramidal side effects seen with this compound although its relatively weak action in the MPFC implies that its NL effectiveness must stem, at least in part, from other actions.

3P PLASMA HVA LEVELS FOLLOWING DEBRISOQUINE ADMINISTRATION DO NOT REFLECT BRAIN DOPAMINE LOSS IN PARKINSON'S DISEASE

S. Rose [1], J.G. Hindmarsh[1], M. J. Steiger[2], N.P. Quinn[2], P. Jenner[1] and C.D. Marsden[2]. [1]Parkinson's Disease Society Experimental Research Laboratories, Pharmacology Group, Biomedical Sciences Division, King's College, Manresa Rd, London,U.K. [2] University Department of Clinical Neurology, Institute of Neurology, The National Hospital, Queen Square, London, U.K.

Debrisoquine (DBQ) administration prevents the peripheral formation of homovanillic acid (HVA) so that plasma HVA (pHVA) levels may be used to assess central dopaminergic activity in animals and man. Importantly, Kopin *et al* (1988) showed that following DBQ treatment, pHVA levels were reduced in monkeys treated with the selective nigral toxin, MPTP, and showing parkinsonism, compared to control animals. This might suggest that pHVA levels following DBQ administration may be used to measure brain dopamine loss in patients with Parkinson's disease. However, pHVA levels have not been studied in patients with Parkinson's disease following DBQ administration. We report the results of an investigation into whether measurement of pHVA can provide an indication of the extent of dopamine neuronal loss in the brain in Parkinson's disease.

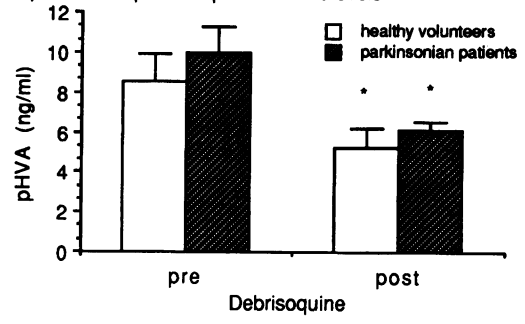
Initially, the protocol for DBQ administration was studied in healthy volunteers (age 31±3yrs). Subjects fasted for 12h prior to DBQ (10mg) administration at 9am. Blood samples were taken just prior to and at hourly intervals for up to 7h following DBQ administration. pHVA concentrations were analysed by HPLC. This protocol was repeated (1) following a loading dose of DBQ (10mg), 10h prior to the morning dose and (2) following DBQ (20mg). Patients with Parkinson's disease (age 58±5; Hoehn and Yahr Scale 2-3; duration of disease 5±1yrs), not previously treated with L-DOPA or dopamine agonists, and healthy volunteers (age 31±3yrs) fasted for 12h prior to DBQ (10mg) administration. Blood samples were taken just prior to and 6h after DBQ administration. pHVA levels were assessed by HPLC.

Following DBQ administration to healthy volunteers, pHVA levels fell with time to a maximum of 61.5% of control values at 6h. The % decrease in pHVA was not affected by loading with DBQ 10h previously (67.6±5.8%) or increasing the dose to 20mg (56.1±11.8%) compared to control (66.5±4.5%). There was no difference in pHVA levels pre- or post-DBQ in *de novo* patients with Parkinson's disease compared to healthy healthy volunteers (Fig 1).

These results suggest that following DBQ administration, pHVA levels cannot be used as an indication of dopamine neuronal loss in *de novo* patients with Parkinson's disease. This could be due to compensation for loss of central dopamine by increased dopamine turnover in the surviving neurones.

Kopin, I.J., Bankiewicz, K.S. and Harvey-White, J. (1988) Neuropsychopharmacol 1: 119-125

Fig 1 The effect of DBQ administration on pHVA levels in parkinsonian patients (n=9) and healthy volunteers (n=5) * p<0.05 compared to pretreatment levels



4P ACTIONS OF THE NOMIFENSINE METABOLITE 8-AMINO-2-METHYL-4-(3-METHOXY-4-HYDROXY-PHENYL)-1,2,3,4-TETRAHYDROISOQUINOLINE ON STRIATAL DOPAMINE EFFLUX AND UPTAKE

J.A. Stamford, S. Hafizi & P. Palij. Anaesthetics Unit, London Hospital Medical College, Alexandra Wing, Royal London Hospital, LONDON E1 1BB.

Several antidepressants are known to have biologically active metabolites, in some cases contributing significantly to the pharmacological profile. Nomifensine, an antidepressant with significant actions on dopamine (DA) neurons, has three main metabolites in humans, designated M1-3 (Kellner *et al.*, 1977). Costall *et al.* (1975) have shown that the M1 metabolite is as potent as the parent drug in behavioural tests of DA function. The purpose of the present study was to establish whether the two other principal human metabolites (M2: 8-amino-2-methyl-4-(3-methoxy-4-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline and M3: 8-amino-2-methyl-4-(3-hydroxy-4-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline) had actions on DA release and uptake.

All experiments were conducted in 350μm slices of rat striatum using the method of Palij *et al.* (1990). Slices were held in a standard brain slice chamber and superfused with artificial cerebrospinal fluid (32°C, 1ml/min). Local DA efflux was evoked by constant-current pulses (0.1ms, 10mA) applied via bipolar tungsten stimulating electrodes. DA efflux and uptake following single pulses or trains (20 pulses, 50Hz) applied every 10 minutes was monitored using fast cyclic voltammetry at carbon fibre microelectrodes implanted 80μm below the slice surface. All drugs were administered via the superfusion fluid and their effects were measured after 2h exposure.

Over the 2h period DA efflux in controls declined to 63 and 64% of pre-drug values on single pulses and trains respectively. The uptake half-life was also slightly prolonged (110%). Nomifensine (0.5μM) significantly ($P<0.05$) increased efflux on both single pulse (289% of pre-drug) and train stimuli (485%) and, as expected, significantly ($P<0.05$) prolonged the uptake half-life (178% of pre-drug). The M2 metabolite (0.5μM) was equipotent with nomifensine on DA efflux (247% : pulse, 470% : train) but did not significantly inhibit uptake. Both nomifensine ($P<0.05$) and the M2 metabolite ($P<0.001$) increased DA efflux to a greater extent on the train than on the single pulse. The M3 metabolite (0.5μM) caused a modest increase in DA efflux, significant ($P<0.05$) only on the single pulse (129%). The selective DA uptake blocker GBR 12909 (0.3μM) blocked DA uptake ($t_{\frac{1}{2}}$: 292%) and increased DA efflux ($P<0.05$) on single pulse (430%) and train stimuli (645%). Conversely, the psychomotor stimulant amfonelic acid (0.1μM) blocked DA uptake ($t_{\frac{1}{2}}$: 234%, $P<0.01$) and elevated DA efflux ($P<0.05$) to a greater extent on trains (934%) than on single pulses (430%).

In summary, the M2 metabolite of nomifensine elevates DA efflux by an action largely independent of uptake blockade. The greater elevation of DA efflux on trains is consistent with an action to mobilise the DA storage pool since it is also exhibited by the stimulant amfonelic acid but not by the pure uptake blocker GBR 12909. It is concluded that some of the apparent activity of nomifensine in humans may be mediated via its metabolites.

This research was funded largely by The Royal Society. We thank Hoechst for the gift of nomifensine metabolites and J. Winyard for technical assistance.

Costall, B., Kelly, D.M. & Naylor R.J. (1975) *Psychopharmacologia*, **41**, 153-164.
Kellner, H.M., Baeder, C., Christ, O., Heptner, W., Hornke, I. & Ings, R.M. (1977) *Br. J. Clin Pharmacol.* **4**, 109S-116S
Palij, P., Bull, D.R., Sheehan, M.J., Millar, J., Stamford, J.A., Kruk, Z.L. & Humphrey, P.P.A. (1990) *Brain Res.* **509**, 172-174.

5P STRIATAL D_2 RECEPTORS ARE UNCOUPLED FROM ADENYLYL CYCLASE FOLLOWING 6-HYDROXYDOPAMINE LESION OF THE NIGROSTRIATAL PATHWAY

K. Thomas, P. Jenner and C.D. Marsden[1]. Parkinson's Disease Society Experimental Research Laboratories, Pharmacology Group, Biomedical Sciences Division, King's College, London SW3 6LX,U.K. [1] University Department of Clinical Neurology, Institute of Neurology, The National Hospital for Nervous Diseases, Queen Square, London WC1N 3BG, U.K.

In rat striatum some D-2 receptors are negatively linked to adenylyl cyclase (AC) (Stoof and Kebabian, 1981). Behavioural studies suggest a breakdown of the linkage following lesioning of the nigrostriatal pathway using 6-hydroxydopamine (6-OHDA) (Arnt and Hyttel, 1984). Using striatal prisms, we measured the accumulation of cyclic AMP in response to DA agonists and antagonists to investigate whether uncoupling also occurs at the DA receptor level.
Cyclic AMP accumulation was measured in cross chopped (0.35 x 0.35mm) striatal slices prepared from either left or right hemispheres of the rat brain according to the method of Lazareno et al. (1985) 28 days following a unilateral 6-OHDA (8µg free base in 0.9% saline/0.1% ascorbate) injection into the medial forebrain bundle (MFB) and a vehicle injection into the contralateral MFB. In sham lesioned striatal slices, DA (1.0-320µM) and the selective D-1 agonist SKF 38393 (0.1-3.2µM) stimulated cyclic AMP accumulation in a concentration dependent fashion, with EC50 values of 34 ± 8µM and 0.4 ± 0.1µM respectively. SCH 23390 (10µM) inhibited the cyclic AMP accumulation produced by 320µM DA and 1µM SKF 38393 (Table 1). The D-2 antagonist (-)-sulpiride (50µM), enhanced the response to DA and the selective D-2 agonist quinpirole (10µM) attenuated the response to SKF 38393. In striatal slices prepared from the 6-OHDA lesioned hemisphere, SCH 23390 inhibited cyclic AMP accumulation produced by DA and SKF 38393. However, (-)-sulpiride did not augment the AC response to DA nor did quinpirole inhibit the response to SKF 38393

Table 1. The accumulation of cyclic AMP (pmol/mg protein + SEM) in response to DA agonists and antagonists in striatal slices after a sham and 6-OHDA lesion of the MFB. (n = 9-12. *P < 0.05. Dunn's test).

	DA (320µM)			SKF 38393 (1µM)	
	Sham	6-OHDA Lesion		Sham	6-OHDA Lesion
Alone	51.1 ± 9.2	48.5 ± 5.2	Alone	45.6 ± 3.3	36.0 ± 4.4
SCH 23390 (10µM)	26.5 ± 2.0*	30.1 ± 2.7*	SCH 23390 (10µM)	19.8 ± 1.8*	24.1 ± 3.8*
Alone	43.0 ± 5.0	56.5 ± 3.5	Alone	34.0 ± 5.5	27.0 ± 2.6
(-)-Sulpiride (50µM)	64.9 ± 4.9*	51.3 ± 4.6	Quinpirole (10µM)	18.8 ± 2.4*	24.5 ± 3.0

The results confirm that in normal striatal slices, the D-2 receptor is coupled to D-1 receptor associated AC in an inhibitory fashion. However, the results suggest that following DAergic deafferentation the inhibitory D-2 receptor becomes uncoupled from the D-1 receptor associated AC.

Arnt J. and Hyttel J. (1984) Eur J Pharmacol. **102**, 345-354.
Lazareno S., Marriott D.B. and Nahorski S. (1985) Brain Research **361**, 91-98.
Stoof J.C. and Kebabian J.W. (1981) Nature (Lond.) **294**, 366-368.

6P THE EFFECTS OF DOPAMINE ON RAT SUBSTANTIA NIGRA ZONA COMPACTA NEURONES STUDIED USING THE WHOLE CELL RECORDING TECHNIQUE

Gareth. A. Hicks and Graeme Henderson, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ

D_2 dopamine receptors present on substantia nigra zona compacta dopaminergic neurones are coupled to an inwardly rectifying potassium conductance (Lacey *et al.*, 1988). Recently it has been suggested that in enzymically dissociated substantia nigra neurones the potassium conductance coupled to the D_2 receptor is in fact an adenosine triphosphate (ATP)-sensitive potassium conductance (Roeper *et al.*, 1990).

We have made whole cell patch recordings from substantia nigra zona compacta neurones contained in a 400 µm thick brain slice prepared from 14-28 day old, male rats. The patch pipette contained (mM): KCl 140, $MgCl_2$ 2, $CaCl_2$ 1, EGTA 10, cAMP 0.1, ATP 0.1, GTP 0.1 and HEPES 5, pH 7.4 with KOH and the fluid superfusing the the slice (flow rate 2ml min^{-1}) contained (mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.24, $MgCl_2$ 1.3, $CaCl_2$ 2.4, Glucose 10, $NaHCO_3$ 26, Ascorbic acid 0.1, gassed with 95% O_2/5%CO_2, pH 7.4 at 26°C.

Neurones in the zona compacta could be divided into two types on the basis of their rates of action potential firing. The most frequently encountered neurones fired slowly at frequencies between 0.25 and 5Hz whereas occasionally neurones firing between 11.5 and 15Hz were observed. These two cell types appear to correspond to the principal and secondary type neurones described by Lacey *et al* .(1989).

Dopamine (30 or 100µM) reduced the rate of cell firing and hyperpolarised all slowly firing zona compacta neurones indicating that these are in fact dopaminergic neurones. When cells were manually clamped at -60mV dopamine (100µM) evoked a fall in input resistance from 353±20 to 217±7MΩ (n=5; mean±s.e.mean). The effects of dopamine were completely reversed by co-application of sulpiride (1 or 3µM n=14). However, the dopamine-induced hyperpolarisation was not reversed by the ATP-sensitive potassium channel inhibitors, tolbutamide (100-300µM, n=7) or glibenclamide (3-30µM, n=8). Furthermore the ATP-sensitive potassium channel activator, cromakalim (3-500µM, n=8) did not slow the firing rate or hyperpolarise dopamine-sensitive neurones either before or after blockade of synaptic transmission by the addition of Mg^{2+} (20mM) to the superfusing solution. To ensure that our drug containing solutions retained activity at the ATP-sensitive potassium channel we demonstrated that cromakalim inhibited in a tolbutamide- and glibenclamide-sensitive manner the nerve-evoked contractions of the guinea-pig ileum. Under our recording conditions the potassium conductance activated by dopamine acting through the D_2-dopamine receptor does not show the pharmacological characteristics of an ATP-sensitive potassium conductance.

G.H. Jr. holds a Glaxo Research Scholarship.

Lacey, M.G., Mercuri, N.B., and North, R.A. (1988). *J. Physiol. (Lond.)* **401**, 437-453
Lacey, M.G., Mercuri, N.B., and North, R.A. (1989). *J. Neurosci.* **9**, 1233-1241
Roeper, J., Hainsworth, A.H., and Ashcroft, F.M. (1990). *Pflugers Arch.* **416**, 473-475

7P DISTINCT ANTAGONIST PROFILES OF TWO D_1 AGONIST-INDUCED BEHAVIOURS: DIFFERENTIAL INVOLVEMENT OF D_1 RECEPTOR SUBTYPES?

Siobhan A. Daly & J.L. Waddington, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2, Ireland.

The increasing availability of drugs acting selectively at the D-1 dopamine receptor now allows further insights into its functional role. Using the new isochroman D-1 agonist A-68930, a partial agonist in the retina but a full efficacy agonist in the striatum (DeNinno et al., 1991), we have recently described the induction not only of intense grooming behaviour that was readily blocked by the selective D-1 antagonist SCH 23390, but also of vacuous chewing behaviour that was insensitive to such blockade (Daly & Waddington, 1991a). We present here a detailed characterisation of these responses using a range of new selective D-1 antagonists.

Using previously described procedures (Daly & Waddington, 1991a,b), rats were challenged s.c. with A-68930 after s.c. pretreatment with vehicle, SCH 23390 or the new selective D-1 antagonists NO 756 (Andersen et al., 1988) and BW 737C (Riddall & Ionides, 1990), and behavioural responses assessed; the selectivities of these agents for D-1 vs D-2 receptors were clarified in binding studies using ^{3}H-SCH 23390 and ^{3}H-spiperone. A-68930 given at 0.25 mg/kg readily induced intense grooming ($P<0.01$ vs vehicle alone) that was readily blocked ($P<0.01$) by pretreatment with 0.01-1.0 mg/kg SCH 23390, with 0.01-1.0 mg/kg NO 756 and with 0.2-5.0 mg/kg BW 737C. This agonist also induced vacuous chewing ($P<0.01$ vs vehicle alone) that was not blocked by such pretreatments with SCH 23390 [which induced significant ($P<0.05$) vacuous chewing when given alone] or with NO 756, but was readily blocked ($P<0.01$) by pretreatment with BW 737C. All agents showed > 100-fold selectivity for D-1 vs D-2 receptors.

On initially reporting the failure of SCH 23390 to antagonise A-68930-induced vacuous chewing, we speculated that this might indicate the involvement of a 'D-1-like' receptor relatively insensitive to SCH 23390 (Daly & Waddington, 1991a). The present results sustain and extend this notion, first by generalising that finding to another benzazepine D-1 antagonist NO 756 and secondly by indicating that such a 'D-1-like' receptor appears sensitive to antagonism by the novel isoquinoline D-1 antagonist BW 737C. How any such site might or might not relate to putative 'D-1-like' receptor subtypes ($D\text{-}1_A$, $D\text{-}1_B$,D-5) identified recently in molecular biological studies remains to be clarified.

This work was supported by the Wellcome Trust; we thank Abbott, Novo, Schering and Wellcome Foundation for gifts of drugs.

Andersen, P.H., Groenvald, F.C., Hohlweg, R. & Braestrup, C. (1988) Soc. Neurosci. Abstr. 14, 935.
Daly, S.A. & Waddington, J.L. (1991a) Br. J. Pharmac. (in press).
Daly, S.A. & Waddington, J.L. (1991b) Br. J. Pharmac. 102 (Suppl.), 10P.
DeNinno, M.P., Schoenleber, R., MacKenzie, R. et al. (1991) Eur. J. Pharmac. 199, 209-219.
Riddal, D.R. & Ionides, J. (1990) XVIIth CINP Abstr. 2, 308.

8P CELLULAR EXPRESSION OF CATECHOL-O-METHYLTRANSFERASE (COMT) IN RAT CNS AND PERIPHERAL ORGANS REVEALED BY *IN SITU* HYBRIDIZATION HISTOCHEMISTRY

J.G. Richards , B. Bertocci, M. Da Prada and P. Malherbe, Pharma Division, Preclinical Research, F.Hoffmann-La Roche Ltd, CH-4002 Basel, Switzerland

COMT (EC 2.1.1.6), a membrane-associated enzyme, catalyzes the metabolism of dopamine, noradrenaline, adrenaline and other catechol derivatives. In the brain, COMT is responsible for the extraneuronal inactivation of released catecholamines. cDNA clones for human and rat COMT have been recently isolated and sequenced (Bertocci et al., 1991; Salminen et al., 1990). In order to identify the cells expressing COMT in rat CNS and peripheral organs, we used ^{35}S-labelled oligonucleotide probes (nucleotide sequences 92–152 and 587–647) for in situ hybridization histochemistry. Transcripts were found in discrete regions of rat brain (ependyma, choroid plexus, circumventricular organs, dentate gyrus granule cells > hippocampal CA1-4 pyramidal cells, cerebellar granule cells, Bergmann glia, brain stem nuclei – facial n., vestibular n., olive n., pontine n., > cerebral cortex, olf. bulb), the meninges, intracranial blood vessels and peripheral tissues including renal pyramids, duodenal villi, vas deferens epithelium and placenta. The results suggest that non-neuronal elements provide an enzymatic barrier to the transport of catecholamines between the CSF or blood and the brain (see also Kaplan et al.,1981). The identity those of neurons in the hippocampal formation and brainstem nuclei expressing COMT mRNA is being further investigated.

Bertocci, B., Miggiano, V., Da Prada, M., Dembic, Z., Lahm, H.-W. & Malherbe, P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1416-1420.
Salminen, M., Lundström, K., Tilgmann, C., Savolainen, R., Kalkkinen, N. & Ulmanen, I. (1990)*Gene* **93**, 241-247.
Kaplan, G.P., Hartman, B.K. & Creveling, C.R. (1981) *Brain Res.* **204**, 353-360.

9P SYSTEMIC ADMINISTRATION OF NICOTINE INCREASES HIPPOCAMPAL NORADRENALINE RELEASE *IN VIVO* LARGELY BY AN ACTION AT THE LOCUS CAERULEUS

S.N. Mitchell & J.A. Gray (introduced by M.H. Joseph), Dept. of Psychology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, U.K.

Using *in-vivo* microdialysis in freely moving rats acute systemic administration of nicotine has been shown to dose-dependently increase hippocampal noradrenaline (NA) release (Brazell et al., 1991). Although this response was central in origin, the precise site of action, i.e. cell body or nerve terminal, was not explored. *In-vitro* studies have indicated a sensitivity of both the locus coeruleus (LC; Egan & North, 1986) and noradrenergic terminal areas (Arqueros et al., 1978; Snell & Johnson, 1989) to nicotine. Experiments were therefore conducted using *in-vivo* microdialysis in both freely moving and anaesthetised animals to ascertain the contribution of both sites to the overall systemic response by combining local and systemic drug administration with on-line measurements of NA release.

Male Sprague-Dawley rats (300-350 g) were used. Dialysis probes (Hospal membrane 3-4 mm in length, 290μm o.d. wet) were implanted into the hippocampus via guide cannulae in freely moving animals (Brazell et al., 1991), or directly in anaesthetised animals (chloral hydrate 600 mg/kg i.p.). Nomifensine (1μM) was included in the Krebs' solution perfusing hippocampus. Methods were essentially as described previously (Brazell et al., 1991). Drugs were administered via the probe over 10 min using a liquid switch, or via a cannula positioned close to the LC (from bregma and dura surface; caudal -9.5 mm, lateral -1.4 mm, vertical -6.0 mm) delivered in 1μl over 1 min. Samples were collected every 10 min; all changes in NA levels are expressed as a percentage of a preinjection control period.

In anaesthetised animals, systemic administration of nicotine (0.4 mg/kg s.c.) increased extracellular levels of NA. The overall response profile was different to that in freely moving animals as there was no response within the first 10 min. However, the remaining response profile was similar, with maximal increases occurring 20-40 min after injection (185%-239%, n=6). In freely moving animals, intrahippocampal administration of nicotine (50-500μM) increased extracellular levels of NA (115±11% - 159±10%, n=3-7); anaesthetised animals were similarly sensitive to local administration. In anaesthetised rats, intrahippocampal adminstration of mecamylamine (25-100μM), a nicotinic channel blocker, inhibited the effect of locally applied nicotine (250μM), but failed to inhibit the response to a systemic injection (0.4 mg/kg, n=6). In contrast, LC administration of mecamylamine (50μM) almost abolished [ANOVA, $F(1,9) = 11.16$, $P < 0.01$] and trimetaphan (50μM), a nicotinic receptor antagonist, significantly reduced [$F(1,10) = 6.14$, $P < 0.05$] the hippocampal response provoked by a systemic nicotine (0.4 mg/kg) challenge. In support of the presence of nicotinic receptors in the LC, local application of nicotine (50μM) in anaesthetised rats elevated levels of NA in the hippocampus to 281 ± 65% (n=5).

In freely moving animals the response to a systemic injection of nicotine includes an initial component which may be associated with the behavioural effects of the drug, although the locus of this action is presently unknown. Noradrenergic neurones are sensitive to local administration of nicotine in both the hippocampus and LC. Using anaesthetised animals, the remaining hippocampal NA response provoked by a systemic injection of nicotine appears to be mediated largely by an action at the LC.

Arqueros, L., Naquira, D. & Zunino, E. (1978) Biochem. Pharmacol., 27, 2667-2674.
Brazell, M.P., Mitchell, S.N. & Gray, J.A. (1991) Neuropharmacology, 30, 23-833.
Egan, T.M. & North, R.A. (1986) Neuroscience, 19, 65-571.
Snell, L.D. & Johnson, K.M. (1989) Synapse, 3, 29-135.

This work was supported in part by The Council for Tobacco Research, U.S.A.

10P IPRINDOLE DOWN-REGULATES β_1–ADRENOCEPTORS IN CULTURED RAT C_6 GLIOMA CELLS

A Ruck , P J Millns, S J Hill and D A Kendall. Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH

Previous studies have shown that chronic *in vivo* administration of tricyclic antidepressants, in particular desipramine, results in a loss of β-adrenoceptors and a reduction in β-adrenoceptor stimulated adenylate cyclase activity in rat brain (Pandey and Davis, 1983). Rat C_6 glioma cells have a high density of β_1-adrenoceptors which are positively coupled to adenylate cyclase and have proven to be a useful *in vitro* model for the investigation of β-adrenoceptor down regulation (Fishman *et al.*, 1981). We have investigated the effect of culturing C_6 glioma cells in the presence of iprindole, a tricyclic antidepressant drug which is similar in clinical potency and efficacy to desipramine but is a weak inhibitor of noradrenaline re-uptake and has no effect on monoamine oxidase activity in rat brain.

Rat C_6 glioma cells were cultured in Dulbecco's modified eagles medium (DMEM) containing 2 mM glutamine and 10% foetal calf serum and maintained at 37°C in a water saturated atmosphere of air: CO_2 (90:10). Cells were exposed to either vehicle or drug (1-10 µM iprindole or 5 µM desipramine) for a period of 28 days. Cells were then plated into either 6 well cluster dishes (binding studies using the hydrophilic β-antagonist [^{3}H] CGP-12177) or 24 well cluster dishes (cAMP accumulation studies using ^{3}H-adenine uptake and sequential Dowex-alumina chromatography (Ruck *et al.*, 1990)).

Rat C_6 glioma cells exposed to the tricyclic antidepressant iprindole showed a dose dependent decrease in isoprenaline-stimulated cyclic AMP accumulation and loss of surface β-adrenoceptors. The maximum isoprenaline stimulated cyclic AMP accumulation in cells treated with 8 µM iprindole was 70 ± 5% of the maximum control cell isoprenaline response (n=3). The cyclic AMP response induced by forskolin was not significantly changed by iprindole treatment. In addition, the EC_{50} (isoprenaline) for control and iprindole treated cells differed by a factor of 10 (9.4 ± 2.4 nM control, n=3; 94.4 ± 33.3 nM iprindole treated, n=3). Cells exposed to 8 µM iprindole for 28 days exhibited a reduction in surface β-adrenoceptors of 67 ± 6% (n=3) when compared with control cells. There was no significant change in the K_D value between control and iprindole treated cells.

In conclusion, it would seem that iprindole, like desipramine, is capable of down regulating β-adrenoceptors in C_6 glioma cells by a mechanism(s) which does not rely upon modulation of noradrenergic neurotransmission.

We are grateful to the MRC for financial support.

References

Fishman, P H, Mallorga, P & Tallman, J F (1981) *Mol. Pharmacol.*, **20**, 310-318.
Pandey, G N & Davis, J M (1983) *Drug Dev. Res.*, **3**, 393-406.
Ruck, A, Millns, P J, Kendall, D A & Hill, S J (1990) *Biochem. Pharmacol.*, **40**, 2371-2375.

11P GABA$_B$ RECEPTOR HETEROGENEITY IN RAT CEREBRAL CORTEX

G. Bonanno & M. Raiteri, Istituto di Farmacologia e Farmacognosia, Viale Cembrano 4,16148 Genova, Italy.

Three GABA$_B$ receptors mediating inhibition of the release of a) endogenous GABA, b) endogenous glutamate, or c) somatostatin-like immunoreactivity (SRIF-LI) have been studied to explore the existence of subtypes of the GABA$_B$ receptor. The experimental set up consisted of rat cerebrocortical synaptosomes depolarized in superfusion with 12 or 15 mM KCl. GABA and glutamate were measured by high performance liquid chromatography and SRIF-LI by radioimmunoassay.

The selective GABA$_B$ receptor agonist (-)-baclofen inhibited in a concentration-dependent manner the K^+-evoked release of GABA (EC_{50}: 1.1 ± 0.1 μM), glutamate (EC_{50}: 1.5 ± 0.1 μM) and SRIF-LI (EC_{50}: 1.3 ± 0.1 μM) with a maximal effect around 45 - 50%. Neither (+)-baclofen nor muscimol mimicked (-)-baclofen.

The GABA$_B$ receptor antagonist phaclofen concentration-dependently reduced the inhibitory effect of 10 μM (-)-baclofen on the release of GABA and SRIF-LI while it was ineffective on the release of glutamate up to 1000 μM. A different pattern was displayed by the GABA$_B$ receptor antagonist 3-amino-propyl(diethoxymethyl)phosphinic acid (CGP 35348). The drug antagonized 10 μM (-)-baclofen on the release of glutamate and SRIF-LI but not on that of GABA. Table 1 reports the IC_{50} values for the two antagonists.

Table 1

	IC_{50} values (μM)		
	GABA release	Glutamate release	SRIF-LI release
Phaclofen	79.18 ± 9.34	> 1000	62.56 ± 7.92
CGP 35348	> 1000	4.15 ± 0.78	3.56 ± 0.71

These preliminary results suggest the existence of different subtypes of GABA$_B$ receptors in the rat cerebral cortex.

This work was supported by grants from the Italian M.U.R.S.T. and from the Italian C.N.R. (Target Project on Biotechnology and Bioinstrumentation).

12P ANTAGONISM OF THE STEROID RECOGNITION SITE OF THE GABA$_A$ RECEPTOR BY EPIPREGNANOLONE

R.J.Prince & M.A.Simmonds, Department of Pharmacology, The School of Pharmacy, University of London, 29-39, Brunswick Square, London WC1N 1AX (U.K.)

Certain pregnane steroids such as pregnanolone (5β-pregnan-3α-ol-20-one) have been shown to be potent potentiators of responses to GABA at GABA$_A$ receptors (Turner & Simmonds, 1989). This phenomenon has a distinct structure activity relationship with a 3α-OH group being required for activity and a 20-one moiety being favoured over 20-ol. Another class of steroids, the 3β-sulphate esters of pregnenolone (PS) and dehydroepiandrosterone (DHEAS) appear to have mixed agonist/antagonist interactions, directly enhancing flunitrazepam (FNZ) binding whilst inhibiting the potentiation of FNZ binding by barbiturates and inhibiting muscimol stimulated chloride flux (Majewska et al, 1990). Recently it has been shown in our laboratory that the 3β-isomer of pregnanolone, epipregnanolone (EP) is a potent antagonist of the potentiation of GABA responses by pregnanolone (Pettey & Simmonds, 1991) We have now investigated the site and mode of this interaction.

The binding of 1nM [^{3}H] FNZ to crude rat brain membranes was shown to be robustly potentiated by GABA (EC_{50} 0.34 ±0.1μM (3)), pentobarbitone (EC_{50} 0.41 ±0.4mM (8)) and pregnanolone (EC_{50} 237 ±27nM (10)). When EP (10-60μM) was co-applied, the dose response curve for pregnanolone was shifted to the right in a parallel fashion, indicative of competitive antagonism, with a Ki of 10.5μM. EP alone, produced a small but significant increase in FNZ binding (105.6±2.41 (9) $P<0.05$) only at the highest concentration used. The presence of 60μM EP did not significantly alter the dose response curve for GABA. When 60μM EP was co-applied with pentobarbitone, the slope of the pentobarbitone dose response curve decreased significantly from 2.18 ±0.16 (8) to 1.27 ±0.21 (4) ($P<0.01$) and there was also an apparent shift in the EC_{50} to 0.29 ±0.027mM. ($0.1<P<0.05$).

These results indicate that EP is a specific and probably isosteric antagonist of the GABA$_A$ receptor pregnane steroid recognition site. The change in slope of the pentobarbitone dose response curve is probably due to weak partial agonist activity of the steroid, lowering the threshold for the barbiturate. The lack of antagonism of pentobarbitone and GABA suggests that EP acts at a site different from that of the 3β-sulphate esters.

Turner, J.P. & Simmonds, M.A. (1989) Br. J. Pharmacol. 96, 409-417
Majewska, M.D., Demirgören, S., Spivak, C.E. & London, E.D. (1990) Brain Res. 526, 143-146
Pettey, C.J. & Simmonds, M.A. (1991) Fund. Clin. Pharmacol. 5, 384

13P INTERACTIONS OF CHLORMETHIAZOLE WITH THE GABA RECEPTOR IN IMR-32 HUMAN NEUROBLASTOMA CELLS

S M P Anderson, R J De Souza and A J Cross, Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ.

We have reported previously that the IMR 32 human neuroblastoma cell line possesses a functional $GABA_A$ receptor (Anderson et al, 1991). The chloride channel of this receptor can be identified in radioligand binding studies using [^{35}S]-TBPS (t-butylbicyclo-phosphorothionate) or by measuring chloride efflux. We have investigated the actions of chlormethiazole at this $GABA_A$ receptor using both ligand binding and chloride efflux measurements.

IMR 32 cells were maintained in culture with minimum essential Eagle's medium supplemented with the following: 10% heat-inactivated foetal calf serum, 1% L-glutamine, 1% non-esssential amino acids and 50$\mu g ml^{-1}$ gentamicin. [^{35}S]-TBPS binding was carried out on freshly prepared membranes according to the method of Cross et al (1989). Chloride flux was measured using changes in quenching of the fluorescent dye 6-methoxy-N-sulphonopropyl quinolinium (SPQ) by intracellular chloride ions. 10mM SPQ and 150 mM Cl^- were loaded into IMR 32 cells for 60 min at 37°C. The cells were transferred to low chloride buffer containing furosemide (1mM) and DIDS (10μM). Chloride efflux increased fluorescence and this was expressed as a percentage of that induced by 100μM GABA.

Table 1 Effects of chlormethiazole and pentobarbital on GABA receptors in IMR 32 cells

	Chloride efflux (% GABA stimulated)		[^{35}S]-TBPS binding $IC_{50}\mu M$
	alone	+10μM PTX	
Chlormethiazole (100μM)	67 ± 5	20 ± 3	97 ± 4
Pentobarbital (100μM)	39 ± 2	10 ± 1	255 ± 29*

Values are mean ± s.e. mean, n=4-12. *data from Anderson et al (1991).

Chlormethiazole at concentrations up to 100μM increased chloride efflux and this was reduced by picrotoxin (PTX: 10μM) and pentobarbital (100μM) also stimulated chloride efflux in a picrotoxinin-sensitive manner (Table 1). Chlormethiazole totally displaced [^{35}S]-TBPS binding to the $GABA_A$ receptor in IMR 32 cell membranes, and was more potent than pentobarbital (Table 1).

We conclude that IMR 32 cells express a $GABA_A$ receptor-chloride channel which can be modulated by the sedative/anticonvulsant drug chlormethiazole, and that pentobarbital and chlormethiazole interact differently with this receptor.

Anderson, S.M.P., Cross, A.J. De Souza, R.J. and Green, A.R. (1991) Br. J. Pharmacol. (In press).
Cross, A.J., Stirling, J.M., Robinson, T.N., Bowen, D.M. and Green, A.R. (1989). Br. J. Pharmacol. 96: 284-290.

14P ETHANOL WITHDRAWAL HYPEREXCITABILITY IN ISOLATED HIPPOCAMPAL SLICES IS NOT DUE TO DECREASES IN GABA-MEDIATED INHIBITION

M. A. Whittington[1], J. D. C. Lambert[2] and H. J. Little[1] (1) Department of Pharmacology, The Medical School, University Walk, Bristol BS81TD and (2) Institute of Physiology, Aarhus University, DK 8000, Aarhus C, Denmark.

The physiological basis of the hyperexcitability seen on cessation of long-term ethanol intake is not yet understood, but it is often suggested to be due to decreased GABA inhibition. Patterns of hyperexcitability in isolated hippocampal slices, after chronic ethanol treatment in vivo, showed different time courses (Whittington and Little, 1990; 1991). An increase in paired pulse potentiation of the population spike reached maximum 2h after ethanol withdrawal, returning to control values 4h from withdrawal. It was followed by decreases in the thresholds for elicitation of single and multiple population spikes, reaching maximum at about 7h from withdrawal. Drugs that prevent the behavioural syndrome decreased these changes. We now investigate the underlying causes.

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, 1990; 1991). They were perfused with Kreb's solution, in the absence of ethanol. Intracellular recordings were made from pyramidal cells in area CA1. Monosynaptic inhibitory postsynaptic potentials (IPSPs) were evoked by stimulation of the stratum radiatum, close to the recording site. The fast component of the IPSP was isolated by including APV, 50 µM, and CNQX, 10 µM, in the perfusing medium (to block synaptic excitation of pyramidal cells and interneurones), and QX314, 50 mM (to block the $GABA_B$ slow-IPSP, Nathan et al., 1990) in the recording electrodes; the electrodes were made with potassium acetate. All times are from the beginning of slice preparation. Comparisons were made by Student's t-test (n=7-8 slices per group, each from a different mouse).

At 2h into withdrawal, the amplitude of the fast (GABA-mediated) IPSP was significantly increased ($P < 0.05$), at high stimulus intensities (control maximum 10 ± 1; ethanol withdrawal 14 ± 2 mV, membrane potential held at -50mV). Duration and time to peak were unaffected. The reversal potential of the fast IPSP was shifted to more negative membrane potentials at this time (control -69 ± 1; ethanol withdrawal -75 ± 2 mV, $P < 0.05$). At later times (4h and 6h into withdrawal) there were no significant differences in the stimulus response curves or the reversal potential. Paired pulse depression of the fast IPSP was significantly increased at 2h into withdrawal, when a 150 msec interpulse interval was used, but the difference was not significant when the interval was 70 msec (the time used in our earlier field potential studies, Whittington and Little, 1990; 1991)

It is possible that the increase in the fast IPSP was due to residual ethanol; the concentrations will be measured. The results indicate that the epileptiform activity seen between 4h and 7h into withdrawal in our earlier studies could not be accounted for by decreases in GABA-mediated inhibition, although changes in the fast IPSP may contribute to the increase in the paired pulse potentiation of the population spike seen earlier in the withdrawal period.

Nathan, T., Jensen, M. S. and Lambert, J. D. C. (1990) Neurosci. Lett., 110, 309-313
Whittington, M. A. & Little, H. J. (1990) Brain Res., 523, 237-244
Whittington, M. A. & Little, H. J. (1991) Br. J. Pharmacol., 103, 1313-1320

15P ACTIONS OF FOUR ENDOGENOUS EXCITATORY AMINO ACIDS ON NMDA RECEPTORS OF RAT VENTROBASAL THALAMIC NEURONES *IN VIVO*

S.A. Eaton & T.E. Salt, Department of Visual Science, Institute of Ophthalmology, Judd Street, London WC1H 9QS, UK.

Excitatory amino acid (EAA) receptors of both the NMDA and non-NMDA types mediate sensory synaptic input to the ventrobasal thalamus (vbt) (Salt and Eaton, 1991), however, the identity of the transmitter is unknown. Since L-glutamate (L-Glu), L-aspartate (L-Asp), L-Homocysteate (L-HCA) and L-cysteine sulphinate (L-CSA) are likely EAA transmitter candidates, we have tested the pharmacology of their excitatory actions on vbt neurones.

In urethane-anaesthetised rats, multibarrel glass electrodes were used to record from 68 vbt neurones and to iontophoretically apply drugs. Reproducible responses to EAAs were challenged with the NMDA receptor antagonist, CPP (3((±)-2-carboxypiperazin-4-yl)propyl-1-phosphonate), and/or the non-NMDA receptor antagonist, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione).

At ejection currents which antagonised responses to NMA (N-methyl-D,L-aspartate) or NMDA (table 1), but not responses to AMPA ((RS)-α-amino-3-hydroxy-5-isoxazolepropionate) (% of control = 98%, n=14), kainate (96%, n=32) or *trans*-(±)-1-aminocyclopentane-1,3-dicarboxylate (t-ACPD, 98%, n=9), CPP greatly reduced the responses to L-HCA, L-CSA, L-Asp and L-Glu (table 1) on 46 neurones. In marked contrast, CNQX had little effect against responses to NMDA or NMA, L-HCA, L-CSA, L-Asp, L-Glu (table 1) or t-ACPD (123%, n=6) at ejection currents which reduced responses to kainate (11%, n=30) and/or AMPA (10%, n=20) on 48 neurones.

Table 1. Effects of CNQX and CPP on responses to NMDA/NMA and endogenous agonists
Results are expressed as mean percentages of control responses of n neurones.

CNQX	%		%	n	CPP	%		%	n
NMDA	97	L-HCA	85	16	NMDA	8	L-HCA	6	16
NMDA	90	L-CSA	80	11	NMDA	1	L-CSA	7	9
NMA	79	L-ASP	80	11	NMA	3	L-ASP	10	11
NMA	95	L-GLU	92	18	NMA	6	L-GLU	12	15

The marked sensitivity of the iontophoretically applied endogenous agonists to CPP indicates that their action on vbt neurones is mediated predominantly via NMDA receptors.

Salt, T.E. & Eaton, S.A. (1991) *Eur. J. Neuroscience* **3**, 296-300.

16P THE EFFECT OF EXCITATORY AMINO ACID ANTAGONISTS ON RETINOGENICULATE NEUROTRANSMISSION IN THE RAT

G. Scott & R. Mason, Department of Physiology & Pharmacology, University of Nottingham Medical School, Queens Medical Centre, Nottingham NG7 2UH.

The thalamic dorsolateral geniculate nucleus (dLGN) relays visual information from the retina to the visual cortex. Visually-driven retinogeniculate transmission is mediated by excitatory amino acid (EAA) receptors in the cat (Sillito *et al*, 1990), however, the functional role of EAA receptors in visually-mediated neurotransmission in the rat dLGN *in vivo* remains unclear.

Male Lister Hooded rats (200-250g) were anaesthetised with urethane (1.3-1.5 $g.kg^{-1}$, i.p.) and seven-barreled silicone-coated micropipettes were used to record extracellular dLGN neuronal discharge activity (Scott and Mason, 1991). Visual stimuli were presented as whole-field flash stimulation (1 second duration) to the contralateral eye. Ionophoretic ejection of drugs was controlled by an electronic clock and retaining currents of ± 10nA were used between ejections with continuous automatic current balancing. The effect of ionophoresed Mg^{2+} ($MgSO_4$ 100mM; 10-80nA), DL-2-amino-5-phosphonopentanoic acid (AP5 10mM; 2-40nA), 7-chlorokynurenate (7-CK 10mM; 0-20nA) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX 10mM; 0-20nA) on NMDA-evoked (10mM; 10s ejections (20-40nA) repeated at 120s intervals) and visually-evoked activity in light-suppressed OFF cells in the dLGN was evaluated from integrated firing rate histograms. Ionophoresis of Mg^{2+} (10-80nA; 60-600s duration; n=5 cells), AP5 (2-40nA; 120s duration; n=5 cells) and 7-CK (0-20nA; 120s duration; n=3 cells) had no effect on visually-evoked activity, however, ionophoresis of Mg^{2+}, AP5 and 7-CK produced a current-dependent and reversible inhibition of the NMDA-evoked response (mean ED_{50}: Mg^{2+} 31.25 ± 3.15 nA, n=4 cells; AP5 6.5 ± 3.5nA, n=2 cells; 7-CK 2.33 ± 0.33nA, n=3 cells). Initial studies have shown that ionophoresis of CNQX in the dLGN produced a current-dependent (2-20nA) and reversible inhibition of visually-evoked firing (mean ED_{50}: 6 ± 1.0nA, n=2 cells). Ionophoresis of Mg^{2+}, AP5, 7-CK and CNQX had no effect on spike waveform.

The observation that the NMDA receptor antagonists Mg^{2+}, AP5 and 7-CK inhibited NMDA-evoked responses in the dLGN but had no effect on the visually-evoked response in OFF cells suggests that under these conditions *in vivo*, the NMDA receptor may contribute little to retinogeniculate transmission in OFF cells after physiological visual stimulation. These results are in agreement with *in vitro* studies (Crunelli *et al.*, 1987) demonstrating that D-2-amino-5-phosphonovalerate (D-APV) had no effect on the excitatory post-synaptic potential (epsp) evoked by electrical stimulation of the optic nerve, whereas τ-D-glutamylglycine, a non-specific EAA receptor antagonist, inhibited the epsp in a competitive manner, thus supporting the view that retinogeniculate transmission in the rat is mediated by non-NMDA receptors.

GS is a MRC collaborative student with Boots Pharmaceuticals.

Crunelli, V., Kelly, J.S., Leresche, N. and Pirchio, M. (1987) *J. Physiol.* **384**, 603-618
Scott, G. and Mason, R. (1991) *J. Physiol.*, in press
Sillito, A.M, Murphy, P.C., Salt, T.E. and Moody, C.I. (1990) *J.Neurophysiol.* **63**, 347-355

17P FURTHER CHARACTERIZATION OF POLYAMINE-SENSITIVE [^{3}H]-IFENPRODIL BINDING TO THE NMDA RECEPTOR

H. Schoemaker (introduced by B. Scatton), Department of Biology, Synthélabo Recherche (L.E.R.S.), 31 av. Paul Vaillant-Couturier, 92220 Bagneux, France

The cerebral anti-ischaemic effects of ifenprodil and its structural analog SL 82.0715 have been attributed to their antagonism towards the N-methyl-D-aspartate (NMDA)-sensitive subtype of glutamate receptors, mediated through a blockade of the polyamine-modulatory site of this receptor complex (Carter *et al.*, 1990). We previously reported that the use of [^{3}H]ifenprodil allows the identification of high affinity binding sites in the rat cerebral cortex that were recognized by different polyamines (Schoemaker *et al.*, 1990). The present study was undertaken to evaluate the relationship between polyamine-sensitive [^{3}H]ifenprodil binding sites and the NMDA receptor complex.

[^{3}H]Ifenprodil binding to the rat cerebral cortex, determined as described by Schoemaker *et al* (1990), is of high affinity (K_d = 37 nM ; B_{max}= 2.14 pmol/mg protein) and sensitive to inhibition by unlabelled ifenprodil (IC_{50} = 46 nM) and SL 82.0715 (IC_{50} = 128 nM). Moreover, [^{3}H]ifenprodil binding is fully and monophasically inhibited by the polyamines spermine, spermidine, arcaine and agmatine with IC_{50}'s of 8.4, 70, 72 and 510 µM, respectively. The competitive NMDA antagonist CPP produces a partial inhibition of [^{3}H]ifenprodil binding to a maximum of 28% of control values with an IC_{50} of 4.5 µM. The inhibitory effect of CPP is stereoselective and essentially due to its dextrogyre enantiomer. L-Glutamate fails to affect [^{3}H]ifenprodil binding under control conditions, but reverses the inhibition of [^{3}H]ifenprodil binding produced by CPP. A reversal of the inhibitory effects of CPP is also observed with NMDA and D-aspartate but not with glycine. Similar results were obtained with the NMDA antagonists D-AP5, CGS19755 and CGP37849.

Although glycine fails to affect [^{3}H]ifenprodil binding under control conditions, the glycine antagonist 7-chlorokynurenate enhances [^{3}H]ifenprodil binding to a maximum of 137% of controls (EC_{50} = 0.36 µM) and in a glycine-reversible manner.

The present data demonstrate that polyamine-sensitive [^{3}H]ifenprodil binding sites are subject to allosteric modulation mediated through the glutamate/NMDA recognition site and the strychnine-insensitive glycine recognition site within the NMDA receptor complex. These data provide evidence that [^{3}H]ifenprodil binding defines a novel site within the NMDA receptor complex, possibly identical to the one through which polyamines modulate NMDA receptor function.

Carter, C.J., Lloyd, K.G., Zivkovic, B. & Scatton, B. (1990) J. Pharmacol. Exp. Ther. **253**, 475-482
Schoemaker, H., Allen, J. & Langer, S.Z. (1990) Eur. J. Pharmacol. **176**, 249-250

18P GLYCINE RECEPTOR STATUS DETERMINES THE EFFECTS OF IFENPRODIL AND SPERMIDINE ON [^{3}H]-TCP BINDING TO THE NMDA RECEPTOR

C. Carter, C. Minisclou and J.P. Rivy. Department of Biology, Synthelabo Recherche (LERS), 31 Avenue Paul Vaillant-Couturier, 92220 Bagneux, France.
(introduced by S. O'Connor)

The NMDA receptor, in addition to its agonist site responsive to glutamate, possesses additional modulatory sites sensitive to glycine and the polyamines (e.g. spermidine). Antagonists at these two modulatory sites include 7-Cl,kynurenate (Kemp et al, 1989) and ifenprodil (Carter et al, 1989) respectively. We have attempted to analyse the interactions between the polyamine and glycine sites using [^{3}H]TCP binding as an index of NMDA channel activation.

[^{3}H]TCP binding to rat forebrain membranes was studied using 2.5 nM [^{3}H]TCP (44 Ci/mmol, NEN) and an incubation time of 1 hr at 25°C with a final protein concentration of 0.5 mg/ml in 5 mM Tris. Hcl Buffer (pH 7.7). Non-specific binding was defined with 100 µM TCP.

The stimulatory effects of spermidine on [^{3}H]TCP binding showed an absolute dependence on the presence of glutamate and were abolished by the competitive NMDA antagonist CGP 37849 (10 µM). High concentrations of 7-Cl, kynurenate (> 10 µM) also abolished the stimulatory effects of spermidine. However, low concentrations of 7-Cl, kynurenate sufficient to abolish the effects of glutamate in the absence of spermidine, did not reduce the effects of combinations of glutamate (10 µM) + spermidine (0.1 - 1000 µM). In fact 7-Cl,kynurenate increased the maximal effects of spermidine at low concentrations (0.1 - 10 µM) in a concentration dependent manner. Ifenprodil displaced [^{3}H]TCP in a biphasic fashion (IC_{50} = 0.11 and 78 µM). The high and low affinity IC_{50}'s of ifenprodil were not influenced by glycine or 7, Cl-Kynurenate but glycine reduced and 7-Cl, kynurenate increased the proportion of [^{3}H] TCP displaced with high affinity by ifenprodil. As high concentrations of 7-Cl, kynurenate are able to totally inhibit the effects of spermidine (and glutamate, (Kemp et al, 1989)), glycine receptor activation seems ultimately an absolute prerequisite for NMDA receptor stimulation by glutamate or spermidine, as widely supposed. However, the potentiating effects at low concentrations of 7-Cl,kynurenate on the stimulatory effects of spermidine and on the inhibitory effects of ifenprodil also suggests that glycine will in fact indirectly reduce the effects of the polyamines and of their antagonists.

One explanation for these latter effects could lie in the fact that spermidine increases the affinity of the glycine site specifically for agonists (Sacaan and Johnson, 1990). Thus, provided the glycine site is occupied but not saturated, the polyamines can potentiate the effects of NMDA, effectively by increasing the potency of glycine. If the glycine site were already saturated it would perhaps be difficult to demonstrate a further potentiating effect of the polyamines or an inhibitory effect of their antagonists on NMDA receptor-mediated responses. In support of this hypothesis we have shown that ifenprodil, which has little effect on NMDA responses on the hemisected rat spinal cord under normal conditions (Carter et al, 1989), blocks the effects of NMDA in the presence of sub-maximal concentrations of 7,Cl-kynurenate. The effects of polyamine antagonists in functional models will thus depend on the tonicity of both the glycine and polyamine systems in the brain.

Carter, C., Rivy, J.P. and Scatton, B. (1989) Eur.J.Pharmacol. 164, 611-612.
Kemp, J.A., Foster, A.C., Leeson, P.D. et al (1988). Proc.Natl.Acad.sci. 85, 6547-6550.
Sacaan, A.I. and Johnson, K.M. (1989). Mol.Pharmacol. 36, 836-839.

19P SOME PHARMACOLOGICAL PROPERTIES OF NON-NMDA RECEPTORS IN RAT SYMPATHETIC PREGANGLIONIC NEURONES *IN VITRO*

D Spanswick, A E Pickering, I C Gibson* and S D Logan. Department of Physiology, University of Birmingham Medical School, B15 2TT.

Sympathetic preganglionic neurones (SPN) are involved in cardiovascular control and are considered the final site of integration of sympathetic activity. We have previously demonstrated that excitatory amino acids (EAA) acting via N-methyl-D-aspartate (NMDA) and non-NMDA subtypes of receptor are involved in synaptic transmission in these neurones (Spanswick & Logan, 1991). The classification of EAA receptor subtypes has recently been expanded to include the metabotropic receptor for which quisqualate and (1S, 3R)-1-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD) are ligands. We have utilised the in vitro spinal cord slice preparation to investigate the effects of selective non-NMDA receptor agonists on SPN.

Intracellular and whole-cell recordings were made from SPN in neonate rat thoracolumbar spinal cord slices as described by us previously (Spanswick & Logan, 1990; Pickering et al, 1991). The selective EAA receptor agonists α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate and quisqualate (500nM-20μM) superfused for 1s to 3 mins induced concentration-dependent depolarisations in all neurones tested. Superfused trans-ACPD (10-200μM) for 2-60s also induced concentration-dependent depolarising responses in SPN. The order of potency of these agonists in SPN was quisqualate $\geq$ AMPA > kainate > trans-ACPD. The selective non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (5-40μM) blocked responses to both AMPA and kainate but only partially blocked responses to quisqualate. Depolarising responses to trans-ACPD were unaffected or enhanced in the presence of CNQX and the selective NMDA receptor antagonist 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (R-CPP). Responses to quisqualate and trans-ACPD were unaffected by DL- or L-2-amino-3-phosphonopropionic acid (AP3) or L-2-amino-4-phosphonobutyric acid (AP4) (30μM-1mM). At concentrations in excess of 100μM, L-AP3 and L-AP4 had depolarising effects on some SPN.

These results suggest that SPN possess AMPA and metabotropic subtypes of EAA receptors. In these neurones CNQX does not discriminate between AMPA and kainate receptors. In our hands L-AP3 and LAP-4 are limited in their usefulness as antagonists at the metabotropic receptor.

This work is supported by the MRC and British Heart Foundation. AEP is in receipt of a Wellcome Prize Studentship.

Pickering A E, Spanswick D and Logan S D (1991). Neurosci lett, 130(2): 237-242
Spanswick D and Logan S D (1991). J Physiol, Lond, 435: 37P.
Spanswick D and Logan S D (1990). Brain Res, 525: 181-188.

20P CHLORMETHIAZOLE INHIBITS THE EFFECTS OF N-METHYL-DL-ASPARTATE IN VIVO BUT DOES NOT INHIBIT NMDA RECEPTOR BINDING

A J Cross, A Misra, M F Snape, T K Murray and A R Green, Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ

Chlormethiazole (CMZ) prevents degeneration of hippocampal neurones following transient ischaemia in the gerbil (Cross et al, 1991), and reduces the neurotoxicity of methamphetamine (Green et al, 1991). Antagonists of excitatory amino acids, particularly N-methyl-D-aspartate (NMDA), are also potent neuroprotective agents (Meldrum, 1990). We have therefore examined the effect of CMZ on NMDLA-induced seizures and on [^{3}H]-dizocilpine binding to the NMDA receptor in rat cortical membranes.

Male TO mice (20-30g) were treated with experimental drugs (injected i.p. in isotonic saline) 15 min prior to NMDLA (500mgkg^{-1} i.p.) and observed for a further 15 min. The latency and frequency of tonic seizures were recorded . [^{3}H]-dizocilpine binding to extensively washed rat cortical membranes was performed as described by Robinson et al (1990), using glutamate (10 μM), glycine (10 μM) and spermidine (100 μM) to stimulate binding.

Table 1 Inhibition of NMDLA seizures and NMDA receptor binding by chlormethiazole and dizocilpine.

	NMDLA seizures ED_{50}(mgkg^{-1})	[^{3}H]-dizocilpine binding pIC_{50} (M)	
		Basal	Stimulated
Chlormethiazole	34 ± 8	<3	<3
Dizocilpine	0.2[a]	9.2 ± 0.4	9.0 ± 0.2

Values are mean ± s.e. mean of at least 3 experiments, [a] mean of 2 experiments

Chlormethiazole was an effective inhibitor of NMDLA induced seizures (Table 1), and completely inhibited seizures at doses (100mgkg^{-1}) previously shown to be neuroprotective. Chlormethiazole (1mM) did not inhibit [^{3}H]-dizocilpine binding to the NMDA receptor under basal or stimulatory conditions (Table 1).

The present data suggest that the inhibition of NMDLA seizures following chlormethiazole treatment is unlikely to be due to a direct NMDA receptor antagonism.

Cross, A.J., Jones, J.A., Baldwin, H.A. & Green, A.R. (1991) Br. J. Pharmacol. 104: 406-411.
Green, A.R., De Souza, R.J., Williams, J.L., Murray, T.K., Cross, A.J. & Jonsson, G. (1991) Br. J. Pharmacol. (In press).
Meldrum, B. (1990) Cardiovasc. Brain Metab. Rev. 2: 27-57.
Robinson, T.N., Robertson, C., Cross, A.J. & Green, A.R. (1990) Mol. Neuropharmacol. 1: 31-36.

21P CEREBROPROTECTIVE EFFECTS OF GYKI 52466 (1-(4-AMINOPHENYL-4-METHYL-7,8-METHYLEN-DIOXY-5H-2,3-BENZODIAZEPINE) AFTER FOCAL ISCHAEMIA IN THE RAT

S.E. Smith & B.S. Meldrum, Institute of Psychiatry, Department of Neurology, De Crespigny Park, Denmark Hill, London SE5 8AF. U.K.

GYKI 52466 (GY) is an anticonvulsant with appreciable non-NMDA antagonist activity (Tarnawa *et al.*, 1990; Smith *et al.*, 1991). We performed proximal left middle cerebral artery occlusion (with intact zygoma) using bipolar coagulation in anaesthetised (2% halothane in 70% N_2O & 30% O_2) male Fischer rats with core temperature maintained between 36-38 ^oC. Animals received either: A) 3 doses of GY (10 mg kg^{-1}, i.p.) or vehicle (veh, 5 ml kg^{-1}) one each at 0, 2 and 4 h post-occlusion, B) 10 mg kg^{-1} of GY or veh over 5 min then 15 mg kg^{-1} h^{-1} for 2 h of GY or veh (blind), C) as B) with a 1h delay before treatment, D) as B) with a 2h delay before treatment, or E) 1.5 mg kg^{-1} of MK801 (MK 1.5, i.p.) or veh (1 ml kg^{-1}) at 30 min post-occlusion. 24 h later a graded neurological test was performed blind: 0 normal, 1 forelimb flexion on lifting, 2 loss of resistance to lateral push, 3 circling. After decapitation, ten 1 mm thick serial brain sections were cut and incubated in 2 % w/v 2,3,5-triphenyl-tetrazolium chloride in saline at 37 ^oC (Non-infarcted tissue becomes red) then stored in 0.1 M phosphate (pH=7.4) 5 % formaldehyde in saline. The sections were photographed and areas of infarction were estimated from the developed film using an image analyser. Infarct volumes were calculated based on ends of spheres and truncated cones integrations. Results are shown in table 1. * $P<0.05$ with control (n=6-13), Student's unpaired *t* test. Neurological deficit was improved in groups treated with GY from 0 to 2h after ischaemia.

TABLE 1 Uncorrected lesion (mm^3)

	A) GY		B) GY Inf		C) GY +1h		D) GY +2h		E) MK 1.5	
	Veh	Drug	Veh	Drug	Veh	Drug	Veh	Drug	Veh	Drug
Cortical	67	61	69	22*	60	31*	41	44	71	21*
Noncortical	74	61	82	49	77	64	59	60	72	41

A role for non-NMDA receptors in the infarction process is suggested.

Tarnawa, I., Engberg, I. & Flatman, J.A. (1990) in Amino acids: Chemistry, Biology & Medicine. eds. Lubec, G. & Rosenthal, G.A. pp 538-546. The Netherlands: ESCOM.
Smith, S.E., Dürmüller, N. & Meldrum, B.S. (1991) Eur. J. Pharmacol. 201, 179-183.

22P EXCITATORY AMINO ACID-STIMULATED ACCUMULATION OF INOSITOL 1,4,5-TRISPHOSPHATE MASS IN GUINEA-PIG CEREBRAL CORTEX IS RAPID AND TRANSIENT

S.P.H. Alexander, Department of Physiology & Pharmacology, University of Nottingham, Nottingham NG7 2UH, UNITED KINGDOM.

Phosphoinositidase C activated by certain hormones and neurotransmitters accelerates the hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate leading to the production of inositol 1,4,5-trisphosphate (1,4,5-$InsP_3$) and diacylglycerol. Recently an assay was established to measure 1,4,5-$InsP_3$ mass utilising competition with [^{3}H]1,4,5-$InsP_3$ binding to a binding protein from bovine adrenal cortex (Challiss *et al.*, 1989). The present report describes the use of this assay to follow the time course of 1,4,5-$InsP_3$ generation induced by L-glutamate and a rigid analogue, DL-1-aminocyclopentane-*trans*-1,3-dicarboxylate (ACPD), in guinea pig cerebral cortical slices.

After pre-equilibration with Krebs-Henseleit medium, guinea pig cerebral cortical slices (350 x 350 μm) were maintained in suspension by light stirring with a magnetic stirrer at 37°C in Krebs Henseleit medium undergoing continuous gassing (O_2:CO_2 95:5). Experiments were carried out in triplicate, on at least three separate occasions. At intervals 300 μL aliquots of the suspension were removed and terminated with perchloric acid. 1,4,5-$InsP_3$ levels in the neutralised supernatant were measured by minor modifications of the radioreceptor methodology of Challiss *et al.* (1989). Protein in the NaOH-digested pellet was measured by the method of Bradford (1978).

Basal levels of 1,4,5-$InsP_3$ in guinea pig cerebral cortical slices were 16.8 ± 2.2 pmol/mg protein (n=11, 6-9 replicates). Addition of L-glutamate (10 mM) elevated 1,4,5-$InsP_3$ levels to 50.2 ± 9.6 pmol/mg (n=4, $P<0.01$) at 20 seconds, subsiding to baseline levels by 150 seconds. Addition of ACPD (100 μM) resulted in a smaller generation of 1,4,5-$InsP_3$ accumulation (26.7 ± 1.3 pmol/mg, n = 4, $P<0.01$) also peaking at 20 seconds but declining more rapidly to baseline levels by 60 seconds. Muscarinic receptor stimulation with carbachol (1 mM) was also observed to elicit rapid, transient accumulations of 1,4,5-$InsP_3$ (peak 30 seconds; 29.5 ± 2.8 pmol/mg, $P<0.01$) falling to baseline levels by 60 seconds.

In summary therefore, activation of both "metabotropic" excitatory amino acid receptors with L-glutamate or ACPD, or muscarinic receptors with carbachol evokes a rapid, transient increase in guinea pig cerebral cortical 1,4,5-$InsP_3$ levels. This contrasts with a study in rat cerebral cortical slices in which 1,4,5-$InsP_3$ levels stimulated by a variety of receptor types was observed at an elevated plateau for up to 300 seconds (Challiss *et al.*, 1989).

The finanical support of the Wellcome Trust is gratefully acknowledged.

Alexander, SPH; Hill, SJ; Kendall, DA (1990) J. Neurochem. 56: 1439-1441
Bradford, MM (1978) Anal. Biochem. 72: 248-254
Challiss, RAJ; Batty I; Nahorski, SR (1989) Biochim. Biophys. Res. Commun. 157: 684-691

23P DIFFERENT PATTERNS OF Ca^{2+}-DEPENDENCE OF HISTAMINE-INDUCED INOSITOL PHOSPHATE FORMATION IN HeLa CELLS AND RAT BRAIN

J.A. Arias-Montaño & J.M. Young, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ.

HeLa cells possess histamine H_1-receptors coupled to phosphoinositide hydrolysis, as indicated by the formation of [^{3}H]-inositol phosphates ([^{3}H]-IP) in [^{3}H]-inositol prelabelled cells. (Tilly *et al.*, 1990; Bristow *et al.*, 1991). However, whether the mechanism of histamine-induced inositol phosphate formation in HeLa cells is a good model of that in mammalian brain remains to be established. A notable characteristic of the response to histamine in brain is the strong dependence on the extracellular Ca^{2+} concentration (Kendall & Nahorski, 1984; Alexander *et al.*, 1990). In this respect the response to histamine differs from that to carbachol, which is unaffected by an increase of extracellular Ca^{2+} from 0.3 to 4.0 mM (Alexander *et al.*, 1990). We have therefore compared the Ca^{2+}-dependence of [^{3}H]-IP formation induced by histamine and carbachol in HeLa cells.

HeLa cells were grown in DMEM supplemented with 10% bovine calf serum, penicillin and streptomycin. For the last 20 h of growth the cells were labelled with [^{3}H]-inositol in inositol-free DMEM containing 10 μM inositol. The cells were washed twice with Krebs-Henseleit medium from which Ca^{2+} had been omitted and incubated for 30 min with 30 mM Li^+, 1 mM histamine or 1 mM carbachol and 0 - 4.0 mM Ca^{2+}. Incubations were terminated by addition of 10% perchloric acid and [^{3}H]-inositol phosphates separated by anion-exchange chromatography.

Increasing the Ca^{2+} added to the medium from 0 to 4 mM had little effect on the basal accumulation of [^{3}H]-IP_1, [^{3}H]-IP_2 or [^{3}H]-IP_3. The amounts of [^{3}H]-IP_2 or [^{3}H]-IP_3 present after 30 min incubation with 1 mM histamine in the presence of 2.5 mM Ca^{2+} were small, 107 ± 1 and 129 ± 1% of basal, respectively, and even when added together were a small proportion of total [^{3}H]-IP (8.4 ± 1.0%, 16 measurements). These higher phosphates were similarly a small proportion of total [^{3}H]-IP stimulated by 1 mM carbachol (6.8 ± 1.1%, 3 measurements). The accumulation of [^{3}H]-IP_1 in response to 1 mM histamine was increased by 2.5 ± 0.4 fold (5 measurements) when Ca^{2+} was increased from 0 (nominal) to 0.3 mM. However, further increasing the Ca^{2+} concentration up to 4 mM caused no further increase in the amount of [^{3}H]-IP_1. A closely similar pattern of Ca^{2+} dependence was observed with 1 mM carbachol-induced [^{3}H]-IP_1 accumulation (5 experiments).

The pattern of calcium dependence of [^{3}H]-IP accumulation observed with both histamine and carbachol in HeLa cells is thus similar to that for carbachol in mouse and rat brain, but is markedly different from that reported for histamine (Alexander *et al.*, 1990). The similarity in the response to the two agonists in HeLa cells suggests that there is no fundamental difference in the interaction of histamine H_1- and muscarinic receptors with G_q/phospholipase C. Histamine-induced [^{3}H]-IP formation in mammalian brain apparently involves an additional step dependent on the entry of extracellular Ca^{2+}.

Alexander, S.P., Hill, S.J. & Kendall, D.A. (1990) *Biochem. Pharmacol.* **40**, 1793-1799.
Bristow, D.R., Arias-Montaño, J.A. & Young, J.M. (1991) *Br. J. Pharmacol.* in press.
Kendall, D.A. & Nahorski, S.R. (1984) *J. Neurochem.* **42**, 1388-1394.
Tilly, B.C., Tertoolen, L.G.J., Lambrechts, A.C., Remorie, R., de Laat, S.W. & Moolenaar, W.H. (1990) *Biochem. J.* **266**, 235-243.

24P INHIBITION OF NORADRENALINE-STIMULATED INOSITOL POLYPHOSPHATE ACCUMULATION BY LITHIUM IN DEPOLARISED RAT CEREBRAL CORTEX SLICES

R.A. John Challiss , Neela Patel & Stefan R. Nahorski, Department of Pharmacology & Therapeutics, University of Leicester, Leicester LE1 9HN, U.K.

Lithium exerts profound effects on the phosphoinositide cycle in cerebral cortex (Nahorski et al., 1991). In addition to its potentiating action on agonist-stimulated inositol monophosphate accumulation, lithium also increases CMP-phosphatidate and causes a time-dependent decrease in inositol 1,4,5-trisphosphate ($InsP_3$) and inositol 1,3,4,5-tetrakisphosphate ($InsP_4$) mass (Kennedy et al., 1990). To date, these complex actions of lithium have only been demonstrated for muscarinic-cholinoceptor agonist stimulation. We have recently reported that whereas noradrenaline stimulation does not significantly increase $InsP_3$ or $InsP_4$ accumulations in cerebral cortex, this agonist does produce significant increases in $InsP_4$ accumulation in the presence of depolarizing concentrations of K^+ (Challiss & Nahorski, 1991). We have now examined the effects of lithium on noradrenaline-stimulated $InsP_3$ and $InsP_4$ accumulations in depolarized slices.

Rat cerebral cortex slices (0.35 x 0.35 mm) were prepared and preincubated as described previously (Kennedy et al., 1990). Packed slices (50 μl) were incubated in 300 μl Krebs-Henseleit buffer equilibrated with O_2:CO_2 (19:1) for 60 min. For time-course studies, LiCl was added, to a final concentration of 1 mM, 10 min prior to simultaneous additions of noradrenaline (100 μM) and/or KCl (final concentration 30 mM). Incubations were terminated by addition of trichloroacetic acid (0.5 M) and mass levels of $InsP_3$ and $InsP_4$ (expressed as pmol/mg of protein) determined as described previously (Kennedy et al., 1990). Results are means ± s.e.mean for (n) experiments performed in triplicate.

Elevation of K^+ per se for 5 min caused a significant increase in both $InsP_3$ (basal: 20.2 ± 0.4 (5); $+K^+$: 32.3 ± 0.6 (5)) and $InsP_4$ (basal: 2.77 ± 0.21 (4); $+K^+$: 12.4 ± 1.5 (3)). At this time-point, these values were further increased by simultaneous addition of noradrenaline ($InsP_3$: 38.2 ± 0.9 (5); $InsP_4$: 38.0 ± 2.9 (4)). Addition of lithium prior to agonist-challenge and depolarization had no effect on the observed initial increases in $InsP_3$ and $InsP_4$ accumulation; however, during longer periods of exposure to noradrenaline and depolarization (15-20 min), significant decreases in the concentrations of these inositol polyphosphates were observed in the presence of 1 mM LiCl. Thus after 20 min, both $InsP_3$ (-lithium: 34.4 ± 0.6 (6); +lithium: 28.8 ± 0.7 (6); $p<0.01$) and $InsP_4$ (-lithium: 26.8 ± 1.4 (4); +lithium: 16.1 ± 0.9 (4); $p<0.001$) accumulations were dramatically affected by the presence of lithium.

These data clearly demonstrate that therapeutically relevant concentrations of lithium affect noradrenaline-stimulated phosphoinositide metabolism in depolarized cerebral cortex slices.

We thank the Wellcome Trust for financial support.

Challiss, R.A.J. & Nahorski, S.R. (1991) J. Neurochem. 57, 1042-1051.
Kennedy, E.D., Challiss, R.A.J., Ragan, C.I. & Nahorski, S.R. (1990) Biochem. J. 267, 781-786.
Nahorski, S.R., Ragan, C.I. & Challisss, R.A.J. (1991) Trends Pharmacol. Sci. 12, 297-303.

25P COUPLING OF PROSTACYCLIN (IP) RECEPTORS TO G_s DURING IP RECEPTOR DESENSITIZATION

A Krane & M Keen, Department of Pharmacology, University of Birmingham, Birmingham B15 2TT, UK.

We have previously shown that pretreatment of NG108-15 or NCB-20 cells with IP receptor agonists or forskolin results in desensitization of the IP receptor coupled adenylate cyclase system (Kelly et al.,1990, Wiltshire et al.,1990). While IP receptor agonist pretreatment of NCB-20 cells and forskolin pretreatment of NCB-20 and NG108-15 cells only produce changes at the IP receptor level, IP receptor agonists produce an additional loss of functional Gs in NG108-15 cells. The ß-adrenoreceptor (ßAR) coupled adenylate cyclase system is the best studied system of desensitization. Following binding of ßAR agonists protein kinase A (PKA) and ßAR-kinase (ßARK) phosphorylate the receptor, leading to rapid uncoupling of the ßAR from Gs (Lefkowitz et al., 1990). In this study we have investigated, whether the coupling of the IP receptors to Gs is similarly affected during IP receptor desensitization.

Confluent NG108-15 (passage 16-25) and NCB-20 (passage 11-25) cells were pretreated with the IP receptor agonist prostaglandin E1 (PGE1) (30nM-25μM) or forskolin (10μM) for 24h. Cells were then harvested in phosphate buffered saline (PBS), washed three times and frozen at -80°C. [3H]-iloprost (10nM) binding was assayed in cell homogenates as previously described (Krane et al., 1990). The % increase in the specific [3H]-iloprost binding in the absence of GppNHp compared to the specific [3H]-iloprost binding in the presence of 0.1mM GppNHp was taken as a measure of the coupling of IP receptors to Gs.

PGE1 pretreatment of NG108-15 cells reduced the specific binding of [3H]-iloprost but had no effect on the coupling of the IP receptor to Gs. The % increase in the specific binding of [3H]-iloprost in the absence of GppNHp was the same in control (106.7±55.6%, mean±sem,n=16) and PGE1 treated (115±17.4%, mean±sem, n=16) NG108-15 homogenates. It is possible that this lack of uncoupling may explain why Gs is down-regulated in parallel with IP receptors. However forskolin pretreatment of NG108-15 cells and PGE1 treatment of NCB-20 cells do not produce a loss of Gs and also had no effect on the coupling of IP receptors to Gs. In forskolin experiments the % increase in the specific binding of [3H]-iloprost in the absence of GppNHp was 167.1±74.7% (mean±sem,n=3) in control and 203.8±32.9% (mean±sem,n=3) in treated NG108-15 homogenates. In PGE1 experiments the % increase in the specific binding of [3H]-iloprost in the absence of GppNHp was 163±64.1% (mean±sem,n=4) in control and 134±93.8% (mean±sem,n=4) in treated NCB-20 homogenates. Thus the lack of uncoupling is not sufficient to explain the loss of Gs that occurs in NG108-15 cells treated with IP receptor agonists.

These results demonstrate, that IP receptors do not uncouple from Gs during agonist-induced and forskolin-induced desensitization. In this respect the IP receptor is clearly different from the ßAR.

We gratefully acknowledge the support of the Wellcome Trust and Schering AG, Berlin.

Kelly, E., Keen, M., Nobbs, P. & MacDermot, J. (1990) Br.J.Pharmacol. 99, 309-316.
Wiltshire, R., Keen, M., Kelly, E. & MacDermot, J. (1990) Br. J. Pharmacol. 100, 425P.
Lefkowitz, R.J., Hausdorff, W.P. & Caron, M.G. (1990) Trends Pharmacol. Sci. 11, 190-194.
Krane, A., Kelly, E., MacDermot, J. & Keen, M. (1990) Eur. J. Pharmacol. 183,2143-2144.

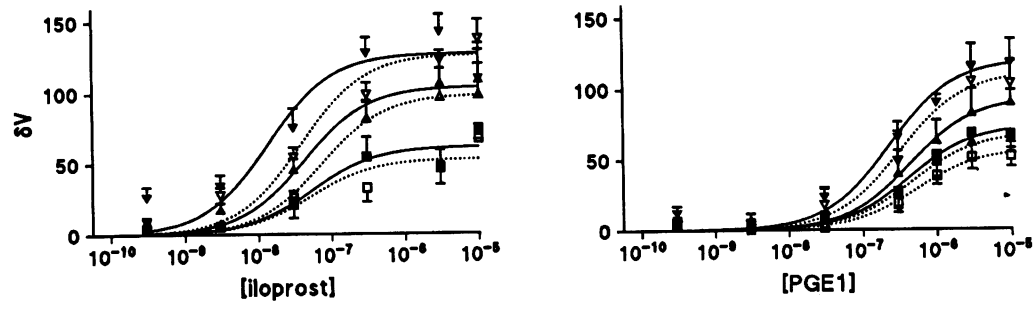
26P THE INFLUENCE OF GTP CONCENTRATION ON THE DOSE/RESPONSE CURVES FOR PROSTACYCLIN RECEPTOR AGONISTS

J. Malkhandi & M. Keen, Department of Pharmacology, University of Birmingham, Birmingham B15 2TT.

The hybrid cell line NG108-15 expresses prostacyclin (IP) receptors linked via Gs to the stimulation of adenylyl cyclase. We have looked at the effects of changes in GTP concentration and receptor number on the dose/response characteristics of the IP receptor agonists iloprost and prostaglandin E1 (PGE1). Pretreatment of NG108-15 cells with forskolin causes a 30% loss of IP receptors without affecting Gs (Wiltshire et al 1990).

Confluent NG108-15 cells were treated overnight in the presence and absence of 10μM forskolin, harvested, washed in phosphate buffered saline and stored at -80°C until required. Adenylyl cyclase activity was measured as described previously (Kelly et al 1990), the concentration of GTP being varied to provide nominal final assay concentrations of 0, 10 and 100μM.

Figure



The figure shows dose/response curves for iloprost and PGE1, each point is the mean ± sem from three separate experiments. The response (δV) is the increase in adenylyl cyclase activity over basal in pmol cAMP/min/mg protein. Filled symbols and unbroken lines indicate the effects of 0(■), 10(▲) and 100(▼) μM GTP on control cells; open symbols and broken lines indicate the same experiments on treated cells.

Increasing the GTP concentration caused a reduction in EC50 accompanied by an increased maximum response, consistent with an increase in efficacy. Reducing receptor number reversed this trend as expected. However, despite the fact that the maximal responses to the two agonists were similar, the pattern of changes appears different, which is inconsistent with current receptor theory.

Kelly, E., Keen, M., Nobbs, P. and MacDermot, J. (1990) Br J Pharmacol. 99 309-316.
Wiltshire, R. Keen, M. Kelly, E. and MacDermot, J. (1990) Br. J. Pharmacol. 100 425p.

27P ADP-RIBOSYLTRANSFERASE ACTIVITY OF NG108-15 CELLS MODIFIES THE STRUCTURE, ACTIVITY AND ABUNDANCE OF $G_s\alpha$

J. MacDermot, L.E. Donnelly & R.S. Boyd. Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 0NN.

Eukaryotic mono(ADP-ribosyl)transferase activity has been reported in many cells and tissues. However, the function of this enzyme activity in eukaryotic cells remains unclear. Recent evidence has suggested that mono(ADP-ribosyl)transferase activity from chicken spleen ADP-ribosylates Gsα (Obara et al 1991). Inhibition of ADP-ribosyltransferase activity in NG108-15 cells by nicotinamide leads to an increase in membrane associated Gsα (Donnelly et al 1991). In the present study we have examined further the possibility that Gsα may be a substrate for an endogenous ADP-ribosyltransferase in NG108-15 cells, and that this covalent modification may play a role in the regulation of Gsα activity.

NG108-15 (approx. 2 X 10^7) cells were permeablized in 300µl PEG 1500 (w/v) and 200µCi [^{32}P]-NAD for 10 min at 20°C. The cells were cultured for 24h, harvested and the cell proteins resolved by SDS polyacrylamide gel electrophoresis. The labelled proteins were identified autoradiographically. Membranes were prepared from NG108-15 cells and incubated with 200 µCi [^{32}P]-NAD in the presence of 10µM GTP at 37°C for 1h. Identification of the labelled substrate as Gsα was performed by immunoprecipitation of the labelled protein (Anderson and Blobel 1983) using anti-Gsα antibody. Adenylate cyclase activity was determined in both membranes and homogenates.

NG108-15 cells have the capacity to ADP-ribosylate many proteins, incuding one which corresponds to the cholera toxin substrate Gsα. Immunoprecipitation of [^{32}P]-labelled ADP-ribosylated NG108-15 membranes with an anti-Gsα antibody has indicated that Gsα is a substrate for endogenous ADP-ribosylation. Treatment of NG108-15 cells with nicotinamide for 18h, leads to a significant increase in both basal [11.6 ±1.2 (n =9) vs 21.2 ±2.7 (n = 12)] and iloprost stimulated adenylate cyclase activity [65.7 ±5.44 (n = 7) vs 83.1 ±5.9 (n = 8)] (measured in the presence of GTP). Similar results were obtained using another inhibitor of ADP-ribosylation, 5-Bromo-2'-deoxy-uridine. Short term treatment of NG108-15 cells with nicotinamide for 1h did not result in any significant changes in either basal or agonist stimulated adenylate cyclase activity.

We conclude that Gsα is a substrate for an endogenous ADP-ribosyltransferase in NG108-15 cells. Inhibition of ADP-ribosylation of Gsα with nicotinamide leads to an increase in the abundance of Gsα in the cell membrane, and also to an increase in its activity.

This work was supported by grants from the Royal Society and the Wellcome Trust.

Anderson, D. J. and Blobel, G. (1983) Methods Enzymol. **96**, 111-112.
Donnelly, L.E., Boyd, R.S. and MacDermot, J. (1991) Br. J. Pharmacol. **102**, 34P.
Obara, S., Yamada, K., Yashimura, Y. and Shimoyama, M. (1991) Eur. J. Biochem. **200**, 75-80.

28P MODULATORY AND DEPOLARISING ACTIONS OF 5-HT IN RAT NEOCORTEX *IN VITRO*

M.H.T.Roberts, J.A.Lopez-Garcia & S.K.Long[1], Department of Physiology, University of Wales College of Cardiff, P.O.Box 902, Cardiff, CF1 1SS and [1]Duphar BV, Weesp, Holland.

In vitro brain slice techniques have been used to study the actions of 5HT and some agonists on neocortical neurones in layers 2/3 of the frontal cortex of the rat. An interface bath of the Haas-type was used, superfused at 0.4 ml/min with artificial cerebrospinal fluid containing (in mM) NaCl (124); $NaHCO_3$ (25.5); glucose (10); KCl (3.3); $MgSO_4.7H_2O$ (1); KH_2PO_4 (1.2); $CaCl_2$ (2.5).

5 barrelled extracellular microiontophoresis electrodes (see Davies et al. 1988) recorded no spontaneous action potentials unless an excitatory amino acid was ejected from one of the barrels. Cells driven in this way responded to 5HT applied iontophoretically with excitation (Ca 30%) or inhibition (Ca 30%) in much the same way as reported by others during in vivo studies (Roberts & Straughan, 1967). Intracellular studies using 3M citrate recording electrodes and 5 barrelled extracellular microiontophoresis electrodes, showed that these effects of 5HT were often not the result of membrane resistance changes or depolarising or hyperpolarising responses to 5HT. 5HT was applied iontophoretically to 40 cells: 16 showed weak depolarisations and increase in resistance, whereas 33 showed a marked potentiation of responses to iontophoretically applied NMDA. There was little relationship between these two effects of 5HT; 12 cells showed both a depolarisation and facilitation of responses to NMDA; 4 cells showed a marked depolarisation but no facilitation of NMDA; however, 15 cells showed no depolarisation, no resistance change and yet a marked facilitation of responses to NMDA. A similar lack of dependence between these two effects of 5HT was also seen when 5HT was superfused at a concentration of 10 uM. Ketanserin, superfused at 1 uM for 10-30 min abolished the depolarisation and resistance increase caused by iontophoretically-applied 5HT, but did not alter the potentiation of responses to NMDA by 5HT. In the presence of 1 uM tetrodotoxin, sufficient to block sodium spikes, iontophoretically-applied 5HT was still able to depolarise and/or potentiate responses to NMDA. This suggests that neither action of 5HT is due to synaptically transmitted effects from nearby cells.

These data strongly imply that 5HT may increase the firing rate of neocortical neurones by two pharmacologically distinct mechanisms. Depolarisation of the cell and increase of membrane resistance may be mediated by ketanserin-sensitive, $5HT_2$ receptors, as reported by Araneda & Andrade (1991). However, a modulatory potentiation of responses to NMDA similar to that reported by Nedergaard et al (1986) is not prevented by 1 uM ketanserin nor by block of synaptic transmission with 1 uM tetrodotoxin.

Araneda,R. & Andrade,R. (1991) Neuroscience 40 399-412
Davies,M., Wilkinson,L.S. & Roberts,M.H.T.(1988) Br.J.Pharmacol. 94 483-491
Nedergaard,S. Engberg,I. & Flatman,J.A. (1986) Acta physiol.scand. 128 323-325
Roberts,M.H.T. & Straughan,D.W. (1967) J.Physiol. 193 269-294

29P SDZ 216-525: A SELECTIVE, POTENT AND SILENT 5-HT$_{1A}$ RECEPTOR ANTAGONIST

D. Hoyer, P. Schoeffter, J.M. Palacios, H.O. Kalkman, A.T. Bruinvels, J.R. Fozard, H. Siegl, M.P. Seiler & A. Stoll.
Preclinical Research, 386/525, SANDOZ Pharma Ltd, CH 4002 Basel, Switzerland.

Although selective 5-HT$_{1A}$ receptor agonists are available, no truly selective silent antagonist has been yet reported. Here we describe the pharmacological properties of SDZ 216-525, methyl 4-{4-[4-(1,1,3-trioxo-2H-1,2-benziosothiazol-2-yl)butyl]-1-piperazinyl}1H-indole-2-carboxylate, a selective, potent and silent 5-HT$_{1A}$ receptor antagonist both in vitro and in vivo.

Radioligand binding studies and measurements of forskolin-stimulated adenylate cyclase activity in calf hippocampus were carried out as described (Schoeffter and Hoyer, 1989). SDZ 216-525 showed high affinity and selectivity for 5-HT$_{1A}$ sites (Table 1). The affinity for α_1, α_2, β_1 and β_2 adrenoceptors, and dopamine D_2 receptors was at least 50-100 times lower than for 5-HT$_{1A}$ sites. SDZ 216-525 potently inhibited the effects of 8-OH-DPAT on adenylate cyclase activity and displayed no intrinsic activity in this test (Table 1).

Table 1: Binding affinities of 8-OH-DPAT and SDZ 216-525 at 5-HT sites and effects on forskolin-stimulated adenylate cyclase activity. (pK_D values, -log M); * Intrinsic activity, 5-HT = 100%; ** pEC_{50}, *** pK_B (-log mol/l). Data are mean values of n > 3.

Compound	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1C}$	5-HT$_{1D}$	5-HT$_2$	5-HT$_3$	Adenylate Cyclase	I. A. % *
8-OH-DPAT	8.7	4.2	5.2	6.0	<5	4.6	8.1 **	96
SDZ 216-525	9.2	6.0	7.2	7.6	5.2	5.4	10.0 ***	0

In autoradiographic studies performed in rat brain, SDZ 216-525 (0.1-10 nM) displaced concentration-dependently the binding to all specific 5-HT$_{1A}$ sites labelled with [^{3}H]8-OH-DPAT. Further, [^{3}H]SDZ 216-525 labelled high affinity sites in pig brain cortex with a profile typical of that of 5-HT$_{1A}$ sites and quantitative autoradiography in rat brain revealed that the distribution of sites labelled by [^{3}H]SDZ 216-525 and [^{3}H]8-OH-DPAT was identical. In vivo, SDZ 216-525 did not induce forepaw treading or flat body posture in lightly reserpinised rats (Tricklebank et al. 1985) at doses up to 1 mg/kg s.c., whereas the response to a submaximal dose of 8-OH-DPAT (0.25 mg/kg; s.c.) was inhibited dose-dependently 1 h following SDZ 216-525 over the range 0.03-1 mg/kg s.c.

These data demonstrate SDZ 216-525 to be a potent, selective and silent 5-HT$_{1A}$ receptor antagonist both in vitro and in vivo.

Schoeffter, P. & Hoyer, D. (1989). Naunyn Schmiedeberg's Arch. Pharmacol. 339, 675-683.
Tricklebank, M.D., Forler, C., Middlemiss, D.N. & Fozard, J.R. (1985). Eur. J. Pharmacol. 117, 15-24.

30P EVIDENCE TO SUGGEST THAT ACTIVATION OF CENTRAL 5-HT$_2$/5-HT$_{1C}$ RECEPTORS CAUSES THE RELEASE OF VASOPRESSIN IN ANAESTHETIZED RATS

I.K. Anderson, G.R. Martin[1] and A.G. Ramage, Academic Department of Pharmacology, Royal Free Hospital School of Medicine, Hampstead, London NW3 2PF; [1]Wellcome Research Laboratories, Beckenham, Kent BR3 3BS

In the anaesthetized rat, right lateral ventricular (i.c.v.) injection of 5-HT caused a rise in blood pressure which was associated with an initial bradycardia and sympathoinhibition followed by a tachycardia and sympathoexcitation. This latter phase was due to activation of forebrain 5-HT$_{1A}$ receptors (Anderson, 1991). The present experiments were designed to investigate the nature of the 5-HT receptors mediating the initial phase of this 5-HT response.

In male Sprague-Dawley rats (250-375g) anaesthesia was induced with halothane and maintained with α-chloralose (80 mg kg^{-1}). Rats were artificially ventilated following neuromuscular blockade with decamethonium iodide (3 mg kg^{-1}). Simultaneous recordings were made of blood pressure, heart rate, and renal nerve activity (RNA). Drugs given i.c.v. were microinjected in a volume of 5 µl over 20s.

5-HT (120 nmol kg^{-1}; n=8) i.c.v. caused an initial rise in BP of 12±2 mmHg and falls in HR and RNA of 15±2 bpm and 40±8 % respectively, after 2 min. Neither the 5-HT$_2$ receptor antagonist cinanserin (300 nmol kg^{-1} i.c.v., n=6) nor the vasopressin V$_1$ receptor antagonist, [ß-mercapto-ß,ß-cyclopentamethylenepropionyl^1, O-Me-Tyr^2,Arg^8]-vasopressin (10 µg kg^{-1} i.v., n=5), prevented the 5-HT induced rise in BP. However, they did reverse the initial bradycardia and renal sympathoinhibition to tachycardia and sympathoexcitation respectively. The 5-HT$_2$/5-HT$_{1C}$ agonist DOI (1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane; 120 nmol kg^{-1}; n=6) given i.c.v. caused a rise in BP of 15±3 mmHg and falls in HR and RNA of 21±8 bpm and 53±7 % respectively, after 5min. This profile of action was similar to the initial phase of the 5-HT response. However, the action of DOI differed from that of 5-HT in that the DOI response (n=4) but not the 5-HT response (n=4) blocked by the peripherally acting 5-HT$_2$ receptor antagonist BW501C67 (0.1 mg kg^{-1} i.v.)

These results suggest that 5-HT can cause activation of forebrain 5-HT$_2$/5-HT$_{1C}$ receptors to release vasopressin which produces a rise in systemic blood pressure and a baroreceptor reflex mediated bradycardia and sympathoinhibition. DOI did not appear to activate central 5-HT$_2$/5-HT$_{1C}$ receptors to cause a release of vasopressin, but appeared to 'leak' out of the brain and activate 5-HT$_2$ receptors on vascular smooth muscle to cause a rise in blood pressure.

I.K.A is in receipt of a SERC CASE Studentship.

Anderson I.K. (1991). Br. J. Pharmacol., 104, 65P

31P MODULATION BY ANAESTHETIC AGENTS OF THE REFLEX CARDIO-INHIBITORY RESPONSES TO ACTIVATION OF THE BAROREFLEX AND THE CARDIOPULMONARY C-FIBRE AFFERENTS IN THE RAT

E. Kirkman, H.W. Marshall & R.A. Little, North Western Injury Research Centre, University of Manchester, Manchester, M13 9PT.

A number of anaesthetic agents are known to modify cardiovascular reflexes and responses e.g. barbiturates can attenuate both the baroreflex and the cardiovascular response elicited by tissue injury (Redfern, 1981). The present study has investigated the effects of three agents, α-chloralose, alphadolone/alphaxolone and propofol on two reflexes which produce a cardio-inhibition, namely the arterial baroreflex and that elicited by stimulation of cardio-pulmonary C-fibre afferents.

Experiments were performed on 18 male Wistar rats (225-261g). The animals were anaesthetized briefly with isoflurane (3.5% and 2.0% in oxygen, respectively, for induction and maintenance). ECG electrodes were attached to the skin and cannulae placed in both lateral tail veins, ventral tail artery and the right external jugular vein. The animals were then allowed to recover and 1.5 hours later the response to stimulation of the cardio-pulmonary afferent fibres was assessed by constructing dose-response curves to phenylbiguanide (PBG; 0.25-32μg) injected into the right atrium via the right external jugular vein (Fastier *et al.*, 1959). The baroreflex was assessed as the slope of the relationship between heart period and mean arterial blood pressure during an infusion of phenylephrine (5-60$\mu g.kg^{-1}min^{-1}$ iv; Redfern, 1981). The animals were then treated with either α-chloralose (70$mg.kg^{-1}$; maintenance 0.26±0.1 $mg.kg^{-1}.min^{-1}$, mean±s.e.mean, 6 rats), alphadolone/alphaxolone (12$mg.kg^{-1}$; maintenance 0.23±0.01 $mg.kg^{-1}.min^{-1}$, 6 rats) or propofol (8.25$mg.kg^{-1}$; maintenance 0.81±0.01$mg.kg^{-1}.min^{-1}$, 6 rats) to produce loss of spontaneous movement and righting reflex. The infusion rate of the three agents was adjusted to allow comparable increases in arterial blood pressure to hindlimb stimulation in each group. Body temperature was maintained with external heating. The dose of PBG required to increase heart period by 220% (1.15±0.17μg) was not affected significantly by α-chloralose, while it was increased significantly ($p<0.05$, Wilcoxon matched-pairs rank-sum test) from 0.94±0.13 to 4.32±1.36μg in the alphadolone/alphaxolone treated group. This increase in the dose of PBG was even greater (from 1.00±0.27 to 11.15±2.53μg; $p<0.05$) in the propofol treated group. By contrast α-chloralose and alphadolone/alphaxolone had no consistent effects on the baroreflex, while propofol significantly reduced baroreflex sensitivity in 3 of the 6 rats, the group mean value being reduced from 0.95±0.03 to 0.76±0.02 $ms.mmHg^{-1}$.

These results indicate that treatment with alphadolone/alphaxolone and propofol differentially modulate the cardio-inhibitory responses elicited by activation of the baroreflex and cardio-pulmonary afferent fibres. By contrast α-chloralose did not modify these reflexes.

Supported by the MRC.

Fastier, F.N., McDowall, M.A. & Waal, H. (1959) Br. J. Pharmacol. **14**, 527-535.
Redfern, W.S. (1981) Ph.D. Thesis, University of Manchester.

32P SHORT- AND LONG-LATENCY EFFECTS OF A BRIEF SWIM TEST ON CENTRAL 5-HT_2 RECEPTOR FUNCTION AND BINDING

S. Davis , D.J. Heal[1], G.P. Luscombe[1] and S.C. Stanford, Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, and [1]Boots Pharmaceuticals Research Department, Nottingham, NG2 3AA.

We have examined central 5-HT2 receptors after a brief swim test to determine whether this procedure produced changes in binding or function. The effects on these changes of prior administration of a putative antidepressant (sibutramine hydrochloride; Buckett et al., 1988) or previous experience of repeated mild stress (saline injection) were also examined.

Male CD1 mice (20-25g) were given sibutramine (3 mg/kg i.p.) or saline (10 ml/kg i.p.) once-daily for 10 days. 24 h after the final injection, these mice, together with a group of unhandled controls, were exposed individually to a 6 min swim. Immobility was recorded during the last 4 min, after which mice were dried and returned to their home cage. 2-4 h or 7 days later, 5-HT2 receptor function was assessed in these animals, and in a group of unstressed ('naive') controls, by counting head-twitches induced by 5-methoxy-N,N-dimethyltryptamine (5-MeODMT; 2 mg/kg i.p.; Goodwin & Green, 1985). Neocortical [3H]ketanserin (0.05-5.5 nM) binding defined by methysergide (5 μM) was studied in parallel groups.

Table 1 Swim-induced immobility and effects on 5-HT2 receptor function and number

	Immobility	Head-twitches		5-HT2 Bmax (pmol/g protein)	
	(sec)	2-4 h after swim	7 days after swim	2-4 h after swim (n=5)	7 days after swim
Naive	N/A	6.2 ± 0.7	5.9 ± 0.5	338.7 ± 24.3	196.2 ± 14.9
Swim only	183 ± 7	4.7 ± 0.4*	9.3 ± 0.8***†††	335.6 ± 37.0	254.0 ± 18.0
Saline + swim	166 ± 6	6.8 ± 0.7	6.8 ± 0.6	376.1 ± 31.3	212.1 ± 15.5
Sibutramine + swim	151 ± 11***	4.3 ± 0.6*	5.9 ± 0.6†	371.2 ± 14.1	248.6 ± 20.9

Unless otherwise stated, all values are mean ± s.e. mean for 10-15 mice. *p<0.05, ***p<0.001: ANOVA, post-hoc t-test (vs. all treatments at each time). † p<0.05 (vs. sibutramine + swim, at 2-4 h), ††† p<0.001 (vs. saline + swim, at 2-4 h): 2-way ANOVA, post-hoc t-test.

Sibutramine, but not saline, injection significantly reduced immobility. Swimming significantly reduced head-twitches at 2-4 h, and this effect was abolished only by previous mild stress. In contrast, 7 days after the swim, the number of head-twitches was significantly increased in the 'swim only' group; this was abolished by both saline and sibutramine pretreatments. These changes in 5-HT2 receptor function were not paralleled by changes in receptor number or affinity in the neocortex. We conclude that swim-induced short- and long-latency changes in 5-HT2 receptors are influenced by previous experience of repeated mild stress or sibutramine, but could involve changes downstream of the 5-HT2 receptor binding site.

S.D. is a SERC/CASE scholar, in collaboration with Boots Pharmaceuticals Research Department.

Buckett, W.R., Thomas, P.C. & Luscombe, G.P. (1988) Prog. Neuro-Psychopharmacol. Biol. Psychiat. **12**, 575-584.
Goodwin, G.M. & Green, A.R. (1985) Br. J. Pharmacol. **84**, 743-753.

33P LABELLING OF SPECIES VARIANTS OF 5-HT$_3$ RECEPTORS BY A NOVEL 5-HT$_3$ RECEPTOR LIGAND, [^{3}H]RS-42358-197

E.H.F. Wong , I. Wu, R.M. Eglen & R.L. Whiting
Institute of Pharmacology, Syntex Research, 3401 Hillview Ave., Palo Alto, CA 94304, USA

The binding characteristics of [^{3}H]RS-42358-197 ((S)-N-(1-azabicyclo[2.2.2]oct-3-yl)-2,4,5,6-tetrahydro-1-H-benzo[de]isoquinolin-1-one hydrochloride) have been studied. The methodology of Sharif et al. (1991) was employed for all studies, and non-specific binding was defined by 0.1 uM (S)zacopride. Dissociation constants (K_d) and number of sites (B_{max}) are quoted in nM and fmol/mg protein, respectively. [^{3}H]RS-42358-197 labelled a uniform population of high affinity and saturable binding sites in rat cortex (0.12 ± 0.01; 16 ± 2), rabbit myenteric plexus (0.10 ± 0.01; 91 ± 12), and NG108-15 cells (0.09 ± 0.02; 565 ± 200), similar to binding characteristics reported for [^{3}H]GR65630 (Wong et al., 1991). [^{3}H]RS-42356-197 also labelled a binding site in guinea pig myenteric plexus (G.P.M.P.; 1.6 ± 0.4; 91 ± 17). The displacement data are summarized in Table 1.

Table 1 Comparison of Binding and Functional Potencies of 5-HT$_3$ Receptor Ligands

Ligands	vs [^{3}H]RS-42358 Rat Cortex, pK_i	vs [^{3}H]RS-42358 G.P.M.P., pK_i	Functional (Butler et al., 1990) G.P.M.P., pK_B	Functional (Eglen et al., 1990) G.P. whole ileum, pA_2
(S)Zacopride	9.7 ± 0.1	8.4 ± 0.1	8.1 ± 0.1(S/R)	8.0 ± 0.2
ICS 205-930	9.0 ± 0.1	7.8 ± 0.1	8.0 ± 0.2	7.6 ± 0.1
(R)Zacopride	8.3 ± 0.1	7.1 ± 0.1	N.D.	7.2 ± 0.1
Ondansetron	8.5 ± 0.1	6.9 ± 0.1	7.3 ± 0.1	7.0 ± 0.1
MDL 72222	7.5 ± 0.2	6.4 ± 0.1	6.7 ± 0.1	N.D.
Metoclopramide	6.4 ± 0.1	5.7 ± 0.1	5.5 ± 0.1	N.D.

All values reported above are means ± s.e. mean, n=3. The G.P.M.P. binding and functional values were significantly ($p<0.05$) correlated ($r= 0.95$).

The pharmacological specificity of [^{3}H]RS-42358-197 binding in rat cortex (Table 1), rabbit ileum and NG108-15 cells (data not shown) was consistent with labelling of a 5-HT$_3$ receptor (Sharif et al., 1991). The selectivity of this ligand was indicated by the lack of affinity of RS-42358-197 for another 28 receptors. The correspondence of the binding and the functional data in the guinea pig (Table 1) suggested that [^{3}H]RS-42358-197 labelled a 5-HT$_3$ receptor that differs from that in rat cortex. The relatively high affinity and specificity of [^{3}H]RS-42358-197, therefore, suggests that it is a useful ligand to characterize 5-HT$_3$ binding sites in a range of membrane preparations.

Butler, A., Elswood, C.J., Burridge, J., Ireland, S.J., Bunce, K.T., Kilpatrick, G.J. & Tyers, M.B. (1990) Br.J.Pharmacol., **101**, 591-598.
Eglen, R.M., Swank, S.R., Walsh, L.K.M. & Whiting, R.L. (1990) Br.J.Pharmacol., **101**, 513-520.
Sharif, N.A., Wong, E.H.F., Loury, D.N., Stefanich, E., Michel, A.D., Eglen, R.M. & Whiting, R.L. (1991) Br.J.Pharmacol., **102**, 919-925.
Wong, E.H.F., Lee, J., Loury, D.N., Eglen, R.M. & Whiting, R.L. (1991) J.Neurochem., **57**(Suppl.), S136A.

34P IS 2-METHYL-5-HT A SELECTIVE 5-HT$_3$ RECEPTOR AGONIST?

S. Tadipatri & P.R. Saxena, Department of Pharmacology, Faculty of Medicine & Health Sciences, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR ROTTERDAM, The Netherlands.

The present research on classification of 5-hydroxytryptamine (5-HT) receptors is based on the scheme proposed by Bradley *et al.* (1986). Recently, the 5-HT receptors mediating contractions in the rabbit isolated renal artery have been identified as 5-HT$_1$-like in nature (Tadipatri *et al.*, 1991). Notwithstanding, we recently observed that 2-methyl-5-HT, reported as a selective 5-HT$_3$ receptor agonist (Bradley *et al.*, 1986), elicited concentration dependent contractions of the rabbit renal artery. Therefore, in the present study, the contractile effects of 2-methyl-5-HT as well as of 5-HT, α-methyl-5-HT and 5-methoxytryptamine were analyzed. Ring segments of the renal artery were suspended in organ baths containing Kreb's solution at 37°C and aerated with 95% O_2 and 5% CO_2. Changes in the tension were monitored isometrically. Cumulative concentration effect curves were established for the agonists in the absence or presence of some 5-HT receptor antagonists.

Table 1 Antagonist pA_2 values against agonist-induced contractions in the rabbit isolated renal artery (means±s.e.mean; n=4-6).

Antagonist	**Conc. (μM)**	**5-HT**	**2-Methyl-5-HT**	**α-Methyl-5-HT**	**5-Methoxytryptamine**
ICS 205-930	3	No antagonism	No antagonism	No antagonism	No antagonism
MDL 72222	1	No antagonism	No antagonism	No antagonism	No antagonism
Ketanserin	0.3, 1	6.6±0.1[a]	6.8±0.1	7.0±0.3	6.9±0.2
Methiothepin	0.01, 0.1	8.6±0.4[a]	8.4±0.2	8.8±0.1	8.7±0.2

Conc., concentration of antagonist used; [a], pK_B values (taken from Tadipatri *et al.*, 1991).

The pD_2 values for the various agonists were: 5-HT (5.2±0.2), 2-methyl-5-HT (5.6±0.3), α-methyl-5-HT (5.7±0.2) and 5-methoxytryptamine (4.3±0.2) (n=4-6); renal vessel segments precontracted with U 46619 were 10-100 fold more sensitive to these compounds. As shown in Table 1, neither ICS 205-930 nor MDL 72222 antagonised the agonist responses, thus ruling out the involvement of 5-HT$_3$ or 5-HT$_4$ receptors. On the other hand, methiothepin proved to be a potent antagonist and ketanserin a weak antagonist of the agonist-induced contractions, which is consistent with an interaction with 5-HT$_1$-like receptors. In conclusion, this study proves that, besides 5-HT, α-methyl-5-HT and 5-methoxytryptamine, 2-methyl-5-HT is an agonist at the rabbit renal artery 5-HT$_1$-like receptor and, therefore, cannot be considered as a highly selective 5-HT$_3$ receptor ligand.

Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P & Saxena, P.R. (1986). *Neuropharmacology*, **25**, 563-576.
Tadipatri, S., Van Heuven-Nolsen, D., Feniuk, W. & Saxena P.R. (1991). *Br. J. Pharmacol.*, **104**, 887-894.

35P BEHAVIOURAL EFFECTS OF THE 5-HT_3 RECEPTOR AGONISTS 1-PHENYLBIGUANIDE AND m-CHLOROPHENYLBIGUANIDE (mCPG) IN RATS

G.A. Higgins & E.M. Sellers, Addiction Research Foundation, 33 Russell Street, Toronto, M5S 2S1, and Departments of Pharmacology and Medicine, University of Toronto, Toronto, Ontario, Canada.

Research suggests that 5-HT_3 receptor antagonists, e.g. ICS205-930 and ondansetron may enhance behaviours suppressed by environmental manipulations, reduce behaviours associated with DA mesolimbic activation, and prevent the emetic episodes resulting from irradiation or cytotoxin treatment (see Kilpatrick et al., 1990a). In each case, the effect of the antagonist was examined in animals whose behaviour was being driven by either a drug or environmental stimulus. However in normal "unstimulated" animals, 5-HT_3 receptor antagonists generally appear to be behaviourally inert, suggesting that under such conditions the endogenous 5-HT tone at this receptor is low. Therefore, in the present study we have examined the overt behavioural and motivational effects of the 5-HT_3 receptor agonists phenylbiguanide (PBG) and m-chlorophenylbiguanide (mCPBG) (Kilpatrick et al., 1990b) in male Wistar rats (230-300g). Drug induced place conditioning was by the unbiased method with 4 x 45 min pairings of drug and vehicle to respective compartments.

Intraperitoneal injection of PBG (3-30 mg/kg) and mCPBG (0.3-10 mg/kg) produced a dose-related incidence of abdominal constriction, writhing, pawshakes and salivation in some, but not all, rats. Both PBG (30 mg/kg) and mCPBG (1-10 mg/kg) produced a significant place aversion (e.g. PBG 30 mg/kg paired side 247 $\pm$ 39s; vehicle paired side 540 $\pm$ 46s; $p<0.05$). Furthermore, PBG (30 mg/kg) induced a significant conditioned taste aversion for a novel 0.1% saccharin solution. Thirty min pretreatment with ICS205-930 (ICS) and its quaternised derivative (Q-ICS205-930) (both 0.1 mg/kg ip) blocked the PBG (30 mg/kg)-induced place aversion (veh/PBG - 417 $\pm$ 57s: ICS/PBG + 45 $\pm$ 112s: Q-ICS/PBG - 67 $\pm$ 94s; both $p<0.05$ vs. veh/PBG group). Following intracerebroventricular injection, PBG (1-30 μg) and mCPBG (0.1-10 μg) tended to increase locomotor and chewing-type behaviours although the effects of PBG appeared more robust with dose-related increases in locomotion ($F[3,21]=10.3$; $p<0.01$) and chewing of wooden blocks. Both behaviours induced by PBG (10 μg) were antagonised by pretreatment with haloperidol (0.03-0.3 mg/kg sc) but not consistently by ICS205-930 (0.1 mg/kg ip) or ondansetron (0.0001-1 mg/kg sc).

It is concluded that peripheral administration of 5-HT_3 receptor agonists elicits aversive type behaviours as shown by the demonstration of place and taste aversions to these treatments. Central injection of PBG and mCPBG produced behaviours indicative of dopaminergic activation. However, they were resistant to blockade by 5-HT_3 antagonists suggesting they may be a consequence of the dopamine releasing properties of each drug (see Kilpatrick et al., 1990b).

Kilpatrick, G.J., Bunce, K.T. & Tyers, M.B. (1990a) Med. Res. Rev. 10, 441-475.
Kilpatrick, G.J., Butler, A., Burridge, J. & Oxford, A.W. (1990b) Eur. J. Pharmacol. 182, 193-197.

36P DIFFERENTIAL ACTIVITIES OF (R)- AND (S)-ZACOPRIDE TO MODIFY EXTRACELLULAR LEVELS OF 5-HT IN THE RAT FRONTAL CORTEX

C.H.K. Cheng, N.M. Barnes[1], B. Costall, J. Ge and R.J. Naylor, Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP, [1]Department of Pharmacology, The Medical School, University of Birmingham, Birmingham, B15 2TT, UK

Previous studies have demonstrated that the isomers of zacopride display a differential activity with respect to possessing anxiolytic-like potential (Barnes et al., 1991). In the present studies we assess the ability of the isomers of zacopride to modify extracellular levels of 5-hydroxytryptamine (5-HT) in the frontal cortex of freely-moving rats using 'in vivo' microdialysis.

Microdialysis probes were implanted into chronically indwelling cannulae located in the frontal cortex (probe tip A+0.7, V-7.0, L-1.5, relative to Bregma at an angle of 45°) of male hooded Lister rats (300-350g) 12 hrs prior to the experiment. Probes were perfused with artificial CSF at a rate of 2 μl/min. 20-min samples were collected and analysed for 5-HT by HPLC-ECD. After assessment of basal levels (time = 0), the animals were treated with either drug or vehicle.

Table 1. The effect of (R)- and (S)-zacopride on the extracellular level of 5-HT in the rat frontal cortex assessed by 'in vivo' microdialysis.

Treatment	Time (min)							
(1.0ml/kg i.p.)	0	20	40	60	80	100	120	340
0.9% NaCl (vehicle)	9.9±0.4	9.9±0.3	9.9±0.5	9.5±0.4	8.6±0.3	9.0±0.2	9.1±0.3	9.3±0.3
(R)-zacopride								
1μg/kg	10.0±0.3	8.1±0.6	8.3±0.5	9.1±0.4	7.3±0.4*	6.8±0.4*	6.0±0.4**	6.2±0.4**
10μg/kg	10.8±0.5	8.2±1.0*	6.2±0.7**	5.2±0.5**	4.2±0.5**	5.0±0.6**	4.3±0.5**	3.9±0.4**
100μg/kg	9.6±0.3	5.7±0.5**	4.4±0.4**	3.8±0.4**	3.0±0.5**	3.8±0.6**	2.8±0.6**	2.3±0.5**
(S)-zacopride								
10μg/kg	10.3±0.4	9.9±0.4	9.7±0.5	9.8±0.4	11.9±1.3	10.5±0.7	9.5±0.4	10.0±0.6
100μg/kg	9.1±0.4	9.3±0.3	9.4±0.4	9.3±0.5	8.6±0.4	8.9±0.5	9.1±0.2	9.3±0.4

Data represent mean ± SEM (pg/40μl sample), n = 5-8, *$P<0.05$, **$P<0.01$ significant reduction compared to the basal level (one way ANOVA, followed by Dunnett's t test).

The failure of the 5-HT_3 receptor antagonist (S)-zacopride to modify extracellular levels of 5-HT in the rat frontal cortex is consistent with the results obtained with the structurally dissimilar 5-HT_3 receptor antagonist ondansetron (Ge et al., 1991). This indicates that the decrease in extracellular 5-HT levels following administration of (R)-zacopride may be a consequence of an interaction at an additional (S)-zacopride-insensitive site. Such sites have previously been identified within the rat and mouse cortex using [^{3}H](R)-zacopride in radioligand binding studies (Barnes et al., 1991).

Barnes, J.M., Barnes, N.M., Costall, B., Domeney, A.M., Johnson, D.N., Kelly, M.E., Munson, H.R., Naylor, R.J., Young, R. (1990) Pharmacol. Biochem. Behav. 37, 717-727
Ge, J., Barnes, N.M., Cheng, C.H.K., Costall, B., Naylor, R.J. (1991) Br. J. Pharmacol. 104 (Suppl), 365P

37P PHARMACOLOGICAL CHARACTERIZATION OF 5-HT-INDUCED EFFECTS ON PERIPHERAL NOCICEPTORS OF THE NEONATAL RAT *IN VITRO*

A. Rueff' & A. Dray, Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN

An increase in the chemosensitivity of nociceptive neurones is an important mechanism in peripheral hyperalgesia. 5-hydroxytryptamine (5-HT) is one of a number of substances released following tissue injury and inflammation which activates or sensitizes nociceptors and may therefore contribute to hyperalgesia (Richardson *et al.*, 1985; Eschalier *et al.*, 1989). In the present study we have characterized 5-HT-receptors involved in the sensitization and activation of peripheral nociceptors. For this we have used an *in vitro* preparation of the neonatal rat spinal cord with functionally connected tail.

The intact spinal cord with tail attached was isolated from 0-2 days old rats. The cord and tail were separately superfused (3 ml/min on cord; 6 ml/min on tail) with a physiological salt solution at 23±2°C and gassed with 95% O_2:5% CO_2. The activation of nerve fibres in the tail was assessed by measuring spinal ventral root potentials (VRP). Recordings were made using a glass micropipette placed in an electrolyte-filled well which contained the selected ventral root (L_3-L_5). Peripheral nociceptors were activated by stimuli applied at regular intervals but sufficient to avoid tachyphylaxis.

Continuous superfusion of the tail for 10 min with 5-HT (0.5-10μM) did not evoke a VRP, but significantly enhanced (by 30-45%) in a concentration related manner the responses evoked by submaximal or subthreshold concentrations of the algogens bradykinin BK (25-150nM) and capsaicin (75-300nM). 5-HT-induced sensitization of peripheral nociceptors was blocked by the selective 5-HT_2-receptor antagonist ketanserin (10nM-1μM) but not by the 5-HT_3/5-HT_4-receptor antagonist ICS 205-930 (1-10μM) or the 5-HT_1/5-HT_2-receptor antagonist methiothepin (1μM). The 5-HT_{1C}/5-HT_2-receptor agonist α-methyl-5-HT (5μM) mimicked the sensitizing effect of 5-HT (35-45% enhancement) but the 5-HT_3-receptor agonist 2-methyl-5-HT (1μM) and the 5-HT_1-receptor agonist 5-carboxamidotryptamine (5-CT; 5μM) did not.

Peripheral thermoreceptors were activated at a threshold temperature of 35±1°C. 5-HT (5-10μM) and α-methyl-5-HT (5μM) enhanced (by 30-50%) the VRP evoked by a threshold thermal stimulus. Concomitant perfusion with 1μM ketanserin blocked the α-methyl-5-HT-induced sensitization to threshold thermal stimuli.

Following continuous perfusion of the tail for 10 min with a threshold concentration of BK (20-25nM) or capsaicin (50-100nM) a brief application of 5-HT (1-10μM) or 5-CT (1μM) but not α-methyl-5-HT (10μM) or 2-methyl-5-HT (10μM) evoked a ventral root potential. These responses were significantly reduced (by 45-65%) in the presence of methiothepin (1μM) but not by ketanserin (1-10μM) or ICS 205-930 (10μM). Neither the sensitizing nor the excitatory effects of 5-HT were blocked by the cyclooxygenase-inhibitor indomethacin (1μM) and they did not show any signs of tachyphylaxis if drug administrations were repeated at intervals of 60 min.

Two types of receptor appear to be involved in the peripheral actions of 5-HT. The increased chemical and thermal responsiveness of polymodal nociceptors involves a 5-HT_2-receptor whereas 5-HT-mediated activation of sensitized nociceptors involves a 5-HT_1like-receptor.

Eschalier, A., Kayser, V. & Guilbaud, G. (1989) *Pain* 36, 249-255
Richardson, B.P., Engel, G., Donatsch, P. & Stadler, P.A. (1985) *Nature* 316, 126-131

38P REDUCTION IN THE ANTINOCICEPTIVE EFFECTS OF INTRATHECAL NORADRENALINE FOLLOWING 5,7 DIHYDROXYTRYPTAMINE LESIONS OF THE DORSOLATERAL FUNICULUS

Z.Ali, G.Foster, S.Barasi,[1]G.Wu &[2]S.Butcher, Department of Physiology, University of Wales College of Cardiff, CF1 1SS. [1]Department of Neurobiology, Shanghai Medical University, China and [2]Department of Pharmacology, University of Edinburgh.

Spinal 5-hydroxytryptamine (5HT) depletion is reported either to potentiate (Archer et al., 1986) or not to affect the antinociceptive action of noradrenaline (NA) (Sawynok & Reid, 1990). In the above studies the neurotoxin 5,7 dihydroxytryptamine (5,7DHT) was applied intrathecally (i.t.). Depletion of both dorsal and ventral horn 5HT may confuse the interpretation of data derived from behavioural experiments. In the present study we used the tail flick test to examine the antinociceptive action of NA in rats with selective depletion of dorsal horn 5HT. Additionally, we investigated the possibility of supersensitivity to i.t. applied 5HT in 5,7DHT lesioned rats.

Male Wistar rats (150g) were pretreated with desipramine (25mg/kg) 30min prior to surgery. Following anaesthesia with halothane, two adjacent vertebrae at C4 were separated by traction. 0.3μg of 5,7DHT in 75nl 0.9% NaCl containing 0.2% ascorbic acid was injected bilaterally into the dorsolateral funiculus at a rate of 50nl/min. The pipette was left in place for 5 minutes before being withdrawn. The neurochemical effects of the lesions were examined in a number of unilaterally lesioned rats using high performance liquid chromatography with electrochemical detection. Results from this preliminary study indicate that ipsilateral to the lesion, 5HT levels were reduced by 58% in the dorsal horn and 34% in the ventral horn. Dorsal horn NA levels were unaffected by the lesion. Following a recovery period of at least three weeks, rats were lightly anaesthetised with Saffan whilst tail flick latencies (TFL) to noxious heat were recorded at four-minute intervals. Following three control TFLs (2.5-3.5 s) 5μl of 15nmol NA or 260nmol 5HT was injected intrathecally and TFLs were recorded until there was recovery from drug effects.

In 5,7DHT lesioned rats, NA elevated TFLs to cutoff levels for a mean duration of 49.27±10.14 min (n=11) which is significantly shorter than in naive rats (122.7±8.24 min) (n=20) $p<0.001$. In contrast there was no significant difference between the period for which 5HT elevated the TFLs in naive (21.1±3 min) (n=6) and lesioned rats (25.5±8.7 min) (n=7). Control TFLs in lesioned rats were not significantly different from those of naive rats.

The reduced antinociceptive action of i.t. NA in 5,7DHT lesioned rats provides further support for an interaction between 5HT and NA in spinal cord nociceptive processing. Differences between these results and those of previous studies (Archer et al.,1986, Sawynok & Reid, 1990) may be explained by the more selective lesions in this preparation, which principally affect 5HT neurones projecting to the dorsal horn.

Archer T., Jonsson G., Minor B.G., Post C. (1986) European Journal of Pharmacology 120, 295-307
Sawynok J., Reid A. (1990) Naunyn-Schmiedeberg's Arch Pharmacol 342, 1-8

Dr G. Wu is in receipt of a K.C. Wong fellowship from the Royal Society

39P STUDIES WITH CI-977 AND ICI 204448 SUPPORT A ROLE FOR PERIPHERAL RECEPTORS IN κ-MEDIATED ANTINOCICEPTION AND DIURESIS

H. Wheeler-Aceto & A. Cowan, Department of Pharmacology, Temple University School of Medicine, Philadelphia, U.S.A.

Peripheral sites may be involved in the modulation of inflammation-induced hyperalgesia by opioids. Here, we compare morphine (M) with the potent and highly selective *kappa* agonist CI-977 (CI; (-)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide; Hunter *et al.*, 1990) and ICI 204448 (ICI; (R,S)-N-[2-(N-methyl-3,4-dichlorophenyl-acetamido)-2-(3-carboxyphenyl)-ethyl] pyrrolidine; Cambridge Research Biochemicals), a selective *kappa* agonist with limited access to the CNS (Shaw *et al.*, 1989), against formalin-induced nociception. Dose-response curves were obtained after s.c., intrapaw (i.paw) or i.th. administration for each compound in the tonic phase of the rat hind paw formalin test (Wheeler-Aceto & Cowan, 1991). On the left side of the following table are antinociceptive A50 values (μg/rat) of the three opioids by each route. The right side of the table compares the relative potency of each agonist when given by the same route (CI-977 = 1).

Morphine	**i.th. > s.c. > i.paw**		*Intrathecal*	**CI = M > ICI**	
	0.1	**18**	**45**	**1**	**1**	**10**
CI-977	**i.th. = i.paw > s.c.**		*Intrapaw*	**CI >> ICI = M**	
	0.3	**0.4**	**1.5**	**1**	**100**	**100**
ICI 204448	**i.th. > i.paw > s.c.**		*Subcutaneous*	**CI > M >> ICI**	
	2	**34**	**550**	**1**	**10**	**350**

Behavioural depression was observed with antinociceptive doses of CI-977 by all routes. ICI 204448 caused behavioural depression after only i.th. dosing. In complete contrast, an A70 dose of ICI 204448 given i.th. caused no diuresis while A70 doses given peripherally elicited a prolonged increase in urine flow; diuresis was even observed when the i.th. A70 dose (6 μg) was given s.c. CI-977 caused diuresis by all routes of administration. Overall, these data support a role for peripheral receptors in *mu*- and especially *kappa*-mediated antinociception. Moreover, they suggest that while it may be possible to separate behavioural depression from antinociception by targeting peripheral *kappa* receptors, peripherally acting *kappa* analgesics are still likely to cause diuresis.

Acknowledgements: Supported by DA03945 and DA07237 from NIDA. CI-977 was kindly supplied by Parke-Davis.

Hunter, J.C.,Leighton, G.E., Meecham, K.G., Boyle, S.J., Horwell, D.C., Rees, D.C. & Hughes, J. (1990) Br. J. Pharmac. **101**, 183-189.
Shaw, J.S., Carroll, J.A., Alcock, P. & Main, B.G. (1989) Br. J. Pharmac. **96**, 986-992.
Wheeler-Aceto, H. & Cowan, A. (1991) Psychopharmacology, **104**, 35-44.

40P EVIDENCE FOR THE INVOLVEMENT OF CENTRAL δ OPIOID RECEPTORS IN THE INTERACTION BETWEEN OPIOID ANTAGONISTS AND AMPHETAMINE

Jones D.N.C. and Holtzman S.G., Dept. of Pharmacology, Emory University School of Medicine, Atlanta, Georgia, 30322, USA. (Introduced by A.M. Domeney).

Naloxone (NX), an opioid receptor antagonist, attenuates the behavioural effects of amphetamine (AMPH) in a wide range of species and procedures (eg Trujillo et al., 1991). NX attenuation of AMPH-induced stimulation of locomotor activity has been especially well documented (eg Holtzman, 1974). The objective of the present study was to determine if the interaction between NX and AMPH is mediated centrally and the type of opioid receptor mediating this interaction. This was accomplished by examining the effects of AMPH on the motor activity of rats pretreated with one of the following opioid antagonists over a range of doses: naloxone methiodide (NX.M, non-selective opioid antagonist which does not pass the blood brain barrier), β-funaltrexamine (β-FNA, μ receptor selective), naltrindole (NTI, δ receptor selective) and nor-binaltorphimine (nBNI, k receptor selective).

Cumulative dose-response curves to AMPH were constructed (SAL, 0.1, 0.4, 1.6 and 6.4mg/kg s.c.) in Sprague-Dawley rats (male, 260-310g, n=9-12) with dosing at 30 min intervals. A recording (20 min) of both gross locomotor and fine movements was made 10 min after each injection. The drug/vehicle pretreatments were injected intracisternally (i.c., 10μl volume) under halothane anaesthesia either 15 min (NX.M and NTI) or 24 hr (β-FNA and nBNI) before testing. NX.M was also tested after systemic administration (5mg/kg s.c.). Cumulative administration of AMPH caused dose-dependent increases in both the fine (eg $F=38.97$, $P<0.0001$) and gross (eg $F=72.11$, $P<0.0001$) counts/20 min. NX.M (20 and 30 μg i.c.) attenuated the AMPH-induced increase in gross activity ($F=9.895$, $P<0.01$), such that counts/20 min for rats treated with 30 μg/kg were reduced from 1561 ± 102 (VEH) to 753.5 ± 139 ($P<0.05$) following 1.6mg/kg AMPH. Similarly, pretreatment with NTI (10 and 30μg i.c.) attenuated the gross activity response to AMPH ($F=9.57$, $P<0.01$). For example, following NTI (10 μg), counts/20min were reduced from 1381.1 ± 99.5 (VEH) to 967.4 ±133.8 ($P<0.01$) following 1.6mg/kg AMPH. The AMPH-induced increase in fine counts was not influenced by either NX.M or NTI administration at doses which attenuated the gross activity response. However, a lower dose of NX.M (2 μg) potentiated the fine activity response to AMPH ($F=4.54$, $P<0.05$) (eg. the fine counts/20 min were increased from 669 ± 114 (VEH) to 1218 ± 142 (NX.M) after 1.6 mg/kg AMPH). Pretreatment with β-FNA (1.25-20 μg i.c.), nBNI (10 and 30 μg i.c.) or NX.M (5mg/kg s.c.) failed to alter the response of rats to AMPH.

That NX.M attenuated the locomotor response to AMPH when it was administered i.c., but not when it was administered s.c., indicates a central site for the opioid antagonist/AMPH interaction. The similarity of the results obtained with NTI to those following i.c. NX.M administration, coupled with the ineffectiveness of β-FNA and nBNI, implicates δ receptors in the opioid mediated modulation of the behavioural stimulant effects of AMPH.

This work was supported in part by Grant DA00541 and Research Scientist Award DA00008 (SGH), both from NIDA, USA.

Holtzman, S.G. (1974). J.Pharm.Exp.Ther. 189 (1):51-60
Trujillo K.A., Belluzi, J.D., Stein L. (1991) Psychopharmacol. 104:265-274

41P CHRONIC EXPOSURE TO MORPHINE DOES NOT INDUCE DEPENDENCE AT THE LEVEL OF CALCIUM CURRENT IN HUMAN SH-SY5Y CELLS

C. Kennedy, Department of Pharmacology, University of Cambridge, Tennis Court Rd., Cambridge, CB2 1QJ.

μ-opioid receptors mediate inhibition of the N-type calcium channel current (I_{Ca}) present in the human neuroblastoma cell line SH-SY5Y following differentiation with retinoic acid (Seward *et al.*, 1991). We have previously shown that chronic exposure to morphine induces homologous tolerance to this effect (Kennedy & Henderson, 1991). Here we have studied whether chronic incubation with morphine (1μM for 3-7 days) also induces physical dependence at the level of I_{Ca}.

I_{Ca} was recorded using the whole cell and perforated-patch variations of the patch clamp technique, with Ba^{2+} as the charge carrier. The recording electrode contained (mM): CsCl 100, EGTA 10, $MgCl_2$ 5, ATP 2, HEPES 40, pH 7.3. Nystatin (200μg/ml) was included to obtain perforated patches. The superfusate contained (mM): NaCl 140, CsCl 5.4, $BaCl_2$ 10.8, $MgCl_2$ 1, D-glucose 10, HEPES 10, TTX 0.5μM & morphine (1μM). The possible expression of a withdrawal syndrome was examined either by adding naloxone (1μM) to the morphine-containing superfusate or by bathing the cells in a morphine-free superfusate and the resulting changes in I_{Ca} recorded.

Initial experiments were performed using the whole cell technique. On washout of morphine I_{Ca} amplitude increased by 13±1% (n=20). This was reversed by readdition of morphine. Naloxone (1μM) elicited a similar increase. This increase may be due to the reversal of a residual inhibitory effect of morphine on I_{Ca} rather than a novel withdrawal response, as in chronically-treated cells acute readministration of morphine (1μM) inhibited I_{Ca} by 11±1% (n=6). Chronic exposure to morphine did not change the voltage-sensitivity of I_{Ca} or induce the appearance of a current sensitive to the L-type calcium channel agonists Bay K 8644 (3μM) and S(+)-PN 202-791 (1μM).

In a further series of experiments the perforated-patch technique was used in order to prevent washout of any L-type activity present in the cells. Under these conditions an L-type I_{Ca} was unmasked. ω Conus Toxin GVIA (ω-CgTx) (1μM) irreversibly depressed peak I_{Ca} by approximately 90% both in control cells and cells chronically exposed to morphine. Now Bay K 8644 (3μM) almost doubled the remaining current in both groups of cells. This effect did not reverse on washout of Bay K 8644.

These results show that whilst chronic exposure to morphine induces tolerance to the inhibitory actions of morphine on the N-type I_{Ca} in the human SH-SY5Y neuroblastoma cell line (Kennedy & Henderson, 1991), it does not induce physical dependence and a withdrawal syndrome at the level of this current. The L-type I_{Ca} revealed by ω-CgTx treament in perforated-patch recordings was also unchanged. Perhaps only certain sub-populations of opioid-sensitive neurones express the cellular mechanisms required for dependence to develop.

C.K. was a Beit Memorial Research Fellow. The collaboration of Dr. G. Henderson throughout this study is gratefully acknowledged.

Kennedy, C. & Henderson, G. (1991) Mol. Pharmacol. *in press.*
Seward, E.P., Hammond, C. & Henderson, G. (1991) Proc. Roy. Soc. Lond. B. **244**, 129-135.

42P HISTAMINE H_3 RECEPTOR-MEDIATED MODULATION OF [^{3}H]-ACETYLCHOLINE RELEASE FROM SLICES OF RAT ENTORHINAL CORTEX

J. Clapham and G.J. Kilpatrick; Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DP.

The histamine H_3 receptor was first characterized as a presynaptic autoreceptor regulating the release of histamine in the central nervous system (CNS; Arrang et al., 1983). Subsequent studies have shown that activation of H_3 receptors inhibits the release of several neurotransmitters in both the peripheral and central nervous systems (see Timmerman, 1990). H_3 receptors modulate cholinergic transmission in the periphery (Poli et al., 1991) but no information is available regarding their effects on acetylcholine release in the CNS. We have therefore examined the effect of the selective H_3 receptor agonist, (R)α-methylhistamine (RAMH), and a number of H_3 receptor antagonists, including the highly selective compound thioperamide, on the release of tritium from slices of rat entorhinal cortex preloaded with tritiated choline.

The entorhinal cortex from male Lister Hooded rats (250-300g) was chopped transversely to produce 0.3mm slices. The slices were depolarized and loaded with [^{3}H]-choline as described by Barnes et al. (1989). They were then placed in chambers and superfused with aerated Krebs solution (37°C; $CaCl_2$ 1.25mM) containing hemicholinium-3 (0.1μM). Tritium release was evoked by exposure to KCl(K^+;20mM) for 4 minutes. Agonists were included 20min prior to the K^+ stimulation and antagonists were present from the start of the equilibration period (60mins) onwards. Drug effects were quantified on their ability to either increase or inhibit the K^+-evoked release as compared with control.

Inclusion of K^+(20mM) in the perfusate resulted in a calcium dependent release of tritium. The selective H_3 agonist, RAMH, inhibited the K^+-evoked release in a concentration-dependent manner with no effect on basal release. The apparent pD_2 value of RAMH was 5.9; this was increased approximately 10-fold to 7.0 when the H_1 antagonist mepyramine (3μM) and the H_2 antagonist ranitidine (10μM) were included in the superfusion medium. These antagonists were therefore present in subsequent experiments. The maximal inhibitory effect of RAMH (3μM) was 48.4±2.5% (n=3). The RAMH concentration response curve was displaced to the right in a parallel fashion by the selective H_3 receptor antagonist thioperamide (0.03μM) with no decrease in the maximum inhibition. A pK_B value of 8.6 was calculated for thioperamide. The non-selective H_3 antagonists impromidine and burimamide also inhibited the response to RAMH and pK_B values of 6.2 and 6.7 respectively were obtained. Thioperamide (1μM) alone enhanced K^+-evoked tritium release by 23.0±6.7% (n=3).

The inhibition of tritium release from slices preincubated with [^{3}H]-choline by RAMH and potent reversal of this by H_3 receptor antagonists indicates that H_3 receptors control acetylcholine release from slices of rat entorhinal cortex. The enhancement of tritium release by the H_3 receptor antagonist thioperamide indicates that there may be some endogenous histamine tone. We therefore conclude that H_3 receptors have a modulatory role in the release of acetylcholine in the central nervous system.

Arrang, J.M., Garbarg, M. and Schwartz, J.C. (1923) Nature, 302, 832-837.
Barnes, J.M., Barnes, N.M., Costall, B., Naylor, R.J. & Tyers, M.B. (1989) Nature **338**, 762-763.
Poli, E., Stark, H. & Bertaccini, G. (1991) Agents & Actions **33**, 167-169.
Timmerman, H. (1990) J. Med. Chem. **33**, 4-11.

43P EFFECT OF GALANIN ON HIPPOCAMPAL ACETYLCHOLINE RELEASE AND PERFORMANCE OF RATS IN THE MORRIS WATER MAZE

Sue Aspley, Kevin C.F. Fone, Geoffrey W. Bennett & Charles A. Marsden
Dept. of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

The neuropeptide galanin (Gal) co-exists with choline acetyltransferase (ChAT) in septohippocampal and basal forebrain neurones which if lesioned impair performance of rats on a test of spatial memory (Hagan & Morris, 1988). Gal inhibits K^+- evoked release of [^{3}H] acetylcholine (ACh) from rat ventral hippocampal (Hip) slices *in vitro* (Fisone *et al.*, 1987) consistent with a role for this neuropeptide in cholinergic regulation. The present study examined the effect of porcine Gal on; i). endogenous ACh release *in vitro* measured using a modified HPLC technique, and ii). the performance of rats in a water maze learning paradigm, coupled with the post mortem levels of selected neurochemical markers

Hip slices from male Hooded Lister rats were washed in Ca^{2+}-free Krebs and suspended in Krebs containing neostigmine (3.8μM), choline (2μM) and Ca^{2+} (1.25mM) to measure the effect of K^+ and Gal on ACh released into supernatants using HPLC with electrochemical detection (Damsma *et al.*, 1987), with the modification that supernatant choline was removed by a choline oxidase loaded pre-column (Aspley *et al.*, 1991). In behavioural experiments, individually housed male Hooded Lister rats (260-340g) were cannulated above the lateral ventricle under Halothane anaesthesia. Following 10 days recovery rats were tested (twice daily, 180s each for 4 or 5 days) on a 2m diameter water maze (25°C, filled with opaque water, Hagan & Morris, 1988), 15min after pretreatment with either Gal (1 or 10μg in 5μl saline, i.c.v., n=8 and 11, respectively) or saline (5μl, n=11 and 12). On the last two days respectively, rats were dosed as before but i). subjected to a 60s transfer test (time spent in the quadrant from which the platform had been removed was measured) and ii). Hip and frontoparietal cortex (FPC) were taken for the analysis of ChAT activity by radioenzymatic assay and 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and dopamine (DA) levels by HPLC with electrochemical detection. Results are presented as mean ± s.e.mean and Student's t-test was used for statistical analysis unless otherwise stated.

In vitro basal ACh release (27 ± 5 pmols/mg protein, n=17) was significantly elevated by the addition of increasing concentrations of K^+ (by 66 ± 40%, $P>0.05$, 62 ±17%, $P<0.01$, and 170 ± 37%, $P<0.001$ by 10, 15 and 50mM K^+), an effect attenuated by removal of Ca^{2+}. K^+-evoked release (15mM) was significantly inhibited by 10 and 100nM Gal (by 26 ± 10%, $P<0.05$ and 20 ± 3%, $P<0.01$ respectively). Control and Gal treated animals showed equal acquisition of the maze task (two-way ANOVA F=0.17, P=0.90, 1μg Gal and F=0.19, P=0.67, 10μg Gal, compared with saline) and similar performance in the subsequent transfer test (25 ± 3s saline and 25 ± 2s 1μg Gal, P=0.87; and 20 ± 3s saline and 20 ± 2s 10μg Gal, P=0.98). Neither dose of Gal changed ChAT activity or 5-HT and 5-HIAA levels in the Hip or FPC but 1μg significantly increased DA in the FPC (from 6.3 ± 0.6 to 11.8 ± 2.5 pmoles/mg protein, $P<0.02$).

In this study *in vitro* Gal inhibited K^+-evoked endogenous Hip ACh release, but Gal i.c.v. failed to alter learning in the water maze despite the fact that previous studies using a different protocol and strain of rat (Wistar) have shown inhibition of the aquisition of a water maze task by 1 and 5μg Gal i.c.v. (Sundstrom *et al.*, 1988). Taken together, these data suggest that the role of Gal in cognition remains unclear.

We thank Merck Sharp & Dohme for financial support.

Aspley, S., Kulkarni, R.S., Fone, K.C.F., Bennett, G.W. & Marsden, C.A. (1991) In *Monitoring Molecules in Neuroscience* eds Rollema, Westerink & Drijfhout, pp297-299. Groningen: University Centre for Pharmacy.
Damsma, G., Westerink, B.H.C., DeVries, J.B., Van den Berg, C.J. & Horn, A.S. (1987) J. Neurochem. **48**, 1523-1528.
Hagan, J.J. & Morris, R.G.M. (1988) In *Handbook of Psychopharmacology* eds. Iversen, Iversen & Snyder, pp 237-325. New York: Plenum.
Fisone, G., Wu, C.F., Consolo, S., Nordstrom, O., Brynne, N., Bartfai, T., Melander, T. & Hokfelt, T. (1987) Proc. Natl. Acad. Sci. USA **84**, 7339-7343.
Sundstrom, E., Archer, T., Melander, T. & Hokfelt, T. (1988) Neurosci. Lett. 88, 331-335.

44P LIPOCORTIN-1 INHIBITS THE NEUTROPHIL INFILTRATION ELICITED BY INTERLEUKIN-1 IN THE MOUSE AIR-POUCH

M Perretti & R J Flower. Dept. Biochemical Pharmacology, St. Bartholomew's Hospital Medical College, Charterhouse Sq, London EC1M 6BQ

Interleukin-1 (IL-1) is a pro-inflammatory cytokine which has been implicated in the migration of polymorphonuclear leukocytes (PMN) in the early stages of the inflammatory response. In particular, PMN migration has been clearly observed after IL-1 injection in localized sites such as the knee joint and the skin of the rabbit and the peritoneal cavity of both mice and rats (Pettipher et al., 1986, Rampart & Williams, 1988, Faccioli et al., 1990). Human recombinant lipocortin-1 (LC1) is a glucocorticoid-inducible protein with anti-inflammatory effects in the rat carrageenin oedema model (Cirino et al., 1989,). In this study we have investigated PMN infiltration into the mouse air-pouch following IL-1 injection, and evaluated the systemic and local effects of either LC1 or dexamethasone (DEX) administration.

Pouches were formed on the back of male T.O. mice (22-25 g) by s.c. injection of 2 ml of air on day 0 and 3. On day 6 animals received either IL-1α or IL-1β (20 ng) injected locally in 0.5 ml of carboxymethylcellulose (0.5% in phosphate buffered saline, PBS). Pouches were washed 4 h after the cytokine administration with 2 ml of PBS (+ heparin 50 Uml^{-1}) and, after centrifugation at 900 rpm x 10 min at 4°C, PMN were counted following staining with Turk's solution (1:10 dilution). In some experiments mice were treated i.v. with either DEX (2 h before IL-1) or LC1 (30 min before IL-1). In other experiments DEX or LC1 were injected directly in the pouch concomitantly with IL-1. In all cases pouches were washed 4 h following IL-1 treatment.

Injection of either IL-1α or IL-1β resulted in an intensive and consistent PMN migration (8.26 ± 0.44 x 10^6 PMN per mouse, n=25 and 7.88 ± 0.41 x 10^6 PMN per mouse, n=30, respectively). The effect of DEX and LC1 treatment is reported in the following Table:

Route	Drug	Dose/Mouse	Migration (% Inhibition ± s.e.m.)	n	Student's t test
Systemic	DEX	5μg	37.6±5.8	6	p<0.05
Systemic	DEX	50μg	63.8±4.1	6	p<0.01
Systemic	LC1	10μg	89.5±2.5	5	p<0.01
Systemic	LC1	5μg	43.6±9.2	6	p<0.05
Systemic	LC1	1μg	24.9±9.0	6	N.S.
Systemic	LC1 Inactived	10μg	11.2±1.8	7	N.S.
Local	DEX	1μg	62.3±5.7	7	p<0.01
Local	LC1	10μg	10.0±0.9	5	N.S.

Inasmuch as specific binding sites for LC1 on human PMN have been recently described (Goulding et al., 1990), the inhibitory activity of LC1 on IL-1 induced migration observed only after systemic treatment might be explained through a direct effect on these leukocytes. By way of contrast, the glucocorticoid hormone DEX was active after both systemic and local administration and may inhibit the cytokine action at level of the endothelial wall, which is activated after treatment with IL-1.

R E Pettipher et al., (1986) PNAS 83: 8749-8753
M Rampart & T J Williams, (1988) Br. J. Pharmacol. 94: 1143-1148
L H Faccioli et al., (1990) Agents Actions. 30: 344-349
G Cirino et al., (1989) PNAS. 86: 3428-3432
N J Goulding et al., (1990) Biochem. Soc. Trans. 18: 1237-1238

The financial support of Ono Pharmaceutical Co. Japan is acknowledged. RJF is funded by an endowment from Lilly. We thank Dr J Browning (Biogen Research Corp. USA) for the generous gift of human recombinant lipocortin 1.

45P PGE_2 AND EP_3 AGONISTS INDUCE CHEMOTAXIS OF HUMAN NEUTROPHILS *IN VITRO*

Roma A Armstrong, Department of Phamacology, University of Edinburgh, Edinburgh EH8 9JZ.

Using the rabbit skin inflammation model, we have recently demonstrated the existence of a leukocyte-dependent mechanism involved in the pro-inflammatory actions of PGE_2, which operates through an EP_3 receptor (Armstrong & Jones, 1991). The aim of this study was to determine if EP_3 agonists are chemotactic for human neutrophils *in vitro*. Fresh heparinised human blood was layered onto mono-poly resolving medium and centrifuged at 450 g for 30 min at 20°C. The neutrophil fraction was removed and washed several times with sterile saline. Cell count and viability were determined using trypan blue. Chemotaxis was measured using blindwell chambers with 3 μm Nuclepore polycarbonate filters, where 200 μl of chemo-attractant was placed in the bottom well, and 400 μl of cells (1.5×10^5 cells ml^{-1} in RPMI medium containing 10 mM HEPES and 1% serum albumin) in the top compartment. After incubation for 45 min at 37°C in a moist, 5% CO_2 atmosphere, filters were stained with Diff Quik and cells on either side of the filter counted. The chemotactic responses are expressed as total number of neutrophils which have migrated through the filter (sum of 5 fields). Results have been compared with those of formyl-methionyl-leucyl-phenylalanine (FMLP) which is an established activator of human neutrophils (Snyderman & Pike, 1984)), and are given in Table 1.

Table 1 Chemotaxis of human neutrophils *in vitro*. (mean ± s.e.m. n =5 - 8) Control values for RPMI buffer have been subtracted (21.2 ± 6.3).

	[cells which have migrated]			[cells adhering to cell-side of filter]		
Concentration (nM)	15	150	1500	15	150	1500
EP_3 agonists						
M&B 28,767	69.0 ± 23.0	50.6 ± 7.5	41.7 ± 8.8	283.7 ± 68.3	314.7 ± 83.9	281.6 ± 99.5
sulprostone	51.8 ± 13.7	51.1 ± 10.8	49.8 ± 8.9	358.9 ± 78.5	424.9 ± 103.6	378.9 ± 99.1
GR 63799X	55.7 ± 14.4	30.7 ± 9.1	19.2 ± 10.2	407.5 ± 194.4	355.0 ± 180.3	308.0 ± 77.0
oxoprostol	63.4 ± 30.2	86.0 ± 27.1	47.8 ± 21.5	183.5 ± 64.2	312.0 ± 82.1	346.5 ± 93.7
EP_3/EP_2 agonists						
PGE_2	35.2 ± 10.9	48.4 ± 28.3	24.7 ± 5.4	415.0 ± 88.7	347.7 ± 121.5	371.0 ± 162.9
misoprostol	129.8 ± 61.8	231.0 ± 97.8	81.3 ± 40.4	167.5 ± 80.5	269.2 ± 77.2	254.0 ±79.3
mexiprostil	120.8 ± 49.1	64.6 ± 20.9	80.8 ± 40.1	319.2 ± 113.2	265.5 ± 106.9	191.5 ± 126.8
enisoprost	70.0 ± 37.5	48.7 ± 30.7	15.0 ± 7.0	373.2 ± 134.0	229.2 ± 136.2	65.3 ± 32.0
nocloprost	103.0 ± 41.0	84.6 ± 29.8	36.6 ± 9.6	168.6 ± 36.3	352.2 ± 73.4	272.2 ± 67.3
FMLP (1 and 5 μM)		128.0 ± 25	166.7 ± 20.7		133.6 ± 23.4	187.1 ± 56.8

EP_3 agonists induce chemotaxis of human neutrophils at very low concentrations, with a maximum effect at 15 nM. Misoprostol was the most effective analogue tested, reaching a similar maximum to FMLP, but at a 70 fold lower concentration. Interestingly, EP_3 agonists promote adhesion of neutrophils to the filter itself. A previous study showed that PGE_2 inhibited chemotaxis (Goetzl & Gorman, 1978), but the authors used concentrations of 3 - 75 μM. It is likely that at high concentrations the stimulatory effect of PGE_2 operating through an EP_3 receptor is masked by an inhibitory effect linked to stimulation of adenylate cyclase, as can be seen with EP_2 agonists, such as enisoprost and nocloprost.

Armstrong, R.A. & Jones, R.L.,(1991) Br. J. Pharmacol Proc. Suppl., **102**, 92P.
Goetzl, E.J, & Gorman, R.R..(1978) J. Immunol., **120**, 526 - 531.
Snyderman, R. & Pike, M.C. (1984) Ann. Rev. Immunol., **2**, 257 - 281.

46P BASAL AND ANGIOTENSIN II-STIMULATED PROSTACYCLIN RELEASE BY PERFUSED RAT LUNG IN EXPERIMENTAL DIABETES: EFFECTS OF INSULIN AND ALDOSE REDUCTASE INHIBITION

E.J. Stevens, G.B. Willars & D.R. Tomlinson, William Harvey Research Institute, Department of Pharmacology, Queen Mary and Westfield College, Mile End Road, London E1 4NS

Macro- and microvascular complications are a major cause of morbidity and mortality in patients with diabetes mellitus but their aetiology is uncertain. As one indicator of endothelial cell function we examined prostacyclin release, measured as immunoreactive 6-keto prostaglandin $F_{1\alpha}$, from isolated perfused rat lung (5ml/min KRB, 1%BSA, pH7.4, 37°C, 95%O_2/5%CO_2). Perfusion pressure was also monitored throughout. A 30min equilibration period was followed by a 3min basal period. A transient increase in perfusion pressure and prostacyclin release was then provoked (peaks at approximately 1min after starting infusion) by a 5min infusion of angiotensin II (AII) (0.125ml/min, final concentration 1.2×10^{-6}M). Prostacyclin was measured in 1min aliquots of perfusate from the basal and stimulated periods. Data are means±SD. Lungs were taken from control rats (n=9, 459±41g) and 4-6 week diabetic rats (streptozotocin 65mg/kg i.p.) either untreated (n=10, 283±50g) or treated twice-daily with insulin (n=9, 417±32g). A diabetic group treated with an aldose reductase inhibitor (ARI) (n=9, 282±26g) (imirestat, Hoechst, 1mg/kg/day p.o.) was included to explore the role of exaggerated polyol pathway flux in any defects. ARI treatment was effective, in that it prevented the accumulation of sciatic nerve sorbitol (nmoles/mg; control 0.14±0.13, diabetic 5.39±2.09, diabetic-ARI 0.39±0.19). This accumulation was also prevented by insulin (0.57±0.61). Lung wet weight, dry weight and protein content were similar in all groups. There were no differences in basal perfusion pressure (for all animals 5.1±3.3mmHg) or the peak response to AII (22.3±6.1mmHg). However, areas under the curves during AII infusion, both total and above basal, were greater in diabetics (3625±1385 and 2145±749 mmHg.s respectively) compared to controls (2581±573 and 1416±188). These elevations were prevented by insulin (2774±947 and 1235±234) but not the ARI (3307±756 and 1954±558). Prostacyclin release was expressed as both a perfusate concentration and in relation to lung protein content and dry weight. Irrespective of the method of expression there was considerable within group variability and there were no significant differences in basal or stimulated release. Despite this, the mean profiles of perfusate concentration suggested a trend toward reductions in basal and stimulated release in untreated and ARI-treated diabetic groups compared to the other groups. Numerical reductions in mean stimulated release were not apparent when basal release was subtracted. Indications of differences were diminished when release was referenced to units of lung mass. These data indicate an enhanced pressure response to AII in perfused diabetic lung which was prevented by insulin but not ARI. Despite these changes and evidence of a trend toward reduced levels of prostacyclin from diabetic and diabetic-ARI rat lung there were no significant differences in prostacyclin release.

This work was funded by Ono Pharmaceutical Co. Ltd., Osaka, Japan.

47P THE OXIDATIVE MODIFICATION OF LOW-DENSITY LIPOPROTEIN BY POLYMORPHONUCLEAR LEUKOCYTES (PMNs)

L A Forster, M Katsura and E E Änggård, The William Harvey Research Institute, St Bartholomew's Hospital Medical College, Charterhouse Square, London, UK

The oxidative modification of low-density lipoprotein (LDL) is seen as a key event in the development of atherogenesis (Steinberg et al, 1989). Endothelial cells, smooth muscle cells and macrophages have been reported to possess the ability to oxidatively modify LDL in vitro. These cells are present in atherosclerotic lesions and hence, their ability to modify LDL could be important. Polymorphonuclear leukoyctes (PMNs) are transient cells and are not present in atherosclerotic lesions. However, they accumulate at sites of endothelial injury (Ramsey et al, 1982) and could, therefore, play a role in the initiation of atherogenesis. Here we report that PMNs can oxidatively modify LDL to a form which is more rapidly taken up by macrophages.

^{125}I-LDL (100 μg/ml) was incubated with PMNs (4 x 10^6 cells/ml) for various time intervals up to 20 h in Ham's F-10 medium and oxidative modification of the LDL was assessed by measurement of thiobarbituric acid reactive substances (TBARS). Uptake of the modified LDL by the macrophage scavenger pathway was assessed by measurement of cholesteryl ester formation and LDL-degradation products in mouse peritoneal macrophages.

Incubation of LDL with PMNs for 4 - 20 h resulted in the appearance of TBA reactive materials. The oxidative modification of LDL was found to be both time and cell number-dependant and plateaued after 15 h incubation and at 6 x 10^6 PMNs/ml respectively. The level of oxidation was similar to that obtained upon incubation of LDL with rabbit aortic endothelial cells (50-70 nmol MDA equivalents/mg LDL protein). The PMN-modified LDL showed increased uptake into mouse peritoneal macrophages as compared to LDL incubated in the absence of PMNs (4.33 $\pm$ 1.00 as compared to 0.27 $\pm$ 0.12 nmol cholesteryl ^{14}C-oleate formed/mg macrophage protein). The PMN induced modifications were completely inhibited by the antioxidants BHT (20 μM) and EDTA (50 μM) but were unaffected by protease inhibitors. Inhibition of oxidative modification by antioxidants also resulted in less degradation by macrophages; PMN modified LDL showed a single band with increased electrophoretic mobility on agarose gel, the mobility was the same as that of copper ion oxidised LDL.

We have observed for the first time that PMNs have the ability to oxidize LDL. The PMN-modified LDL was incorporated faster by macrophages and could, therefore, be a contributory factor in the initial stages of atherogenesis. ***(This work was supported by a grant from the ONO Pharmaceutical Company, Japan)***

Ramsay M M et al (1982); Atherosclerosis 43 (2-3), 233-243
Steinberg D et al (1989); N. Eng. J. Med. 320 (14) 915-924

48P IMPORTANCE OF ENDOGENOUS CORTICOSTEROIDS IN THE CONTROL OF THE CELLULAR IMMUNE RESPONSE IN THE BROWN NORWAY RAT

S H Peers, G S Duncan, C Bolton* & R J Flower, Dept. Biochemical Pharmacology, St Bartholomew's Hospital Medical College, Charterhouse Sq, London EC1M 6BQ, *Pharmacology Group, University of Bath, Claverton Down, Bath BA2 7AY.

Corticosteroids (CCS) are widely used anti-inflammatory and immunosuppressive drugs, however control of the immune system by endogenous CCS is less well understood. It has been suggested that the susceptibility of the Lewis rat to cell-mediated autoimmune adjuvant arthritis or experimental autoimmune encephalomyelitis (EAE) may be due to a 'dysfunctional' hypothalamo-pituitary-adrenal (HPA) axis (Mason et al, 1990, Sternberg et al, 1989) although we have shown that the antibody response in this strain is limited by endogenous CCS (Peers et al, 1991). The Brown Norway (BN) rat is less susceptible to EAE (Levine & Sowinski, 1975). We have investigated the importance of endogenous CCS in control of the cellular immune response in this strain by investigating the effects of in vivo treatment with the steroid antagonist RU486 (mifepristone).

Male Lewis and BN rats (Olac, UK, 250g) were injected s.c. with 0.2ml complete Freund's adjuvant containing 0.5mg ovalbumin (OA). Dexamethasone sodium phosphate (0.01mg/kg s.c.) or RU486 (20mg/kg p.o.) was administered daily on days 0-4 and 7-11 post inoculation (p.i.). Rats were killed on day 14-16 p.i. (2 per experiment) spleens removed and leukocytes prepared by conventional techniques. Cells were incubated at 2 x 10^6/ml for 48h with stimuli as appropriate (6 replicates) before being pulsed with ^{3}H-thymidine (1μCi/well) and incubated for a further 16-20h before harvesting. Results are shown as cpm incorporated/well.

Table 1: Proliferation of Lewis and BN splenic leukocytes, cpm mean $\pm$ s.e.m. (n experiments)

Stimulus	Lewis (4)	BN Control (7)	RU (4)	dex (6)
nil	24158±1498[a]	6238 ± 951	6892± 371	7997±1939
OA 1μg/ml	33534±3557[ab]	6219 ± 860	10403± 426[ab]	7052±1776
10μg/ml	42846±3857[ab]	6309 ±1218	11269±1821[ab]	6296±1724
PHA 2μg/ml	NT	22803±4338[b]	32462±2301[b]	17462±5544
PWM 0.1μg/ml	71065±5761[ab]	28746±5070[b]	23893±5511[b]	11343±1498[ab]

a P <0.05 vs BN con, appropriate stimulus b P <0.05 vs no stimulus; unpaired t test.

Unstimulated cells from control BN rats proliferate less than those from the Lewis in vitro. Furthermore, cells from control sensitised BNs do not respond in vitro to antigen (Table 1). Although the cpm incorporated in response to lectins phytohaemagglutinin (PHA) and pokeweed mitogen (PWM) are less than in the Lewis, the stimulation index (S.I.) is higher (4.61 and 2.94 BN and Lewis respectively, with PWM). Unstimulated proliferation is not affected by RU486 treatment in vivo, however the response to PHA is greater (S.I. 4.71 vs 3.66) and in addition, cells from these rats proliferate in response to antigen. Dexamethasone treatment in vivo tends to reduce proliferation occurring in response to stimuli.

In conclusion, splenic leukocytes from the BN rat show lower proliferative responses in vitro than do cells from the Lewis, and do not respond to antigen. This response is revealed by treatment with RU486 suggesting that in this strain, endogenous corticosteroids may influence the development of a cellular response to antigen in vitro. This supports observations made concerning the development of EAE in this strain.

We acknowledge the financial support of the Ono Pharmaceutical Co, Japan. RJF is funded by an endowment from Lilly and CB by the Multiple Sclerosis Society. We thank Roussel-Uclaf for kind gifts of RU486.
Levine S & Sowinski R (1975) J.Immunol. 114: 597-601
Mason D, MacPhee I & Antoni F (1990) Immunology 70: 1-5.
Peers SH, Duncan GS & Flower RJ (1991) Br.J.Pharmac. 104: 92P
Sternberg EM, Hill JM, Crousos GP, Kamilaris T, Listwak SJ, Gold PW & Wilder RL (1989) Proc.Natn.Acad.Sci. USA 86: 2374-2378

49P THE ROLE OF COMPLEMENT, PAF AND LEUKOTRIENE B_4 IN THE REVERSED PASSIVE ARTHUS REACTION IN RABBIT SKIN

A.G. Rossi, D. Donigi-Gale[1], T.S. Shoupe[1], R. Edwards, K.E. Norman & T.J. Williams, Department of Applied Pharmacology, National Heart & Lung Institute, Dovehouse Street, London SW3 6LY, UK and [1]Department of Molecular Discovery, Purdue Frederick, Norwalk, CT, USA.

We investigated the involvement of complement, platelet activating factor (PAF) and leukotriene B_4 (LTB_4) in the reversed passive Arthus reaction (RPA) and the response to zymosan in the rabbit skin. The compounds used were recombinant soluble human complement receptor type 1 (sCR1; Yeh et al., 1991); the PAF antagonists, WEB 2086 and PF10040 [1-(3,4-dimethoxyphenylethyl)-6-methyl-3,4-dihydroisoquinoline hydrochloride] and the LTB_4 antagonist, LY-255283.

Rabbit dorsal skin was shaved and ^{125}I-albumin with Evans blue dye injected i.v. After 10 min, anti-bovine-γ-globulin (BGG) antiserum or zymosan with or without sCR1 (10μg/site) were injected i.d (0.1ml vol). Alternatively, antiserum, PAF or LTB_4 were injected i.d. with or without their antagonists. After a further 5 min an i.v. injection of antigen (BGG; 5mg/kg) was administered and oedema formation measured after 4h in 17mm diameter skin punch samples. The results are expressed as the mean$\pm$s.e.mean μl plasma leakage subtracted for counts in saline-injected sites; responses to PAF and LTB_4 were measured in the presence of the vasodilator, PGE_2 ($3x10^{-10}$mol/site) (Wedmore & Williams, 1981). The effect of sCR1 (10μg/site) on oedema formation produced by different titres of antisera and by zymosan (300μg/site) in 7-9 rabbits was examined; 100, 50, 25, and 12.5% antiserum induced responses of 40.8 ± 5.9, 33.3 ± 6.1, 20.4 ± 2.9, and 12.3 ± 1.8, which were inhibited by sCR1 to 28.8 ± 3.4 ($p<0.05$), 22.2 ± 3.0 ($p<0.05$), 9.1 ± 1.9 ($p<0.01$) and 4.9 ± 1.4 ($p<0.01$) respectively. Similarly, sCR1 reduced the response to zymosan from 45.2 ± 8.6 to 28.2 ± 4.8 ($p<0.01$). PAF (10^{-10}mol/site) induced 24.5 ± 4.3 (n=9 rabbits), a response that was reduced by WEB 2086 (10^{-7}mol/site) and PF10040 (10^{-7}mol/site) to 3.8 ± 0.7 ($p<0.01$) and 12.0 ± 2.3 ($p<0.05$). WEB 2086 (10^{-7}mol/site) and PF10040 (10^{-7}mol/site) suppressed oedema in the RPA reaction induced by undiluted antiserum by 34.3 ± 9.1% ($p<0.05$; n=7 rabbits) and 56.9 ± 13.9% ($p<0.01$; n=7 rabbits) in accordance with previous results using the PAF antagonist, L-652731 (Hellewell & Williams, 1986). In 6 additional rabbits we showed that although the PAF antagonists inhibited RPA and PAF induced oedema formation, they did not inhibit the zymosan (300μg/site) induced response. The LTB_4 antagonist, LY-255283 (10^{-7}mol/site), inhibited LTB_4 ($5x10^{-10}$mol/site) induced oedema from 40.7 ± 8.2 to 13.9 ± 3.0 ($p<0.01$; n=7 rabbits), whereas the compound did not inhibit PAF or RPA induced responses.

Thus sCR1, administered locally in rabbit skin, suppresses oedema formation in the RPA and induced by zymosan suggesting that complement plays a fundamental role in these responses. Furthermore, the PAF antagonists implicate PAF in the RPA but not in the response to zymosan. LY-255283 inhibited LTB_4-induced oedema formation but not the leakage induced in the RPA reaction suggesting that LTB_4 does not play a direct role in increased vascular permeability in the RPA.

We thank Purdue Frederick, USA for supplying PF10040; SmithKline & Beecham, UK for sCR1; Lilly, USA for LY-255283 and Boehringer Ingelheim, UK for WEB 2086. AGR is Purdue Frederick Research Fellow.

Hellewell, P.G. & Williams, T.J. (1986) J. Immunol. **137**, 302-307
Wedmore, C.V. & Williams, T.J. (1981) Nature **289**, 646-651
Yeh, C.G., Marsh, H.C., Carson, G.R., Berman, L., Concino, M.F., Scesney, S.M., Kuestner, R.E., Skibbens, R., Donahue, K.A. & Ip, S.H. (1991) J. Immunol., **146**, 250-256

50P INHIBITION OF ARACHIDONIC ACID-INDUCED RABBIT PLATELET AGGREGATION *IN VITRO* BY LIGANDS WHICH SELECTIVELY BIND PERIPHERAL-TYPE BENZODIAZEPINE SITES

C.N. Berry & A.Choppin, Synthelabo Recherche (L.E.R.S.), 31 Ave. P.-V. Couturier, 92220 Bagneux, France.

Peripheral-type benzodiazepine binding sites (BZp) have been found on platelets from various species (O'Beirne & Williams, 1984, inter alia). However, as yet, no functional role has been clearly ascribed to them. Recently, Fonlupt et al. (1990) showed that arachidonate (AA)-induced human platelet aggregation can be inhibited by ligands acting at this site, we therefore decided to test a series of BZp-selective ligands on AA-induced rabbit platelet aggregation in-vitro.

Citrated platelet rich plasma (300 μl) was incubated at 37°C with test compound or vehicle (DMSO 0.3% final concentration) for one minute before adding a submaximal concentration of arachidonic acid (30-40 μg/ml). Platelet aggregation was followed for 5 minutes in a 4 channel aggregometer and the area under the curve was determined, after which time, the samples were acidified and thromboxane B_2 (TXB_2) was extracted twice into ethylacetate (2 x 1 ml). The dried extracts were assayed for TXB_2 by radioimmunoassay. The inhibition of each parameter was quantified by determination of the mean IC_{50} $\pm$ s.e.mean (μM) of 4-5 separate experiments.

The BZp-selective ligands Ro 5-4864 (Ro, Schoemaker et al., 1983), PK-11195 (PK, Le Fur et al., 1983) and PK-14067 (Q(-), Dubroeucq et al., 1986) inhibited aggregation and TXB_2 synthesis with IC_{50} values of 0.19 $\pm$ 0.06 and 0.22 $\pm$ 0.11 (Ro), 0.31 $\pm$ 0.05 and 0.38 $\pm$ 0.07 (Q-), and 0.71 $\pm$ 0.52 and 0.60 $\pm$ 0.20 (PK) respectively; whereas, clonazepam (2.9 $\pm$ 1.6 versus aggregation), diazepam (3.3 $\pm$ 0.5 and 3.5 $\pm$ 0.7) and the + enantiomer of Q(-), PK-14068 (op. cit. 2.4 $\pm$ 0.8 and 3.2 $\pm$ 0.4) were less active against aggregation and TXB_2 synthesis with flumazenil being completely inactive (> 600 μM in both tests). There was a significant ($P < 0.01$) correlation between the inhibition of aggregation and TXB_2 synthesis suggesting cyclooxygenase to be the site of action. When, under standard conditions, the concentration of aggregant was increased, the log-dose inhibition curve of PK-11195 was shifted to the right. The inhibition of aggregation by PK-11195 at 0.2 μM (9 $\pm$ 4 %, n=5) was significantly ($P<0.01$) increased to 56 $\pm$ 9 % (n=5) under conditions which favoured intracellular penetration, i.e. doubling the solvent volume and 20 minutes pre-incubation (the appropriate controls were performed).

We conclude that platelet cyclooxygenase possesses certain characteristics of BZp receptors such as intracellular location, preferential sensitivity to BZp-specific ligands and stereoselectivity.

Dubroeucq, M.C. et al. (1986) Eur. J. Pharmacol. 128, 269-272
Fonlupt, P. et al. (1990) Br. J. Pharmacol. 101, 920-924
Le Fur, G. et al. (1983) Life Sci. 32, 1849-1856
O'Beirne, G.B. & Williams, D.C. (1984) Biochem. Pharmacol. 33, 1568-1571
Schoemaker, H. et al. (1983) J. Pharmacol. Exp. Ther. 225, 61-69

51P COPPER CHELATORS INHIBIT CYCLOOXYGENASE AND LIPOXYGENASE ACTIVITY BY WASHED HUMAN PLATELETS, *IN VITRO*

J.Y. Jeremy, G. Sifakis, J. Gill & [1]G. Kontoghiorghes, Department of Chemical Pathology and Human Metabolism and [1]Department of Haematology, Royal Free Hospital and School of Medicine, Pond Street, London NW3 2QG

Copper imbalance is associated with cardiovascular disease (CVD). Divalent copper has also been shown to modulate eicosanoid synthesis (Elliot *et al*, 1987; Mitchell *et al*, 1988). Platelet thromboxane synthesis and lipoxygenase activity has been implicated in the pathophysiology of CVD. In order to explore the role of copper in modulating eicosanoid synthesis by platelets, the effect of the Cu^{2+} chelators, dimethyldithiocarbamic acid (DMtC), diethyldithiocarbamic acid (DEtC) and tetraethylthiuram disulphide (TETD) on TXA_2 synthesis and hydroxy-eicosatetraenoic acid (HETE) synthesis by isolated human platelets was investigated. Platelets were obtained from healthy volunteers. Platelets were washed with HEPES containing apyrase and resuspended in HEPES buffer. Aliquots of platelets were incubated with DMtC, DEtC and TETD for 15 min at 37 °C prior to the stimulation of TXA_2 synthesis with the following stimulators: arachidonic acid (AA; 10 μM), calcium ionophore A23187 (10 μM), NaF (10 μM), phorbol ester dibutyrate (1 μM); and spontaneous release. Platelets were further incubated for 20 min at 37°C. The reaction was stopped by addition of ethanol, and aliquots of supernatant taken for measurement of TXB_2 by radioimmunoassay. Conversion of AA to HETE was assessed by the addition of [^{14}C]-AA to platelets suspended in HEPES containing various concentrations of Cu^{2+} chelators. Following incubation for 15 min at 37 °C, the reaction was stopped and HETE and unchanged AA extracted with chloroform/methanol (2:1). HETE was separated from AA by thin layer chromatography, counted for radioactivity and percentage inhibition of AA conversion to HETE calculated.

DMtC inhibited TXA_2 synthesis in a concentration-dependent manner. IC_{50}s (concentration at which DMtC inhibited TXA_2 synthesis by 50%; derived from 10 experiments) were similar for all stimulators (AA, 33 μM; A23187, 35 μM; NaF, 34 μM; phorbol ester, 30 μM; spontaneous, 28 μM). The similarity in the IC_{50} is indicative of an effect of chelator on cyclooxygenase, rather than on other enzymes or systems (phospholipases, protein kinase C, calcium mobilisation). DMtC also inhibited the conversion of [^{14}C]-AA to [^{14}C]-HETE (IC_{50}, 38.5 μM). DEtC and TETD inhibited TXA_2 synthesis and lipoxygenase activity in a similar fashion to DMtC and at similar concentrations. The inhibitory effect of all Cu^{2+} chelators was reversed by the addition of approximately equimolar concentrations of Cu^{2+} but not by Fe^{3+}, Fe^{2+}, Zn^{2+}, Al^{3+} or Sn^{2+}. These data indicate that Cu^{2+} (as is the case with Fe^{2+}) may play an obligatory role in cyclooxygenase and lipoxygenase activity. The present data also support the concept that copper imbalance may be involved in the pathophysiology of CVD and that eicosanoids may play a role in mediating this relationship.

Elliot, G.R., van Batenburg, M.J. & Bonta, I.L. Prostaglandins, 1987; 34: 657-668.
Mitchell, L.L., Allen, K.G.D. & Mathias, M.M. Prostaglandins, 1988, 35: 977-986.

52P MODULATION OF THE ACTIVATION OF PLATELETS BY GENISTEIN, AN INHIBITOR OF TYROSINE KINASE(S)

C.T. Murphy & J. Westwick, Department of Pharmacology, University of Bath, Claverton Down, Bath, Avon, BA2 7AY

We have explored the role of tyrosine kinases in the signal transduction of PAF-activated rabbit platelets. Rabbit platelets were prepared, signal molecules quantitated and dense granule release determined as described previously (Murphy et al, 1991). Tyrosine phosphorylation of rabbit platelet proteins was determined by Western blotting of platelet lysates with PY20, a monoclonal anti-phosphotyrosine antibody (Glenney et al, 1988). Resting platelets exhibited at least four tyrosine phosphorylated (TP)-proteins of 52-62 kDa. Stimulation with 300 nM PAF induced a rapid increase in TP-proteins visible within 5 secs of both low (35-45 kDa) and high (66-90 kDa) molecular weight. Pretreatment of platelets with genistein (10-300 μM) for 20 min produced a dose related inhibition of tyrosine phosphorylation of a number of the 66-150 kDa and 35-45 kDa proteins, although was much less effective against the proteins of 52-62 kDa. Genistein (Akiyama et al, 1987) was an effective inhibitor of PAF-induced calcium elevation ($[Ca^{++}]_i$), inositol 1,4,5-trisphosphate (IP_3) formation, thromboxane (Tx) B_2 generation and the release of dense granules (^{14}C-5HT) see table.

Treatment	$[Ca^{++}]_i$ (nM)	IP_3(pmol/10^9pl)	TxB_2(pmol/10^8pl)	^{14}C-5HT (%)
Unstimulated	107 ± 12	7.5 ± 1.5	<0.1	<0.1
Vehicle + PAF	490 ± 22	34 ± 5.5	44 ± 3.2	58 ± 1.5
10 μM Genistein + PAF	434 ± 17.5	-	25.5 ± 0.31	46 ± 0.7
30 μM Genistein + PAF	421 ± 15	22 ± 2	3.4 ± 1.6	39 ± 2.8
100 μM Genistein + PAF	341 ± 24	15 ± 0.8	<0.1	13.8 ± 2.3
300 μM Genistein + PAF	135 ± 13	10.2 ± 2.7	<0.1	3 ± 0.5

We have demonstrated that PAF induces a rapid tyrosine phosphorylation of approximately 17 proteins in three molecular weight ranges. This probably involves a number of tyrosine kinases as genistein is an effective inhibitor of only a limited number of proteins. However, these TP proteins appear to be crucial in the very early signal transduction events of PAF activated platelets as genistein is an effective inhibitor of these events.

We are grateful to the British Heart Foundation for support.

Akiyama, T., Ishida, I., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M. & Fukami, Y. (1987) *J. Biol. Chem.* **262**, 5592-5595.
Glenney, J.R. Jr., Tokas, L. & Kamps, M.P. (1988) *J. Immunol. Meth.* **109**, 277-285.
Murphy, C.T., Elmore, M., Kellie, S. & Westwick, J. (1991) *Biochem. J.* **278**, 255-261.

53P DISSOCIATION OF AGONIST-INDUCED INTRACELLULAR FREE CALCIUM ELEVATION FROM HYDROGEN PEROXIDE PRODUCTION IN GUINEA-PIG EOSINOPHILS

A.D. Bourne, M.L. Watson, D. Smith & J. Westwick, Department of Pharmacology, University of Bath, Avon BA2 7AY.

The physiological activators of eosinophil function and their transduction mechanisms are poorly characterised. We have compared the abilities of LTB_4, Paf, recombinant human C5a and IL-8, fMLP and lipopolysaccharide (LPS) to elevate intracellular free calcium ($[Ca^{2+}]_i$) and stimulate hydrogen peroxide production (H_2O_2) in guinea pig eosinophils. Horse serum - elicited cells were purified to >90% eosinophils. $[Ca^{2+}]_i$ was monitored using fura 2 - loaded cells in a Deltascan fluorimeter (PTI, London) and H_2O_2 release from $2x10^5$ cells/well was measured in a Fluoroskan multiwell plate reader (Flow, Herts, UK) using scopoletin (Nathan, 1987). Maximal increases in $[Ca^{2+}]_i$, and H_2O_2 production after 60 min, are shown below (means ± SEM from n=4 - 12 different eosinophil preparations. Basal $[Ca^{2+}]_i$ was 59 ± 9 nM and cells produced 0.6 ± 0.2 nmoles H_2O_2/well over 60 min in the absence of added agonist.

	Paf			C5a			LTB_4		
nM	1.0	10	100	1.0	10	100	1.0	10	100
$\Delta[Ca^{2+}]_i$ nM	61±13	196±20	252±67	18±4	130±18	185±26	130±15	180±24	256±110
H_2O_2 nmol	0.8±0.4	1.4±0.2	1.4±0.2	0.9±0.5	2.8±0.7	7.8±0.3	2.6±0.4	5.1±0.8	7.9±0.4

Hence, the order of potency for $[Ca^{2+}]_i$ elevation is LTB_4 > Paf > C5a, whereas for the release of H_2O_2 it is LTB_4 > C5a > > Paf. The ability of agonists to elicit H_2O_2 release was apparently unrelated to their ability to stimulate eosinophil adhesion, since the above agonists caused similar adhesion of eosinophils to laminin-coated plastic, monitored using Rose Bengal uptake (Gamble & Vadas, 1988). Minimal or no responses were stimulated in these assays by fMLP (0.1 - 1μM), IL-8 (1.0 - 10nM) or LPS (E.coli 026:B6, 0.1 - 10μg/ml). These data suggest that receptors for different eosinophil activators are coupled to cellular responses by differing transduction mechanisms. This may provide scope for selective pharmacological control of eosinophil function.

This work was supported by the Wellcome Trust.

Nathan, C.F. (1987) *J. Clin. Invest.* **80**, 1550-1560.
Gamble, J.R. & Vadas, M.A. (1988) *J. Immunol. Methods* **109**, 175-184.

54P THROMBIN STIMULATES ACTIN POLYMERIZATION IN U937 HUMAN MONOCYTIC CELLS

S.Joseph & J. MacDermot.Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 0NN.

Exposure of leukocytes to chemotactic agents activates a series of biochemical pathways which mediate the functional response. One such process is the conversion of globular (G)- to filamentous (F)-actin (polymerization). The signalling pathways linking receptor activation by chemoattractants such as PAF or FMLP to F-actin formation in leukocytes have been studied in some detail [Bengtsson et al,1990]. In contrast, little if anything is known about how the serine protease thrombin, a chemotactic agent for monocytes and macrophages [Bar-Shavit et al,1983], causes F-actin formation in these cells. In the present study we have characterised the formation of F-actin by thrombin in the U937 human pro-monocytic cell line differentiated into macrophage-like cells.

U937 cells were differentiated into macrophage-like cells by culture at 37° C for 48 h in RPMI-1640 medium containing 20 % conditioned medium in an atmosphere of CO_2:air (5:95 v/v) [Joseph and MacDermot,1991]. The cells were subsequently washed and resuspended in Hepes-buffered tyrodes solution, pH 7.4 containing 1 mM $CaCl_2$. F-actin formation in U937 cells was detected by fluorescence-activated cell sorting (FACS) analysis following the binding of the fluorescent toxin NBD-phallacidin to cytoplasmic F-actin [Bengtsson et al,1990].

Stimulation of U937 cells with bovine thrombin caused a dose and time dependent increase in F-actin content, the maximal response (≈1.5 fold increase above basal) occurring with ≥10 U/ml thrombin (EC_{50} ≈2 U/ml). A significant increase in F-actin content (1.20±0.05 fold above basal, n=4 from 2 separate experiments) was detected as early as 10 sec after thrombin (50 U/ml) stimulation. This response peaked by 2 min and remained above basal levels (1.22±0.05 fold above basal) 10 min post-stimulation. The kinetics of the thrombin response was however different from that seen with 1 μM PAF or FMLP, both agents causing a maximal increase in F-actin content (1.4-1.5 fold above basal) 10-30 sec after stimulation, which then returned to basal levels by 2 min. The thrombin (10 U/ml) stimulated increase in F-actin content was unaffected by the presence by 5 mM EGTA, EGTA inhibiting by 80 % the rise in intracellular Ca^{2+} by thrombin [Joseph and MacDermot,1991]. The thrombin response was also unaffected by pretreatment with pertussis toxin (0.5 μg/ml P.T., 3 h, 37°C) which was in marked contrast to that seen with PAF or FMLP, P.T. inhibiting by >60 % the latter two responses. The thrombin response was however inhibited by ≈ 70 % by 0.5 μM staurosporine, a non-specific protein kinase C and tyrosine kinase inhibitor. The present results suggest that thrombin causes F-actin formation in monocytes/macrophages by a pathway dependent upon protein phosphorylation, but independent of an influx of extracellular Ca^{2+}. Further, in contrast to the chemoattractants PAF and FMLP, thrombin causes a pertussis toxin-insensitive increase in F-actin content.

Bengtsson,T, Sarndahl,E, Stendahl,O and Andersson,T. (1990) P.N.A.S.(USA) 87, 2921
Bar-Shavit, R Kahn,A. and Wilner,G.D. (1983) Science 220, 728
Joseph,S and MacDermot,J (1991) Eur.J.Pharm (Mol.Pharm Section). 208, 149

55P HISTAMINE RELEASE FROM MAST CELLS DERIVED FROM OBESE AND LEAN ZUCKER RATS

M. Ennis & E. R. Trimble, Department of Clinical Biochemistry, Institute of Clinical Science, The Queen's University of Belfast, Belfast BT12 6BJ

Zucker rats inherit obesity as an autosomal recessive trait and the obese animals are hyperinsulinemic although their blood glucose levels are in the normal range. In this study histamine secretion from peritoneal mast cells derived from lean and obese Zucker rats was compared.
Mast cells from obese and lean rats (1 year old) released similar amounts of histamine after challenge with the calcium ionophore A 23187 (0.1-1 μM), compound 48/80 (0.05-10 μg/ml), substance P (1-10 μM) or dextran (0.06-1.2 mg/ml) (Table 1). In contrast, peritoneal mast cells obtained from lean Zucker rats were significantly more responsive to concanavalin A (0.25-100 μg/ml) than those derived from obese animals (Table 1). The low response to dextran was further investigated by using mast cells from 6 and 12 week old rats. Cells from obese animals were more responsive than those from lean animals at both ages (e.g. dextran 1.2 mg/ml released 37.2±1.8% and 26.1 ±0.9% of the total histamine (n=4) from 6 week old obese and lean rats respectively and 28.4±2.4% and 15.8±1.3% (n=3) from 12 week old animals).

Table 1

	Histamine Release (% of total cellular content of the amine) induced by				
	A 23187 (1 μM)	Compound 48/80 (0.25 μg/ml)	Substance P (10 μM)	Concanvalin A (10 μg/ml)	Dextran (1.2 mg/ml)
Source of cells					
Lean Zucker rats	76.3±4.6 (5)	72.8±3.4 (5)	39.8±2.8 (5)	40.6±5.0 (8)	9.0±3.8 (5)
Obese Zucker rats	72.1±7.4 (5)	67.7±2.4 (5)	33.7±2.5 (5)	15.1±2.6 (6)	8.2±1.2 (5)

All values are given as means ± SEM, for the number of experiments (n) shown in brackets

These results demonstrate that mast cells from lean and obese Zucker rats differ in their response to those secretagogues thought to act via glucose receptors (dextran) or through binding to glucose/mannose molecules on the cell membrane (concanavalin A).

56P DEXAMETHASONE INHIBITS FIBRINOLYSIS IN VIVO BY MODULATION OF t-PA AND PAI-1 LEVELS

J.J.J. van Giezen & J.W.C.M. Jansen, Department of Vascular Pharmacology, Solvay Duphar B.V., P.O.Box 900, 1380 DA Weesp, The Netherlands. (Introduced by S.K. Long)

Dexamethasone decreases the fibrinolytic activity in the cultured medium of several cell-types (Laugh, 1983; Loskutoff et al., 1986). It appeared that this phenomenon is caused by affecting both the plasminogen activator inhibitor type 1 (PAI-1) and the tissue-type plasminogen activator (t-PA) synthesis (Gelehrter et al., 1987). In primary cultures of rat hepatocytes the lowered fibrinolytical activity in the cultured medium was due to an increased PAI-1 and a decreased t-PA synthesis (Heaton & Gelehrter, 1989). These in vitro observations could be confirmed by in vivo experiments. Oral administration of dexamethasone to rats resulted in a reduced blood fibrinolytic activity as measured with the blood clot lysis test (Jansen et al., 1989). Here we report that the reduced fibrinolytic activity in vivo is due to a decreased t-PA and an increased PAI-1 level resulting in a functional inhibition of the blood fibrinolytic activity.

Rats were treated for a period of five consecutive days with dexamethasone in a dose range of 0.1 to 3 mg/kg/day. This dose range has been shown to inhibit blood clot lysis in a dose dependent manner up to 44±15% (±SD; n=7) at the highest dose tested (Jansen et al., 1989).

The t-PA activity in plasma was measured after SDS-PAGE electrophoresis in 0.5 cm cut gel-slices with the spectrophotometric t-PA test using the chromogenic substrate S2251 as described by Verheijen et al. (1982). Both the free t-PA activity and the activity obtained by the t-PA-PAI complex were reduced by resp. 39±11% and 53±14% (±SEM; n=3) in rats treated with 3 mg/kg dexamethasone. The plasma PAI-1 level was measured by titration with human t-PA. It appeared that dexamethasone treatment caused a dose dependent increase in blood PAI-1 level from 5.6±0.9 to 15.3±2.6 IU/ml (±SD;n=5) for rats treated in the dose range 0.1-3.0 mg/kg/day.

To study the in vivo functional effect of the reduced fibrinolytic activity the ^{125}I-fibrin coated aorta loop model as described by Jansen (1986) was used. The rate of label disappearence from the aortic loop, used as a measure of fibrinolytic activity, was decreased from 2.8±0.4%/min for vehicle treated rats to 1.9±0.2%/min (±SD; n=5) for dexamethasone treated rats at a dose of 1 mg/kg.

These results clearly demonstrate that dexamethasone treatment reduced the fibrinolytic activity in rats. Based on the obtained data it may be concluded that the lowered fibrinolytic activity in vivo is caused by a combination of an increased PAI-1 and a decreased t-PA level which correlates with in vitro measured effects.

Gelehrter, T.D., Sznycer-Laszuk, R., Zeheb, R. and Cwikel, B.J. (1987) Mol. Endocrinol. 1, 97-101.
Heaton, J.H. & Gelehrter, T.D. (1989) Mol. Endocrinol. 3, 185-192.
Jansen, J.W.C.M. (1986) Fibrinolysis suppl., 223.
Jansen, J.W.C.M., van Giezen, J.J.J. & Reinders, J.H. (1989) Thromb. Haemostas. 62, 475.
Laugh, W.E. (1983) Thromb. Haemostas. 50, 888-892.
Loskutoff, D.J., Roegner, K., Erickson, L.A., Schleef, R.R., Huttenlocher, A., Coleman, P.L. & Gelehrter, T.D. (1986) Thromb. Haemostas. 55, 8-11.
Verheijen, J.H., Mullaart, E., Chang, G.T.C., Kluft, C. & Wijngaards, I. (1982) Thromb. Haemostas. 48, 266-269.

57P EVIDENCE THAT LIPOCORTIN 1 MEDIATES THE INHIBITORY EFFECT OF DEXAMETHASONE ON THE SECRETION OF PROLACTIN BY THE RAT ANTERIOR PITUITARY GLAND

A.D. Taylor, H.D. Loxley, A-M. Cowell, [1]R.J. Flower & J.C. Buckingham, Dept. of Pharmacology, Charing Cross & Westminster Medical School, London W6 8RF. [1]Dept. of Biochemical Pharmacology, St. Bartholomew's Hospital Medical College, London EC1M 6BQ.

Lipocortin 1, a 37kd glucocorticoid inducible Ca^{++} and phospholipid binding protein, has been strongly implicated as a mediator of certain aspects of the antiflammatory actions of corticosteroids (Flower, 1988). The extent to which this protein contributes to the many other pharmacological actions of these steroids is unknown. Reports that lipocortin 1 (LC1) is present in appreciable quantities in the anterior pituitary gland and that its expression in this tissue is glucocorticoid regulated (Smith et al, 1991) raise the possibility that the protein may be involved in the steroid-regulation of pituitary hormone secretion. Accordingly, we have used an in vitro model to examine the influence of LC1 and a neutralizing monoclonal LC1 antibody (LC1-Ab) on the secretion in vitro of immunoreactive prolactin (ir-PRL) in the presence and absence of hypothalamic extracts (HE) and/or dexamethasone.

Anterior pituitary tissue, collected post-mortem from male rats (≈ 200g), was incubated as described previously (Hadley et al, 1990). PRL release was determined by radioimmunoassay and the data analysed by Duncan's multiple range test. Using a 30 min contact time, HE (0.05-0.2HE/ml, n = 6/group) caused significant ($p<0.01$) concentration dependent increases in ir-PRL release with the highest concentration (0.2HE/ml) producing an approximately 12-fold increment in peptide release. The significant ($p<0.01$), increase in prolactin release evoked by a sub-maximal concentration of the extract (0.1HE/ml) was reduced by ≈ 70% ($p<0.01$, n = 6/group) when dexamethasone (0.1μM) was included in the medium throughout the 2h pre-incubation period. Simultaneous inclusion of LC1-Ab (Zymed, diluted 1:15000) in the medium completely overcame the inhibitory effects of dexamethasone on hypothalamic extract-induced ir-PRL release whereas a control monoclonal antibody (anti-myosin light chain kinase Sigma Chemical Co. Ltd., diluted 1:15000) was without effect. Neither recombinant human LC1 (r-hu-LC1, Biogen Inc., USA, 100fg-100ng/ml) nor an N-terminal fragment of the protein ($LC1_{1-188}$, ICI plc, 10ng-1μg/ml) influenced the spontaneous release of prolactin from the pituitary segments (n = 6/group) although at one concentration (1ng/ml) a small increase in peptide release was apparent ($p<0.01$). By contrast, r-hu-LC1 (100fg-100ng/ml) reduced by ≈ 40-50% ($p<0.01$, n = 6/group) the significant ($p<0.01$), approximately 8-fold increase in prolactin release induced by HE (0.1 HE /ml). Similarly $LC1_{1-188}$ (10pg-1ng/ml) reduced ($P<0.01$) by ≈ 50% the ir-PRL response to HE (0.1HE/ml) although higher concentrations (100ng & 1μg/ml) were without effect. The results suggest that the inhibitory effects of dexamethasone on prolactin release are mediated, at least in part, by lipocortin 1.

We are grateful to Dr. J. Browning, Biogen Inc., USA for r-hu-LC1, to Drs F. Carey & R. Forder, ICI plc for $LC1_{1-188}$ and to Ono Pharmaceuticals Co., Japan for financial support.
R.J. Flower is supported by an endowment by Lilly

Flower, R.J. (1988) Br. J. Pharmacol. **94**, 987-1015
Hadley, A.J., Flack, J.D. & Buckingham, J.C. (1990) Br. J. Pharmacol. **100**, 337P
Smith, T., Smith, S., Flower, R.J. & Buckingham, J.C. (1991) Br. J. Pharmacol. **104**, 218P

KEY WORDS: Lipocortin, Glucocorticoids, Pituitary gland.

58P EVALUATION OF THE α_1-ADRENOCEPTOR SUBTYPES IN THE RENAL VASCULATURE OF THE STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RAT

A.S.Munavvar & E.J. Johns, Department of Physiology, Medical School, Birmingham, B15 2TT

The sympathetic nervous system exerts an important control over blood flow in the kidney which is mediated by α_1- adrenoceptors. The question arises as to the sub-types of α_1-adrenoceptors which may be involved in regulating renal haemodynamics in hypertension. The present study was undertaken in anaesthetised (sodium pentobarbital, 60mgkg^{-1}i.p.) male stroke-prone spontaneously hypertensive rats, 280-310g. After tracheostomy, a carotid artery and jugular vein were cannulated for the measurement of blood pressure and infusion of saline. The renal nerves were isolated and dissected for stimulation. The iliac artery was cannulated for close renal arterial administration of all drugs. The renal vasoconstrictor responses to direct renal nerve stimulation (RNS), (1, 2, 4, 6, 8, 10 Hz at 15V and 0.2ms), bolus doses of phenylephrine (10, 20, 30, 40 μg) and methoxamine (20, 40, 60, 80 μg) were determined before and after the administration of bolus doses of amlodipine (200μgkg^{-1} and 400 μgkg^{-1}), chloroethylclonidine (CEC) and 5-methylurapidil (5MeU) at 5μgkg^{-1} and 10μgkg^{-1} in three different groups of rats. The rate of infusion was fixed to deliver 6mlh^{-1} and contained 25% of the doses of amlodipine, CEC and 5MeU besides 12.5 mgkg^{-1} sodium pentobarbital.

Table 1 — Mean % decrease in renal blood flow ± s.e.m.

GROUP	Treatment	RNS	PHENYLEPHRINE	METHOXAMINE
A	Control	34.90 ± 9.00	53.26 ± 9.92	41.66 ± 9.07
(n=8)	200 μgkg^{-1} amlodipine	25.41 ± 6.45 *	38.22 ± 8.48 *	30.58 ± 6.93 *
	400 μgkg^{-1} amlodipine	18.88 ± 4.59 *	29.92 ± 5.88 *	29.10 ± 6.06 *
B	Control	48.11 ± 12.15	67.68 ± 11.44	48.66 ± 11.11
(n=11)	5 μgkg^{-1} 5 MeU	41.00 ± 9.17	44.57 ± 10.63 *	29.67 ± 8.41 *
	10 μgkg^{-1} 5 MeU	34.39 ± 7.62 *	33.21 ± 7.05 *	19.04 ± 3.06 *
C	Control	37.82 ± 9.70	55.65 ± 16.82	46.56 ± 14.35
(n=8)	5 μgkg^{-1} CEC	28.42 ± 7.28	51.70 ± 14.69	41.03 ± 11.85
	10 μgkg^{-1} CEC	28.53 ± 7.28	50.34 ± 13.58	41.92 ± 12.10

* $p<0.05$ (ANOVA)

The results obtained in this study indicated a predominance of the extracellular calcium dependent and 5MeU sensitive receptors i.e. the proposed α_{1a} sub-type over the CEC sensitive receptors which are thought to be the α_{1b} sub-type. This situation is similar to that previously observed in the normotensive Wistar rats (Munavvar & Johns, 1991).

Munavvar, A.S. & Johns, E.J. (1991). Br. J. Pharmacol. Proceedings September meeting.

59P EVIDENCE FOR AN $\alpha 2_A$-LIGAND BINDING SITE IN RAT SUBMANDIBULAR GLAND

Karen Smith and James R. Docherty, Department of Physiology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland.

Alpha$_2$-adrenoceptors have been subdivided into 4 subtypes based on radioligand binding studies (Bylund et al.,1991), and at least 3 genes code for alpha$_2$-adrenoceptors (Lorenz et al.,1990). The alpha$_{2D}$-ligand binding site has been identified in the bovine pineal gland (Bylund et al.,1991), but it may be identical to the ligand binding site in rat submandibular gland (Michel et al.,1989). The object of this study was to investigate the alpha$_2$- ligand binding site of rat submandibular gland.

In prostatic portions of rat vas deferens, antagonist potency was expressed as a K_B from the ability to antagonise the inhibition by xylazine of the isometric contraction to single pulse stimulation. Preparation of human platelet and rat kidney membranes was carried out exactly as described by Connaughton & Docherty (1990), and rat submandibular gland membrane preparation was identical to that for rat kidney. Antagonist K_i values were obtained from the displacement of [^{3}H]-yohimbine binding.

The alpha$_2$-ligand binding site of rat submandibular gland showed a poor correlation with the alpha$_{2B}$ site in rat kidney ($r=0.68$, $n=9$, $P<0.05$), but is not identical with the alpha$_{2A}$ site of human platelet ($r=0.83$, $n=9$, $P<0.01$). The functional prejunctional alpha$_2$-adrenoceptor of rat vas deferens is alpha$_{2A}$-like since there was good correlation with affinity for the alpha$_{2A}$-ligand binding site in human platelet ($r=0.90$, $n=9$, $P<0.001$). However, there was a better correlation between affinity for the alpha$_2$-ligand binding site of rat submandibular gland and prejunctional potency in rat vas deferens ($r=0.97$, $n=9$, $P<0.001$).

It is concluded that the alpha$_2$-ligand binding site of the rat submandibular gland may be an alpha$_{2A}$ site which is not identical to the alpha$_{2A}$ site of human platelet.

Supported by the Health Research Board (Ireland), the Irish Heart Foundation and RCSI.

Bylund, D.B., Blaxall, H.S., Murphy, T.J. & Simmoneaux, V. (1991) in *Adrenoceptors: Structure, Mechanisms, Function.* ed. Szabadi, E. & Bradshaw, C.M. pp 27-36. Basel:Birkhauser Verlag.
Connaughton, S. & Docherty, J.R. (1990) Br. J. Pharmacol. **101**, 285-290
Lorenz, W., Lomasney, J.W., Collins, S., Regan, J.W., Caron, M.G. & Lefkowitz, R.J. (1990) Mol. Pharmacol. **38**, 599-603
Michel, A.D., Loury, D.N. & Whiting, R.L. (1989) Br. J. Pharmacol. **98**, 890-897

60P EVIDENCE THAT SYMPATHETIC NERVE ENDINGS OF RAT KIDNEY POSSESS α_2-AUTORECEPTORS OF THE α_{2A}-SUBTYPE

L.C. Rump & C. Bohmann, 'introduced by J.R. Docherty', Department of Internal Medicine IV, University of Freiburg, Hugstetter Straße 55, D-7800 Freiburg, FRG.

On the basis of radioligand binding studies α_2-adrenoceptors have been divided into α_{2A}- and α_{2B}-subtypes and it has been suggested that prejunctional α-adrenoceptors belong to the α_{2B}-subtype (Bylund et al., 1988). Activation of α_2-adrenoceptors inhibits noradrenaline release in rat kidney. Moreover, α_2-adrenoceptor antagonists enhance renal noradrenaline release at a frequency of 1 Hz which suggests that endogenous noradrenaline activates prejunctional inhibitory α_2-autoreceptors (Rump & Majewski, 1987). The aim of the present study was to test whether the α_{2B} - or the α_{2A}-adrenoceptor subtype mediates inhibition of the stimulation induced (S-I) outflow of radioactivity in rat isolated kidney preincubated with [3H]-noradrenaline. The S-I outflow of radioactivity was taken as an index of noradrenaline release. There were six short trains of stimulation (S_1-S_6), each at 100 Hz for only 60 ms, to avoid autoinhibition. In this situation, none of the α_2-adrenoceptor antagonists (see below) used enhanced S-I outflow of radioactivity by itself. Thus, this method avoids autoinhibition by endogenous noradrenaline, so that apparent pK_B values for α_2-adrenoceptor antagonists can be determined by the method of Furchgott (1972). Cumulative concentration response curves for the α-adrenoceptor agonist clonidine were constructed. Clonidine induced a maximal inhibition of the S-I outflow of radioactivity of 90% with an EC_{50} of 7.8 nM. The following α-adrenoceptor antagonists shifted the concentration response curve for clonidine to the right: Idazoxan (0.1 μM, non-selective for α_{2A} and α_{2B}) - $pK_B=8.3$; 2-[2H-(1-methyl-1,3-dihydroiso-indole)methyl]-4,5-di-hydroimidazole (BRL 44408, 0.3 μM, α_{2A}-selective) - $pK_B=7.7$; 1,2,-dimethyl-2,3,9, 13b-tetrahydro-1H-dibenzo[c,f]imidazo[1,5-a]azepine (BRL 41992, 0.3 μM, α_{2B}-selective) - $pK_B=7.0$; prazosin (0.1 μM; α_{2B}-selective) - $pK_B=7.1$; imiloxan (0.3 μM, α_{2B}-selective) - $pK_B=6.7$. The rank order of affinities for the α-adrenoceptor antagonists (BRL 44408 > BRL 41992 > imiloxan) argues against the notion that prejunctional α_2-adrenoceptors belong to the α_{2B}-subtype. In contrast, α_{2A}-adrenoceptors seem to modulate noradrenaline release in rat kidney but the possibility that a less important population of prejunctional α_{2B}-adrenoceptors coexists needs to be clarified in future receptor protection experiments.

Bylund. D.B., Ray-Prenger, C. & Murphy, T.J. (1988) J. Pharmac. Exp. Ther. **245**, 600-607.
Rump, L.C. & Majewski, H. (1987). J. Cardiovasc. Pharmacol. **9**, 500-507.
Furchgott, R.F. (1972) in Catecholamines. ed. Blaschko & Muscholl. vol. XXXIII, pp 283-335. Berlin: Springer.

61P THE CONTRACTILE EFFECT OF SOME α-ADRENOCEPTOR ANTAGONISTS IN THE RAT PORTAL VEIN AND THE PUTATIVE INVOLVEMENT OF ATP-SENSITIVE K^+ CHANNELS

H.R. Schwietert, D. Wilhelm[1], B. Wilffert[1] & P.A. van Zwieten, Department of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands. [1]Department of Experimental Medicine, Janssen Research Foundation, Neuss, F.R.G.

We have previously reported on the ability of some α-adrenoceptor antagonists to increase the spontaneous myogenic activity in the rat portal vein (Schwietert *et al.*, 1991). We hypothesized that this action, which we demonstrated to be independent of α-adrenoceptor blockade, might be related to a blockade of glibenclamide-sensitive K^+-channels, since α-adrenoceptor antagonists, notably phentolamine and yohimbine, have recently been shown to interact with glibenclamide/ATP-sensitive K^+-channels (Plant & Henquin, 1990).

Portal veins of male Wistar rats (350-400 g) were mounted longitudinally under a passive force of 1 g in thermostatically controlled organ baths (37°C). Tissues were bathed in Tyrode's solution (NaCl 124, KCl 4.0, $MgCl_2$ 1.1, $NaHCO_3$ 24.9, Na_2HPO_4 0.42, Glucose 5.5, Ca^{2+} 0.9 mM or 2.5 mM) and gassed with 95 % O_2 and 5 % CO_2. Isometric contractile activity was measured via force displacement transducers.

The K^+-channel openers cromakalim and diazoxide decreased the contractile amplitude of the rat portal vein with pD_2 values of 6.74 ± 0.08 -logM (n=7) and 4.84 ± 0.04 -logM (n=8), respectively. Phentolamine (1 μM - 10 μM) shifted the concentration-response curves of cromakalim and diazoxide concentration-dependently to the right. Yohimbine showed only a modest effect in the highest concentration (100 μM) applied. All other α-adrenoceptor antagonists (prazosin, corynanthine and idazoxan) when tested in concentrations which maximally stimulated spontaneous myogenic activity failed to influence the relaxations by cromakalim and diazoxide. Glibenclamide was inactive as a contractile agent when applied in concentrations (0.3 μM - 3 μM) which markedly shifted the concentration-response curves of cromakalim and diazoxide. E-4031 (10 nM - 0.3 μM), a sotalol derivative and one of the most selective blockers of the delayed rectifier current (I_k) in cardiac tissue, exerted a potent contractile action in the rat portal vein. Antazoline (1 μM - 100 μM) exhibited similar effects on spontaneous myogenic activity as its structurally closely related analogue phentolamine.

In conclusion, except for phentolamine, no correlation was found in the rat portal vein between the effects of the α-adrenoceptor antagonists on spontaneous myogenic activity and their antagonism of the relaxations by cromakalim and diazoxide. We suggest that other mechanisms than the blockade of K^+-channels sensitive to glibenclamide/ATP play a role in the contractile effect of some α-adrenoceptor antagonists in the rat portal vein.

Schwietert, H.R., Wilhelm, D., Wilffert, B. & van Zwieten, P.A. (1991) *Blood Vessels*, **28**, 332-333
Plant, T.D. & Henquin, J.C. (1990) *Br. J. Pharmacol.*, **101**, 115-120

62P INTERACTION OF POTASSIUM CHANNEL OPENERS WITH [^{3}H]-IDAZOXAN BINDING AT THE NON-ADRENERGIC IDAZOXAN BINDING SITE IN THE RAT LIVER

T. Ibbotson, & A.H. Weston, Smooth Muscle Research Group, Department of Physiological Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT.

Zonnenschein et al., (1990) reported that a homogenate prepared from rat liver contains a dense population of [^{3}H] idazoxan binding sites but is totally devoid of any α_2-adrenoceptors. The ligands for this site may be classified by the possession of either an imidazoline or a guanidine moiety within their structures (Michel and Insel, 1989). Furthermore, based on the findings of Zonennschein et al., (1990) it is possible that a potassium channel is involved in the physiological functioning of the non-adrenergic idazoxan binding site (NAIBS). In the present study the effect of imidazoline/guanidine based compounds on [^{3}H] idazoxan binding was examined. In addition, the ability of a series of potassium channel openers to displace [^{3}H] idazoxan binding was also investigated. The effect of certain NAIBS ligands on [^{3}H] glibenclamide binding in a rat brain homogenate was also evaluated.

Rat liver homogenate prepared according to Zonennschein et al., (1990) was incubated with [^{3}H] idazoxan in Tris/HCl buffer + 2mM $MgCl_2$, pH=7.4 for 40min at room temperature. [^{3}H] glibenclamide binding was performed on a homogenate derived from whole rat brain in Krebs Tris/HCl buffer, pH=7.4 for 60min. Non-specific binding was defined as that detected in the presence of 10^{-5}M idazoxan or 10-5M glibenclamide, respectively.

The rank-order of potency for NAIBS ligands was cirazoline (IC_{50}=5nM) > RX801023 (IC_{50}=12nM) > idazoxan (IC_{50}= 15nM) > guanabenz (IC_{50}=21nM). In addition UK14304, amiloride, clonidine and guanethidine were found to be relatively potent each with IC_{50} values < 10μM. The α-adrenoceptor ligands RX821002, prazosin, adrenaline and noradrenaline were also tested and found to be inactive (IC_{50} values > 10^{-5}M). None of the potassium channel openers tested (BRL38227, RP52891, P1060 and diazoxide) with the exception of pinacidil (IC_{50}=4.1μM) had any effect on the binding of [^{3}H] idazoxan. All agents identified as potent NAIBS ligands were inactive at displacing [^{3}H] glibenclamide from the rat brain homogenate.

The rat liver NAIB site is sensitive to imidazoline/guanidine type ligands but is unaffected by all but one of the potassium channel modulators. The ability of pinacidil to displace idazoxan binding may be attributable to the guanidine moiety within its structure and not to any potassium channel modulating properties. The lack of effect of any other potassium channel opener coupled with the inability of the NAIBS ligands to displace [^{3}H] glibenclamide binding suggests that if the NAIB site is associated with a potassium channel it is unlikely to be blocked by glibenclamide or opened by agents like BRL38227.

TI was supported by an MRC collarborative studentship in conjunction with Pfizer Central Research.

Michel,M.C. and Insel,P.A. (1989) Trends Pharmacol Sci.,10, 342-344
Zonnenschein,R., Diamant,S. and Atlas,D. (1990) Eur J Pharmacol.,190, 203-215

63P PREJUNCTIONAL α_2-ADRENOCEPTORS IN MOUSE ATRIA FUNCTION THROUGH G-PROTEINS WHICH ARE SENSITIVE TO N-ETHYLMALEIMIDE, BUT NOT PERTUSSIS TOXIN

T.V. Murphy*, S. Foucart[1] & H. Majewski[2], Department of Physiology and Pharmacology, University of Southampton, Southampton, S09 3TU, [1]Department of Pharmacological and Physiological Sciences, University of Chicago, 947 East 58th Street, Chicago Il. 60639, U.S.A. and [2]Prince Henry's Institute of Medical Research, 68 Wells Street, South Melbourne 3205, Australia

α_2-Adrenoceptors function through GTP-binding proteins (G-proteins; Harrison *et al.*, 1991). Prejunctional α_2-adrenoceptors on sympathetic nerves inhibit noradrenaline release, however the identity of the G-protein involved in signal transduction of prejunctional α_2-adrenoceptors is unclear. In a previous study, pertussis toxin, which inactivates the G-proteins G_i and G_o, did not alter the effects of α_2-adrenoceptor ligands on noradrenaline release from mouse atria (Musgrave *et al.*, 1987). The aim of the present study was to further investigate the G-proteins involved in prejunctional α_2-adrenoceptor signal transduction in mouse atria.

Mouse atria were incubated with [^{3}H]noradrenaline and then subjected to electrical field stimulation (5, 2.5, 1.75 Hz, 30 mA, 0.1 ms, 60 s). The stimulation-induced outflow of radioactivity was taken as an index of endogenous noradrenaline release. At a stimulation frequency of 5 Hz, the α_2-adrenoceptor agonist clonidine (0.03 μM) inhibited noradrenaline release from the mouse atria by 29.5 %. This effect was not altered by pretreatment of the mice with pertussis toxin (1.5 μg). The α_2-adrenoceptor antagonist idazoxan (0.1 μM) enhanced noradrenaline release from the mouse atria by 65.7 %, and this effect was also not altered by pertussis toxin. The possibility that the ineffectiveness of pertussis toxin was due to the presence of spare prejunctional α_2-adrenoceptors was investigated using St 363, an α_2-adrenoceptor agonist of lower efficacy than clonidine (Medgett and McCulloch, 1980). At a stimulation frequency of 2.5 Hz, St 363 (10 μM) inhibited noradrenaline release from mouse atria by 27.2 %, and this effect was unaltered by pertussis toxin.

Following incubation of the mouse atria with another, less selective G-protein inactivator, N-ethylmaleimide (NEM; 3 μM, 60 min), neither clonidine nor St 363 inhibited noradrenaline release from this tissue. The facilitatory effect of idazoxan on noradrenaline release was reduced by approximately 80 % in atria incubated with NEM. These results suggest that prejunctional α_2-adrenoceptors on sympathetic nerves in mouse atria function through G-proteins which are sensitive to N-ethylmaleimide, but not pertussis toxin. The identity of these G-proteins remains to be elucidated.

Harrison, J.K., Pearson, W.R. & Lynch, K.R. (1991) *Trends Pharmacol. Sci.*, **12**, 62-67.
Musgrave, I.F., Marley, P. & Majewski, H. (1987) *Naunyn-Schmiedeb. Arch. Pharmacol.*, **339**, 48-53.
Medgett, I.C. & McCulloch, M.W. (1980) *J. Pharm. Pharmacol.*, **32**, 137-138.

64P CHRONIC β_1-ADRENOCEPTOR ANTAGONIST TREATMENT DOES NOT AFFECT ACTIVITY OF G_S-PROTEIN OR ADENYLATE CYCLASE IN HUMAN RIGHT ATRIUM

M.C. Michel, A. Broede, M. Michel-Reher, H.-R. Zerkowski & O.-E. Brodde, Dept. Medicine, University of Essen, Hufelandstr. 55, D-4300 Essen, Germany

It has previously been demonstrated that β_2-adrenoceptor-mediated positive inotropic effects are enhanced in atria of patients chronically receiving β_1-selective antagonists although no increases of β_2-adrenoceptor density were detected (Hall et al. 1990, Motomura et al. 1990). We have now studied whether alterations distal to the β_2-adrenoceptor such as G-proteins or the catalytic subunit of adenylate cyclase may be involved in this sensitization.

Right atria were obtained from patients without apparent heart failure undergoing coronary artery bypass grafting which had or had not been treated with β_1-selective adrenoceptor antagonists (atenolol, metoprolol, bisoprolol) for at least 3 weeks prior to surgery. Adenylate cyclase was assessed in atrial membranes as conversion of [^{32}P]ATP to [^{32}P]cAMP during a 10 min incubation at 30°. G_s function was assessed as 10 mM NaF-stimulated adenylate cyclase activity following reconstitution of four concentrations of atrial membrane extracts into cyc$^-$ cell membranes. The α-subunits of the G-protein G_s and $G_{11/2}$ were quantified by quantitative Western blotting with the specific RM/1 and AS/7 antisera, respectively, followed by [^{125}I]-protein A detection. $G_{i\alpha}$ was also quantified using pertussis toxin-catalyzed ADP-ribosylation. At least 8 patients were studied in each group.

Adenylate cyclase stimulation by GTP (100 μM) or NaF (10 mM), which directly activate G-proteins, and by forskolin (10 μM) or $MnCl_2$ (10 mM), which directly activate adenylate cyclase, was not significantly different between β_1-antagonist-treated and control patients. We also failed to detect significant differences between the two groups for reconstituted G_s activity, immunodetectable $G_{s\alpha}$ and $G_{i\alpha}$ or pertussis toxin substrates (1213±178 control and 980±145 fmol/mg protein β_1-antagonist-treated patients).

We conclude that altered amounts of $G_{s\alpha}$ or $G_{i\alpha}$ or changes in the functional activity of G_s or the catalytic subunit of adenylate cyclase may not contribute to the enhanced β_2-adrenoceptor function in atria from patients chronically receiving β_1-adrenoceptor antagonist treatment.

Hall, J.R., Kaumann, A.J. & Brown M.J. (1990) Circ. Res. 66, 1610-1623.
Motomura, S., Deighton, N.M., Zerkowski, H.-R., Doetsch, N., Michel, M.C. & Brodde, O.-E. (1990) Br. J. Pharmacol. 101, 363-369.

65P DESENSITISATION OF RECOMBINANT MUSCARINIC M_3 RECEPTOR RESPONSES FOLLOWING SHORT TERM EXPOSURE TO AGONIST

A.B. Tobin , D.G. Lambert and S.R. Nahorski, Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, LEICESTER. LE1 9HN. U.K.

Of the five cloned muscarinic receptor subtypes three (m1, m3 and m5) are coupled efficiently to the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2). Previous studies have demonstrated that desensitisation of m1 and m3 muscarinic receptor mediated responses following prolonged (>2hr) agonist exposure are associated with receptor sequestration and/or down regulation (1). However, muscarinic receptors coupled to PIP_2 hydrolysis appear to be relatively insensitive to desensitisation following short exposures to agonist. In this study we describe desensitisation of m3 muscarinic receptor mediated responses following a short 5 min pre-exposure to agonist.
The effect of carbachol on inositol 1,4,5-trisphosphate ($InsP_3$) levels in Chinese Hamster Ovary cells expressing transfected m3 muscarinic receptors (Bmax = 2384 fmol/mg protein, Kd = 0.157nM for [^{3}H]N-methyl scopolamine) was monitored using an $InsP_3$ mass assay. Application of 1mM carbachol results in a rapid increase in $InsP_3$ rising to an initial peak 6.82 ± 0.36 fold over basal (± SE, n=3) within 10 secs. In the following 50 secs $InsP_3$ fell to 4.09 ± 0.18 fold (n=3) over basal, a level which is maintained for at least 5 min and which constitutes the plateau phase of the response. A 5 min pre-exposure to 1mM carbachol followed by a 2 min wash eliminates the peak $InsP_3$ response of a subsequent agonist application without affecting the later plateau phase. This effect is reversible with the complete recovery of the carbachol response occurring within 15 min of the desensitising pulse of agonist. Since the primary function of $InsP_3$ is to mobilise intracellular stores of calcium the anticipated cellular consequence of desensitisation of the $InsP_3$ peak would be a reduction in agonist-mediated calcium mobilisation. This was investigated using the calcium chelating fluorophore FURA-2 in conjunction with standard epifluorescence microscopy. In control cells the application of 1mM carbachol results in a rapid peak of free intracellular Ca^{2+} (basal F_{340}/F_{380} ratio = 0.74 ± 0.05 and peak ratio = 2.72 ± 0.22 (±SE, n=12) a response that correlates with the peak of $InsP_3$ production. Pre-exposure of the cells to 1mM agonist for 5 minutes followed by a 2 min wash period reduced the Ca^{2+} peak stimulated by a subsequent application of carbachol by >50%. As in the case of $InsP_3$ mass desensitisation the Ca^{2+} response was completely restored within 15 min of the initial agonist exposure. This desensitisation phenomena cannot be accounted for by receptor sequestration or down regulation since no change in [^{3}H]N-methyl-scopolamine binding is observed following a 5 min exposure to agonist. Other mechanisms of desensitisation, such as receptor uncoupling, are currently under consideration.

1. Hu, J., Wang, S.-Z. & El-Fakahany, E. (1991) J. Pharm. Exp. Therap., 257, 938-945.

66P BRADYKININ-INDUCED PHOSPHOINOSITIDE HYDROLYSIS AND CALCIUM MOBILISATION IN CULTURED BOVINE TRACHEAL SMOOTH MUSCLE CELLS

K.A.Marsh & S.J.Hill, Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

It is widely accepted that intracellular calcium ions are the primary regulators of smooth muscle contraction, an increase in which can be triggered by agonist-induced phospholipid hydrolysis. Bradykinin-B_2 receptor stimulation has been shown to mediate smooth muscle contraction in several isolated tissues (Regoli *et al*, 1990). In this study we report the effects of bradykinin on phosphoinositide hydrolysis and calcium ion mobilisation in cultures of bovine tracheal smooth muscle cells.

Cell cultures were prepared from fresh strips of bovine tracheal smooth muscle. Briefly tissue was chopped into 1mm^3 pieces which were then transferred to 75cm^2 tissue culture flasks. 10ml of D-val MEM (Gibco) supplemented with 10% FCS was added to each explant culture which was then incubated at 37°C in a 10% CO_2 humidified atmosphere. A complete change of media was performed every 3-4 days until confluency when explants were discarded. Cells were subcultured using a 0.05% trypsin/EDTA solution. All experiments were performed on cells between passage 3 and 9. Identification of smooth muscle cells was confirmed by indirect immunocytochemistry using a monoclonal antibody to alpha smooth muscle actin. Cell monolayers grown in 24-well plates were loaded with ^{3}H-myo-inositol (1μCi/well) for 72 hr in inositol-free DMEM supplemented with 0.5% FCS and 2mM glutamine. Cells were then washed twice with 1ml Hanks/HEPES buffer, pH7.4 and incubated for 30 min in 0.7ml Hanks/HEPES with 20mM LiCl. Where appropriate antagonists were added after 28 min incubation. Agonists were added in 10μl of medium for incubation times of 2.5 to 45 min. Stimulation was stopped by the addition of 1ml ice cold methanol/0.12ml HCl (1:1 v/v). Cells were left overnight at -20°C before total inositol phosphates in the supernatant were separated by anion-exchange chromatography (Hall & Hill, 1988). For image analysis, cells were sparsely seeded and grown for 48 hr on 22mm circular glass coverslips. These were then loaded with 5μM Fura-2 AM and placed in a holder with 900μl of physiological salt solution containing 2mM $CaCl_2$ and maintained at 37°C by means of a heated chamber. Agents were added in a volume of 100μl directly onto the coverslip and changes in calcium ion concentration within single cells were monitored using a Nikon fluorescent microscope and the Joyce Loebl Magical image analysis system.

Bradykinin (0.1μM) produced an increase in ^{3}H-inositol phosphate accumulation which was evident after 2.5 min and began to plateau between 25 and 45 min after agonist stimulation. The response to bradykinin (0.1μM) increased with increasing concentrations of LiCl (up to 50 mM) in the preincubation solution. Concentration response analysis of the bradykinin response revealed a log EC_{50} (M) of -9.43 ± 0.20 (n=8) at 10 min incubation and an E_{max} of 5.53 ± 0.99 fold over basal levels. The bradykinin B_1-receptor agonist, Des-Arg^9-bradykinin was without significant effect on inositol phosphate accumulation at concentrations up to 10μM. The bradykinin-B_1 receptor antagonist Des-Arg^9 -[Leu^8]-bradykinin showed no antagonism of the bradykinin response. However, the bradykinin-B_2 receptor antagonists D-Arg-Hyp^3-D-Phe^7-bradykinin (K_D = 40 ±14 nM n=3) and D-Arg-Hyp^3,Thi^{5,8}-D-Phe-bradykinin (K_D = 8.58 ± 2.80 nM; n=3) both produced parallel shifts of the bradykinin dose-response curve to higher agonist concentrations indicating competitive antagonism at bradykinin-B_2 receptors. Bradykinin (1nM - 10μM) produced a partially sustained increase in intracellular calcium ion concentration $[Ca^{2+}]_i$ in 80% of the bovine tracheal smooth muscle cells studied in Ca^{2+}-containing buffer (n=45). In Ca^{2+} -free buffer 67% of cells responded to bradykinin at a concentration of 10nM (n=6). The addition of forskolin (10μM), a known activator of adenylate cyclase was able to return the increased $[Ca^{2+}]_i$, in response to bradykinin (1nM), to a basal level in calcium-containing buffer. These results indicate that cultured bovine tracheal smooth muscle cells are a useful tool in the investigation of the control of phospholipid hydrolysis and calcium mobilisation by bradykinin-B_2 receptors in airway smooth muscle.

We would like to thank the AFRC for their financial support of this work.

Hall, I.P. & Hill, S.J. (1988) Br. J. Pharmacol. **95**, 1204-1212.
Regoli, d., Rhaleb, N.E., Dion, S. & Drapeau, G. (1990) Trends Pharmacol. Sci. **11**, 156-161.

67P HISTAMINE H_1 RECEPTOR-MEDIATED Ca^{2+} INFLUX IN THE HAMSTER VAS DEFERENS CELL LINE DDT_1MF-2

John M. Dickenson & Stephen J. Hill. Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

Agonist induced Ca^{2+} influx into cells can occur by at least two mechanisms: one controlled by the Ca^{2+} content of the intracellular store and one which is dependent upon continued receptor activation (Meldolesi *et al.*, 1991). In DDT_1MF-2 cells, histamine H_1-receptor activation can stimulate a release of intracellular Ca^{2+} and an influx of extracellular Ca^{2+} (Dickenson & Hill, 1991). In this study, we have investigated the nature of the histamine (HA) stimulated Ca^{2+} influx in DDT_1MF-2 cells.

Monolayers of DDT_1MF-2, were grown on glass coverslips and loaded with fura-2/AM. $[Ca^{2+}]_i$ changes elicited by HA stimulation were then measured essentially as described previously (Dickenson & Hill, 1991). Stimulation with HA (100 μM: EC_{50} 7.9 x 10^{-6}M ± 0.2, n=4) in Ca^{2+}-free medium (containing 0.1mM EGTA) caused a rapid and transient rise in $[Ca^{2+}]_i$. The subsequent readdition of exogenous Ca^{2+} (2mM) resulted in a further increase in $[Ca^{2+}]_i$ which appeared to be due to Ca^{2+} influx (N=5). Addition of mepyramine (10 μM; N=3), applied 8 min prior to 2mM $CaCl_2$, attenuated the rise in $[Ca^{2+}]_i$ observed when Ca^{2+} was reapplied. Pretreatment with Ni^{2+} (1mM; N=3) or Co^{2+} (1mM; N=3) inhibited the Ca^{2+} influx, whereas, the organic voltage-operated Ca^{2+} channel antagonists nifedipine (10 μM; N=3) and PN-200-110 (10 μM; N=3) were without effect. After stimulation with HA (100 μM) in Ca^{2+}-free medium, the response to a subsequent addition of bradykinin (BK; 100nM) was markedly reduced. However, if exogenous Ca^{2+} (2mM) was applied for a short period (5 min, in the presence or absence of 10 μM mepyramine) between the HA and BK stimuli, a maximal BK response was re-established (n=5). Furthermore, during this refilling of the internal Ca^{2+} stores in the presence of mepyramine, there was no observable rise in $[Ca^{2+}]_i$.

The results of this study suggest that H_1-receptor activation in DDT_1MF-2 cells stimulates both release of Ca^{2+} from internal stores and Ca^{2+} influx which may be through a receptor-activated Ca^{2+} channel. Furthermore, the subsequent refilling of the internal Ca^{2+} store appears to be independent of H_1-receptor activation and can occur without a noticeable rise in cytosolic free Ca^{2+}.

We thank the Wellcome Trust for financial support.

Dickenson, J.M. & Hill, S.J. (1991) *Biochem. Pharmacol.* **42**, 1545-1550
Meldolesi, J., Clementi, E., Fasolato, C., Zacchetti, D. & Pozzan, T. (1991) *Trends Pharmacol. Sci.* **12**, 289-292

68P PROTEIN KINASE C MODULATION OF THE SODIUM PUMP OF PERIPHERAL NERVE

D.R. Tomlinson & C.B. Ettlinger, Department of Pharmacology, Queen Mary and Westfield College, Mile End Road, London E1 4NS, U.K.

Circumstantial evidence has linked *myo*-inositol metabolism to basal activity of ouabain-sensitive ATPase in sciatic nerve homogenates and phosphoinositide-derived kinase activation has been suggested as the link (Simmons *et al.* 1982). We have therefore examined the capacity of activators and inhibitors of protein kinase C (PKC) to modulate activity of the sodium pump in cells of the endoneurium of the rat sciatic nerve. These preparations are made by desheathing the nerve and then fenestrating the perineurium of the major fascicle by microdissection. Activity of the sodium pump was measured as ouabain-sensitive uptake of [^{86}Rb] in preparations incubated in Krebs-Henseleit bicarbonate-buffered saline, containing 3% defatted bovine serum albumin, at 37°C equilibrated with 95%O_2/5%CO_2 over a 30 min period, during which uptake was linear without intervention. Permeation of extracellular space was monitored by inclusion of [^{3}H] sucrose in the incubate. Uptake was therefore calculated with reference to nerve protein and [^{3}H] content and is expressed below as % basal activity, derived from control preparations, ±1 SD, with significance values calculated by one-way ANOVA with Duncan's multiple range tests. Preparations were incubated either in the presence (ouabain-resistant uptake) or absence (total uptake) of 2 mM ouabain and the ouabain-sensitive activity (Na^+/K^+ pumping) inferred by subtraction. Total [^{86}Rb] uptake was increased by 47±14% ($p<0.01$) in the presence of phorbol-12,13-dibutyrate (PDBu) at 20 μM. This effect was prevented completely when 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7) was added to the incubate at 50 μM 10 min before addition of PDBu. Addition of PDBu 10 min before H-7 nullified the capacity of the latter to block the action of the phorbol ester. These findings implicated PKC in the activation of the Na^+ pump, however when we examined the effect of a specific PKC antagonist, Ro31-8220 (see Davis *et al.* 1989), we found that it also activated the pump, causing a 70±29% ($p<0.001$) increase in [^{86}Rb] uptake at 0.1 μM. In a separate experiment effects of PDBu and Ro 31-8220 were studied together. Thus, Ro 31-8220 (0.1 μM) alone increased basal total [^{86}Rb] uptake by 66±39% ($p<0.01$) and PDBu (20 μM) by 47±15% ($p<0.01$). In combination these agents increased activity by 46±11% ($p<0.05$), indicating no additive effect. None of the above interventions affected [^{86}Rb] uptake in the presence of ouabain (2 mM), indicating that all changes were mediated by the Na^+/K^+ pump. We therefore conclude that PKC exerts a tonic inhibition on the Na^+/K^+ pump in rat sciatic endoneurium, that PDBu stimulates the pump by a mechanism other than PKC activation and that H-7 is by no means specific as a blocker of activation of protein kinase C.

Davis, P.D., Hill, C.H., Keech, E., Lawton, G., Nixon, J.S., Sedgwick, A.D., Wadsworth, J., Westmacott, D. & Wilkinson, S.E. (1989) FEBS Lett. **259**, 61-63.
Simmons, D.A., Winegrad, A.I. & Martin, D.B. (1982) Science **217**, 848-851.

69P CROMAKALIM INHIBITS [^{3}H]ACETYLCHOLINE RELEASE FROM PARASYMPATHETIC NERVES OF THE ISOLATED RAT TRACHEA VIA AN EPITHELIUM-DEPENDENT MECHANISM

I. Wessler*, D. Pohan, J. Maclagan[1] & K. Racké, Department of Pharmacology, University of Mainz, Germany and [1]Department of Pharmacology, Royal Free Hospital Medical School, London NM3 2PF, UK.

The airway epithelium plays a crucial role in regulating the responsiveness of the airway smooth muscle (see Goldie et al., 1990) and mediates a substantial inhibition of evoked [^{3}H]acetylcholine (ACh) release (Wessler et al., 1990). Recently, it has become possible to measure the release of ACh elicited by preganglionic stimulation of the parasympathetic nerves innervating the trachea (Wessler et al., 1991). Using this radiotracer method the effects of potassium channel openers on the release of ACh were analysed.

Isolated rat tracheae remained intact or were opened by cutting the cartilage. The left laryngeal recurrent nerve and the right vagal nerve were prepared for electrical stimulation. Epithelium was mechanically removed in some experiments. Tracheae were incubated (30 min) in a physiological salt solution (2 ml) that contained [^{3}H]choline (5μCi/ml); electrical nerve stimulation (10 Hz, 0.2 ms pulse width, 45 mA) was applied during labelling. After a subsequent washout and the addition of 10 μM hemicholinium-3 two (S1, S2) stimulation periods (four or six 20 s stimulations at 15 Hz with 10 s intervals) were applied; potassium channel openers were present from 18 min before S2 and their effects on evoked ACh release were estimated by comparing the respective S2/S1 ratios.

Electrical stimulation of the parasympathetic nerves caused an increase in tritium outflow that was abolished by tetrodotoxin or by removal of extracellular calcium; hexamethonium (0.3 mM) and tubocurarine (0.1 mM) reduced evoked tritium efflux by 85%. Analysis by reverse phase HPLC showed the evoked tritium efflux to be exclusively caused by the release of ACh. When tracheae (with or without the epithelium) were opened and thus, allowing a rapid exchange between the luminal surface and the incubation medium, BRL 34915 ((±)-cromakalim) and BRL 38227 ((-)-cromakalim; 1 and 10 μM) did not affect evoked ACh release. In intact tracheae, however, BRL 34915 concentration-dependently inhibited ACh release; the maximal effect (inhibition by 50%) occurred at a concentration of 1 μM. BRL 38227 (0.01 and 0.1 μM) reduced ACh release by about 40%; concentrations higher than 0.1 μM were ineffective. The inhibitory effects of both potassium channel openers were abolished by removal of the epithelium or blocked by 0.1 μM glibenclamide.

In conclusion the present experiments show potassium channel openers to inhibit ACh release from postganglionic parasympathetic neurones in the airways via an indirect, epithelium-dependent pathway.

Supported by the Deutsche Forschungsgemeinschaft (We 1165/2-1).

Goldie, R.G., Fernandes, L.B., Farmer, S.G. & Hay, W.P. (1990) Trends. Pharmacol. Sci. **11**, 67-70
Wessler, I., Hellwig, H. & Racké, K. (1990) Naunyn-Schmiedeberg's Arch. Pharmacol. **342**, 387-393
Wessler, I., Klein, A., Pohan, D., Maclagan, J. & Racké, K. (1991) Naunyn-Schmiedeberg's Arch. Pharmacol., in press

---

70P COMPARISON OF THE *IN VITRO* AIRWAY SMOOTH MUSCLE RELAXANT PROPERTIES OF ROLIPRAM, ISOPRENALINE AND SALBUTAMOL

A. Tomkinson*, A. Dupuy, J-A. Karlsson & D. Raeburn, Rhône-Poulenc Rorer Ltd., Dagenham Research Centre, Dagenham, Essex

A number of cyclic nucleotide phosphodiesterase (PDE) isoenzymes have been identified in inflammatory and airway smooth muscle cells. Although the type IV cAMP specific PDE may be the major isoenzyme in inflammatory cells its role in airway smooth muscle is less distinct (Torphy & Undem, 1991). In this study we have compared the airway smooth muscle relaxant properties of the selective type IV cAMP PDE inhibitor rolipram with the non-selective and β_2-selective bronchodilators isoprenaline and salbutamol in guinea-pig and bovine trachea and in porcine bronchi *in vitro*.Tissues were suspended under an optimum applied load (L_o) in modified Krebs-Henseleit solution at 37° C gassed with 5 % CO_2 in O_2. After equilibration (90 min), tissues were precontracted with methacholine (EC_{30}) and cumulative concentration-response curves to the relaxants were constructed. Results are expressed as mean ± s.e.mean of the pD_2 values (*P<0.05 *vs* rolipram).

Table 1 pD_2 values for rolipram, isoprenaline and salbutamol in different species

	guinea-pig trachea	n	bovine trachea	n	porcine bronchus	n
rolipram	7.01 ± 0.04	6	7.71 ± 0.23	5	4.25 ± 0.02	5
isoprenaline	8.43 ± 0.15*	5	8.52 ± 0.08*	7	7.22 ± 0.20*	5
salbutamol	8.06 ± 0.10*	9	8.40 ± 0.10*	4	2.94 ± 0.08*	4

Rolipram was significantly less potent than isoprenaline and salbutamol in bovine and guinea-pig tissues, although the same maximum relaxations were produced. Salbutamol was much less active than isoprenaline in porcine bronchus, indicating that β_1 - but not β_2 - adrenoceptors mediate relaxation (Goldie et al.,1983) . The profile of PDE isoenzymes in the pig bronchus is not known but the low potency of rolipram suggests that it is different from guinea-pig and bovine trachea and that other PDE isoenzymes may be important in the regulation of bronchial tone in this species. The possible relationship between the ß-adrenoceptor subtype and the functional importance of the type IV cAMP PDE isoenzyme is intriguing and warrants further study.

Goldie,R.J., Paterson,J.W. & Wale,J.L.(1983) Br.J.Pharmacol. **79**, 177-180.
Torphy,T.J. & Undem,B.J.(1991) Thorax. **46**, 512-523.

71P CONTROL OF TISSUE CYCLIC AMP CONTENT IN PRIMARY CULTURES OF HUMAN AIRWAY SMOOTH MUSCLE CELLS

I. P. Hall, P.Townsend, K. Daykin, S. Widdop (introduced by P. C. Rubin), Department of Therapeutics, University Hospital of Nottingham, Nottingham NG7 2UH

Elevation of tissue cyclic AMP content leads to relaxation of airway smooth muscle preparations. In order to study the mechanisms underlying control of tissue cyclic AMP content in human airway smooth muscle we have established primary cultures of human airway smooth muscle cells (Hall & Widdop, this meeting). The formation of [^{3}H]-cyclic AMP was measured using a modification of the assay previously described (Ruck et al 1991).

Isoprenaline produced concentration related [^{3}H]-cyclic AMP formation (EC_{50} 2.0 $\pm$ 0.4 x10^{-7}M, n=13, fold stimulation to 1µM isoprenaline 9.5$\pm$1.3,n=47) via stimulation of a beta 2 receptor as reported at this meeting (Hall & Widdop). Forskolin (1nM-100µM) also produced concentration related [^{3}H]-cyclic AMP formation. The response to forskolin failed to reach a maximum with the highest concentration (100µM) used (n=5). The response to 1µM forskolin was 2.0$\pm$0.3 fold over basal (n=13). NaF (10mM) also increased [^{3}H]-cyclic AMP levels (fold stimulation 1.6$\pm$0.3, n=4) presumably through a direct action on Gs.

The nonselective phosphodiesterase (PDE) inhibitor 3-isobutyl-1-methylxanthine (0.1-100µM) produced concentration related [^{3}H]-cyclic AMP formation in both the absence (1.8$\pm$0.3 fold) and presence (2.1$\pm$0.2 fold) of isoprenaline, (n=9). The type IV (Nicholson et al, 1991) PDE isozyme inhibitor rolipram (100µM) also elevated tissue cyclic AMP content (fold stimulation 1.5$\pm$0.2, n=9) but did not significantly potentiate the response to isoprenaline (fold stimulation 1.0$\pm$0.1 compared with 1µM isoprenaline, n=9). The type III PDE isozyme inhibitor SK&F 94120 (0.1-100µM) was without significant effect upon basal cyclic AMP levels (n=4).

These results demonstrate that cell cyclic AMP content can be elevated in primary cultures of human airway smooth muscle by direct receptor stimulation, activation of Gs and adenylate cyclase, and inhibition of PDE activity using nonselective and type IV isozyme selective PDE inhibitors.

Ruck, A., Kendall, D.A., & Hill, S.J. (1991) Biochem. Pharmacol. 42., 59-69
Hall, I.P. & Widdop, S. (1991) This meeting.
Nicholson, C.D., Challiss, R.A.J. & Shahid, M. (1991) Trends in Pharmacol. Sci. 12., 19-27.

72P DESENSITISATION OF ISOPRENALINE-INDUCED [^{3}H]-CYCLIC AMP FORMATION IN PRIMARY CULTURES OF HUMAN AIRWAY SMOOTH MUSCLE CELLS

I. P. Hall & S. Widdop. (introduced by P. C. Rubin), Department of Therapeutics, University Hospital of Nottingham, Nottingham NG7 2UH

Controversy exists over the long term effects upon the airways of administration of beta adrenoceptor agonists (Hall & Tattersfield, in press). The initial bronchodilator response to beta agonists is due to their ability to elevate tissue cyclic AMP content and hence to relax airway smooth muscle. The aim of this study was to define the effects of these agents upon cyclic AMP responses in human airway smooth muscle. Primary cultures of human airway smooth muscle were prepared from explants of human trachealis muscle obtained at post mortem. For experiments, cells were plated in 24 (x1ml) well plates. Formation of [^{3}H]-cyclic AMP was measured as previously described (Ruck et al 1991). Agonists were added for 10 mins unless otherwise stated.

Isoprenaline (EC_{50} 2.0 $\pm$ 0.4 x10^{-7}M, n=13) induced concentration dependent [^{3}H]-cyclic AMP formation (fold stimulation to 1µM isoprenaline 9.5 $\pm$ 1.3, n=47) in cultured human airway smooth muscle cells. This response was inhibited by the beta 2 selective antagonist ICI 118551 (Bilski et al 1983) with a K_A value of 1.9 $\pm$ 0.1 x $10^8 M^{-1}$ (n=4). Prior exposure of cells to isoprenaline (1µM) for 16h followed by washing and repeat challenge with 1µM isoprenaline resulted in marked desensitisation of the response (85 $\pm$ 3% reduction compared with control cells, n=8, $p<0.001$). When cells were incubated for 16h with varying concentrations of isoprenaline, dose dependent desensitisation of the [^{3}H]-cyclic AMP response to subsequent challenge with 1µM isoprenaline was observed (IC_{50} 3.3 $\pm$ 0.2 x10^{-8}M, n=4). Incubation of cells for 16h with 1µM forskolin also resulted in marked attenuation of the subsequent response to 1µM isoprenaline (63 $\pm$ 6% reduction, $p<0.05$ compared with control cells, n=5), suggesting the involvement of cyclic AMP in desensitisation of the beta-2 adrenoceptor in human airway smooth muscle cells.

These results demonstrate that the cyclic AMP response to beta-2 receptor stimulation by isoprenaline desensitises following prior exposure to either isoprenaline or forskolin in primary culture of human airway smooth muscle cells.

Bilski, A.J., Halliday, S.E., Fitzgerald, J.D. & Wale, J.L. (1983). J. Cardiovasc. Res. 5., 430-437
Hall, I.P. & Tattersfield, A.E. (in press) in Asthma, (3rd edition) ed. Clark, Godfrey & Lee. London: Chapman & Hall
Ruck, A., Kendall, D.A., & Hill, S.J. (1991) Biochem. Pharmacol. 42., 59-69

73P BRL 55834: A NOVEL POTASSIUM CHANNEL ACTIVATOR (KCA) THAT IS MORE SELECTIVE FOR THE AIRWAYS IN THE GUINEA-PIG AND RAT COMPARED WITH BRL 38227

N.E. Bowring, D.R. Buckle, J.F. Taylor & J.R.S. Arch, SmithKline Beecham Pharmaceuticals, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ

The KCA's cromakalim and its active enantiomer BRL 38227 are potent relaxants of vascular and airways smooth muscle, and have shown potential in the therapy of both hypertension and asthma. Whilst these compounds have little effect on blood pressure in normotensive man, compounds with improved selectivity for the airways would be preferred for asthma. In this study, a novel KCA, BRL 55834 [(3S,4R)-3,4-dihydro-2,2-dimethyl-4-(2-oxopiperidin-1-yl)-6 pentafluoroethyl-2H-1-benzopyran-3-ol], is shown to have more potent and selective airways/vascular relaxant activity in both guinea-pigs and rats compared to BRL 38227. Methods were as described by Bowring *et al.*(1991).

BRL 55834 (0.5mg/kg, p.o.) was maximally effective in protecting conscious guinea-pigs from histamine-induced bronchospasm when given 60 min before challenge. This is also the Tmax for BRL 38227. Dose-response studies conducted at this time showed that half the animals were protected throughout the 4 min observation period by 0.25mg/kg BRL 55834 or 1.5mg/kg BRL 38227.

In urethane-anaesthetized guinea-pigs, BRL 55834 [ED_{25}=2.5 (2.1-2.9)μg/kg, i.v.] was 4.5-fold more potent than BRL 38227 [ED_{25}=11.3 (8.7-14.6)μg/kg] in inhibiting the increase in airways resistance following i.v. histamine (geometric means with 95% CI, n=6-8). Since the anaesthetic abolished reflex tachycardia, selectivity for the airways relative to the vasculature can be assessed from baseline blood pressure changes. Although more potent on the airways, BRL 55834 (ED_{17}=8.4μg/kg, i.v.) was 1.5-fold less potent than BRL 38227 (ED_{17}=5.6μg/kg) in lowering mean blood pressure. By the intraduodenal route, 0.1mg/kg BRL 55834 and 0.5mg/kg BRL 38227 elicited similar (34±7% at 25 min) inhibitions of histamine-induced bronchoconstriction and 0.25mg/kg BRL 55834 had a greater (45±11%) effect, but 0.5mg/kg BRL 38227 elicited a greater fall in blood pressure than 0.25mg/kg BRL 55834 (32±2 vs. 11±2%, n≥5, at 25 min). In rats, BRL 55834 (ED_{35}=3.7μg/kg, i.v.) was 4.3-fold more potent than BRL 38227 (ED_{35}=16μg/kg) in inhibiting the increase in airways resistance following inhaled methacholine, and the effect of BRL 55834, but not that of BRL 38227, was well-sustained. However, the compounds had very similar potencies as blood pressure-lowering agents (ED_{11}=8μg/kg).

Thus BRL 55834 is four- to sixfold more potent than BRL 38227 in the airways. It was sevenfold more airways selective than BRL 38227 in the guinea-pig and fourfold more airways selective than BRL 38227 in the rat.

Bowring, N.E., Buckle, D.R., Clarke, G.D., Taylor, J.F. & Arch, J.R.S. (1991) Pulmonary Pharmacol. **4**, 99-105.

74P EFFECT OF STIMULATION-FREQUENCY ON INHIBITION BY NEUROPEPTIDE Y OF ELECTRICALLY-EVOKED TWITCHES IN RAT ISOLATED VAS DEFERENS

R.M. Khan* & M.A. Zar Department of Pharmacological Sciences, The Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH.

Neuropeptide Y (NPY), a 36 amino acid peptide is present in large quantities in rodent vas deferens (Lundberg *et al.* 1984) and is known to inhibit the electrically-evoked neurogenic twitches of the vas (Wahlestedt *et al.* 1986). The rich presence of NPY-containing nerves within the vas deferens, raises the possibility that endogenous NPY, released from the intrinsic nerves as a result of electrical field stimulation (EFS), may contribute to the observed inhibitory effect of exogenous NPY on the EFS-evoked contractions. This possibility has been examined in the present investigation by determining the inhibition produced by a fixed concentration (30nM) of exogenous NPY of the neurogenic twitches of the rat vas deferens, evoked by EFS at different frequencies ranging from 0.008 Hz to 0.5Hz.

Male Wistar rats (200-250g) were killed by decapitation; 1cm long segments of vas deferens from prostatic end were prepared and 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. The bathing solution contained phentolamine (5μM) + propranolol (5μM) in order to exclude involvement of endogenous noradrenaline. Isometric contractile responses to EFS (0.08ms pulse duration, supramaximal voltage, frequency = 0.008, 0.016, 0.16, 0.25 and 0.5Hz) were tetrodotoxin-sensitive (0.5μM) and therefore neurogenic. NPY (30nM) inhibited submaximally the EFS-evoked twitches at each of the five frequencies of stimulation, but both the degree of maximum inhibition as well as the time-course of the onset of inhibition varied with stimulation-frequency (maximum % twitch inhibition: mean ± s.e. mean = 29 ± 3, 31 ± 3, 48 ± 3, 39 ± 2, 31 ± 1.5 at 0.5, 0.25, 0.16, 0.016, and 0.008 Hz respectively; n = 10); onset of inhibition was quickest at the highest stimulation-frequency and slowest at the lowest (mean + s.e. mean inhibition after 2 min exposure to NPY expressed as % maximal inhibition = 100, 90 ± 3, 86 ± 3, 56 ± 4 and 52 ± 4 at 0.5, 0.25, 0.16, 0.016 and 0.08Hz respectively; n = 10). The minimum duration of exposure to NPY, needed to obtain maximal inhibition was also inversely related to the stimulation-frequency (Mean + s.e. mean = 1.8 ± 0.1min at 0.5Hz and 7.4 ± 0.3 min at 0.008 Hz; $P < 0.001$; n = 10)

The results indicate that twitch-frequency affects the inhibitory action of exogenous NPY; since it is reasonable to believe that the quantity of NPY released from intrinsic NPY innervation in response to EFS will be proportional to stimulation-frequency, rising with increasing frequency (Lundberg *et al.*, 1989) the results lend support to the possibility that release of endogenous NPY by EFS contributes to the action of exogenous NPY on the neurogenic twitches.

Lundberg, J.M., Terenius, L., Hokfelt T. & Tatemoto, K. (1984). *J. Neurosci.* **45**, 2376-2386
Wahlestedt, C., Yanaihara, N. & Hakanson, R. (1986) *Regul. Pept.* **13**, 307-318
Lundberg, J.M., Rudehill, A., Sollevi, A., Fried, G. & Wallin, G. (1989) *Neuroscience*, **28**, 475-486.

75P COMPARISON OF THE RELAXANT AND HYPERPOLARISING ACTIONS OF CALCITONIN-GENE RELATED PEPTIDE (CGRP) AND BRL38227 IN RABBIT ISOLATED BASILAR ARTERY: STUDIES USING GLIBENCLAMIDE

D.J.Trezise & A.H.Weston, Department of Physiological Sciences, University of Manchester, U.K. M13 9PT

In rabbit small mesenteric arteries the relaxant effects of CGRP, a highly potent peptide vasodilator, and BRL38227, the active enantiomer of the potassium (K) channel opener cromakalim, are reported to involve the opening of glibenclamide-sensitive K channels (McHarg et al., 1990; Nelson et al., 1990). However, several other cellular mechanisms underlying the relaxant effects of CGRP in other blood vessels have been proposed (see Prieto et al., 1991 for references). In the present experiments we compared the effects of CGRP and BRL38227 on tension and membrane potential in rabbit isolated basilar arteries, and studied the sensitivity of these responses to the K channel blocker glibenclamide.

Basilar arteries were removed from pentobarbitone-overdosed (300mg/kg), male New Zealand White or half-lop rabbits (2-4kg). 2mm length arterial rings were either mounted between fine L-shaped wires for the recording of isometric tension, or pinned to the Sylgaard base of a 4ml volume, perfused chamber for the measurement of membrane potential using standard microelectrode techniques (electrode tip resistances 50-100mΩ). All experiments were performed in normal Krebs solution, at 37°C, constantly gassed with 95% O_2/ 5% CO_2. Both rat CGRP and BRL38227 evoked concentration-dependent relaxations of endothelium-intact arterial rings precontracted with 20mM KCl, with respective geometric mean IC_{50} values of 5.13nM (95% confidence intervals; 4.77-5.88; n=5) and 74.1nM (44.7-123; n=7).Concentrations of 30nM CGRP and 1μM BRL38227 caused similar maximal effects, amounting to 90% relaxation of the KCl-induced tone. Glibenclamide (0.3-3μM) had no effect on the concentration-response curve to CGRP (mean dose ratio shift; 1.03 in the presence of glibenclamide 3μM), but caused a concentration-dependent rightward displacement of the curve to BRL38227 (mean dose ratio shifts; 2.61 (0.3μM), 16.39 (1μM), 48.19 (3μM)). Established relaxations to CGRP (30nM) were not reversed by glibenclamide (3μM). The electrophysiological studies showed the mean resting membrane potential of rabbit basilar arterial smooth muscle cells to be -65.1 ±1.1mV (n=32 cells from 18 arteries). CGRP (30nM) consistently evoked a small hyperpolarisation (-3.1±0.4mV, n=6), whilst BRL38227 (1μM) had a much larger hyperpolarising effect (-12.6±1.9mV, n=6). Glibenclamide (3μM), but not its vehicle (100% ethanol), caused a slowly developing membrane depolarisation (+6.7±1.8mV after 15min, n=7), and prevented the response to subsequently administered CGRP (-0.2±0.4mV, n=3, $p<0.01$ when compared with control; Students t-test, unpaired analysis) and largely inhibited the response to BRL38227 (-4.2±2.5mV, n=5, $p<0.01$).

These results suggest that both CGRP and BRL38227 are capable of opening glibenclamide-sensitive K channels in rabbit basilar artery. However this effect of CGRP is relatively small, and importantly, appears not to be the primary mechanism underlying relaxation. In contrast, BRL38227-evoked relaxation is almost certainly a consequence of the opening of K channels sensitive to glibenclamide. The depolarising effect of glibenclamide may suggest that this channel is open under the experimental conditions.

DJT is supported by a Glaxo Group Research Scholarship

McHarg, A.D., Southerton, J.S. & Weston, A.H. (1990). Eur. J. Pharmacol. **185**, 137-146
Nelson, M.T., Huang, Y., Brayden, J.E., Hescheler, J. & Standen, N.B. (1990). Nature **344**, 770-773
Prieto, D., Benedito, S. & Berg Nyborg, N.C. (1991). Br. J. Pharmacol. **103**, 1764-1768

76P THE EFFECT OF CALCITONIN GENE RELATED PEPTIDE ON BLOOD FLOW AND PLASMA EXTRAVASATION IN THE RAT KNEE JOINT

H. Cambridge and S.D. Brain, Pharmacology Group, Biomedical Sciences Div., King's College, Manresa Rd., London, SW3 6LX.

Recent studies suggest the existence and release of neuropeptides, including substance P and CGRP, in rheumatoid synovial tissue (Mapp *et al.*,1989). We have investigated the activity of CGRP in the rat knee using methods adapted from the study of blood flow and protein extravasation in the skin (Brain *et al.*, 1985). Male Wistar rats (300-400g) were anaesthetised with pentobarbitone, (60mgkg^{-1}, i.p.) and prepared for either intra-articular (i.a.) injection (to measure blood flow by ^{133}Xenon clearance) or i.a. perfusion of both knees (to measure protein extravasation by the local accumulation of i.v.^{125}I albumin in the synovial space). CGRP, 0.1pmoles and 10pmoles, increased ^{133}Xenon clearance by 106.6±33.4% and 329.0±90.9% respectively, calculated as % difference between clearance over 5 min in CGRP (0.1ml) treated joints, compared to contralateral joints which received 0.1ml Tyrodes solution (mean±s.e.mean, n=5-8, $p<0.05$, modified t-test).

Table 1 shows that CGRP, at concentrations up to 10^{-4}M, did not increase protein extravasation into treated joints. In experiments where histamine (HA) and HA+CGRP were infused into opposite joints in the same rats however, the effect of HA on protein extravasation was potentiated by CGRP. Substance P (SP) had little effect in the treated joint even at doses sufficient to cause systemic effects including protein extravasation into skin of the hind paw.

Table 1 Protein extravasation into knee joints (μl/joint, mean±s.e.mean) and skin (μl100mg^{-1}) after i.a. perfusion of mediators (0.1mlmin^{-1} for 4 min into treated joint) or Tyrode (into control joint). *treated v control joint. **Tyrode v mediator-perfused rats. #HA v HA+CGRP (modified t test).

	Control joint	Treated joint	n	p*	skin	p**
Tyrode	0.76±0.22		6		1.89±0.31	
HA(10^{-4}M)	0.71±0.03	2.36±0.55	10	<0.05		
HA(2x10^{-4}M)	0.59±0.09	3.93±0.72	10	<0.01	1.77±0.02	NS
CGRP(10^{-4}M)	0.98±0.06	0.73±0.39	6	NS	2.68±0.58	NS
SP(10^{-5}M)	1.32±0.4	1.37±0.52	6	NS	2.22±0.03	NS
SP(10^{-4}M)	1.51±0.38	2.55±0.91	6	NS	19.58±2.47	0.05
HA(10^{-4}M)		1.73±0.31	10	<0.05#		
HA(10^{-4}M)+CGRP(10^{-7}M)		4.83±1.03				

Our results suggest that CGRP, if released from nerve terminals in the rat synovium, could modulate inflammation by increasing blood flow and potentiating oedema formation induced by mediators of increased microvascular permeability, such as histamine.

This study was supported by the Arthritis and Rheumatism Council.

Brain S.D., Williams, T.J., Tippins, J.R., Morris, H.R. & MacIntyre, I. (1985) *Nature* 313, 54-56
Mapp, P., Kidd, B., Merry, P., Gibson, S., Polak, J. & Blake, D. (1989) *Br J Rheumatol* 28, (suppl 3):8

77P SR 48968, A POTENT AND SELECTIVE NON-PEPTIDE ANTAGONIST OF THE NEUROKININ A (NK_2) RECEPTOR

C. Advenier[1*], X. Emonds-Alt[2], P. Vilain[2], P. Goulaouic[2], V. Proietto[2], D. Van Broeck[2], E. Naline[1], G. Neliat[3], G. Le Fur[2] & J.C. Brelière[2]. [1]Laboratoire de Pharmacologie, Faculté de Médecine Paris-Ouest, F-75270 Paris Cedex 06,France; [2]Sanofi Recherche, F-34184 Montpellier Cedex 04 France & [3]CEREP, F-86600 Celle l'Evescault, France.

Neurokinin A (NKA), substance P (SP) and neurokinin B (NKB) have been shown to act on three different receptors, denoted as NK_2, NK_1 and NK_3 respectively. Neurokinin A induces the contraction of smooth muscles of the cardiovascular, gastrointestinal, respiratory and urinary systems. We report here the biochemical and pharmacological properties of a novel potent and selective non-peptide antagonist, SR 48968 ((S)-N-methyl-N[4-acetylamino-4 phenylpiperidino)-2-(3,4-dichlorophenyl) butyl] benzamide).

SR 48968 inhibited the binding [^{125}I]-NKA to its receptor from rat duodenum membranes with an inhibition constant (K_i) of 0.51 ± 0.09 nM. Its (R)-enantiomer was less active (K_i of 945 ± 58 nM) indicating a stereoselective action of SR 48968. Scatchard analysis of the binding curves of [^{125}I]-NKA in the presence or in the absence of SR 48968 demonstrated that SR 48968 inhibition was competitive as the dissociation constant (K_d) of the ligand was modified in the presence of the compound, without significant reduction of the number of receptor binding sites (B_{max}). In addition, in classical binding assays for NK_1 and NK_3 receptor, SR 48968 did not significantly inhibit (K_i > 5000 nM) either the binding of [^{125}I]-SP to its receptor from rat cortex membranes and human IM9 cells, or the binding of [^{125}I]-eledoisin to its receptor from rat cortex (NK_3 receptor). Thus, SR 48968 selectively binds to the NK_2 receptor.

In vitro, the activity of SR 48968 was first examined on the endothelium-deprived rabbit pulmonary artery, a preparation that contain only the NK_2 receptor type. SR 48968 produced a concentration-dependent parallel rightward shift of the concentration-response of [ßAla^8]-NKA(4-10) (a specific NK_2 agonist). pA_2 value was 10.48 ± 0.06 (slope: 1.1 ± 0.3, n = 6). SR 48968 did not greatly antagonize the [Sar^9, Met $(O_2)^{11}$]-SP-induced endothelium-dependent relaxation of the rabbit pulmonary artery, an NK_1 receptor system (pA_2 = 6.40 ± 0,01, n = 6). No receptor-dependent activity of SR 48968 was observed on the [MePhe^7]-NKB-induced contraction of the rat portal vein, an NK_3 receptor assay. Finally, SR 48968 induced a concentration-dependent parallel rightward shift of the concentration-response of [Nle^{10}]-NKA(4-10) (a specific NK_2 agonist) on the human isolated bronchus. pA_2 value was 9.18 ± 0.18 (slope: 1.0 ± 0.1, n = 12). These results suggest that SR 48968 was a competitive antagonist of the human NK_2 receptor.

The *in vivo* pharmacological activity of SR 48968 was investigated in the anesthetized guinea-pig. SR 48968 produced dose-dependent inhibition of the [Nle^{10}]-NKA(4-10)-induced bronchoconstriction. One hour after administration, ID_{50} were 37 µg/kg (n = 6) or 350 µg/kg (n = 6) for intravenous and intraduodenal administration respectively.

These results demonstrate that SR 48968 is an effective, competitive and highly selective antagonist of the NKA (NK_2) receptor, without any agonist activity. SR 48968 might be a useful probe for studying the physiological or pathophysiological role of NKA.

78P DEMONSTRATION OF A FACILITATORY ROLE OF ENDOGENOUS TACHYKININS IN CHOLINERGIC NEUROTRANSMISSION IN GUINEA-PIG TRACHEA

N. Watson, [1]J. Maclagan and P.J. Barnes. Dept. Thoracic Medicine, National Heart and Lung Institute, Dovehouse St., London, SW3 and [1]Academic Dept. Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill St., London, NW3.

Exogenous tachykinins have previously been shown to facilitate contractions of the guinea-pig trachea induced by cholinergic nerve stimulation (Hall et al., 1989). Depletion experiments with capsaicin have demonstrated the involvement of endogenous tachykinins in cholinergic control of trachea smooth muscle tone in guinea-pigs (Watson et al., 1991). The aim of the present study was to investigate the nature of this tachykinin involvement, using a selective, peptidase resistant NK_1 receptor agonist (GR73632) and antagonist (GR71251). Male Dunkin Hartley guinea-pigs (250-300g) were killed by cervical dislocation and the tracheas removed with vagi and recurrent laryngeal nerves intact, for *in vitro* experiments. Contractions of the trachea, were recorded as increases in intraluminal pressure, and were induced by: (1) pre-ganglionic stimulation of the vagus nerves (PGS: 30V, 0.2ms, 2-50Hz), (2) stimulation of post-ganglionic intramural nerves via transmural stimulation in the presence of 75µM hexamethonium (TMS: 60V, 0.2ms, 2-50Hz) and (3) exogenouslly applied ACh (0.1-300µM). The effects of phosphoramidon (10µM), the NK_1 receptor agonist GR73632 (0.1 and 0.3nM) and the NK_1 receptor antagonist GR71251 (1µM), were investigated on contractions of the trachea induced by each of these methods. The effects of phosphoramidon and GR73632 on PGS- and TMS-induced contractions were then re-examined, across the frequency range (2-50Hz), in the presence of GR71251.

DRUGS PRESENT	CONTRACTION AT 8Hz (% Change from control) PGS	TMS
(a) Phosphoramidon (10µM)	+11 ± 4*	+1 ± 3
(b) NK_1 agonist (GR73632) (#)	+31 ± 9*	+38 ± 1*
(c) NK_1 antagonist (GR71251) (1µM)	- 6 ± 2*	-3 ± 1

(#) [GR73632] for PGS was 0.3nM and for TMS was 0.1nM.
(* $p < 0.02$, according to Wilcoxon ranked-sign test).

Phosphoramidon significantly facilitated contractions of the trachea induced by PGS but not TMS (a). This effect of phosphoramidon on PGS-induced contractions was significantly inhibited by the NK_1 receptor antagonist GR71251 (-3 ± 3 % at 8 Hz). TMS-induced contractions were not significantly altered (+1 ± 2 % at 8Hz). Both PGS- and TMS-induced contractions were facilitated by the NK_1 receptor agonist GR73632 (b) and this effect could be significantly antagonisted by pre-incubation with the NK_1 antagonist GR71251 (PGS +12 ± 3, TMS +2 ± 2 % at 8Hz). The NK_1 receptor antagonist GR71251 applied alone during both PGS and TMS caused a slight, but significant inhibition of PGS-induced contractions, while TMS-induced contractions were not significantly altered compared to the vehicle/time control (c). There was no significant effect of GR73632, GR71251 or the vehicle on responses to exogenous ACh.

These results suggest that NK_1 receptors are present on the final nerve terminals and in the paratracheal ganglia of cholinergic nerves. Activation of these receptors, by exogenously applied agonist, causes facilitation of cholinergic nerve-induced contractions. The finding that phosphoramidon facilitates, and the NK_1 receptor antagonist reduces, PGS-induced contractions suggests an involvement of endogenous tachykinins in ganglionic neurotransmission in pulmonary parasympathetic nerves.

Hall, A.K., Barnes, P.J., Meldrum, L.A. and Maclagan, J. (1989). Br. J. Pharmacol., 97, 274-280.
Watson, N., Maclagan, J. and Barnes, P.J. (1991). Respir. Med., (in press).

79P NK_1 TACHYKININ RECEPTORS ON THE U373MG CELL LINE: ANTAGONIST EFFECTS OF RP 67580

L. PRADIER, M. LAVILLE, S. LE GUERN, J.P. HUBERT, A. DOBLE, J.C. BLANCHARD. Rhône-Poulenc Rorer, CRVA, 13 Quai Jules Guesde, BP 14, 94403 Vitry sur Seine, France.

RP 67580 (7,7-diphenyl-2(1-imino-2-(2-methoxyphenyl)ethyl)perhydroisoindole) has recently been characterized as a potent non-peptide antagonist of substance P (SP) in the rat (Garret et al. 1991.).

This study reports on the functional properties of RP 67580 in a human astrocytoma cell line (U373MG). In this system, SP activates phospholipase C, mobilizes intracellular calcium pools (Ireland et al. 1991) and elicits ion channel responses (Pradier et al. 1991). We have studied the functional responses to SP using calcium microfluorimetry and patch-clamp techniques. Cells were cultured in standard conditions and used 3 to 7 days after plating (non-confluent monolayer).

Free intracellular calcium was monitored in individual cells using the fluorescent indicator indo-1 at room temperature. SP (EC_{50} = 2 x 10^{-9} M) and the NK_1-selective agonist [Pro^9]-SP (EC_{50} = 3 x 10^{-9} M) produced a reproducible transient intracellular calcium elevation, which was independent of extracellular calcium. RP 67580 displayed a potent, reversible antagonist effect on responses to SP (10^{-7} M) with an IC_{50} of 10^{-8} M, as did (±)-cis-3-(2-chlorobenzylamino)-2-benzhydrylquinuclidine (IC_{50} = 10^{-9} M).

Electrophysiological responses were recorded using the whole cell configuration of the patch-clamp technique at 32°C with a fast application system (U-tube). SP (10^{-8} M; 4 s pulses) reproducibly elicited a biphasic response with an initial activation of a potassium channel. Again, RP 67580 was a potent inhibitor of SP responses with an IC_{50} of 10^{-7} M as compared to 3 x 10^{-8} M for the quinuclidine compound. These values were in good correlation with the results obtained by intracellular calcium measurements.

In conclusion, RP 67580 has been demonstrated to be a potent functional antagonist of NK_1 receptors in a human model.

Garret,C.,Carruette,A.,Fardin,V.,Moussaoui,S.,Peyronel,J.F.,Blanchard,J.C. and Laduron, P.M. (1991) Proc. Natl. Acad. Sci., in press.
Ireland,S.J.,Hagan,R.M.,Bailey,F.,MacBride,C.,Jordon,C.C.,Ward,P. and Eistetter,H. (1991) European Neuropeptide Club, European meeting of functional and pharmacological aspects of neuropeptides (Austria).
Pradier, L., Laville, M., Le Guern, S. and Doble, A. (1991) Soc. Neurosci. Abst.,in press.

80P BINDING PROFILE OF RP 67580, A NEW NON-PEPTIDE SUBSTANCE P ANTAGONIST: COMPARISON WITH CP-96,345

V. Fardin, F. Foucault, M.D. Bock and C. Garret (Introduced by D. Girdlestone), Rhône-Poulenc Rorer, CRVA, 13 quai Jules Guesde, B.P. 14, 94403 Vitry-sur-Seine Cedex, France.

To date, only one non-peptide Substance P (SP) antagonist, CP-96,345, from the quinuclidine chemical series has been reported (Snider et al.,1991). This study describes the affinities for the three mammalian tachykinin receptor sites, NK_1, NK_2 and NK_3, of RP 67580 (Figure 1), a new potent, selective non-peptide SP antagonist in vitro and in vivo (Garret et al.,in press). As species variations in affinity for SP receptor sites exist, the ability of RP 67580 to inhibit binding of [^{3}H]SP in homogenates of subcortical structures of rat and mouse brains and in a human astrocytoma cell line (U373MG) has been compared. IC_{50} values (in nM) are given in Table 1 for RP 67580 (3aR,7aR), its (3aS,7aS) enantiomer RP 68651, and the two quinuclidines CP-96,345 and CP-II [(±)cis-3-(2-chlorobenzylamino)-2-benhydrylquinuclidine].

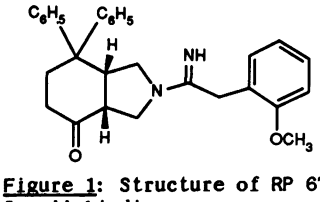


Figure 1: Structure of RP 67580

Table 1: Inhibition of [^{3}H]SP binding (0.3-0.4 nM) in various homogenates

	RAT BRAIN	MOUSE BRAIN	U373MG
Substance P	0.32 ± 0.03 (3)	0.42 ± 0.01 (3)	0.49 ± 0.08 (4)
RP 67580	7.0 ± 1.1 (3)	11.4 ± 0.9 (3)	39.8 ± 1.3 (4)
RP 68651	> 10000 (3)	> 10000 (3)	3214 ± 679 (3)
(±) CP-96,345	117 ± 17 (3)	144 ± 25 (3)	0.8 ± 0.1 (3)
(±) CP-II	142 ± 14 (3)	148 ± 19 (3)	6.2 ± 0.8 (3)

In all binding assays, a clear stereoselective action is found between RP 67580 and its enantiomer RP 68651. In rat and mouse, RP 67580 inhibits [^{3}H]SP binding with high affinity in comparison to the quinuclidine compounds. Saturation studies performed in rat brain membranes,with or without RP 67580, show that this compound is a competitive inhibitor of [^{3}H]SP binding. Although less marked than with CP-96,345, reverse species variation in affinity is observed with RP 67580 and the order of potency of antagonists differed in U373MG, in comparison to rat and mouse. In addition, RP 67580 displays high selectivity for the SP receptor, since it does not inhibit, at concentrations up to 10 µM, the binding of [^{3}H]propionyl-NKA to rat duodenum (NK_2 sites), nor that of [^{3}H]senktide to guinea-pig cortex homogenates (NK_3 sites). It does not displace binding to any other receptors studied.

In conclusion, RP 67580 appears to be the most potent SP antagonist described so far in rat and mouse. It should be a powerful tool for the investigation of the roles of SP in physiology and pathology, since it has strong SP antagonist activity in rodents and human cell lines (this meeting). In addition, the relative affinities of both RP 67580 and CP-96,345 should be very useful to further characterize tachykinin receptors.

Garret,C., Carruette,A., Fardin,V., Moussaoui,S., Peyronel,J.F., Blanchard,J.C. & Laduron,P.M. (in press) Proc. Natl. Acad. Sci. (USA).
Snider,R.M., Constantine,J.W., LoweIII,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. (1991) Science 251, 435-437.

81P BEHAVIOURAL EFFECTS OF DIRECT INTRATHECAL ADMINISTRATION OF NEUROKININS IN THE WEANLING RAT

H. Rogers, S.M. Harrison, and P.J. Birch. Department of Neuropharmacology, Glaxo Group Research Ltd, Ware, Herts, SG12 0DP, U.K.

The behavioural effects of neurokinins administered intrathecally in the mouse have been well documented (e.g. Wilcox, 1988). However, there are relatively few reports of the effects of intrathecal administration of neurokinins in the rat, except using indwelling intrathecal cannulae. We describe here the effects of direct, intrathecal administration of neurokinin receptor agonists in conscious weanling rats.

Male, Glaxo-bred, Random Hooded rats (weight range 40-70g) were used. Intrathecal injections of neurokinins or vehicle were made directly into the lumbar region of conscious rats (dose volume 10μl) via a 30 guage needle. The appearance of caudally-directed biting/scratching (CDBS) was recorded up to 5min post-injection. Drugs were made up in 0.9% saline at pH 7.4 and each rat received a single agonist dose. Each dose was given to at least 6 rats. The ED_{50} value, calculated from dose-response curves, was the dose which produced 50% responding amongst the rats.

Intrathecal injection of the NK_1 agonist, GR73632 (Hagan et al., 1989), substance P methylester (SPOMe), eledoisin, or physalaemin induced a dose-dependent CDBS. The relative order of potency, comparing ED_{50} values (pmol/rat) calculated from the proportion of rats responding, was: GR73632 (ED_{50}: 2.5 (0.8-4.3)) = physalaemin (ED_{50}: 2.8 (1.3-6.2)) > eledoisin (ED_{50}: 12.1 (0.01-1600) >> SPOMe (ED_{50}: 323 (83-748)). Substance P induced CDBS over the dose range 16-10000pmol/rat, but the dose-response relationship was multiphasic. The selective NK_2 agonist, GR64349 (Hagan et al., 1989) and the selective NK_3 agonist, senktide were injected intrathecally. GR64349 or senktide, up to 1nmol/rat, did not induce significant CDBS (10 min observation period). However, senktide did produce behaviours that were not observed with any of the other agonists including tail-lifting (ED_{50}: 29 (8-54) pmol/rat) and wet dog shakes (ED_{50}: 73 (42-184) pmol/rat). Wet dog shakes were observed 5-10 min post-injection. Pretreatment of rats with the selective NK_1 antagonist, GR82334 (Hagan et al., 1990); 1 nmol/rat; 5 min pretreatment) caused a rightward shift in the dose-response for GR73632 giving a dose-ratio of 7.5 (3.4-18.2). The CDBS produced by substance P was also antagonised by GR82334.

In conclusion, CDBS can be evoked in conscious, weanling rats by direct intrathecal injection of a range of neurokinin agonists, and is similar to that previously described in mice (Wilcox, 1988). CDBS is probably mediated by the activation of NK_1 receptors because i) CDBS is not produced by intrathecal injection of agonists selective for the NK_2 or NK_3 receptors, and ii) the response to GR73632 or substance P is antagonised by the selective NK_1 antagonist, GR82334.

Wilcox, G.L. (1988) Ann. N. Y. Acad. Sci., **525**, 228-236.
Hagan, R.M., Ireland, S.J., Jordan, C.C., Bailey, F., Stephens-Smith, M., Deal, M., and Ward, P. (1989) Br.J. Pharmacol., **98**, 71P.
Hagan, R.M., Ireland, S.J., Bailey, F., McBride, C., Jordan, C.C., and Ward, P. (1990) Br.J. Pharmacol., 168P.

82P ANGIOTENSIN II INDUCES DNA SYNTHESIS IN CULTURED VASCULAR SMOOTH MUSCLE CELLS

V. Briand*, L. Riva and A.M. Galzin (introduced by S. Arbilla), Synthélabo Recherche (L.E.R.S.), 31 Avenue Paul Vaillant Couturier, 92200 Bagneux, France

Alterations in vascular smooth muscle cell functions are believed to play a key role in the development of hypertension and atherosclerosis (Schwartz et al., 1986). The ability of angiotensin II (AII) to induce the expression of genes implicated in the regulation of cell proliferation (c fos, c myc) (Araki et al., 1990) or the induction of platelet derived growth factor (PDGF) -A chain mRNA (Naftilan et al., 1989) as well as the existence of a renin-angiotensin system at the vascular smooth muscle cell level (Egleme et al., 1990) support the view that AII, in addition to its vasoconstrictor effect, may have mitogenic properties in vivo.

The aim of the present experiments was therefore to study the effects of AII and PDGF on DNA synthesis in cultured rat aortic smooth muscle cells following serum deprivation (Travo et al., 1980). AII (0.01 nM - 0.1 µM), PDGF-BB (1 - 100 ng/ml) and PDGF-AA (1 - 100 ng/ml) increased [^{3}H]-thymidine incorporation in a concentration-dependent manner with ED_{50} of 2.8 nM, 25 ng/ml and 17 ng/ml, respectively. Maximum effects were reached at 0.1 µM (AII) and 100 ng/ml (PDGF-AA and BB) and corresponded to a 4 (AII), 15 (PDGF-BB) and 4.9 (PDGF-AA) fold increase over basal [^{3}H]-thymidine incorporation. The stimulatory effect of AII (3 nM) was antagonised in a concentration-dependent manner by the selective AII receptor antagonists sarcosin 1-isoleucin-8 AII (IC_{50} = 1 nM) and DUP 753 (IC_{50} = 10.5 nM) and by the reducing agent dithiothreitol (DTT, IC_{50} = 0.4 mM), but not by the selective AT-2 receptor antagonist CGP 42112A (IC_{50} > 1 µM).

Taken together, these results support the view that AII can induce vascular smooth muscle cell growth through receptors which could correspond to the AT-1 subtype (Chiu et al., 1989). Therefore, AII could participate in the proliferation of vascular smooth muscle cells observed in some forms of hypertension and atherosclerosis, through a mechanism which remains to be defined. In particular, the role of the PDGF-A chain expression in the mitogenic effect of AII warrants further investigation.

Araki S.I., Kawahara Y. et al. (1990) Biochem. Biophys. Res. Comm. **168** (1), 350-357.
Chiu A.T., McCall D.E., Nguyen T.T. et al. (1989) Eur. J. Pharmacol. **170**, 117-118.
Egleme C., Cressier F. and Wood J.M. (1990) Br. J. Pharmacol. **100**, 237-240.
Naftilan A.J., Pratt R.E. and Dzau V.J. (1989) J. Clin. Invest. **83**, 1419-1424.
Schwartz S.M., Campbell G.R. and Campbell J.H. (1986) Circ. Res. **58**, 427-444.
Travo P., Barrett G. and Burnstock G. (1980) Blood Vessels **17**, 110-117.

83P ICI D8731: A NOVEL, POTENT AND ORALLY-EFFECTIVE ANGIOTENSIN II ANTAGONIST

A.A. Oldham, C.P Allott, J.S. Major, R.J. Pearce[1], D.A. Roberts[1], S.T. Russell[1],
Bioscience II and Chemistry II[1] Departments, ICI Pharmaceuticals, Alderley Park, Macclesfield, SK10 4TG.

ICI D8731 (2-ethyl-4-[(2'-(1H-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl)-methoxy]quinoline hydrochloride) is one of a series of novel biphenylylmethoxy-quinolines which antagonise the pharmacological actions of angiotensin II. This study concerns the in vitro effects of ICI D8731 in guinea-pig and rabbit tissues and its in vivo effects in the rat.

In guinea-pig adrenal gland membranes, ICI D8731 displaced (^{125}I)-angiotensin II from its binding sites in a concentration-related manner, with an IC50 of 30.7 nM (n=7). Similarly, the compound was a potent, competitive antagonist of angiotensin II-mediated contractions in isolated rabbit aorta with a pA_2 of 8.3 (n=5-10 at each concentration). In contrast, ICI D8731 at a concentration of $1x10^{-7}$M had no effect on responses to noradrenaline in this preparation (dose ratio of 1.0, n=5).

Conscious male Alderley Park Wistar rats were prepared with indwelling carotid artery and jugular vein cannulae. Continuous angiotensin II infusion at 1.0 $\mu g\ kg^{-1}\ min^{-1}$ i.v. increased mean arterial pressure by 48.9 ± 2.7 mmHg and cumulative i.v. dosing of ICI D8731 inhibited this response with an ID50 of 1.0 mg kg^{-1} (n=10). In further experiments, rats prepared as above were given intermittent angiotensin II infusions of 1.0 $\mu g\ kg^{-1}\ min^{-1}$. ICI D8731 dosed at 5.0 mg kg^{-1} p.o. inhibited the angiotensin II pressor responses by 70.0 ± 5.4% and 51.0 ± 5.5% at 1 and 5 hrs after dosing, respectively (n=8). The effectiveness of ICI D8731 was maintained when administered daily at 5.0 mg $kg^{-1}\ day^{-1}$ p.o. for 10 days in this model.

Conscious male Alderley Park Wistar rats were prepared with a partial occlusion of the left renal artery, using a platinum clip of 0.25 mm i.d. After 12-14 days, carotid artery cannulae were implanted and, the following day, blood pressure measurements were taken before and for up to 24 hours after administration of ICI D8731 (5.0 mg kg^{-1} p.o.). Mean arterial pressure was reduced from 175.9 ± 3.4 mmHg at the start of the experiment to 146.0 ± 4.8 mmHg, 7 hrs after dosing ICI D8731. Blood pressure at 24 hrs after dosing (161.7 ± 2.9 mmHg) was significantly reduced compared with rats prepared as above but treated with dosing vehicle alone (173.8 ± 4.4 mmHg p=0.031, n=6). In contrast, ICI D8731 dosed at 5.0 mg kg^{-1} p.o. had only small effects in normotensive, sham operated rats. Mean arterial pressure was 122.1 ± 2.9 mmHg at the start of the experiment and was reduced to 111.3 ± 1.8 mmHg, 7 hrs after dosing the compound.

The results of the above studies are consistent with the conclusion that ICI D8731 is a potent, specific, long-acting and orally effective angiotensin II receptor antagonist. Hence ICI D8731 may have therapeutic potential in the treatment of hypertension and heart failure.

84P PHARMACOLOGICAL PROPERTIES OF SR 47436, A NON-PEPTIDIC ANGIOTENSIN II RECEPTOR ANTAGONIST

D. Nisato, C. Cazaubon, C. Lacour, J. Gougat, P. Guiraudou, C. Bernhart, P. Perreaut, J.C. Brelière & G. Le fur, SANOFI Recherche, 371, rue du Professeur J. Blayac, 34184 Montpellier, Cédex 04 France.
(Introduced by A.S. Manning)

SR 47436, 2-n-butyl-4-spirocyclopentane-1-[((2'-tetrazol-5-yl)biphenyl-4-yl)methyl]2-imidazolin-5one, is a new highly potent and specific angiotensin II (AII) antagonist without any agonistic properties. It inhibits the binding of [125I]AII in rat liver membranes (AII-1 subtype) its IC_{50} being 1.3 nM, and does not interact with AII-2 subtype receptors up to 10 µM (rat adrenal cortical membranes). In isolated rabbit aorta, SR 47436 inhibits AII-induced contractions (IC_{50}=4.0 nM) and shifts AII curves to the right, in a parallel fashion, without total recovery of the maximal response (80 % at 100 nM SR 47436). It is more potent than the most developed compound in this new class of drugs, DuP 753 (binding to rat liver preparation : IC_{50}=14nM; rabbit aorta IC_{50}=26nM) and equivalent to saralasin (IC_{50}=2.4 and 2.7 nM respectively).

The high specificity of SR 47436 was demonstrated (up to 10 µM) in many different receptor systems (e.g. α_1, α_2, $5HT_{1D}$, muscarinic M_1 and M_2, histamine, neurotensin, endothelin, neuropeptide Y, substance P, bombesin,calcium channels, Na^+/Ca^{++} and Na^+/H^+ pumps). In addition, it does not inhibit renin nor converting enzyme. Furthermore, in functional preparations, the compound (up to 10 µM) did not interfere with Ca^{++} channels, norepinephrine in rabbit aorta and vasopressin in caudal rat artery, nor did it inhibit guinea-pig ileum contractions induced by bradykinin and acetylcholine.

In conscious rats, SR 47436 (0.1 to 3 mg/kg given i.v. and 0.3 to 30 mg/kg given orally) antagonizes the pressor responses to AII in a dose-related manner (maximal effect=29 to 87 % and 20 to 89 % respectively).At the same dosages, DuP 753 produced equivalent inhibition but the maximal effect was delayed (180 min versus 15 min for SR 47436). In conscious cynomolgus monkeys, 1 mg/kg SR 47436 strongly antagonizes the AII-induced hypertension (89 % and 66 % after i.v. and oral administration respectively). Under similar conditions DuP 753 at 10 mg/kg inhibits this hypertension by 83 % and 20 % respectively. Thus, in monkeys, SR 47436 is at least ten times more potent than DuP 753; this result is in good agreement with those obtained in in vitro studies. Conversely, DuP 753 is extensively metabolized in rats, and it is known that an active metabolite contributes extensively to its activity. Thus the discrepancy observed in rats could be explained by differences in the metabolization of both compounds.

From these results, we conclude that SR 47436 is a new chemical entity displaying potent and specific AII antagonistic properties and a good oral absorption.

85P CHARACTERIZATION OF TWO NOVEL NONPEPTIDE ANGIOTENSIN II ANTAGONISTS

J.S. Zhang[1] and P.A. van Zwieten[2], 1. Pharma. Research, Dr. Karl Thomae, Birkendorferstrasse 65, 7950 Biberach/Riss, Germany. 2. Department of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands.

Two novel nonpeptide AII-receptor antagonists, 4'-[(2-n-butyl-6-cyclohexylaminocarbonylamino-benzimidazole-1-yl)-methyl] biphenyl-2-carboxylic acid (BIBS39) and 2-n-butyl-1-[4-(6-carboxy-2,5-dichlorbenzoylamino)-benzyl]-6-N-(methylaminocarbonyl)-n-pentylamino-benzimidazole (BIBS222), were characterized in vivo and in vitro. Radioligand binding studies revealed that in rat lung homogenates, BIBS39 and BIBS222 both display high affinity for AT_1 binding sites with Ki values of 29 and 20 nM, respectively. Unlike DuP753 (selective for AT_1) and other AT_1 or AT_2 selective antagonists so far developed, both agents also showed considerable affinity for AT_2 binding sites, as indicated by their Ki values of 480 and 730 nM, established in a rat adrenal medulla preparation. The ratios (Ki AT_2/Ki AT_1) for BIBS39 and BIBS222 were 17 and 37, respectively. These compounds did not exhibit relevant affinity for various other receptor types.

In isolated rabbit aortic rings, AII concentration-contractile response curves were displaced by BIBS39 (10^{-8} to 10^{-6} M) to the right in a parallel manner without a significant depression of the maximal response. The pA_2 value and slope derived from the Schild plot were 8.2 and 1.00, respectively, suggesting competitive antagonism. BIBS222 caused nonparallel shifts to the right and decreased the maximal response to AII yielding a log K_B value of 7.1, indicating noncompetitive antagonism. At 10^{-5} M, both compounds did not alter the contractile response to noradrenaline and KCl.
In pithed rats, BIBS39 dose-dependently shifted the dose-pressor response curves of AII to the right without altering the maximal response. BIBS222 also caused rightward shifts of the dose-pressor response curves to AII, but a significant reduction of the maximal response was observed at 3 to 10 mg/kg i.v.. However, the dose-pressor response curves for noradrenaline or vassopressin were not significantly changed by both antagonists at a dose of 3 mg/kg i.v., which displaced AII dose-pressor response curves by a factor 55-65.
In conscious renal hypertensive rats, BIBS39, BIBS222 and Dup753 i.v. caused a substantial dose-dependent antihypertensive effect with very similar ED_{30} values of approximately 2 mg/kg. The fall in blood pressure was accompanied by mild and transient reflex tachycardia. BIBS222 had a longer duration (50 min) of antihypertensive action than BIBS39 (25 min), whereas DuP753 lowered blood pressure for more than 60 min. It seems very likely, that the hypotensive activity of the three compounds is mediated by AT_1 receptor antagonism.

In conclusion, BIBS39 and BIBS222 are potent and specific nonpeptide AII-receptor antagonists with substantial antihypertensive activity, in spite of their additional AT_2-receptor affinity.

86P PD 123177-EVOKED INHIBITION OF THE HAEMODYNAMIC EFFECTS OF ANGIOTENSIN II IN CONSCIOUS RATS AFTER EXPOSURE TO EXP 3174

R. E. Widdop, S. M. Gardiner, P. A. Kemp and T. Bennett. Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham.

Recently AT_1 and AT_2 subtypes of the angiotensin II (AII) receptor have been identified by the binding of the non-peptide antagonists, DuP 753 and PD 123177, respectively (Chiu *et al.*, 1989). However, all the known effects of AII seem to be mediated through the AT_1 receptor, even in tissues where AT_2 receptors predominate (Wong *et al.*, 1990a). We have measured the haemodynamic effects of AII in conscious, male, Long Evans rats to determine whether or not an effect of PD 123177 can be shown following pretreatment with the non-competitive AII receptor antagonist, EXP 3174 (the active metabolite of DuP 753, see Wong *et al.*, 1990b).

Under sodium methohexitone anaesthesia (60 mg kg^{-1} i.p.), pulsed Doppler probes were implanted to monitor renal (R), mesenteric (M) and hindquarters (H) blood flows. After at least 7 days, intravascular catheters were implanted under brief anaesthesia (sodium methohexitone 40 mg kg^{-1} i.p.). The following day, continuous recordings were made of mean arterial pressure (MAP) and R, M and H blood flow signals; vascular conductances were calculated from the latter divided by MAP. AII (125 pmol kg^{-1} i.v.) and noradrenaline (NA, 1 nmol kg^{-1} i.v.) were injected before and after 10 mg kg^{-1} PD 123177 (day 1), before and after 1 mg kg^{-1} EXP 3174 (day 2), and before and after 10 mg kg^{-1} PD 123177 (day 3). PD 123177 alone caused no significant blockade of AII effects (n = 4, $P>0.05$, ANOVA). Fifteen min after EXP 3174 there was significant ($P<0.01$, ANOVA, n = 6) blockade of the pressor (3 ± 1 vs 41 ± 4 mmHg, mean ± s. e. mean) and R (-6 ± 2 vs -96 ± 2%) and M (-14 ± 4 vs -84 ± 2%) vascular conductance responses to AII, but not those to NA. Compared with the pre-EXP 3174 response, the effects of AII were still significantly attenuated 24 h after EXP 3174 (MAP, 24 ± 2 mmHg; R conductance, -72 ± 1%; M conductance, -68 ± 3%; $P<0.01$ ANOVA). Administration of PD 123177, 24 h after EXP 3174, caused a significant reduction in the AII-induced R vascular conductance responses assessed 15 and 60 min later (-37 ± 2 and -57 ± 6%, respectively), and an attenuation of the M vascular conductance response at 15 min (-48 ± 3%) ($P<0.01$, ANOVA, n = 6). Two h after PD 123177, its effects had disappeared; at this juncture EXP 3174 (1 mg kg^{-1}) totally abolished the responses to AII (n = 3). There was no significant blockade of NA responses at any time after PD 123177. These results indicate that administration of PD 123177 produced a selective, reversible blockade of the R and M haemodynamic effects of AII, but only after the prior administration of EXP 3174. These data appear to be consistent with functional expression of PD 123177-sensitive (AT_2) receptors following exposure to EXP 3174, and/or an interaction between PD 123177 and EXP 3174.

Chiu, A. T., Herblin, W. F., McCall, D. E. *et al.* (1989). *Biochem. Biophys. Res. Comm.*, **165**, 196-203.
Wong, P. C., Hart, S. D., Zaspel, A. M., Chiu, A. T., Ardecky, R. J., Smith, R. D. and Timmermans, P. B. M. W. M. (1990a). *J. Pharmacol. Exp. Therap.*, **255**, 584-592.
Wong, P. C., Price, W. A., Chiu, A. T. *et al.* (1990b). *J. Pharmacol. Exp. Ther.*, **255**, 211-217.

87P PHARMACOLOGICAL ANALYSIS OF ANGIOTENSIN-II RECEPTOR LIGAND INTERACTIONS IN THE ISOLATED RABBIT AORTA ASSAY

Y.J. Liu, N.P. Shankley[1], N.J. Welsh & J.W. Black, Department of Analytical Pharmacology, King's College School of Medicine & Dentistry, Rayne Institute, 123 Coldharbour Lane, London SE5 9NU. 1 - James Black Foundation, 68 Half Moon Lane, London SE24 9JE.

The complex profile of antagonism obsrved with peptide analogues of angiotensin II (AII) has been attributed to either allosteric (Timmermans *et al.*, 1991; Koziarz & Moore, 1989) interactions and/or non-equilibrium conditions (Koziarz & Moore, 1989; Scanlon & Moore, 1988). For example, it has been suggested that the combination of rightward shift and depression of the maxima of AII concentration-effect curves is due to these ligands behaving as "essentially-irreversible" or "slowly-reversible" antagonists.

On the isolated rabbit aorta assay (Stollak & Furchgott, 1983) a concentration-dependent, non-saturable, rightward shift and saturable depression of the AII concentration-effect curves was obtained with each of eight peptide ligands. The degree of depression was not related to the partial agonist activity observed with two of the ligands. The non-peptide ligand, DuP 753, which behaved as a simple competitive antagonist (pK_B=8.44±0.05), was used as reference antagonist in a combined dose-ratio analysis. All the peptide ligands behaved as though they had attained concentration equilibrium with the AII-receptor and acted syntopically with DuP 753. Moreover, the depression of the AII curve maxima, reversed both by DuP 753 and by washout, was not separable from the rightward shift and was specific to the AII-receptor. The data could be accounted for by a model, developed from a model of agonism (Black & Leff, 1983), in which the peptide ligands act in a reversible, competitive manner to reduce not only the apparent affinity of AII but also its apparent efficacy. A speculated molecular mechanism for this operational combination has been proposed.

Black, JW & Leff, P (1983). Proc. Roy. Soc. Lond. B, 200, 141-162.
Koziarz, P & Moore, GJ (1989). Proc. West. Pharmacol. Soc., 32, 79-84.
Scanlon, MN & Moore, GJ (1988). J. Pharmacol., Meth., 20, 207-224.
Stollak, JS & Furchgott, RF (1983). J. Pharmacol. Exp. Ther., 224, 215-221
Timmermans, PB, Wong, PC, Chiu, AT & Herblin, WF (1991). Trends Pharmacol. Sci., 12, 55-62.

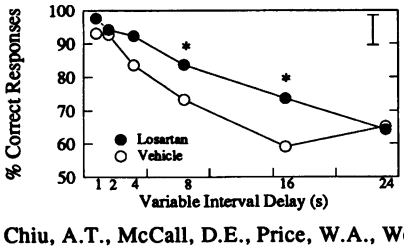
88P THE EFFECT OF THE AT_1 RECEPTOR ANTAGONIST, LOSARTAN (DuP 753) ON COGNITIVE PERFORMANCE IN THE RADIAL MAZE AND IN A DELAYED NON-MATCHING TO POSITION TASK IN THE RAT

R.P. Dennes, J.C. Barnes, A.D. Michel & M.B. Tyers, Neuropharmacology Department, Glaxo Group Research Ltd., Ware, Herts., SG12 0DP.

Studies using the AT_1 selective antagonist, losartan (DuP 753) have revealed the importance of AT_1 receptors in mediating many of the peripheral effects of angiotensin II (AII) (Chiu et al, 1990). However, in view of the localisation of AT_1 sites in the forebrain (Song et al, 1991), and the ability of ACE inhibitors to enhance cognitive processes (eg Dennes & Barnes, 1991), we have investigated the ability of losartan to modify cognitive performance in the rat. In addition, to measure in vivo potency, we have investigated the ability of losartan to inhibit the dipsogenic response induced by icv AII. Memory studies used a 12-arm radial maze and a delayed non-matching to position (DNMTP) task.

All experiments used male LH rats (300-350g). To evaluate losartan in the dipsogenic studies, AII (30pmol in 2ul) was administered icv in animals with guide cannulae, implanted at the level of the lateral ventricles. Following icv AII, the volume (ml) of water consumed in 20 min was measured. Losartan (3, 10 & 30 $mgkg^{-1}$ sc, administered 2h prior to AII) dose-dependently reduced the response to AII by 35%, 80% & 95% of control respectively. From these experiments, 10$mgkg^{-1}$ losartan was selected for evaluation in the cognitive studies. In the radial maze, animals were trained to retrieve food from three arms. Each day, rats received 4 trials and the number of errors (entries into unbaited arms) were recorded. When criterion was reached, antagonists and/or scopolamine (scop, 0.1$mgkg^{-1}$ sc) were administered in a latin-square design, prior to testing. In the DNMTP task, animals were trained as described by Dunnett (1985) and losartan treatment commenced when a criterion of >90% accuracy at the 1 and 2 s delays was reached.

Fig 1: Effect of losartan on DNMTP, Day 7



In the radial arm maze, losartan (10$mgkg^{-1}$ sc, administered 18 and 2h before test) partially reversed the scopolamine-induced performance deficit. (Mean errors ± s.e.mean: vehicle 5.0±0.8, scop alone 32.4±2.3, losartan alone 4.1±0.4, scop + losartan 27.3±2.7, P<0.05 vs scop alone). In the DNMTP, losartan (10$mgkg^{-1}$ sc od) improved performance above vehicle, particularly at the 8 and 16 s delays (eg Day 7. Fig1, *P<0.05 vs vehicle).

These results show that the selective AT_1 antagonist, losartan can facilitate learning and memory in the rat, at doses sufficient to produce a block of an icv AII-induced dipsogenic response, suggesting a role of angiotensin II in cognitive processes.

Chiu, A.T., McCall, D.E., Price, W.A., Wong, P.C., Carini, D.J., Duncia, J.V., Wexler, R.R., Yoo, S.E., Johnson, A.L. & Timmermans, P.B.M.W.M. (1990) J.Pharmacol. Exp. Ther. **252**, 711-718.
Dennes, R.P. & Barnes, J.C. (1991) Br. J. Pharmacol. **102(4)**, Proceedings 278P
Dunnett S.B. (1985) Psychopharmacology **87**, 357-363.
Song, K., Allen, A.M., Paxinos, G. & Mendelsohn, F.A.O. (1991) Clin. Exp. Pharmacol. Physiol. **18**, 93-96.

89P EFFECT OF THE ANGIOTENSIN RECEPTOR ANTAGONIST DuP 753 ON HYPERLOCOMOTION INDUCED BY D_1 AND D_2 RECEPTOR AGONISTS IN THE RAT

R.J.A. Banks, M.F. O'Neill[1] & C.T. Dourish[1], Department of Psychology, University of Sheffield, Sheffield S10 2TN. [1]Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR.

Recent data suggests a functional interaction exists between the brain angiotensin system and dopaminergic neurotransmission. Thus, the angiotensin converting enzyme inhibitors enalapril and captopril block apomorphine-induced stereotypy (Banks *et al.*, 1991). Furthermore, apomorphine-induced stereotyped behaviour is abolished by the non-peptide angiotensin II receptor antagonists DuP 753 and WL19. Dopamine receptor subtypes have been defined according to their ability to modulate the activity of adenylate cyclase in rat brain. Thus, adenylate cyclase activity is increased by D_1 receptor stimulation and inhibited or unchanged by D_2 receptor stimulation (Stoof and Kebabian, 1984). We have now assessed the effects of DuP 753 on hyperlocomotion induced by the selective D_1 receptor agonist SKF 82958 and the selective D_2 agonist (+)-PHNO in the rat.

To assess locomotor activity, stainless steel grill cages (37 x 23 x 24 cm) were used, each equipped with two pairs of photocells positioned 2 cm from the cage floor and 12.5 cm apart. Activity was measured by total photocell interrupts during the test period, and recorded via an interface linked to a CUBE microcomputer. Male S.D. rats (220-300g) were habituated to the cages for 90 min and injected with SKF 82958 (0.01, 0.03, 0.1, 0.3 or 1 mg/kg s.c.), (+)-PHNO (0.003, 0.01, 0.03, 0.1, 0.3 or 1 mg/kg s.c.) or 0.9% saline. The rats were returned to the boxes and locomotor activity recorded for 120 min, beginning 30 min after injection. In the drug interaction studies, rats were habituated to the cages for 60 min and injected with 0.9% saline or DuP 753 (10, 30 or 100 mg/kg s.c.). The rats were returned to the cages for 30 min and injected with SKF 82958 (0.03 or 0.1 mg/kg s.c.), (+)-PHNO (0.03, 0.1 or 0.3 mg/kg s.c.) or 0.9% saline. Locomotor activity was recorded for 120 min, beginning 30 min after second injection.

SKF 82958 significantly increased locomotor activity at doses of 0.03, 0.1 and 0.3 mg/kg : $F(2, 62) = 12.71$, $P < 0.001$. Similarly, (+)-PHNO significantly increased locomotion at doses of 0.01, 0.03, 0.1 and 0.3 mg/kg : $F(5, 85) = 8.24$, $P < 0.0001$. In the drug interaction studies, locomotor activity induced by 0.1 mg/kg SKF 82958 was blocked by DuP 753 at doses of 30 mg/kg : $F(2, 21) = 47.64$, $P < 0.0001$ and 100 mg/kg : $F(2, 21) = 14.27$, $P < 0.0001$. In contrast, locomotion induced by (+)-PHNO (0.01 mg/kg) was potentiated by 100 mg/kg DuP 753 : $F(2, 21) = 21.4$, $P < 0.0001$. Furthermore, activity induced by 0.03 mg/kg (+)-PHNO was potentiated by 100 mg/kg DuP 753 : $F(2, 21) = 18.67$, $P < 0.005$.

These data suggest that hyperlocomotion can be produced by selective activation of either D_1 or D_2 dopamine receptors in the rat. Interactions between angiotensin II and dopamine receptors in the control of behaviour are complex as blockade of D_1 mediated stimulation of locomotion, and enhancement of D_2 mediated stimulation of locomotion were observed in the present study. Furthermore, the results suggest that the blockade of apomorphine-induced stereotypy by angiotensin antagonists, observed in a previous study (Banks and Dourish, 1991) may involve an interaction with D_1 dopamine receptors.

Banks, R.J.A., Mozley, L. & Dourish, C.T. (1991). Neuroscience, submitted.
Banks, R.J.A. & Dourish, C.T. (1991). Br. J. Pharmacol. in press.
Stoof, J.C. & Kebabian, J.W. (1984). Life Sci. **35**, 2281-2296.

90P A COMPARISON OF NANC- AND NITROSOTHIOL-INDUCED RELAXATIONS OF THE MOUSE ANOCOCCYGEUS MUSCLE

A.J. Hobbs , J.F. Tucker & A. Gibson.
Smooth Muscle Pharmacology Group, Biomedical Sciences Division, King's College London, SW3 6LX.

Non-adrenergic, non-cholinergic (NANC) relaxations of several tissues, including the anococcygeus, are reduced by inhibitors of nitric oxide (NO)-synthase; the NANC responses are unaffected by the free radical scavenger hydroquinone, in concentrations which greatly reduce relaxations to NO, suggesting that the actual NANC transmitter may not be free NO, but rather a NO-containing or NO-generating molecule (Hobbs *et al.*, 1991). S-nitrosothiols are possible candidates and therefore we have investigated the effect of five S-nitrosothiols on the mouse anococcygeus, *in vitro*: these were S-nitrosocysteine (CYS-NO), S-nitroso-N-acetylcysteine (NAC-NO), S-nitrosoglutathione (GS-NO), S-nitrosocoenzyme A (CoAS-NO) and S-nitroso-N-acetyl-D,L-penicillamine (SNAP).

Anococcygeus muscles from male mice were set up for the recording of isometric tension responses (Hobbs *et al.*, 1991). S-nitrosothiols were synthesized according to the methods of Kowaluk & Fung (1990).

All five S-nitrosothiols produced concentration-related relaxations of carbachol (50μM)-induced tone, the order of potency being CYS-NO (0.13-50μM) > SNAP (0.1-100μM) > GS-NO (0.2-100μM) > CoAS-NO (1-64μM) > NAC-NO (1-64μM). The guanylate cyclase inhibitor N-methyl-hydroxylamine (NMH; 2mM), which reduces NANC relaxations of the mouse anococcygeus by about 50% (Gibson & Mirzazadeh, 1989), also reduced submaximal relaxations produced by all the S-nitrosothiols; % relaxations of carbachol-tone in the absence and presence of NMH were: 2μM CYS-NO (44±5%; 25±4%), 2μM SNAP (40±5%; 21±7%), 20μM GS-NO (54±8%; 27±6%), 32μM CoAS-NO (28±7%; 9±2%) and 64μM NAC-NO (56±5%; 24±4%; $n>6$; $p<0.05$ in each case). In contrast, the NO-synthase inhibitor L-N^G-nitro-arginine (50μM), which reduces NANC responses by about 90% (Gibson *et al.*, 1990), had no effect on relaxations induced by any of the S-nitrosothiols. Hydroquinone (100μM) reduced responses to 2μM CYS-NO (control relaxation = 48±5%; in the presence of hydroquinone = 6±2%; $n=8$; $p<0.05$), but had no effect on those to SNAP, GS-NO, CoAS-NO or NAC-NO.

Thus, S-nitrosothiols mimic NANC stimulation by inducing NMH-sensitive relaxations. Of those studied here, only CYS-NO was inhibited by hydroquinone, which is consistent with the finding that it most readily releases NO in solution (Kowaluk & Fung, 1990). Relaxations to the other S-nitrosothiols, like NANC responses, were resistant to hydroquinone suggesting that physiologically-relevant nitroso compounds (like GS-NO, CoAS-NO or the proposed intermediate in NO biosynthesis, nitrosoarginine) may be of interest in terms of the NANC transmitter.

A.J.H. is an SERC student.

Gibson, A. & Mirzazadeh, S. (1989). *Br. J. Pharmacol.*, **96**, 637-644.
Gibson, A., Mirzazadeh, S., Hobbs, A.J. & Moore, P.K. (1990). *Br. J. Pharmacol.*, **99**, 602-606.
Hobbs, A.J., Tucker, J.F. & Gibson, A. (1991). *Br. J. Pharmacol.*, (in press).
Kowaluk, E.A. & Fung H-L. (1990). *J. Pharmacol. exp. Ther.*, **255**, 1256-1264.

91P INDUCTION OF L-ARGININE TRANSPORT IN ACTIVATED MACROPHAGES AND ITS ROLE IN NITRIC OXIDE PRODUCTION

Richard G. Bogle, Anwar R. Baydoun, Jeremy D. Pearson and Giovanni E. Mann
Vascular Biology Research Centre, Biomedical Sciences Division, King's College London, Campden Hill Road, London, W8 7AH. U.K.

Macrophages synthesize nitric oxide during the metabolism of L-arginine to nitrite (NO_2^-) and nitrate and it is the NO radical which mediates many of the cytotoxic effects of activated macrophages (Stuehr & Nathan, 1989). Since macrophage NO production is entirely dependent on extracellular L-arginine in the present study we have characterised the transport of L-arginine in normal and activated macrophages. Experiments were performed on the macrophage cell line J774. Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum and 4mM glutamine. In all experiments cells were seeded into 96 well plates (10^5 cells/well), and allowed to adhere for 2 h after which time the medium was replaced with fresh medium containing lipopolysaccharide (LPS) and/or other compounds. NO_2^- release into the medium was measured using the Greiss reagent (Di Rosa et al., 1990) and L-arginine transport (over 5 min) was assessed as previously described (Morgan, 1990).

Unstimulated macrophages did not synthesize NO_2^-. Following activation with LPS (0.1-10 μg/ml) there was a time and concentration dependent stimulation of NO_2^- formation which became detectable following 8 h of LPS treatment. Addition of interferon-γ (100 U/ml) during the incubation period resulted in an increase in the formation of NO_2^- at all doses of LPS tested. LPS-treated macrophages did not synthesize NO^{2-} in the absence of L-arginine. The EC_{50} for the generation of NO_2^- from L-arginine was 40 ± 4 μM with maximal effects seen at concentrations of L-arginine > 300 μM. In unstimulated J774 cells L-arginine was transported by a saturable carrier system which exhibited an apparent K_t of 0.12 ± 0.02 mM and V_{max} of 14 ± 0.8 nmoles/10^6 cells/h (n=4). Following activation with LPS (1μg/ml, 24 h) there was a significant increase ($p < 0.05$) in the V_{max} of L-arginine transport to 31 ± 1.8 nmoles/10^6 cells/h (n=4), with little change in the apparent K_t 0.19 ± 0.02 mM. Transport of L-arginine (100 μM) was competitively inhibited by either L-lysine or L-ornithine with K_i's of 0.37 ± 0.21 and 0.40 ± 0.08 mM respectively (n=4). L-lysine or L-ornithine (0 - 50 mM) also inhibited NO_2^- formation by activated macrophages in a dose-dependent manner when present during the 24 h incubation period. D-mannitol (50 mM), an osmotic control, did not inhibit the generation of NO_2^- by activated macrophages.

These results demonstrate that macrophages transport L-arginine via a saturable carrier inhibited by other cationic amino acids. Following macrophage activation both L-arginine transport and NO_2^- production are stimulated. These results are consistent with a requirement for induction of the L-arginine transporter to provide a mechanism for increased substrate supply following activation of the L-arginine-nitric oxide pathway.

Supported by the Medical Research Council and British Heart Foundation. RGB holds an SERC/CASE-Wellcome Award.

Di Rosa, M., Radomski, M.W., Carnuccio, R. and Moncada, S. (1990). *Biochem. Biophys. Res. Commun.*, **172** 1246-1252.
Morgan, D.M.L. (1990). *Biochem. Soc. Trans.*, **18** 1211-1222.
Stuehr, D.J. and Nathan, C.F. (1989). *J. Exp. Med.*, **169**, 1543-1555.

92P L-N$^{\omega}$-NITRO ARGININE METHYL ESTER AND HAEMOGLOBIN EXPOSE OR ENHANCE PRESSOR RESPONSE TO 5-HT IN THE RAT ISOLATED PERFUSED LUNG

A.M. Shaw , D. Pollock$^+$, J.C. McGrath* and J.E. Thomson$^+$, Departments of physiology* and Pharmacology$^+$, University of Glasgow, G12 8QQ, Scotland.

5-Hydroxytryptamine (5-HT) has complex and potentially opposing effects in the vasculature. For example 5-HT not only has direct vasoconstrictor and indirect sympathomimetic effects but also releases nitric oxide (NO) (Newby & Henderson, 1990). In the lung, 5-HT is synthesised and stored within dense granules of the chemoreceptive cells that make up neuro-epithelial bodies. These cells degranulate in response to acute hypoxia (Lauweryns & Van Lommel, 1982). Pulmonary endothelium is also the principle site of uptake and inactivation of vascular 5-HT. This study examined the possibility that pressor responses to 5-HT in the rat pulmonary vasculature might be attenuated by endogenous NO. Responses to 5-HT in the rat pulmonary vasculature were investigated in the absence and presence of L-Nω nitro arginine methyl ester (L-NAME) and haemoglobin (Hb).

Male Wistar rats (230 - 280 g.) were anaesthetised with pentobarbitone sodium (60 mg kg-1, i.p.) and heparinised (2000 i.u. kg-1, i.p.). The trachea was cannulated and the rat was respired artificially (stroke volume = 2.5 ml, rate = 55 min.-1). Rats were killed by exanguination. A stainless steel cannula was inserted through the right ventricle and into the pulmonary artery. Krebs buffer (22°C) was allowed to run through the pulmonary vasculature and out of the cut left atrium to remove blood cells. The pulmonary vasculature was then perfused at 2 ml per minute for 1 hour with Krebs buffer (35°C, gassed with 95%O_2/5%CO_2) before drugs were administered either as bolus injections or by perfusion to equilibrium.

In the absence of L-NAME, 5-HT (0.1μM - 1mM) either had no effect or caused only small increases in perfusion pressure. L-NAME (500μM) did not affect the basal perfusion pressure but either exposed pressor responses to 5-HT (EC_{50} = 2.83±1.07μM, means±SEM n=8), where previously none occurred, or enhanced pressor responses to 5-HT. These responses were abolished or greatly reduced by L-arginine (1mM). Hb (100μM) increased the baseline perfusion pressure and also exposed the pressor response to 5-HT. Since the 5-HT_1 receptor agonist, 5-carboxamidotryptamine (0.1μM - 1mM) had little or no effect even after administration of L-NAME, pressor responses to 5-HT are not mediated via 5-HT_1 receptors.

The results suggest that endogenous NO normally prevents the appearance of pressor responses to 5-HT in the pulmonary vasculature, perfused with Krebs buffer. NO may be important in the pulmonary vasculature, where its significance may be more obvious in lungs perfused with saline rather than blood, since erythrocytes inactivate NO (Gillespie & Sheng, 1988).

Gillespie, J.S. & Sheng, H. (1988) Br. J. Pharmacol. **95**, 1151-1156.
Lauweryns, J.J. & Van Lommel, A. (1982) Cell Tissue Res. **2216**, 201-214.
Newby, A.C. & Henderson, A.H. (1990) Annu. Physiol. 52, 661-674.

93P RENAL VASODILATOR RESPONSES TO ACETYLCHOLINE IN BRATTLEBORO RATS BEFORE, DURING AND AFTER CHRONIC ORAL INGESTION OF N^G-MONOMETHYL-L-ARGININE (L-NMMA)

S. M. Gardiner, P. A. Kemp, T. Bennett, R. M. J. Palmer* and S. Moncada*. Department of Physiology and Pharmacology, Queen's Medical Centre, Nottingham and *Wellcome Research Laboratories, Langley Court, Beckenham, Kent.

Polydipsic, Brattleboro (i.e. vasopressin-deficient) rats given L-NMMA or N^G-nitro-L-arginine methyl ester (L-NAME) in their drinking water become hypertensive and remain so until the L-NMMA or L-NAME is withdrawn (Gardiner *et al.*, 1990, 1991b). Previously we found that acute i.v. treatment with L-NAME inhibited, but did not abolish, vasodilator responses to acetylcholine (ACh) infusions (Gardiner *et al.*, 1991a). In the present work we assessed vasodilator responses to ACh in Brattleboro rats before, during and after oral ingestion of L-NMMA. Under sodium methohexitone anaesthesia (60 mg kg^{-1} i.p., supplemented as required), pulsed Doppler probes were implanted to monitor renal, mesenteric and hindquarters blood flows. At least 7 days later, animals were re-anaesthetized (sodium methohexitone 40 mg kg^{-1} i.p.) and had intra-arterial and intravenous catheters implanted. Following a recovery period of at least 24 h, a 3 min infusion of ACh (4 μg min^{-1}) was given. L-NMMA was then added to the drinking water (1 mg ml^{-1}), and 6 h and 3 days later animals were re-challenged with ACh. Plain drinking water was then returned and an infusion of ACh was given 6 h later. Resting values and responses to ACh (assessed from areas under or over curves (AUC and AOC, respectively) were compared using Friedman's test. Since, with the protocol we used, only the renal vascular bed showed an increase in flow during ACh infusion, this report concerns those results. Before ingestion of L-NMMA, resting mean arterial blood pressure (MAP) was 111 ± 2 mmHg (mean ± s. e. mean), renal flow was 8.2 ± 0.9 kHz and renal conductance was 74 ± 8 [kHz $mmHg^{-1}]10^3$. Infusion of ACh caused hypotension (AOC, 20 ± 2 units) and increases in renal flow (AUC, 7.4 ± 1.0 units) and conductance (AUC, 83 ± 10 units). Six h after addition of L-NMMA to the drinking water, MAP was increased (132 ± 3 mmHg, $P<0.05$) and renal flow and vascular conductance were decreased (7.8 ± 0.8 kHz and 59 ± 6 [kHz $mmHg^{-1}]10^3$, respectively, $P<0.05$). The hypotensive response to ACh was not changed significantly (AOC, 36 ± 6 units), but the increases in renal flow and conductance were reduced (AUC, 5.2 ± 0.7 and 59 ± 8 units, respectively, $P<0.05$). Three days after addition of L-NMMA to the drinking water, MAP was still increased (128 ± 5 mmHg, $P<0.05$) although renal flow (8.6 ±1.1 kHz) and conductance (66 ± 6 [kHz $mmHg^{-1}]10^3$) were not significantly reduced. ACh elicited an hypotensive response (AOC, 35 ± 8 units) not different from control, but the increases in renal flow and conductance were still decreased (AUC, 4.9 ± 0.6 and 59 ± 9 units, respectively, $P<0.05$). Six h after removal of L-NMMA from the drinking water, resting variables were not different from control (MAP, 110 ± 4 mmHg, renal flow, 7.9 ± 0.6 kHz, renal conductance 72 ± 5 [kHz $mmHg^{-1}]10^3$). Furthermore, the hypotensive response (AOC, 18 ± 4 units), and the increase in renal flow (AUC, 7.1 ± 0.9 units) and vascular conductance (AUC, 79 ± 11 units) with infusion of ACh were not different from the control responses.

Hence, chronic oral ingestion of L-NMMA causes sustained, but reversible, inhibition of the renal vasodilator effect of ACh in conscious, Brattleboro rats.

Gardiner, S. M., Compton, A. M., Bennett, T., Palmer, R. M. J. & Moncada, S. (1990). *Br. J. Pharmacol.*, **101**, 10-12.
Gardiner, S. M., Kemp, P. A. & Bennett, T. (1991a). *Br. J. Pharmacol.*, **103**, 1725-1732.
Gardiner, S. M., Kemp, P. A., Bennett, T., Palmer, R. M. J. & Moncada, S. (1991b). *Br. J. Pharmacol. Proc. Suppl.* (in press).

94P ENDOTHELIAL FUNCTION AND SENSITIVITY TO VASOCONSTRICTOR AGENTS IN ISOLATED SMALL HUMAN FETO-PLACENTAL ARTERIES

A.L. McCarthy , K.S. Raju, R.G. Woolfson[1] & L. Poston[1], UMDS Smooth Muscle Group, Divisions of Physiology[1] and Obstetrics, United Medical and Dental Schools, St.Thomas' Campus, London SE1 7EH.

Little is known of the functional role of the endothelium in the control of feto-placental blood flow. However, in the absence of autonomic control in this circulation, the endothelium may play a critical role in the maintenance of low vascular resistance. It has been established in the perfused placenta that inhibitors of nitric oxide (NO) synthesis cause an increase in basal perfusion pressure (Gude *et al*, 1990). In this study we have investigated endothelium-dependent relaxation in isolated feto-placental arteries, and have studied the effect of NO synthase inhibitors on tension development in response to the thromboxane analogue, U46619.

Feto-placental arteries dissected from human placentae (normalised internal diameter 141-481μm) were mounted on a small vessel myograph (Mulvany & Halpern, 1975). The arteries were stretched to achieve an approximate transmural pressure of 37mmHg, and maintained in physiological buffer gassed with 5%CO_2 in nitrogen. The measured oxygen tension in the organ bath was 30-35mmHg.

Arteries were pre-constricted with 10^{-7}M U46619 and concentration responses to acetylcholine, bradykinin and histamine were performed. Histamine alone caused concentration dependent relaxation but the maximum relaxation ($5x10^{-5}$M) achieved was only 49.26 ± SEM 9.07%, n=7 arteries. In the presence of L-NAME this was significantly inhibited. In further experiments, concentration response curves were performed to U46619 in the presence of indomethacin (10^{-5}M), and repeated with indomethacin and Nω-nitro-l-arginine methyl ester (L-NAME, 10^{-4}M). The addition of L-NAME resulted in enhanced sensitivity to U46619, which achieved significance between $5x10^{-7}$M - 10^{-6}M. At the highest concentration of U46619 (10^{-6}M) the tension in the presence of L-NAME + indomethacin was 3.23 ± SEM 0.44mN/mm artery length compared to that with indomethacin alone,
2.77 ± SEM 0.38mN/mm (n=10 arteries, $P=0.005$).

This study suggests that NO may modify the response to constrictor agents in this circulation, but that feto-placental arteries do not demonstrate marked receptor-mediated relaxation to compounds which effect endothelium-dependent relaxation in other vascular beds.

Gude NM, King RG, Brennecke SP.(1990) Lancet **336**,1589-1590
Mulvany MJ, Halpern W. (1977). Circ Res **41**,19-26

95P EFFECTS OF CLOSE-ARTERIAL INFUSION OF NITROVASODILATORS ON RAT GASTRIC MUCOSAL INTEGRITY

J. Lopez-Belmonte*, B.J.R. Whittle & S. Moncada. Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS, U.K.

Intravenous administration of S-nitroso-N-acetyl-penicillamine (SNAP), a nitrovasodilator (Ignarro et al, 1981) which spontaneously generates nitric oxide (NO), protects the rat small intestine from vascular damage induced by acute endotoxin shock (Boughton-Smith et al, 1990). In the present study, the actions of locally administered SNAP on the damage induced in the rat gastric mucosa by intra-arterial infusion of endothelin-1 (ET-1) have been investigated. In addition, the effects of the 'organic' nitrovasodilator, glyceryl trinitrate (GTN) have been evaluated.

Rats (240g) were anaesthetised with pentobarbitone, the stomach exposed and the left gastric artery cannulated for the intra-arterial administration of the agents (Whittle & Esplugues, 1988). Acid saline (100mM HCl; 2ml) was instilled into the gastric lumen. Local infusion of ET-1 (10pmol kg^{-1} min^{-1} for 10 min) induced extensive haemorrhagic injury in the mucosa, assessed macroscopically 20 min later, with $54 \pm 6\%$ of the total mucosal area ($n=8$; $P<0.01$) being involved. This damage was dose-dependently inhibited by concurrent local infusion of SNAP (5-10 μg kg^{-1} min^{-1} throughout the 30 min experimental period) with a $46 \pm 10\%$ reduction ($n=6$, $P<0.05$) in the area of damage with SNAP (10μg kg^{-1} min^{-1}). However, higher doses of SNAP (20 and 40μg kg^{-1} min^{-1}) failed to alter significantly this damage. Furthermore, local infusion of SNAP at doses of 20 and 40μg kg^{-1} min^{-1} for 30 min, itself induced dose-dependent gastric mucosal injury, with SNAP (20μg kg^{-1} min^{-1}) inducing haemorrhagic damage involving $20 \pm 6\%$ of the total mucosal area ($n=6$; $P<0.01$) which was confirmed histologically. GTN (10-40 μg kg^{-1} min^{-1} i.a.) dose-dependently inhibited ET-1 induced damage, with a $65 \pm 6\%$ reduction ($n=8$, $P<0.001$), at the higher dose, while GTN itself did not injure the mucosa. SNAP or GTN (20 or 40 μg kg^{-1} min^{-1} i.a. respectively) significantly ($P<0.01$) reduced systemic arterial blood pressure by 38 ± 3 and 30 ± 2 mmHg respectively.

These findings indicate that close arterial administration of the nitrovasodilators, SNAP and GTN, can protect the gastric mucosa from the damage induced by the vasoconstrictor peptide, ET-1, which may reflect local microcirculatory interactions. However, SNAP in high doses can itself bring about mucosal injury, whereas GTN, which requires metabolic transformation to release NO, had no such effect. Thus, the unregulated or excessive local release of NO may initiate microvascular changes leading to tissue damage.

Boughton-Smith, et al (1990). Eur. J. Pharmacol., 191, 485-488.
Ignarro, L.J. et al (1981). J. Pharmacol. Expt. Ther. 218, 739-749.
Whittle, B.J.R. & Esplugues, J.V. (1988). Br. J. Pharmacol., 95, 1011-1013.

96P DIFFERENTIAL METABOLISM OF NITROVASODILATORS BY CARDIAC MYOCYTES: NITROPRUSSIDE REDUCES MYOCYTE CONTRACTILITY

A.J.B.Brady, J.B.Warren,[1] P.A.Poole-Wilson & S.E.Harding, Departments of Cardiac Medicine and [1]Applied Pharmacology, National Heart & Lung Institute, Dovehouse Street, London SW3 6LY

We have investigated the contractile response of isolated cardiac myocytes to three different nitrovasodilators. These drugs act by generating nitric oxide or a nitric oxide containing intermediate. However, they differ in their metabolism and subsequent release of this intermediate. There are also tissue differences; thus vascular smooth muscle *in vitro* is capable of metabolising glyceryl trinitrate (Benjamin et al., 1991) whereas endothelium is not. Nitrovasodilators have profound actions on coronary smooth muscle but their effect on cardiac myocyte contractility has not been described.

Guinea-pig cardiac myocytes were isolated as previously described (Harding et al., 1990) and superfused in Krebs-Henseleit buffer, containing 2mM calcium, at 32°C. The contraction amplitude and velocity of shortening of electrically-stimulated (0.5 Hz) myocytes was recorded using a videomicroscopy-length detection system. Myocyte contractility was calculated as the percentage shortening of the resting length. In control recordings myocytes shortened by $5.3\% \pm 0.29$ (mean$\pm$s.e.mean). Control readings were taken before and after the myocytes were exposed to either glyceryl trinitrate (GTN), isosorbide dinitrate (ISDN) or sodium nitroprusside (SNP). GTN and ISDN had no effect on contractility over the dose range 10^{-6} - $3x10^{-5}$M. SNP caused dose-related reductions in myocyte shortening. At 10^{-6}, $3x10^{-6}$, 10^{-5} and $3x10^{-5}$M SNP reduced shortening by $12.7\% \pm 5.3$, $15.0\% \pm 2.6$, $15.4\% \pm 4.4$ and $23.0\% \pm 3.9$ (mean$\pm$s.e.mean, n=4-10 cells at each concentration, $P<0.05$ at each concentration c.f. control). Maximal contraction velocity and time from peak contraction to 90% relaxation were not affected by nitrovasodilators. The effect of SNP on myocyte contractility was inhibited by methylene blue $5x10^{-6}$M.

The contractility of cardiac myocytes is reduced by the presence of SNP. This effect was likely mediated by nitric oxide or a related intermediate since it was prevented by the addition of methylene blue. The lack of response to GTN or ISDN suggests that myocytes lack the enzyme(s) responsible for the release of nitric oxide from these compounds which require additional biotransformation. Nitrovasodilators thus differ in their ability to affect cardiac myocyte contractility.

AJBB is a Medical Research Council Clinical Training Fellow, JBW is supported by the British Heart Foundation

Benjamin, N., Dutton, J.A. & Ritter, J.M.(1991).*Br J Pharmacol.* **102**: 847-50.
Harding, S.E., O'Gara, P., Jones, S.M., Brown, L.A., Vescovo,G. & Poole-Wilson, P.A.(1990).*Cardioscience.* **1**: 49-53.

97P METABOLISM OF GLYCERYL TRINITRATE TO NITRIC OXIDE IN ENDOTHELIAL AND SMOOTH MUSCLE CELLS AND THE EFFECT OF *E. Coli* LIPOPOLYSACCHARIDE

D. Salvemini, V, Mollace, A. Pistelli, E. Anggard & J. Vane, The William Harvey Research Institute, St Bartholomews Hospital Medical College, Charterhouse Square, London EC1M 6BQ.

The vasodilator effect of glyceryl trinitrate (GTN) is mediated through bioconversion to nitric oxide (NO; Ignarro et al., 1981). GTN also inhibits platelet aggregation although the concentrations required for this effect are much greater in vitro than in vivo. NO is also produced endogenously from L-arginine (L-Arg) by various cells including endothelial cells (EC) and smooth muscle cells (SMC); this release can be enhanced by E. coli lipopolysaccharide (LPS; Radomski et al., 1990; Salvemini et al., 1991). We have now investigated whether LPS affects the metabolism of GTN to NO by bovine aortic SMC or EC and whether this pathway is affected by the formation of endogenous NO from L-Arg.

Two bioassay systems were used; inhibition of platelet aggregation and measurements of cGMP after stimulation by NO of guanylate cyclase in SMC or EC. In addition, nitrite (NO_2^-) was measured as another index of NO formation. The inhibitory effects of GTN (22-352μM) or sodium nitroprusside (NaNp;4-64μM) on platelet aggregation induced by thrombin (40mU/ml) were measured either alone or in the presence of SMC ($0.24x10^5$) or EC ($0.4x10^5$) non-treated or treated with LPS. Cells and platelets were always treated with indomethacin (10μM).

SMC or EC enhanced the platelet inhibitory activity of GTN. Thus, the concentration of GTN required to inhibit platelet aggregation by 50% (IC_{50}) was 110±2μM for GTN alone, 21±1μM for GTN in the presence of SMC and 69±1μM in the presence of EC (n=4). This effect was abrogated by oxyhaemoglobin (oxyHb, 10μM; n=4). LPS (0.5μg/ml, 18h) treatment increased further the anti-platelet potency of GTN so that the IC_{50} for SMC decreased to 9±1μM and for EC to 31±1μM (n=4). This enhancement was attenuated by cycloheximide (10μg/ml). Incubation of non-treated or LPS-treated cells with N^G-monomethyl-L-arginine (300μM for 60 min) did not influence the metabolism of GTN to NO (n=4). Furthermore, when incubated with GTN (200μM), SMC or EC (10^5) treated with LPS (0.5μg/ml, 18h) caused an enhanced oxyHb (10μM)-sensitive increase (8-9 fold, n=4) in the levels of cGMP. Exposure of SMC to GTN (600μM) for 30 min led to a cell-dependent increase in the levels of NO_2^- which was enhanced (2-3 fold) in SMC treated with LPS (n=4). When the cells were boiled for 15 min the generation of NO_2^- from GTN was strongly suppressed (n=4). Non-treated or LPS-treated SMC failed to enhance the anti-platelet activity of sodium nitroprusside (n=4). An intraperitoneal injection of LPS (20mg/kg) 18h beforehand into rats enhanced the hypotensive responses of the anaesthetized animals to GTN (0.25-1mg/kg; n=12). These effects were blocked by methylene blue (10mg/kg) but not by indomethacin (3mg/kg). LPS did not alter the hypotensive responses to phentolamine (0.5mg/kg), verapamil (2mg/kg) or SIN-1 (0.25mg/kg) (n=12).

Thus, cultured EC as well as SMC can convert GTN to NO. The biotransformation of GTN both in EC and SMC is induced by pretreatment with LPS, an effect which was shown also in vivo. Metabolism of GTN by EC may be responsible for the antiplatelet effects of the drug in vivo and, along with SMC for its vasodilator activity. ***(This work was supported by a grant from Glaxo Group Research Ltd).***

Ignarro, L.J. et al., (1981). J. Pharmacol. Exp. Ther., 218, 739.
Radomski, M.W. et al., (1990). Proc. Natl. Acad. Sci. USA., 87, 10043.
Salvemini, D. et al., (1991). Br. J. Pharmacol., in press.

98P A 5-HT_1-LIKE RECEPTOR MEDIATES AN ENDOTHELIUM-DEPENDENT RELAXATION IN GUINEA-PIG ISOLATED JUGULAR VEIN

Paul Gupta (introduced by K.F. Rhodes)
Wyeth Research (UK) Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berks. SL6 0PH

In a variety of vascular preparations, 5-HT can evoke relaxation by a mechanism dependent upon the functional integrity of endothelial cells (Cohen, 1989). In this paper, such a mechanism is described in guinea-pig isolated jugular vein.

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. Agonist-evoked relaxant responses were measured isometrically in rings precontracted with U-46619 (30 nM), in the presence of ketanserin and mesulergine (both 3 μM) to antagonise respectively 5-HT_2 and 5-HT_1-like receptors located on the smooth muscle. At these concentrations, ketanserin produced an approximate 10 fold rightward displacement of agonist concentration-response curves which was considered to be in accordance with a possible interaction at a 5-HT_1 receptor; mesulergine was without effect. Biphasic concentration-response curves were observed to both 5-HT and 5-CT, only monophasic curves were seen to sumatriptan and α-methyl-5-HT. In endothelium-denuded preparations, relaxant responses to sumatriptan, α-methyl-5-HT and the first phase of relaxant responses to 5-HT and 5-CT were abolished, thus demonstrating the dependence upon a functional endothelium. All subsequent data refer to endothelium-dependent relaxant responses (see Table). Due to the weak antagonist action of ketanserin at the endothelial 5-HT receptor, the stated EC_{50} values are underestimated (approximately 10 fold).

Agonist	EC_{50} ($-\log_{10}$ M)	Emax (%)	n
5-HT	7.04 ± 0.06	40.4 ± 3.3	20
5-CT	7.51 ± 0.07	55.8 ± 4.8	16
Sumatriptan	5.50 ± 0.10	49.0 ± 5.4	11
α-Methyl-5-HT	5.87 ± 0.05	31.5 ± 6.7	3

Data are the mean ± s.e.mean data for n experiments. Emax refers to maximal relaxation expressed as a % of U-46619 precontraction.

In antagonist studies, 5-HT mediated relaxations in the presence of mesulergine (10 μM; ketanserin absent) were unaffected by ondansetron (1 μM). However, ligands with affinity toward 5-HT_1 receptors such as methiothepin and PAPP (1-[-2-[4-aminophenyl]ethyl]-4-[3-trifluoromethylphenyl]piperazine ; Schoeffter & Hoyer, 1990) blocked relaxations to both 5-HT and sumatriptan with similar potency (respective apparent pA_2 values : methiothepin 8.1 & 8.6; PAPP 8.2 & 8.2, all n=4-7). These data suggest that this endothelial receptor should presently be classified as 5-HT_1-like in accordance with the proposals of Bradley *et al.* (1986).

Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P. & Saxena, P.R. (1986). *Neuropharmacology*, **25**, 563-576.
Cohen, R.A. (1989). In. *The Peripheral Actions of 5-Hydroxytryptamine* (Fozard, J.R. ed.) pp 182-200, Oxford University Press, Oxford.
Schoeffter, P. & Hoyer, D. (1990). *J. Pharmacol. Exp. Ther.*, **252** , 387-395.

99P 5-HT RECEPTORS AND A RECEPTOR RESEMBLING THE 5-HT$_{1D}$ RECEPTOR SUBTYPE MEDIATE CONTRACTIONS IN HUMAN SAPHENOUS VEIN

W.A. Bax, [2]D. van Heuven-Nolsen, [1]E. Bos, [1]M.L. Simoons, P.R. Saxena. Department of Pharmacology and [1]Thorax Centre, Erasmus University Rotterdam, P.O.Box 1738, 3000 DR Rotterdam and [2]Department of Pharmacology, University of Utrecht, the Netherlands.

Human saphenous vein is used in coronary bypass surgery. Postoperative complications include vasospasm in which 5-HT seems to be involved via an action on 5-HT$_1$-like and 5-HT$_2$ receptors (Victorzon *et al.*, 1986). In this study we have attempted to further characterize 5-HT receptors involved using a number of 5-HT receptor agonists and antagonists. Leftover saphenous vein was obtained intraoperatively from 42 patients aged 44-77 years. Ring segments were mounted in 8 ml organ baths and tension was measured isometrically. After an initial 5-HT concentration response curve a second curve with various agonist antagonist combinations was made. All agonists except flesinoxan caused dose dependent contractions.

Table 1. Mean (±s.e.m.) of pD$_2$ (-Log EC$_{50}$) and E$_{MAX}$ (% of max 5-HT contraction). n=3-18.

	5-CT	5-HT	Methysergide	Sumatriptan	DOI	Ru24969
pD$_2$	7.07 (0.24)	6.80 (0.08)	6.29 (0.38)	6.11 (0.09)	5.71 (0.29)	5.80 (0.20)
E$_{MAX}$	89.3 (3.5)	93.0 (3.3)	16.4 (2.8)	37.2 (3.8)	56.9 (7.1)	62.3 (6.0)

Table 2. Mean of pK$_B$ values against sumatriptan. n=4-6.

Antagonist	μM	pK$_B$(± s.e.m.)
Methiothepin	0.1	7.26 (0.11)
Metergoline	1	7.29 (0.25)
Rauwolscine	1	6.68 (0.20)
Cyanopindolol	1	6.46 (0.30)

Ketanserin mainly antagonized the effect of high concentrations of 5-HT. Concentration-effect curves to DOI and α-methyl-5-HT were potently antagonized in a parallel manner by ketanserin, indicating the presence of 5-HT$_2$ receptors. Contractions to sumatriptan were antagonized by methiothepin (10^{-7} M), metergoline (10^{-6} M), rauwolscine (10^{-6} M) and cyanopindolol (10^{-6} M) but not by ketanserin (10^{-6} M). This indicates that sumatriptan contracts human saphenous vein through a 5-HT$_1$-like specific mechanism. A high Pearson's correlation coefficient of functional pD$_2$values to 5-HT$_{1D}$ receptor binding pK$_D$ values obtained in human caudate membrane (Waeber *et al.*,1988) for 5-HT$_1$-like receptor agonists (5-CT, 5-HT, sumatriptan, Ru24969, methysergide and 8-OH-DPAT) was calculated (r=0.98 p<0.01). For antagonist pK$_B$ values (Table 2) the correlation coefficient to 5-HT$_{1D}$ receptor pK$_D$ values was calculated as r=0.94, p<0.05 which is larger than the correlation obtained for pK$_B$ values against binding pK$_D$ values for the 5-HT$_{1A}$, 5-HT$_{1B}$ or 5-HT$_{1C}$ receptor subtype. We therefore conclude that 5-HT contracts human saphenous vein through 5-HT$_2$ receptors and a receptor resembling the 5-HT$_{1D}$ receptor. (Study supported by the Netherlands Heart Foundation, grant 89.252).

Victorzon, M., Tapparelli, C. & Müller-Schweinitzer, E.(1986), Eur. J. Pharmacol., **124**, 107-111.
Waeber, C., Schoeffter, P., Palacios, J.M. & Hoyer, D. (1988) Naunyn Schmiedeberg's Arch. Pharmacol., **337** , 595-601.

100P CHARACTERISTICS OF MUSCARINIC RECEPTORS IN THE RAT ISOLATED PORTAL VEIN

M. Pfaffendorf and P.A. van Zwieten. Department of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands.

The rat isolated portal vein displays spontaneous myogenic activity. When stimulated by muscarinic agonists the amplitude of the rhythmic contractions increase and, at higher doses a concentration dependent contracture develops. This study was undertaken to characterize the muscarinic receptor subtype, mediating the effect on the amplitude of the spontaneous contractions and the subsequent contracture, respectively. Furthermore we investigated whether the two responses reflect the stimulation of distinct receptors or whether the response is mediated by one receptor subtype probably indicating two different signaling pathways coupled to one receptor.

Portal veins of male Wistar rats (350-400 g) were mounted longitudinally at a load of 1 g in thermostatically controlled organ baths (37^0 C). The preparations were suspended in a Tyrode's solution (in mmol/l: NaCl 124, KCl 4.0, $MgCl_2$ 1.1, $NaHCO_3$ 24.9, Na_2HPO_4 0.42, $CaCl_2$ 0.9, glucose 5.5) and gassed with 95% O_2 and 5% CO_2. Isometric contractile activity was measured via force displacement transducers. After a two hour period of equilibration with or without an antagonist cumulative concentration-response curves for carbachol (CCh) were constructed. The pA$_2$-values for the antagonists were obtained from Schild plots. The slopes did not differ from unity.

The EC$_{50}$ values of CCh for the amplification of the rhythmic contractions and the sustained contracture were found to be 0.22 μmol/l and 2.65 μmol/l, respectively.

The pA$_2$ values for the antagonists are given in table 1 (means ± s.e. mean, n=6).

Table 1	atropine	pirenzepine	AF-DX 116	4-DAMP	p-FHHSiD
increase of amplitude	9.68 ± 0.07	6.90 ± 0.15	6.54 ± 0.06	9.55 ± 0.14	7.53 ± 0.10
contracture	9.98 ± 0.11	7.25 ± 0.25	6.78 ± 0.17	9.84 ± 0.08	7.87 ± 0.15

For all antagonists used, a competitive interaction with CCh was observed. Although the contracture was influenced slightly more than the enhancement of the amplitude, the rank order of potency was found to be identical for both types of response: atropine (unsp.) > 4-DAMP (M$_1$=M$_3$) > p-FHHSiD (M$_3$) > pirenzepine (M$_1$) > AF-DX 116 (M$_2$). According to the pA$_2$-values of the antagonists found in other tissues (Eglen & Whiting, 1990), we conclude that both, the increase of contractile amplitude and the sustained contracture are mediated predominantly by the muscarinic M$_3$-receptor subtype.

R.M. Eglen & R.L. Whiting (1990), J.Auton.Pharmacol. **19**, 233-245

101P CHARACTERIZATION OF MUSCARINIC RECEPTORS IN ISOLATED PERFUSED MESENTERIC VASCULAR BED OF SHR AND NORMOTENSIVE CONTROL RATS

M.G.C. Hendriks, M. Pfaffendorf & P.A. van Zwieten. Department of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands.

Characterization of the muscarinic receptor mediating vasodilation, via the release of endothelium derived relaxing factor (EDRF) has been performed in several conduit arteries of several species. However, identification of this muscarinic receptor subtype has not yet been carried out in resistance vessels, which are highly relevant for the control of blood pressure. The present study was undertaken to characterize the muscarinic receptors mediating vasodilatation of the perfused mesenteric vascular bed preparation of the spontaneously hypertensive rat (SHR) and its normotensive control the Wistar-Kyoto rat (WKY). We also assessed the possible contribution of muscarinic receptor changes on the endothelium that may be responsible for potentially altered dilator responses of rat arteries, caused by hypertension.
Isolated vascular preparations were perfused with oxygenated Tyrode solution (37°C) at a constant flow of 5 ml/min. In order to detect the relaxant effect of acetyl-ß-metacholine (MCh) the α_1-adrenoceptor agonist methoxamine was added as a stimulus to the perfusion medium. We used equieffective concentrations of methoxamine (0.3-5.0 μM) to precontract the mesenteric arteries because shear stress enhances EDRF release.
We determined the pA_2-values of several specific muscarinic antagonists, versus the relaxant effect of the muscarinic agonist MCh. No differences were found for the E_{max} (WKY; 89.6%, n=7 versus SHR; 85.4%, n=7) and EC_{50} (44nM versus 41nM MCh) of the relaxing effect for MCh between the two groups. As selective antagonists we used pirenzepine (M_1), AF-DX 116 and AQ-RA 741 (M_2), p-FHHSiD and 4-DAMP (M_3). Atropine (non selective) was used for comparison. The pA_2-values and slopes obtained are listed in the table 1 (mean ± s.e.mean, n=6).

Table 1	WKY	SHR		WKY	SHR
	pA_2 (slope)	pA_2 (slope)		pA_2 (slope)	pA_2 (slope)
atropine	9.84±0.18 (1.06±0.10)	9.96±0.01 (1.03±0.01)	pirenzepine	7.05±0.05 (1.10±0.04)	7.01±0.03 (0.90±0.02)
4-DAMP	9.39±0.11 (1.05±0.04)	8.90±0.10 (1.02±0.15)	AQ-RA 741	6.43±0.06 (1.16±0.12)	6.66±0.10 (0.86±0.11)
p-FHHSiD	8.01±0.49 (0.93±0.22)	7.73±0.74 (0.96±0.40)	AF-DX 116	6.15±0.03 (0.90±0.02)	6.12±0.53 (0.77±0.30)

The pA_2-values were in the expected range: atropine > 4-DAMP > p-FHHSiD > pirenzepine > AQ-RA 741 = AF-DX 116, as established for other arterial vessels (Eglen & Whiting, 1990). These findings indicate the prevalence of muscarinic M_3 receptors in resistance vessels. In this respect no significant difference was found between the SHR and WKY groups.

Eglen R.M. and Whiting R.L., J. Auton. Pharmacol., **19:** 233-245, 1990

102P THE EFFECTS OF (±)-, (+) AND (-)-PINACIDIL ON THE MEMBRANE POTENTIAL OF THE RAT AORTA

S.A. Doggrell & B.E. Bishop, Department of Pharmacology, School of Medicine, University of Auckland, Private Bag, Auckland, New Zealand.

Cromakalim and pinacidil act at a glibenclamide-sensitive site in vascular smooth muscle causing hyperpolarization (cromakalim, Doggrell et al., 1989; pinacidil, Mulvany et al., 1990). Cromakalim and pinacidil have asymmetric carbons and (+)- and (-)-isomers. In the presence of depolarization with KCl, (-)-cromakalim (BRL 38227) hyperpolarizes, and in the presence of hyperpolarization with isoprenaline, (+)-cromakalim (BRL 38226) reverses the hyperpolarization of rat aorta (Bishop et al., 1991). The aim of the present study was to determine the effects of (±)-, (+)- and (-)-pinacidil on membrane potential. An endothelial intact rat aorta was pinned endothelial side uppermost in a recording bath and using standard electrophysiological techniques the membrane potential measured following deliberate electrode withdrawal. The membrane potentials of 10 cells were measured for each condition from each aorta.

The resting membrane potential of the rat aorta is -60mV. At 1µM, (±)-and (+)-pinacidil had no effect and (-)-pinacidil hyperpolarized the rat aorta by 7mV ± 1 (mean mV ± s.e. mean; 40 determinations from 4 aortae). At 10µM, (±)-pinacidil hyperpolarized the aorta by 4mV, (+)-pinacidil depolarized by 17mV and (-)-pinacidil hyperpolarized by 10mV. Addition of KCl (15mM) or isoprenaline at 1µM to the bathing medium caused a 36mV depolarization and a 9mV hyperpolarization, respectively. In the presence of a KCl-induced depolarization, (±)- and (-)- but not (+)-pinacidil at 1µM caused hyperpolarization by 17 and 13mV, respectively. In the presence of isoprenaline-induced hyperpolarization, (±)- and (+)- but not (-)-pinacidil at 1µM produced depolarization by 25mV. Glibenclamide, a blocker of ATP-sensitive potassium channels in pancreatic ß-cells and cardiac cells, at 1µM caused a 14mV depolarization of the rat aorta and prevented (±)-pinacidil from producing hyperpolarization but did not alter the depolarizing action of (+)-pinacidil. In the presence of the (+)-isomer, (-)-pinacidil was still able to cause the membrane to hyperpolarize. In a further series of experiments the time course of the effects of (+)- and (-)-pinacidil at 10µM was determined. The depolarizing action of (+)-pinacidil was maximal after 10.2 min and the hyperpolarizing action of (-)-pinacidil was maximal after 2.7 min.

In conclusion, (+)-pinacidil causes depolarisation of the rat aorta and this effect is probably not due to the opening or blocking of a glibenclamide-sensitive potassium channel.

Supported by the Auckland Medical Research Foundation.

Bishop, B.E., Doggrell, S.A., Pockett, S. & Slack, J.R. (1991) N.Z. Med. J. In press.
Doggrell, S.A., Smith, J.W., Downing, O.A. & Wilson, K.A. (1989) Eur. J. Pharmacol. **174**, 131-133.
Mulvany, M.J., Videbaek, L.M., Hughes, A.D. & Aalkjaer, C. (1990) Blood Vessels **27**, 314-318.

103P VERATRIDINE-INDUCED [$^{45}Ca^{2+}$] UPTAKE AS AN INDICATION OF ELECTRICALLY SILENT Na^+ CHANNELS IN ISOLATED RAT AORTA

Detlef Wermelskirchen*, Bob Wilffert, Ute Nebel, Andrea Wirth and Thies Peters
Janssen Research Foundation, Raiffeisenstrasse 8, D-4040 Neuss 21, F.R.G.

It was shown that various cell types possess Na^+ channels which are electrically silent under normal conditions but can be activated by Na^+ channel openers i.e. veratridine. Hence, it was investigated whether such an electrically silent Na^+ channel could also be found in isolated rat aorta. Since veratridine-induced Na^+ uptake is accompanied by Ca^{2+} uptake the latter, measured as $^{45}Ca^{2+}$ uptake, was taken as an indication of veratridine-induced Na^+ uptake thereby avoiding the use of $^{22}Na^+$. Moreover, ^{14}C-guanidinium uptake was used as another indirect approach to measure Na^+ channel activity.
$^{45}Ca^{2+}$ uptake was measured as described previously (Wermelskirchen et al. 1988), with minor modifications. Briefly, rat isolated aortic strips were equilibrated in Tyrode-solution (37°C) gassed with 95% O_2 and 5% CO_2. After pretreatment for 60 minutes, the strips were labelled for 5 minutes (depolarization-induced uptake), 10 minutes (Na^+ removal-induced uptake) or 30 minutes (veratridine-induced uptake) in $^{45}Ca^{2+}$ (37kBq/ml) containing Tyrode-solution in the presence or absence of the stimulus. The experiments under Na^+-free conditions were carried out in TRIS buffer and Na was substituted by choline. For the determination of the ^{14}C-guanidinium uptake aortic strips were incubated for 30 minutes in Na^+-free TRIS buffer containing ^{14}C-guanidinium (4mM, 74 kBq/ml) in the absence or presence of veratridine (10^{-4}M). Thereafter, the strips were washed for 45 minutes with a 4°C cold Tyrode-solution and the residual radioactivity was detected. The $^{45}Ca^{2+}$ uptake was increased by veratridine (10^{-4}M) from 401 ± 24 to 538 ± 32 dpm/mg ww (n=6). The veratridine-induced $^{45}Ca^{2+}$ uptake was inhibited by tetrodotoxin (10^{-6}M), but not by amiloride (10^{-3}M). Activation of Na^+/Ca^{2+} exchange by Na^+ removal increased $^{45}Ca^{2+}$ uptake from 183 ± 11 to 240 ± 13 dpm/mg ww (n=9), which was suppressed by amiloride (10^{-3}M). Nifedipine (10^{-8}M) and verapamil (10^{-7}M) suppressed the depolarization-induced $^{45}Ca^{2+}$ uptake but not veratridine-induced $^{45}Ca^{2+}$ uptake. The Na^+ channel modulators R 56865 (N-[1-[4-(4-fluorophenoxy)butyl]-piperidinyl]-N-methyl-2-benzothiazolamine) (10^{-7}M) and R 59494 (N-methyl-N-[1-(4-phenoxybutyl)-3-pyrrolidinyl]-2-benzothiazolamine) (10^{-6}M) suppressed the veratridine-induced but not the depolarization-induced $^{45}Ca^{2+}$ uptake. Veratridine (10^{-4}M) increased ^{14}C-guanidinium uptake from 412 ± 8 to 638 ± 51 dpm/mg ww (n=6). These results suggest that the rat aorta possesses a Na^+ channel, which is electrically silent under normal conditions, but could be activated by veratridine. The relevance of this Na^+ channel remains to be further investigated.

Wermelskirchen, D., Wilhelm, D., Wilffert, B., Pegram, B.L., Hunter, J., Nebel, U. & Peters, T. (1988) Pharmacology 36, 84-90.

104P INDOMETHACIN IMPAIRS *EX VIVO* AORTA CONTRACTIONS TO PHENYLEPHRINE AND KCl OF LIPOPOLYSACCHARIDE TREATED RATS

G. Cirino, C.Cicala, R. Sorrentino, L. Miranda and A. Pinto.
Dept of Experimental Pharmacology, University of "Federico II", Via D. Montesano,49, 80131 Naples, Italy.

Cyclooxygenase products are major factors in determining the impaired responsiveness to catecholamines and other vasoconstrictors in isolated vessels from lipopolysaccharide (LPS)-treated animals (Gray et al., 1990). Rat aorta rings were prepared 60 min following LPS administration (25 mg/kg i.v.) or saline in vivo and mounted in an isolated organ bath for measurement of isometric contractions in response to phenylephrine (PE; 0.01 - 10 μM) or KCl (10mM). Contractions were induced in the presence or absence of indomethacin (INDO, 10μM). Aorta rings from LPS-treated rats showed reduced contractility to PE compared to those from saline-treated rats. The reduction was 17.6 ± 3.4% ($P<0.05$, n=6) at 10μM PE. In the presence of INDO, this reduction was significantly greater (48.3 ± 5.2%, $P<0.01$, n=6), whereas INDO had no effect on rings from saline treated rats. Contractions in response to KCl were also reduced in rings from LPS-treated rats compared to those from saline-treated rats (47.5 ± 5.6%, $P<0.05$, n=6) and were further reduced by INDO (82.4 ± 7.8% $P<0.05$, n=6). A similar effect to that of INDO was achieved by the TxA_2 receptor antagonist SQ 29,548 (0.1μM; Regal, 1988).

In another set of experiments, the aorta rings were incubated in vitro with LPS (0.4 mg/ml) for 60 min and then contracted. In these experiments, LPS reduced contractions to PE (26.7 ± 4.5%, $P<0.05$, n=6), but INDO had no further effect. LPS-treated rings also showed reduced contraction to KCl (41.2 ± 6.7%, $P<0.05$, n=6,) and again, INDO had no further effect.

The difference in the effect seen following LPS in vivo and in vitro is not readily explained, but could be partially due to the synthesis of different cyclooxygenase products (Bigaud et al., 1990).

Our results suggest that under these experimental conditions, synthesis of cyclooxygenase products (most likely TxA_2) occurs as a compensatory mechanism against hypocontractility measured ex vivo.

We thank Squibb (Princeton, NJ, USA) for kind gifts of SQ 29,548.

Bigaud, M., Julou-Scaeffer, G., Parratt, J.R., Stoclet, J.C. (1990). Eur. J. Pharmac. 190: 185-192
Gray, G.A., Furman, B.L., Parratt, J.R. (1990) Circ. Shock 31: 395-406
Regal, J.F. (1988). J. Pharm. Exp. Ther. 246: 542-547

105P CHARACTERIZATION OF THE INTERACTION OF IMPERIALINE AT MUSCARINIC RECEPTORS *IN VITRO*

R.M. Eglen, G.C. Harris, H. Cox, E. Stefanich, A. Sullivan & R.L. Whiting*
Institute of Pharmacology, Syntex Research, 3401 Hillview Ave., Palo Alto, CA 94304, USA

Differentiation of muscarinic receptor subtypes (M_1, M_2 and M_3) is hampered by low selectivity of antagonists, allosteric interactions (particularly at the M_2 subtype) and lack of antagonists for m4 and m5 gene products (Hulme et al. 1990). The action of the cervane alkaloid, imperialine, at muscarinic receptors in vitro has been assessed in the present study. Although a muscarinic receptor antagonist (Mir & Ghatak, 1965) it has yet to be characterized with respect to an interaction at the putative subtypes.

Contractions of guinea-pig (Dunkin-Hartley, 300-350 g) isolated left, paced atria (M_2), uterus (M_2), ileum (M_3), oesophageal muscularis mucosa (M_3), trachea (M_3) and relaxations of rat (Sprague-Dawley, 200-250 g) isolated aorta (M_3) were prepared (Eglen et al. 1990). Guinea-pig isolated fundus was also used, a preparation yet to be pharmacologically defined with respect to the muscarinic receptor subtype. (+)cis Dioxolane was used as the agonist and apparent affinities were estimated for imperialine using an equilibration period of 60 min.

Imperialine antagonized, in a surmountable fashion, responses to (+)cis dioxolane. In all tissues, the concentration-response curves were dextrally shifted in a parallel fashion, and the apparent affinities (-log K_B) were: left atria (7.7 ± 0.08), uterus (7.4 ± 0.08), ileum (6.7 ± 0.10), oesophageal muscularis mucosa (6.6 ± 0.12), trachea (6.7 ± 0.10), rat aorta (5.9 ± 0.12) and fundic strip (6.8 ± 0.13). In atria and ileum, Schild analysis revealed pA_2 values of 8.0 ± 0.17 and 6.2 ± 0.10, respectively with Schild slopes of 0.84 ± 0.04 and 1.31 ± 0.16, respectively. The fundic strip contracted in response to M_3 receptor stimulation since the following -log K_B values were seen: atropine (9.0 ± 0.17), pirenzepine (7.0 ± 0.14), 4-diphenyl acetoxy-N-methyl piperidine methiodide (9.0 ± 0.06), (±) para-fluoro-hexahydro-siladifenidol (7.3 ± 0.12), methoctramine (6.6 ± 0.21) and himbacine (6.8 ± 0.14). In radioligand binding studies at M_1 (rat cerebral cortex), M_2 (rat myocardium), M_3 (rat submaxilliary gland) and putative m4 (rabbit lung) sites, imperialine exhibited the following apparent affinities (-log K_i; Hill coefficients in parenthesis) 6.1 (0.9), 7.2 (1.2), 5.7 (0.9) and 6.9 (0.9), respectively.

In summary, imperialine acted as an M_2 selective antagonist with respect to M_1 and M_3 receptors but did not distinguish between M_2 and m4 sites. The order of selectivity appeared to be similar to himbacine (10 fold), but less than that for methoctramine (150 fold; Hulme et al. 1990).

Eglen, R.M., Michel, A.D., Montgomery, W.W., Kunysz, E.A., Machado, C.A. and Whiting, R.L. (1990) Br. J. Pharmacol. 99, 637-642.
Hulme, E.C., Birdsall, N.J.M. & Buckley, N.J. (1990) Ann. Rev. Pharmacol. Toxicol. 30, 633-673.
Mir, G.N. & Ghalak, B.J.R. (1965) Ind. J. Exp. Biol. 3,249-252.

106P SEQUENTIAL MEASUREMENT OF PLASMA DIHYDROXYPHENYLALANINE AND DIHYDROXYPHENYLGLYCOL FROM DOGS WITH PACING-INDUCED HEART FAILURE

C. Forster, G. Naik and P.W. Armstrong, Division of Cardiology, University of Toronto, St Michael's Hospital, 30 Bond Street, Toronto, Canada

Rapid ventricular pacing in the dog reliably produces congestive heart failure (HF) associated with a sequential rise in plasma noradrenaline (NA) and depletion of cardiac NA (Armstrong et al., 1986). Caution should be taken in interpreting these changes in terms of sympathetic nervous activity. A more accurate attempt to examine the role of the sympathetic nervous system in experimental HF, in the absence of performing surgical, interventional studies, would be to assess circulating levels of a NA-precursor and a major NA-metabolite. We therefore developed an ion-pair HPLC with electrochemical detection assay to measure 3,4-dihydroxyphenylalanine (DOPA: the product of tyrosine hydroxylation) and 3,4-dihydroxyphenylglycol (DHPG: the major intraneuronal metabolite of NA) simultaneously with NA and adrenaline (Ad).

Seven male mongrel dogs were included in the study. Ventricular pacing (250 beats min^{-1}) commenced 1 week after pacemaker implantation and continued for 3 weeks, after which time the pacemaker was programmed "off" and the dogs were allowed to recover for a further 4 weeks. We serially measured DOPA, NA, DHPG and Ad (all measured as pg ml^{-1}) obtained from venous plasma collected at control, 1 week pacing, 3 week pacing and 4 week discontinued pacing. From these data we calculated the DHPG:NA as an estimate of NA turnover (Howes et al., 1989). Left ventricular NA (ng g^{-1}) from these 7 animals and data from 3 other series of animals (control, 1 week paced and 3 week paced) was also measured. The results (mean ± s.e. mean) are shown in the table where * represents $P < 0.05$ versus control; ANOVA).

Concentration of DOPA, NA, DHPG and Ad at various times of pacing

	n	DOPA	NA	DHPG	Ad	DHPG:NA	ventricular NA
control	7	3856 ± 705	202 ± 16	388 ± 35	162 ± 27	2.0 ± 0.2	684 ± 51
1 week paced	7	3968 ± 542	528 ± 62*	429 ± 46	164 ± 21	0.9 ± 0.1*	226 ± 23*
3 week paced	4	4432 ± 720	750 ± 139*	513 ± 48*	232 ± 40	0.8 ± 0.1*	316 ± 58*
4 week recovery	7	2879 ± 752	176 ± 27	299 ± 67	80 ± 18	2.0 ± 0.4	557 ± 39

These data show 1) although DHPG rises at 3 week pacing, it does not increase to the same extent, nor at the same rate as NA. 2) There is ventricular depletion of NA as early as 1 week pacing and no further depletion occurs. 3) There are no temporal changes associated with DOPA and Ad as HF progresses. 4) All parameters returned to basal levels 4 weeks following discontinued pacing. We conclude that as HF progresses there is an increased turnover which cannot be compensated for by increased synthesis and this may be due to decreased storage capacity or deficiency in neuronal uptake. Furthermore, normal sympathetic activity is restored following discontinuation of pacing.

Supported by the Heart and Stroke Foundation of Ontario.
Armstrong, P.W., Stopps, T.P., Ford, S.E. & de Bold, A.J. (1986) Circulation 74, 1075-1084.
Howes, L.G., Hodsman, G.P., Maccarrone, C., Kohzuki, M. & Johnston, C.I. (1989) J. Cardiovasc. Pharmacol. 13, 348-352.

107P DIFFERENCES BETWEEN DPI 201-106 AND BDF 9148 IN POSITIVE INOTROPIC RESPONSES OF CARDIAC PREPARATIONS FROM GUINEA-PIG, RAT AND MAN

A. Hoey, A. Lehmkuhl, E. Wettwer & U. Ravens, Department of Pharmacology, University of Essen, Hufelandstraße 55, D-4300 Essen 1

The positive inotropic agents DPI 201-106 (4-[3'-(4"-diphenylmethyl-1"-piperazinyl)-2'-hydroxypropoxy]-1H-indole-2-carbonitrile) and BDF 9148 (4-[3'-(1"-diphenylmethyl-azetidine-3"-oxy)-2'-hydroxypropoxy]-1H-indole-2-carbonitrile) inhibit inactivation of the cardiac Na^+ current thereby inducing a persisting Na^+ influx in heart muscle. This mechanism is considered to produce the positive inotropic action via the Na/Ca exchanger as well as prolonging the action potential duration (Ravens *et al.*, 1991). Because of the potential clinical relevance, we have compared the effects of the two drugs in isolated human tissue obtained from patients undergoing coronary bypass surgery and in atrial tissue from guinea-pig and rat hearts. Electrophysiological experiments were conducted in order to elucidate possible differences in action. - Right auricular trabeculae from man and left atria from guinea pigs and rats were suspended in modified Tyrode solution for force measurement (stimulation frequency 1 Hz). Both compounds enhanced force of contraction with similar efficacy but different potencies (see Table 1).
In papillary muscles from guinea-pig and rat hearts, BDF 9148 tended to be slightly, though not significantly, more potent than DPI 201-106. In the guinea-pig, DPI 201-106 prolonged the action potential duration to a greater extent than BDF 9148, whereas in the rat, BDF 9148 was more effective than DPI 201-106. We have also studied the effects of DPI 201-106 and BDF 9148 in voltage clamped myocytes in which both compounds produced a clear inhibition of the inactivation of Na^+ current and depressed the cardiac Ca^{2+} current. In preliminary experiments, DPI 201-106 caused a reduction of the inward rectifier whereas BDF 9148 did not have this effect. Although we cannot as yet explain the differences in potencies of the two compounds observed in atrial tissue, our results suggest that the differences in prolongation of the APD induced by BDF 9148 and DPI 201-106 may be caused by an additional inhibitory effect of DPI 201-106 on the inward rectifier as can be simulated in a computer model of the action potential.

Table 1 Positive inotropic effects of DPI 201-106 and BDF 9148 in atrial muscle

		man		guinea pig		rat	
DPI 201-106	Control force (mN)	1.6 ± 0.3	(6)	2.7 ± 0.5	(8)	2.1 ± 0.3	(7)
	Increase in force (mN)	1.8 ± 0.4		4.6 ± 0.7		2.7 ± 0.7	
	pD_2	5.85 ± 0.09		5.60 ± 0.09		5.86 ± 0.12	
BDF 9148	Control force (mN)	1.6 ± 0.3	(9)	1.5 ± 0.3	(7)	1.3 ± 0.3	(8)
	Increase in force (mN)	1.5 ± 0.2		3.1 ± 0.6		2.5 ± 0.5	
	pD_2	6.90 ± 0.08		6.50 ± 0.13		6.05 ± 0.10	

Ravens, U., Pfeifer, T., Himmel, H., Wettwer, E. & Armah, B. (1991). *Br. J. Pharmacol.*, **104**, 1019-1021.

108P RECEPTOR SYSTEMS MEDIATING POSITIVE INOTROPIC EFFECT IN ISOLATED HUMAN RIGHT ATRIUM

O.-E.Brodde, S.Bals, A.Broede, K.Kunde, E.Schäfer & H.-R.Zerkowski, Dept. Medicine, University of Essen, Hufelandstraße 55, D-4300 Essen, Germany

Catecholamines acting via the β-adrenoceptor (AR)-adenylate cyclase (AC)-cAMP pathway cause positive ino- and chronotropic effects in the human heart. It has been recently shown, however, that also other receptor systems such as histamine (HIS, Ginsburg et al., 1980), 5-HT (Kaumann et al., 1991), α_1-AR (Brückner et al., 1985), angiotensin II (A II, Schomisch et al., 1990) and endothelin (ET, Davenport et al., 1989) can increase force of contraction (FC). In this study we compared the effects of these agents on second messenger generation and FC-increases in human right atria with those caused by β-AR stimulation.

Right atrial appendages were obtained from patients without apparent heart failure undergoing coronary artery bypass grafting. None of the patients had been treated with β-AR agonists or -antagonists for at least 6 weeks before surgery. AC activity in atrial membranes was assessed by conversion of [^{32}P]ATP to [^{32}P]cAMP, inositol phosphate (IP) generation as accumulation of total [^{3}H]IP in [^{3}H]myo-inositol labelled atrial slices during a 30 min incubation at 37°C in the presence of 10mM LiCl. Cumulative concentration-effect curves for FC-increases were assessed on electrically driven atrial strips equilibrated in Krebs-Henseleit solution at 37°C.

5-HT (pEC_{50}=5.5), HIS (pEC_{50}=6.0) and Isoprenaline (ISO, pEC_{50}=6.6) caused concentration-dependent AC-activation, while A II, ET and phenylephrine (PE, in the presence of 10μM propranolol) did not activate AC. Maximal AC-activation (100 μM ISO=100%) was 36±8% HIS (n=5) and 53±7% 5-HT (n=7). HIS (pD_2=5.6), 5-HT (pD_2=6.3), A II (pD_2=8.2) and ET caused concentration-dependent increases in atrial FC. Maximal effects were (10μM ISO or 12.6 mM Ca^{++}=100%): 61±9% 5-HT (n=7); 88±5% HIS (n=8); 43±7% A II (n=5); 32±5% ET (at 0.1μM, n=3). PE failed to induce any FC-increases. The HIS-effect was antagonized by ranitidine (pK_B=6.92), but not by diphenhydramine suggesting that it is mediated by H_2-receptors; the 5-HT-effect was antagonized by ICS 205930 (pK_B=6.43) indicating that it is mediated by a "5-HT_4-like" receptor (Kaumann et al., 1991), and the A II-effect was antagonized by DuP 753 (pK_B=8.55) suggesting that it is mediated by AT_1-receptors.

We conclude that in human right atrium FC-increases can be induced by cAMP-dependent (HIS, 5-HT, β-AR) and -independent (A II, ET) pathways. At least for ET we could obtain IP-accumulation in atrial slices suggesting that the ET-receptor might couple to the phospholipase C-inositol trisphosphate-diacylglycerol pathway.

Brückner,R., Mügge,A. & Scholz,H. (1985). J.Mol.Cell.Cardiol 17: 639-645.
Davenport,A.P., Kaumann,A.J., Hall,J.A., Nunez,D.J. & Brown, M.J. (1989). Br.J.Pharmacol. 96 (Suppl): 102P.
Ginsburg, R.M., Bristow, M.R., Stinson, E.B. & Harrison, D.C. (1980). Life Sci. 26: 2245-2249.
Kaumann,A.J., Sanders,L., Brown,A.M. Murray,K.J. & Brown, M.J. (1991). N.-S. Arch. Pharmacol. 334: 150-159.
Schomisch,C., Schluchter,M.D., Paranandi,L., Czerska,B., Stewart, R.W., Rosenkranz,E. & Bond,M. (1990). Circulation 82: 1973-1984.

109P IMPAIRED RESPONSE OF SHR HEARTS TO α_1-ADRENOCEPTOR STIMULATION CAUSED BY CHANGES IN POST-RECEPTOR EVENTS

M.J.F. Mertens, H.D Batink, M. Pfaffendorf & P.A. van Zwieten
Department of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands.

In a programme on the responsiveness to inotropic drugs in hearts of hypertensive animals, we investigated the functional response of paced (5 Hz.), isolated hearts (Langendorff) to the α_1-stimulants cirazoline, methoxamine and phenylephrine. Changes in contractile force were quantified by means of E_{max} values, obtained from concentration-response curves for each drug (table 1). Initial values of contractile force and coronary flow were the same for both SHR and WKY. All α_1-agonists were administered in the presence of 10 μM propranolol.

table 1 E_{max}-values in % of the initial contractile force (dP/dt_{max}) (means ± s.e.mean, n=6)

	WKY	SHR	
Cirazoline	53.65 ± 11.72	15.99 ± 6.18	*
Methoxamine	45.68 ± 11.27	28.78 ± 8.64	*
Phenylephrine	50.18 ± 4.09	43.21 ± 13.82	

Except for phenylephrine, the SHR-hearts showed a marked decrease in response to α_1-stimulation. Radioligand binding studies with ^{3}H-prazosin, however, showed the same density (B_{max}: 41.4 ± 3.8 and 61.2 ± 10.2 fmol/mg protein resp.) and affinity (K_D: 0.42 ± 0.08 and 0.40 ± 0.04 nM resp.) of α_1-adrenoceptors in the cardiac tissues from WKY and SHR. These results show that in SHR-hearts there is a decreased response to α_1-stimulation without a concomitant change in receptor density or affinity. We subjected the discrepancy between phenylephrine and the other α_1-agonists to a further analysis by means of the intracellular Ca^{++}-release antagonists ryanodine and TMB-8 in order to rule out the contribution of intracellular Ca^{++} release to the cardiac contractions. In the presence of both intracellular Ca-antagonists we now observed a marked reduction in contractile response in the SHR-hearts when compared to WKY organs (table 2) to all three of the α_1-adrenoceptor agonists investigated.

Table 2 E_{max}-values in % of the initial contractile force (dP/dt_{max}) (means ± s.e.mean, n=6)

	+ $3x10^{-5}$M ryanodine		+ $3x10^{-6}$M TMB-8	
	WKY	SHR	WKY	SHR
Cirazoline	91.6 ± 14.9	17.2 ± 3.5 *	48.2 ± 4.8	15.3 ± 1.4 *
Methoxamine	57.6 ± 9.2	34.7 ± 1.1 *	28.5 ± 1.8	12.8 ± 1.4 *
Phenylephrine	51.9 ± 8.6	10.8 ± 1.1 *	53.3 ± 5.2	9.6 ± 3.6 *

The fact that the effect of phenylephrine in SHR-hearts is clearly modulated by the intracellular Ca-antagonists substantiates our view that the impaired inotropic response to α_1-adrenoceptor stimulation is caused by an altered signal transduction at the post receptor level, rather than by changes in receptor density or affinity.

110P Na^+/Ca^{++} EXCHANGER FROM BOVINE HEART: CLONING AND EXPRESSION IN *XENOPUS* OOCYTES AND MAMMALIAN CELLS

J.F. Aceto*, M. Condrescu, H. Nelson, N. Nelson, D. Nicoll[1], K.D. Philipson[1] and J.P. Reeves.
Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110 and [1]Departments of Medicine and Physiology, UCLA School of Medicine, Los Angeles, CA 90024, USA. (Introduced by A. Cowan)

Na^+/Ca^{++} exchange is a carrier-mediated process which is the principle mode of calcium translocation across the cardiac membrane. The bovine cardiac Na^+/Ca^{++} exchanger has been cloned and sequenced from a cDNA library and expressed in both *Xenopus* oocytes and the COS-7 cell line. To clone the bovine cardiac exchanger, a λgt 10 cDNA library (Clontech) from bovine heart was screened with probes from the 5' and 3' regions of the canine clone (A4), previously described (Nicoll *et al.*, 1990). Two clones that hybridized to both probes were subcloned into Bluescript II SK (Stratagene) for sequence and expression analysis. These clones, p13 and p17, contained inserts of 5.3 kb and 4.5 kb, respectively. Within the coding region, p17 had a nucleotide sequence of 2.9 kb corresponding to 970 amino acids. The untranslated 5' and 3' regions had approximate lengths of 0.27 kb and 1.1 kb, respectively. The nucleotide sequence within the coding region was 92.1% identical with the canine Na^+/Ca^{++} exchanger, differing by only 22 amino acids of which 9 were located within a 32-amino acid signal sequence at the N-terminal region of the protein. The Na^+/Ca^{++} exchanger is unique among membrane transport proteins in encoding a cleaved signal sequence. RNA transcribed from these clones was injected into *Xenopus* oocytes and Na^+/Ca^{++} exchange activity expressed. When nystatin was used to internally load oocytes with Na^+, clone p17 showed an increase in Ca^{++} uptake activity. No Na^+/Ca^{++} exchange activity was observed in water-injected oocytes or in RNA-injected oocytes loaded internally with Li^+ instead of Na^+. In addition, clone p13 was unable to elicit Na^+/Ca^{++} exchange activity. While most of the coding sequence for p13 was identical to p17, p13 had an additional 0.3 kb segment containing several stop codons and located approximately 2300 bp from the initiator methionine. The additional segment in p13 is probably a cloning artifact since it did not correspond to the usual consensus pattern for eukaryotic introns. A 3.9 kb portion of p17 was subcloned into mammalian expression vectors (pSVL and pcDNA) and transfected into COS-7 cells. Microsomal vesicles prepared from these COS-7 cells showed a 2-7 fold increase in Na^+/Ca^{++} exchange activity 72 hr after transfection compared to cells transfected with vector alone. This report provides additional evidence for a functional bovine cardiac Na^+/Ca^{++} exchange carrier and establishes a basis for studying regions of the protein.

Nicoll, D., Longoni, S. & Philipson, K.D. (1990) Science **250**, 562-565.

111P NIFEDIPINE, LACIDIPINE AND NISOLDIPINE IN THE RAT ISOLATED WORKING HEART: A COMPARATIVE STUDY

A.J. Pijl, M. Pfaffendorf and P.A. van Zwieten. Department of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands.

The present study was undertaken to compare two newer dihydropyridine-calcium antagonists (lacidipine and nisoldipine) with the classic prototype of this group nifedipine, in the working heart preparation of the rat. The hearts were paced at a frequency 5 Hz, (pulse width: 5 ms, voltage: 10% above threshold, 2-4 V) using platinum electrodes. Tyrode solution was used at 37°C with a calcium concentration of 2.23 mmol/l.
The calcium antagonists were washed-in for 120 minutes at 4 different concentrations(10^{-9}M, 10^{-8}M, 10^{-7}M, $3x10^{-7}$M). The five parameters Left Ventricular Pressure(LVP), dP/dt, Aortic Output (AO), Coronary Flow (CF), and Cardiac Output (CO) were determined every 30 minutes. All calcium antagonists reduced LVP, dP/dt, AO, and CO in a concentration dependent manner. At 120 minutes the EC_{50} was calculated for each antagonist (see table).
There was no difference in the effect of lacidipine (3×10^{-7}M) between a wash-in period of 30 min. and 120 min respectively, whereas a substantial difference was found for the effect of nifedipine, which had disappeared within 30 minutes.
Subsequently the calcium antagonists were washed in during 30 minutes at a concentration that reduced contractile force by 40-50% at t=30 minutes, and then washed out in the next 90 minutes. The same parameters were determined at the end of the wash-in period and at t=10,20,30,60 and 90 minutes of the wash-out period. The effect of lacidipine did not diminish during wash out up to 90 minutes , whereas the effects of nifedipine disappeared completely within 10 minutes. The effects of nisoldipine, however, disappeared partly within 10 minutes.

Table 1 -logEC_{50} values (M) for the effects of the calcium antagonists at 120 min. (means ± s.e.mean)

	LVP	dp/dt	AO	CO
nifedipine (n=6)	6.78±0.06	6.83±0.05	6.98±0.14	6.86±0.14
lacidipine (n=6)	7.48±0.17*	7.60±0.18*	7.31±0.24	7.37±0.14*
nisoldipine (n=6)	8.58±0.28*	8.27±0.17*	8.40±0.02*	8.30±0.16*

The values marked with * are significantly different from those found for nifedipine ($p<0.05$).

In conclusion:
1) Nisoldipine and lacidipine are more potent calcium antagonists than nifedipine with respect to all 5 parameters measured in rat isolated working hearts, as reflected by the EC_{50} values;
2) Compared to nisoldipine and nifedipine, lacidipine displays a different kinetic pattern as reflected by its persisting effects during a long period of wash-out.

112P INHIBITION OF VERATRINE-INDUCED CONTRACTURE BY DOPROPIDIL, A NEW ANTIANGINAL DRUG

J. Leboeuf , J. Baissat, G. John, R. Massingham. Department of Pharmacology, RL-CERM, 63202 Riom Cedex, France.

The aim of the present study was to determine the ability of dopropidil (Massingham and Monteil, 1990) to antagonize veratrine-induced contracture (linked to increase in intracellular Na^+ and consequent Ca^{++} overload) and induce a direct negative inotropic effect (NIE) in isolated electrically stimulated (4 Hz) rat atria. In this model, 30 minutes superfusion (10 ml/min) with veratrine (100 µg/ml) caused an initial increase followed by decrease in systolic tension with subsequent development of a marked contracture (VIC) which slowly returned to baseline upon washout. Superfusion of dopropidil (1-10 µM) for 60 min before and during veratrine exposure, markedly slowed the onset of VIC, significantly reduced its maximum from a control value of 1057 ± 59 mg (n=16) to 828 ± 78 (n=6), 417 ± 88 (n=8) and 291 ± 75 mg (n=8) with 2, 3, and 10 µM of the drug respectively giving and IC50 (v) = 2.82 µM and facilitated (time to attain baseline tension) recovery upon washout. The direct NIE of dopropidil gave an IC50 (F) = 15.85 µM, and consequently an F/V ratio of 5.62. The respective F/V ratios were 5.19 and 5.36 for bepridil and flunarizine but only 0.08 for verapamil and nifedipine and 0.31 for diltiazem. Added 5 min after initiation of veratrine superfusion, dopropidil also decreased VIC. Thus at the end of veratrine superfusion, the amplitude of contracture was 963 ± 70 mg (n=8) in the control group and 732 ± 45 and 531 ± 41 mg in the presence of 3 and 10 µM of dopropidil respectively. The protective effect of dopropidil was not markedly modified in the presence of atropine + propranolol (1.4 and 0.3 µM respectively) suggesting that its action was not related to an effect on catecholamine release from sympathetic nerves. Since the effect of dopropidil (3 µM) and those of lidocaine (30 µM) were additive, the protective effects of dopropidil might be due to an action at the veratrine modified sodium channel although other possibilities (e.g. intracellular effects) remain to be investigated. These results distinguish dopropidil from the slow calcium channel blockers and suggest that dopropidil may have potential for protecting the heart from injury following ischaemia and reperfusion injury.

Massingham R. & Monteil A. (1990) Drugs of the Future, 15, 453-456.

113P TWO DISTINCT POTASSIUM CURRENTS ARE ACTIVATED BY DEPOLARIZATION IN ADULT RAT VENTRICULAR MYOCYTES

N. A. Castle (introduced by D. G. Haylett), Department of Anesthesia Research Laboratories, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, U.S.A.

Transient outward K^+ currents (I_{TO}) are present in atrial and ventricular tissue of most mammalian hearts. I_{TO} is believed to be the dominant current underlying action potential repolarization in adult rat ventricle. The present study has used the whole cell patch-clamp technique to further examine the outward currents activated by membrane depolarization in single adult rat ventricular myocytes. All experiments were performed in the presence of 20μM tetrodotoxin and 3mM Co^{2+} to block Na^+ and Ca^{2+} currents.

Membrane depolarization to potentials greater than -40mV from a holding potential of -70mV resulted in the activation of I_{TO} (see Fig. 1). This current was characterized by rapid activation (time to peak 10ms at +40mV) and a biphasic decay consisting of a fast phase (τ= 60ms) and a slow phase (τ = 1500-2000ms). The contribution of the fast and slow inactivating components to the overall current varied from cell to cell. The voltage-dependence of steady state inactivation of I_{TO} was half maximal at -45.3mV. I_{TO} was almost completely abolished following exposure to 10mM 4-aminopyridine (4-AP; IC_{50}= 0.29mM) but was essentially unaffected by 10mM tetraethylammonium (TEA). The antiarrhythmic agent, quinidine produced a time-dependent inhibition of I_{TO} (IC_{50}= 6μM) which similar was in nature to the inhibition produced by the local anesthetic, bupivacaine (see Castle, 1990).

When the membrane potential was held more negative than -80mV another outward current could be activated by depolarization to potentials more positive than -40mV (Fig.1 shows 30mV depolarizing steps between -60mV and +60mV). Indeed this current (termed I_K) could be maximally activated by holding the membrane potential at -150mV (half maximal inactivation occuring at -114mV). In contrast to I_{TO}, I_K could be almost completely abolished by 10mM TEA but was insensitive to inhibition by 10mM 4-AP (see Fig.1). I_K activated relatively slowly (time to peak 50ms at +40mV) and inactivated only 25% during a 3s depolarizing pulse to +40mV. Like I_{TO}, I_K was inhibited by quinidine (IC_{50}=10μM) and could be completely abolished by 1mM bupivacaine.

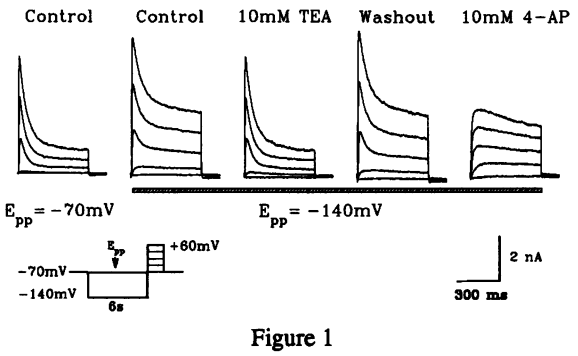


Figure 1

Castle, N. A. (1990) J. Pharmacol. Exp. Ther. **255**, 1038-1046

114P INHIBITION OF [^{125}I]-ENDOTHELIN-1 BINDING TO RAT CARDIAC MEMBRANES BY BRL 38227

C.J. Waugh, M.E.C. Dockrell, W.G. Haynes, †H.J. Olverman, B.C. Williams & D.J. Webb, University Departments of Medicine, Western General Hospital, Edinburgh, EH4 2XU, UK, and †Pharmacology, 1 George Square, Edinburgh EH8 9JZ.

Rat cardiac membranes were incubated with ^{125}I-labelled endothelin-1 (ET-1) in the presence of the two enantiomers of the K^+ channel opener cromakalim (BRL 38227 and BRL 38226) and the calcium channel antagonist nifedipine. Male Sprague-Dawley rats (250-300 g) were sacrificed by cervical dislocation and the hearts removed. The ventricles were coarsely minced, placed into 10 ml of ice cold 20 mM $NaHCO_3$, and homogenised using two 15 s bursts of a Polytron homogeniser working at 4/5 maximum speed. The homogenate was centrifuged at 1000 x g for 10 min, and the supernatant diluted to give a final concentration of 2 mg/ml protein in ice cold 50 mM Tris-HCl containing 0.1 mM phenylmethylsulfonylfluoride, pH 7.4. Protein (0.5 mg) was incubated with ^{125}I-labelled ET-1 at 0.5 nM and either cold ET-1 (10^{-12}-10^{-7}M), the K^+ channel opener BRL 38227, its inactive isomer BRL 38226 or nifedipine (all 10^{-11}-10^{-5} M). The preparations were incubated for 120 minutes at 37°C. The incubation was terminated by the addition of 3 ml of ice cold 10mM Tris-HCl containing 6.6% polyethyleneglycol 6000, pH 7.4. Bound and free ^{125}I-labelled ET-1 were separated by rapid filtration across Whatman GF/C glass microfibre filters and three 10 ml washes with the above buffer. The filters were counted in a multiwell gamma counter. Non-specific binding was defined in the presence of 10^{-6} M ET-1 and was subtracted from the total bound activity to give specific binding.

Table 1. Rat cardiac membrane ^{125}I-ET-1 binding [%B/Bo (SEM); n=5]

Drug concentration (M)	ET-1	Nifedipine	BRL 38226	BRL 38227
10^{-12}	97 (1.6)			
10^{-11}	90 (3.3)	87 (11.0)	90 (3.3)	87 (2.5)
10^{-10}	83 (3.1)	117 (20.3)	89 (4.4)	74 (7.2)
10^{-9}	69 (5.0)	108 (11.0)	89 (6.7)	75 (4.0)
10^{-8}	34 (9.2)	111 (17.6)	108 (5.4)	65 (7.6)
10^{-7}	9 (7.9)	122 (16.6)	102 (5.4)	69 (8.5)
10^{-6}		101 (13.5)	91 (7.2)	64 (10.4)
10^{-5}		107 (12.3)	92 (11.0)	49 (9.4)

Rat cardiac membranes showed a significant ($p<0.05$, ANOVA) inhibition of ^{125}I-labelled ET-1 binding when incubated with increasing doses of cold ET-1. Incubation of membranes with the active isomer, BRL 38227, caused a significant inhibition of ^{125}I-labelled ET-1 binding ($p<0.05$, ANOVA). The inactive isomer, BRL 38226, and nifedipine had no effect upon ^{125}I-labelled ET-1 binding. These observations indicate that the K^+ channel opener, BRL 38227, may antagonise vasoactive effects of ET-1 by an interaction with an ET-1 binding site. The nature of this interaction remains to be elucidated.

115P CHARACTERIZATION OF THE ADENOSINE RECEPTORS IN DOG CORONARY ARTERY, GUINEA-PIG AORTA AND GUINEA-PIG FUNDUS

M.F. Gurden and I. Kennedy. Peripheral Pharmacology Department, Glaxo Group Research, Ware, Herts, SG12 0DP.

Bruns *et al.* (1986) suggested that adenosine A_2 receptors can be divided into two subtypes, one, termed A_{2a}, is present in rat striatum, the other, termed A_{2b}, in human fibroblasts. Key compounds for differentiating the two subtypes include 2-substituted analogues such as 2-phenylaminoadenosine (CV1808) which is a selective A_{2a} agonist. The objective of the present study was to characterize the A_2 receptors mediating relaxation of the dog isolated coronary artery, guinea-pig isolated aorta and fundus. Compounds studied were the A_{2a} agonists, CV1808 and CGS21680 (Hutchinson *et al.*, 1989), the A_1 agonist, N^6-cyclopentyladenosine (CPA, Bruns *et al.*, 1986) and the non-selective agonist, 5'-N-ethylcarboxamidoadenosine (NECA, Bruns *et al.*, 1986), the latter being used as a standard.

Preparations were suspended in modified Krebs solution containing indomethacin ($2.8\text{-}5.6\times10^{-6}$M) at 37°C gassed with 5% carbon dioxide in O_2. Coronary artery rings were contracted with the thromboxane A_2 mimetic U-46619 (1×10^{-8}M), aortic rings with phenylephrine (3×10^{-6}M) and fundic strips with prostaglandin E_2 (1×10^{-8}M). A single cumulative concentration-effect curve for one agonist was constructed on each preparation and compared with a curve to NECA obtained in another preparation from the same animal. Each of the agonists caused concentration-related relaxations of the spasmogen-induced tone in all three preparations. These data are summarised in Table 1.

Table 1:

PREPARATION	NECA EC_{50}nM	EMCR+(where NECA =1)		
		CPA	CV1808	CGS21680
Dog coronary artery	42 (27-65)	20.8 (8-51)	0.75 (0.37-1.52)	1.23 (0.40-5.50)
Guinea-pig aorta	250 (218-286)	29 (20-41)	102 (52-199)	626 (430-912)
Guinea-pig fundus	140 (56-351)	122 (28-533)	67 (7-675)	845 (117-6086)

+Geometric mean equipotent molar concentration ratio; 95% Confidence limits shown in parentheses; n = 11-20 for NECA and 4-8 for other agonists.

Both CGS21680 and CV1808 were potent agonists on the dog coronary artery, whilst CPA was only weakly active. These results are consistent with the adenosine receptors mediating relaxation of this tissue being of the same A_{2a} subtype as those present in rat striatum (Bruns *et al.*, 1986; Hutchinson *et al.*, 1989). However, all three agonists were weakly active relative to NECA on the guinea-pig aorta and fundus. Similar findings have recently been reported by Hargreaves *et al.*, (1991) on the guinea-pig aorta. The receptors mediating relaxation of the guinea-pig aorta and fundus are clearly different from those present on the dog coronary artery and may be of the A_{2b} subtype. However confirmation of this hypothesis must await the development of potent, selective agonists and antagonists for this receptor.

Bruns, R.F., Lu, G.U. & Pugsley, T.A. (1986). Mol. Pharmacol., *29*, 331-346.
Hargreaves, M.B., Stoggall, S.M. & Collis, M.G. (1991). Br. J. Pharmacol., *102*, 198P
Hutchinson, A.J., Webb, R.L., Oei, H.H., Ghai, G.R., Zimmerman, M.B. & Williams, M. (1989). J. Pharmacol. exp. Ther., *251*, 47-55.

116P HALOGEN SUBSTITUTED N^6-ENDONORBORNYL-9-METHYLADENINES ARE POTENT AND SELECTIVE A_1-ADENOSINE RECEPTOR ANTAGONISTS

P.L. Martin and N.J. Cusack, Whitby Research, Inc., 2801 Reserve Street, Richmond, VA 23220, U.S.A.

N^6-endonorbornan-2-yl-9-methyladenine (N-0861) is a potent and selective antagonist for A_1-adenosine receptors (May et al, 1991). The effects of halogenation of N-0861 on potency and selectivity for the A_1 adenosine receptors was tested. Antagonist dissociation constants (K_B's) were determined for inhibition of the negative inotropic responses induced by 5'-N-ethylcarboxamidoadenosine (NECA) in the guinea-pig left atrium (A_1-receptors) and for NECA-induced relaxation of the guinea-pig phenylephrine (3μM)-precontracted aorta (A_2-receptors). The results are illustrated in table 1.

TABLE 1		pK_B (A_1)	pK_B (A_2)	$A_2(K_B)/A_1(K_B)$
C^2-	C^8-		adenine substitution	
-	-	‡6.24±0.06	‡4.25±0.08	98
Cl	-	6.15±0.08	*	>100
F	-	6.07±0.12	*	>100
-	Cl	7.54±0.06	5.69±0.06	71
-	F	6.66±0.07	5.27±0.10	25
-	Br	7.61±6.01	6.01±0.06	40
Cl	Cl	7.24±0.06	5.96±0.08	19
Cl	Br	7.33±0.07	6.05±0.06	19
5'/6'	3'		norbornyl substitution	
Cl	-	6.56±0.06	*	>300
-	Cl	*	*	-

* - no blockade at concentrations up to 1 x 10^{-4}M. ‡ - May et al, 1991.

The substitution of a halogen group at the C^2-position did not affect the potency or the selectivity for A_1 adenosine receptors. The substitution of either a Cl or a Br at the C^8-position increased the potency at A_1 and A_2 receptors by approximately 10-fold, thus leaving the selectivity unchanged. The potency of the 8-F compound, however, was only 3-fold greater than that of N-0861. Substitution of Cl group at the 5'/6' position of the norbornyl group resulted in a 2-fold increase in potency at A_1 receptors and a reduced potency at A_2 receptors, increasing A_1 receptor selectivity. Substitution of Cl on the 3' position of the norbornyl group completely abolished activity at the A_1-adenosine receptor. In conclusion, the A_1 potency and selectivity of these adenosine antagonists is highly dependent upon the position of the halogens on N-0861.

May, J.M., Martin, P.L. and Miller, J.R. (1991). FASEB. J. 5, A1572.

117P THE ANTIARRHYTHMIC EFFECTS OF THE A_1-ADENOSINE AGONIST R-PIA ARE MEDIATED BY BRADYCARDIA

C.L. Wainwright & J.R. Parratt, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow Gl lXW

A_1-adenosine receptors in the heart mediate a sinus bradycardia and a modification of both the release of noradrenaline from sympathetic nerve endings and the intracellular increases in cAMP resulting from β-adrenoceptor stimulation. As these actions would improve metabolic status and increase homogeneity within the ischaemic heart the aim of this study was to determine the effects of a selective A_1-adenosine receptor agonist, R-PIA (R(-)N6-(2-Phenylisopropyl)adenosine; Daly et al, 1981) on the severity of ischaemic arrhythmias in open-chest anaesthetised pigs. Furthermore, to elucidate the importance of the decreased heart rate in the antiarrhythmic action of R-PIA the effect of atrial pacing on the protective action of R-PIA was also determined.

Large white/Landrace cross breed male pigs (28-38 kg) were sedated with azaperone, anaesthetized with chloralose (100 mg kg^{-1} i.v.) and prepared for occlusion of the left anterior descending (LAD) coronary artery following a mid-sternal thoracotomy. Following stabilization animals were randomly allocated into one of three groups: i) Control (solvent vehicle i.v.; n=10) ii) R-PIA (5 μg kg^{-1} i.v.; n=10) iii) R-PIA (5 μg kg^{-1} i.v. plus atrial pacing at 100±5 beats min^{-1}; n=8). Ten minutes after drug intervention the LAD was occluded for a period of 30 minutes and the subsequent arrhythmias were recorded from a Lead II ECG. All standard haemodynamic and electrocardiographic (ST-segment and QT-interval) variables were continuously recorded on a computerised data logging system. Blood samples were withdrawn from the coronary sinus at regular intervals for blood gas and pH analysis and for platelet and leukocyte aggregation studies by impedence aggregometry.

Prior to coronary occlusion R-PIA induced a marked sinus bradycardia (heart rate decreased from 102±7 to 74±3 beats min^{-1}) which was sustained for the duration of the experiment. In the group of pigs which were subject to atrial pacing, pacing at the pre-drug heart rate was commenced 5 minutes after drug administration and maintained for the duration of the experiment. R-PIA alone also caused a reduction in mean arterial blood pressure (from 95±4 to 81±3 mmHg) which was presumably due to the decreased heart rate as pacing restored blood pressure to 92±7 mmHg. There was no increase in coronary sinus oxygen content following drug administration (7.1±0.4 pre-drug vs 7.0±0.4 post-drug ml 100 ml^{-1}), suggesting no effect of the drug on coronary flow. Ex-vivo aggregation in response to phorbol myristate acetate of isolated leukocytes from blood samples withdrawn following drug administration did not differ significantly from controls. Similarly the maximum platelet aggregation response to collagen after drug administration (12.3±2.3 ohms) was not significantly different from the response in blood sampled before R-PIA (11.9±2.4 ohms). Administration of R-PIA alone resulted in a significant reduction in total number of ventricular arrhythmias (121±30 vs 326±71 in untreated controls $P<0.05$) and in the incidence of ventricular fibrillation (20% vs 70% in controls; $P<0.05$) during the 30 minute observation period. However, in the pigs which were subjected to atrial pacing following the adminsitration of R-PIA, the number of arrhythmias (222±69) and the incidence of ventricular fibrillation (88%) was similar to the control group. These results demonstrate that, in a dose which appears devoid of A_2-adenosine receptor actions, the A_1-agonist R-PIA exerts an antiarrhythmic effect which is mediated by a bradycardia.
This work was supported by the Scottish Home and Health Department.

Daly, T.W. et al. (1981) Life Sci. **28**, 2083-2091.

118P Ca^{2+}-DEPENDENT ACTIONS OF PALMITOYL-DL-CARNITINE ON CULTURED RAT DRG NEURONES

R.H. Scott, S.R. Stapleton & K.P.M. Currie, Department of Physiology, St. George's Hospital Medical School, London, SW17 ORE.

Palmitoyl carnitine (PC) is a lipid metabolite which collects in cell cytoplasmic membranes during ischaemia (Knabb et al 1986). It has previously been shown that PC prolongs myocardial action potentials (Inoue and Pappano 1983), and interacts with Ca^{2+} channels to enhance Ca^{2+} influx, and inhibit nitrendipine, verapamil and diltiazem binding (Spedding and Mir 1987). We have investigated the actions of PC on Ca^{2+}-dependent whole cell currents recorded from cultured rat dorsal root ganglion (DRG) neurones. Application of 1mM PC for 2 to 3 minutes reduced the peak amplitude of high voltage-activated Ca^{2+} currents from -1.63 ± 0.15 nA to -1.12 ±0.12 nA (Mean ± S.E.M. n=8) in cells held at -90mV and depolarized to 0mV for 100ms. PC (1mM) also greatly enhanced Ca^{2+} current inactivation, which increased from 43 ± 7% to 71 ± 10% (Mean ± S.E.M. n=8). These actions of PC on Ca^{2+} currents were not readily reversible. Ba^{2+} currents were also reduced by PC (1mM) but to a more modest extent and no change in inactivation was observed. Inspite of marked reductions in Ca^{2+} current, the Ca^{2+}-activated Cl^- tail current (Scott et al 1988) was greatly prolonged by PC (1mM), (n=3), (Figure 1). Application of PC (1mM) to cells held at -90mV lead to activation of calcium-dependent non-selective cation currents (n=5). These PC-induced currents were attenuated by raising intracellular EGTA levels from 1.1 to 20mM. Additionally, in some cells in which PC application had apparently no effect, oscillating inward currents were seen to follow a series of 10 to 20 calcium currents. Our data suggests that PC (1mM) may alter Ca^{2+} homeostasis possibly by inhibiting Ca^{2+} ATPase, so that Ca^{2+} entering cells remains free in the cytoplasm for longer periods. PC-activated oscillating inward currents may result from PC mobilizing Ca^{2+} stores or gradual build up of intracellular Ca^{2+} which then leads to Ca^{2+}-induced Ca^{2+} release from stores.

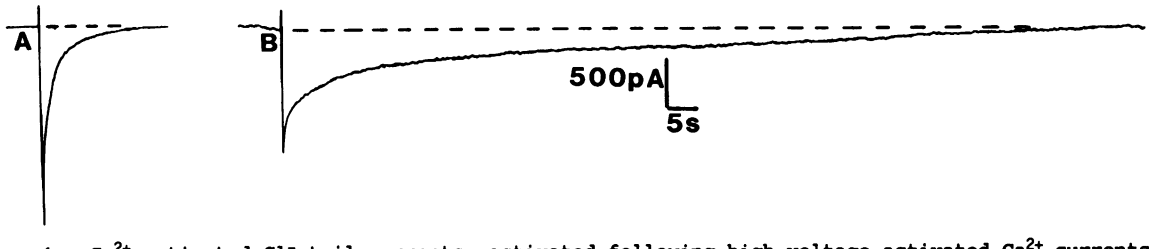


Figure 1. Ca^{2+}-activated Cl^- tail currents, activated following high voltage-activated Ca^{2+} currents in a cell loaded with CsCl based patch solution. A) Control current and B) The current prolonged by 3 minutes application of 1mM PC. We thank the MRC and Wellcome Trust for support.
Inoue, D. & Pappano, A.J. (1983) Circulation Res. 52, 625-634.
Knabb, M.T. Saffitz, J.E., Corr, P.B. & Sobel, B.E. (1986) Circulation Res. 58, 230-240,
Scott, R.H., McGuirk, S.M. & Dolphin, A.C. (1988) Br. J. Pharmacol. 94, 653-662.
Spedding, M. & Mir. A.K. (1987) Br. J. Pharmacol. 92, 457-468.

119P COMPARISON OF CALCIUM MOBILISATION RESPONSES TO DIFFERENT AGONISTS IN UNDIFFERENTIATED NIE-115 CELLS: MODULATORY EFFECTS OF PHORBOL ESTERS

P.A. Iredale, D.A. Kendall, K.F. Martin and S.J. Hill, Department of Physiology and Pharmacology, Queen's Medical Centre, Nottingham, NG7 2UH, UK.

A number of agents have previously been shown to increase $[Ca^{2+}]_i$ in undifferentiated N1E-115 cells (Iredale *et al.*, 1990). In this communication we report that the maximal signals to these agents are not equal in size. Further, we report the effects of pre-incubation with phorbol dibutyrate, a phorbol ester known to activate protein kinase C, on these differential responses.

N1E-115 cells (passages 17-25) were cultured as previously described and loaded with 5 μM fura-2 ester (Iredale *et al.*, 1990). Transient changes in $[Ca^{2+}]_i$ were observed following the addition of maximal doses of bradykinin (100 nM), histamine (300 μM) and carbachol (100 μM) (all $p<0.01$). The differences between the sizes of response can be expressed as a percentage of the bradykinin signal (used as an internal standard at the end of each run) as follows: histamine, 55 ± 23 (n=20); angiotensin II, 53 ± 13 (n=7) and carbachol, 36 ± 14 (n=7). Pre-incubation with the phorbol ester, phorbol dibutyrate (PDBu:1.5 μM) resulted in substantial reductions in responses to all agents with the exception of bradykinin to 10 ± 10%, histamine (n=6); 6 ± 9%, angiotensin II (n=5); and 22 ± 16%, carbachol (n=4) of the control agonist responses. No significant effect was seen on the response to 100 nM bradykinin, but the response to 50 nM bradykinin was reduced to 79 ± 10% (n=4) of control levels ($P<0.05$). When the concentrations of bradykinin and angiotensin II were adjusted to produce the same sized calcium signals, phorbol dibutyrate abolished the response to angiotensin II but only partially inhibited the response to bradykinin. 4α phorbol (1.5 μM), a poor activator of protein kinase C, did not significantly affect the transient response to any of the agonists studied.

These data suggest that a number of agonists are able to mobilise calcium in undifferentiated N1E-115 cells, however, with different maximal effects. In addition, all of the agonists used were significantly affected by pre-incubation with phorbol dibutyrate. The results are in agreement with previous reports of the effects of phorbol esters on cyclic GMP and inositol phosphates production (Kanba *et al.*, 1986; Lai *et al.*, 1987).

Iredale, P.A., Kendall, D.A., Martin, K.F. & Hill, S.J. (1990) Biochem. Soc. Trans. 19, 6S-6S.
Kanba, S., Kanba, K.S. & Richelson, E. (1986) Eur. J. Pharmacol. 125, 155-156.
Lai, W.S. & El-Fakahany, E. (1987) J. Pharmacol. Exp. Ther. 241, 366-373.

120P ANTICONVULSANT EFFECTS OF CALCIUM CHANNEL ANTAGONISTS IN COMBINATION WITH ESTABLISHED ANTI-EPILEPTIC DRUGS

C. Rodger & B.J. Pleuvry, Department of Physiological Sciences, University of Manchester, Manchester, M13 9PT.

The anticonvulsant effects of the calcium channel antagonists flunarizine (FLN) and nifedipine (NIF) were studied alone and in combination with the clinically established anti-epileptic drugs carbamazepine (CBZ) and phenytoin (PHT). Pentylenetetrazole (PTZ, 15mgml^{-1}) was administered as an intravenous infusion to groups of 10 mice, each having received single drug, combination, or vehicle pre-treatment. The amount of PTZ (mgkg^{-1} weight of mouse) required to induce the first myoclonic jerk (MJ) and the subsequent full clonic seizure (FS) was calculated and expressed as mean ± s.e.mean. Flunarizine (20-80mgkg^{-1}) and nifedipine (25-200mgkg^{-1}) produced a dose-related anticonvulsant effect against the PTZ-induced seizures. This was significant at 80mgkg^{-1} flunarizine against its vehicle of 20% ethanol in saline and 0.5% Tween, and 50mgkg^{-1} nifedipine against its vehicle of saline and Tween (ANOVA and Dunnett, $p<0.05$). When a submaximal dose of flunarizine was administered in combination with carbamazepine or phenytoin, an additive anticonvulsant effect was observed in both cases. This was not seen with nifedipine in combination with either carbamazepine or phenytoin. In this case, the anticonvulsant effect was no different from the effects of the individual drug treatments.

	CBZ	CBZ & FLN veh	CBZ & FLN	PHT	PHT & FLN veh.	PHT & FLN	CBZ & NIF	PHT & NIF
MJ	40.7 ±2.2	44.0 ±1.8	59.8* ±2.7	37.4 ±1.8	35.8 ±2.5	47.1 ±1.7	37.4 ±1.5	41.6 ±1.4
FS	100.7 ±5.9	121.1 ±4.2	155.1* ±10.2	104.4 ±10.0	99.6 ±11.2	147.1* ±8.9	123.3 ±8.2	110.8 ±2.2

Values denote mean dose PTZ (mgkg-1)±s.e.mean required to induce seizure stage. *$p<0.05$

The results highlight a difference in action between the two calcium channel antagonists. The additive anticonvulsant effect of the combination of flunarizine with either carbamazepine or phenytoin and the lack of any increase in effect due to the combination of nifedipine with either of the two anti-epileptic drugs may be due to a mechanistic difference between flunarizine and nifedipine. Flunarizine has been suggested to act on L, N and T-type calcium channels as well as sodium channels (Pauwels *et al.*, 1991) whereas nifedipine has been reported to act primarily on the L-type calcium channel (Triggle, 1990). These differences in mechanism of action may account for the observed differences in anticonvulsant activity when the drugs are given in combination with established anti-epileptic drugs in this model of epilepsy.

Acknowledgement: C.R. is an S.E.R.C. scholar.

Pauwels, P.J., Leysen, J.E. & Janssen, P.A.J. (1991) *Life Sciences* **48**, 1881-1893.
Triggle, D.J. (1990) *Can. J. Physiol. Pharmacol.* **68**, 1417-1481.

121P [^{3}H]-PN200-110 BINDING SITES ARE REDUCED IN DISCRETE REGIONS OF THE HIPPOCAMPUS IN ALZHEIMER'S DISEASE

M. Ikeda, D. Dewar, and J. McCulloch, Wellcome Neuroscience Group, University of Glasgow, Glasgow G61 1QH, United Kingdom.

Abnormalities of Ca^{2+}-depdendent processes have been suggested to occur in Alzheimer's Disease (AD) (Mattson, 1990). We have examined the distribution of L-type voltage-sensitive Ca^{2+} channels in the hippocampal region of human postmortem brain by means of [^{3}H]-PN200-110 binding autoradiography. [^{3}H]-PN200-110 binding in AD subjects and controls was compared to hippocampal cell densities and choline acetyltransferase activity.

Serial 20μm cryostat sections of the hippocampal regions from 7 control subjects (age = 76 ± 5 years; postmortem delay = 8.8 ± 2.3; mean ± s.e. mean) and 8 patients with AD (age = 78 ± 1; postmortem delay = 6.9 ± 1.8) were used. Sections were incubated with 0.5nM [^{3}H]-PN200-110 and autoradiography was performed as previously described (Cortés et al. 1984). Autoradiograms were analysed by image analysis and binding values calculated by reference to [^{3}H]-microscales. Subicular and CA1 cells were counted in adjacent cresyl violet sections. Choline acetyltransferase activity was determined in samples of the same tissue used for autoradiographic studies.

	PHG	SUB	CA1	CA3	CA4	DG
Control	7.7 ± 1.8	9.8 ± 1.4	8.7 ± 1.4	4.8 ± 1.4	4.7 ± 1.5	27.3 ± 2.6
AD	5.2 ± 0.8	3.4 ± 0.5*	6.0 ± 1.1	5.3 ± 2.2	3.0 ± 1.5	21.2 ± 2.3

[^{3}H]-PN200-110 binding values are expressed as mean ± s.e. mean pmol/g tissue. *P<0.01, unpaired, two-tailed Student's *t*-test. Abbreviations: PHG = parahippocampal gyrus; SUB = subiculum; DG = dentate gyrus.

Cell density in the subiculum was significantly reduced in the AD patients (94 ± 11 cells/mm^2, mean ± s.e. mean) compared to controls (135 ± 10 cells/mm^2) but not in CA1 (control 58 ± 5; AD 51 ± 6 cells/mm^2). The ratio of binding sites to cell density in the AD group was significantly reduced in the subiculum (control 0.146 ± 0.013; AD 0.038 ± 0.009) but not in CA1 (control 0.170 ± 0.029; AD 0.122 ± 0.018). Choline acetyltransferase activity was reduced in AD (controls 6.9 ± 1.0; AD 2.7 ± 0.9 mean ± s.e. mean nmol/hr/mg protein). There was a strong positive correlation between choline acetyltransferase activity and the binding levels both in the subiculum (r = 0.802) and CA1 (r = 0.661).

The discrete losses of [^{3}H]-PN200-110 binding within the hippocampal region are consistent with the selective cellular vulnerability in Alzheimer's disease. Moreover, the disproportionate reduction of [^{3}H]-PN200-110 binding sites compared with cell loss suggests the precedence of voltage-sensitive calcium channel loss over neuronal fallout.

This work was supported by the Wellcome Trust.

Cortés, R., Supavilai, P., Karobath, M. and Palacios, J.M.(1984) J. Neural Transm. 60, 169-197.
Mattson, M.P. (1990) Neuron, 2, 105-117.

122P DIFFERENTIAL CELLULAR EXPRESSION OF MAO-A AND MAO-B IN CONTROL VERSUS ALZHEIMER BRAINS

J. Saura, J.C. Shih[1], M. Da Prada, V. Chan-Palay, J. Ulrich[2], G. Huber, J. Löffler and J.G. Richards*, Pharma Division, Preclinical and Clinical Research, F.Hoffmann-La Roche Ltd, CH-4002 Basel, Switzerland, [1]John Stauffer Pharmaceutical Sci. Center, USC, Los Angeles CA, and [2]Pathology Institute, Kantonsspital Basel, Switzerland

Monoamine oxidases (MAO) oxidatively deaminate neurotransmitter and xenobiotic amines in the CNS and peripheral organs. Two forms (MAO-A and MAO-B) have been identified by substrate selectivity, inhibitor sensitivity and primary structure. The physiological roles of the isoenzymes are two-fold: to indirectly facilitate the inactivation of released neurotransmitter amines – by maintaining a low cytosolic level of amines after their re-uptake – and as scavengers to prevent various natural substrates from accumulating in monoaminergic neurons to act as false transmitters.

Quantitative enzyme radioautography and hybridization histochemistry have now revealed the distribution, abundance and cellular localization of the isoenzymes and their mRNAs in human postmortem brain. Whereas MAO-A (protein and mRNA) is expressed by noradrenergic neurons of the locus coeruleus, MAO-B is expressed by serotoninergic neurons of the raphé nuclei and histaminergic neurons of the posterior hypothalamus. In the substantia nigra, MAO-A is restricted to the zona compacta whereas MAO-B was found mainly in the zona reticulata. To date, mRNA for neither MAO-A nor MAO-B could be detected in dopaminergic neurons of the substantia nigra or in glial cells of the brainstem. Since the genes of both enzymes contain 15 exons (Grimsby et al., 1991) there is a possibility that different gene products could be formed by alternative splicing. Therefore exon-specific probes are now being tested, for example to determine whether a glial-specific isoform of MAO-B exists.

In cerebral cortex – but not cerebellum – from Alzheimer individuals, MAO-B was markedly increased in astrocytes surrounding senile plaques (identified by ß-amyloid- and GFAP-antigenicity); MAO-A was unchanged. MAO-B mRNA could not be detected in plaques.

Grimsby, J., Chen, K., Wang, L.-J., Lan, N.C. & Shih, J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3637-3641.

123P EFFECT OF OKADAIC ACID ON HISTAMINE RELEASE FROM ACTIVATED HUMAN LUNG MAST CELLS AND BASOPHILS

M.J. Peirce*, J.A. Warner & P.T. Peachell[1] Dept of Physiology and Pharmacology, University of Southampton, Southampton SO9 3TU and [1]Dept of Pharmaceutical Chemistry, School of Pharmacy, Brunswick Sq. London WC1. Introduced by P.J. Roberts

In contrast to the protein kinases, the role of protein phosphatases in the regulation of cellular processes has not been widely studied. The recent identification of okadaic acid(OA) as a cell permeant inhibitor of phosphatases 1 and 2A, has promoted progress in this area. We have investigated the effects of OA (0.001-1μM) on histamine release (HR) from activated human lung mast cells (HLMC) and basophils. Cells were incubated in the presence or absence of 1μM OA for 4 hours, washed, challenged with the appropriate stimulus and HR measured. Both cell types were stimulated with anti-IgE (either 0.1% rabbit anti-human IgE serum for basophils or 0.3% for HLMC) and the calcium ionophore, A23187 (1μg/ml). In addition, the basophils were challenged with f-met peptide (1μM), and the phorbol ester, phorbol myristate acetate (PMA) (100 ng/ml), stimuli which do not initiate degranulation in HLMC. Results are expressed as percent inhibition of HR induced by each secretagogue in the absence of OA.

	Anti-IgE	A23187	F-met peptide	PMA
Lung mast cells	89±3 (n=5, p<0.005)	42±7 (n=4, p<0.05)	Not tested	Not tested
Basophils	90±4 (n=9, p<0.0005)	65±8 (n=5, p<0.05)	17±7 (n=5, p<0.05)	58±8 (n=5, p<0.0005)

The effect of 1μM OA on anti-IgE induced histamine release was half maximal after 1 hour and reached a maximum after a 2 hour pre-incubation (n=4 basophils, n=5 for HLMC). The effect of OA was also dose dependent in both cell types with micromolar concentrations of OA required to produce inhibition (n=4 basophils, n=5 HLMC). In summary, our data suggest that protein phosphatases may be important in regulating exocytosis from both HLMC and basophils.

124P NEUTROPHIL RECRUITMENT TO THE AIRWAYS OF NORMAL HORSES IN RESPONSE TO INTRAVENOUS PAF

S.M. Fairbairn, P. Lees, *C.P. Page & F.M. Cunningham, Department of Veterinary Basic Sciences, The Royal Veterinary College, Hertfordshire and *Department of Pharmacology, King's College, London.

Antigen challenge of horses with allergic respiratory disease causes airways obstruction and radiolabelled neutrophil accumulation in the lung (Fairbairn et al., 1991). In other species, PAF causes bronchoconstriction and leucocyte accumulation in the lung (Mencia-Huerta et al., 1989), and may therefore be implicated in the response of allergic horses to inhalation of allergen. Since the effects of systemically administered PAF have not been reported in the horse, in this initial study, PAF has been given intravenously to 5 normal horses.

Equine neutrophils were radiolabelled with 111-indium-merc and reinfused into the donor horse (Fairbairn et al., 1991). An automated isotope monitoring system (AIMS) was used to monitor the distribution of radiolabelled cells following administration of 5ng/kg PAF in PBS/0.25%ESA. Peripheral total and differential leucocyte counts were also obtained. Airways function was assessed by monitoring respiratory rate and maximum changes in pleural pressure (ΔPplmax), measured indirectly by an oesophageal balloon.

PAF, 5ng/kg, caused an immediate increase in radioactive counts in the lung, which reached a maximum of 21.3 ± 3.0% above control values (mean ± s.e.mean) at 1min. There was a corresponding decrease in radioactive counts in the leg of 15.5 ± 3.3%. In addition, the peripheral leucocyte count decreased significantly at this time, from 6.56 ± 0.77 to 3.15 ± 0.26 x 10^6 cells/ml ($p<0.01$, paired Student's t test). This decrease in the total leucocyte count was accounted for by a fall in neutrophil numbers from 3.39 ± 0.38 to 0.93 ± 0.17 x 10^6 cells/ml ($p<0.005$). The small decreases in mononuclear cell and eosinophil numbers were not significant. PAF also affected airways function causing maximal increases in both ΔPplmax (3.7 ± 0.7 cmH_2O pre and 7.6 ± 1.3cmH_2O post PAF, $p<0.05$) and respiratory rate (11 ± 1.4 breaths/min pre and 44 ± 9.7 breaths/min post PAF, $p<0.05$) at 1 and 2 min respectively. The vehicle had no effect on peripheral leucocyte number or ΔPplmax.

Since PAF causes impaired respiratory function and neutrophil recruitment to the lungs of normal horses, if released during antigen challenge, this mediator could play a role in the pathogenesis of equine allergic respiratory disease.

Fairbairn S.M., Cunningham F.M., Foster A.P., Lees P. and Page C.P. (1991) Am Rev Respir Dis 143, A43.
Mencia-Huerta J.M., Hosford D. and Braquet P. (1989) Clin Exp Allergy 19, 125-142.

We are grateful to the Horserace Betting Levy Board for financial support .

125P EXTENT OF ANTIGEN-INDUCED EOSINOPHILIA AND ENHANCED PRODUCTION OF REACTIVE OXYGEN SPECIES IN AEROSOL AND PARENTERALLY SENSITIZED GUINEA-PIGS

V.P. Russell*, A.M. Thompson, R.G. Sturton and M.F. Fitzgerald.
Bayer UK Ltd., Pharma Research, Stoke Poges, SL2 4LY.

Pulmonary infiltration by activated eosinophils is a characteristic of human asthma and these cells may play a central role in the pathogenesis of this disease. Antigen-challenge of sensitized guinea-pigs also results in an eosinophilia which is associated with an enhanced capacity of bronchoalveolar lavage (BAL) cells to generate reactive oxygen species (ROS) (Fitzgerald et al., 1991). In this study the extent of antigen-induced eosinophilia and enhanced ROS generation by BAL cells has been compared in animals sensitized either by exposure to aerosol or by parenteral injection.

Guinea-pigs were sensitized to ovalbumin (OA) by inhalation (1% w/v for 5 min) on days 0 and 7 and used from day 14, or by parenteral injection (50mg i.p. and 50mg s.c.) on day 0 and used from day 21. All animals were pre-treated with mepyramine (10mg/kg i.p) 1hr prior to challenge with a saline or OA aerosol (2% w/v for 5 min). BAL was performed 24 hr after challenge, cells isolated and total and differential cell counts performed. ROS production was measured as peak phorbol myristate acetate (PMA)-stimulated luminol enhanced chemiluminescence. After OA-challenge the total number of cells recovered by BAL was significantly increased only in animals sensitized parenterally ($15.9 \pm 2.8 \times 10^6$ vs $4.7 \pm 0.04 \times 10^6$; $p < 0.001$, n = 14-16). However, the numbers of eosinophils in the BAL fluid of both the aerosol ($19.3 \pm 3.3 \times 10^5$; $p < 0.01$, n = 32) and the parenterally sensitized group ($43.3 \pm 4 \times 10^5$; $p < 0.001$, n = 14) were significantly elevated following OA challenge when compared to their saline matched controls ($5.3 \pm 0.5 \times 10^5$, n = 29, and $5.5 \pm 0.8 \times 10^5$, n = 16, respectively). OA-challenge also elevated the number of macrophages recovered from the BAL fluid of guinea-pigs from both groups but only elevated neutrophils and lymphocytes in those from the parenterally sensitized group. Following OA-challenge the ability of BAL cells to generate ROS was significantly enhanced in animals sensitized either by aerosol (31.6 ± 3.1 mV/2.5 x 10^5 cells; $p < 0.01$, n = 30) or by parenteral injection (35.7 ± 3.7 mV/2.5 x 10^5 cells; $p < 0.001$, n = 14). ROS generating capacity was similar for cells recovered from saline-challenged animals from both the aerosol (15.0 ± 1.2 mV/2.5 x 10^5 cells, n = 27) and the parenterally sensitized group (18.3 ± 1.7 mV/2.5 x 10^5 cells, n = 16). In the saline-challenged groups there was no correlation between the ability of BAL cells to generate ROS and the numbers of eosinophils. However, following OA-challenge there was a significant correlation between ROS generation and eosinophil number in both the aerosol ($r = 0.44$; $p < 0.01$) and the parenteral group ($r = 0.6$; $p < 0.05$).

In conclusion, antigen challenge results in a 3-fold increase in the number of eosinophils recovered by BAL in animals sensitized by aerosol and an 8-fold increase in animals sensitized parenterally. This difference in eosinophil infiltration probably reflects the differing sensitization schedules employed. BAL cells recovered from these animals demonstrate an enhanced capacity to generate ROS and this appears to be related to the increase in the proportion of eosinophils in the BAL fluid.

Fitzgerald, M.F., Thompson, A.M., Sturton, R.G. & Russell, V.P. (1991). Am.Rev.Resp.Dis., 143, A544.

126P CATIONIC PROTEINS INDUCE AIRWAY HYPERRESPONSIVENESS DEPENDENT ON CHARGE INTERACTIONS

Anthony J. Coyle[1], Steven J. Ackerman[2] Charles G. Irvin[1](Introduced by Clive P. Page). [1]Dept of Medicine, National Jewish Center for Immunology and Respiratory Medicine. 1400 Jackson St., Denver CO 80206, USA.[2]Infectious Diseases Division, Dept. of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA, USA

The role of eosinophil-derived cationic proteins such as Major Basic Protein (MBP) in the pathogenesis of bronchial asthma is unclear. We have previously reported that MBP induces AHR in the rat, an effect mimicked by synthetic polycations such as poly-L-lysine (Uchida et al, 1990), suggesting that charge interactions may be important in the development of AHR. Accordingly, we have investigated whether two other native, structurally diverse cationic proteins, namely Platelet Factor 4 (PF4) and Cathepsin G (CG), were also capable of inducing AHR. Furthermore, the effect of a variety of agents with the ability to neutralise the cationic charge of these proteins was also determined.

Adult rats (200-250g) were anaesthetised with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), intubated and placed in a whole body plethysmograph. A cannula was placed in the oesophagus to estimate intrapleural pressure and measurements of total lung resistance (RL) recorded. Increasing concentrations of methacholine (MCh) (0.156-5.0 mg/ml) were generated by aerosol to assess airway responsiveness and the concentration of MCh required to increase RL by 100 % calculated (PC100). MBP (100 μg) was instilled intratracheally and airway responsiveness determined 1 hr later. Similarly, airway responsiveness was measured 1 hr after instillation of CG (1000 U), PF4 (4000 U) or poly-L-lysine (100 μg). To determine if charge interactions were important, each of these cationic proteins were combined with an equal volume of low molecular weight heparin (LMWH) (1 mg/ml), instilled into the airways and airway responsiveness determined. In addition, the effects of two other negatively charge molecules, albumin and dextran sulphate on poly-L-lysine induced AHR were investigated.

MBP induced a significant increase in airway responsiveness (PRE PC100 Mean ± (GSEM): 1.82 mg/ml, (1.51-2.19), n = 4; POST: 0.63 mg/ml, (0.54-0.74), n = 4, $p < 0.05$). The effect of MBP was inhibited by LMWH (PRE: 1.51mg/ml, (1.41-1.62), n = 4, $P > 0.05$). Similarly, PF4 and CG increased airway responsiveness (PRE PF4: 1.10 mg/ml, (0.97 - 1.27); POST PF4: 0.50 mg/ml, (0.45-0.56), n = 6, $p < 0.05$; PRE CG: 1.09 mg/ml, (0.88-1.37), POST CG: 0.49 mg/ml (0.41-0.59), n = 4 $p < 0.05$). Mixing either PF4 or CG with LMWH inhibited the development of AHR (PRE: 1.23 mg/ml, (1.44 - 1.05); POST PF4 + LMWH: 1.33 mg/ml, (1.08-1.63), n = 4, $p > 0.05$ and PRE: 1.62 mg/ml, (1.44-1.82); POST CG + LMWH: 1.51 mg/ml, (1.45-1.58), n = 4, $p > 0.05$). AHR induced by these native cationic proteins was similar to that induced by poly-L-lysine (PRE: 1.25 mg/ml, (1.14-1.39), POST: 0.25 mg/ml, (0.20-0.31), n = 6, $p < 0.01$). Poly-L-lysine induced AHR was inhibited by LMWH, albumin and dextran sulphate; In contrast, neutral dextran was ineffective.

These observations support the hypothesis that charge interactions in the airways are important in the development of AHR. Moreover, an array of inflammatory cells appear to be capable of inducing AHR by a similar mechanism.

Uchida D, Ackerman S, Weller P, Larsen G, Irvin CG (1990). Am Rev Respir Dis 141:A174

Supported by NIH Grants HL 36577 and AI 22660.

127P [^{111}IN]-EOSINOPHIL ACCUMULATION DURING PASSIVE CUTANEOUS ANAPHYLAXIS IN THE GUINEA-PIG

V.B. Weg, M.L. Watson[1], L.H. Faccioli & T.J. Williams, Department of Applied Pharmacology, National Heart & Lung Institute, London SW3 6LY. [1]Present address: Department of Pharmacology, University of Bath, Avon BA2 7AY.

Eosinophil accumulation and plasma extravasation are features of type I allergic responses. In an attempt to characterise the mediators of these responses, we have examined the local accumulation of ^{111}In-eosinophils and leakage of ^{125}I-albumin during passive cutaneous anaphylaxis (PCA) reactions and in response to defined inflammatory mediators in the guinea pig. Animals were passively sensitised by intradermal injection of anti-bovine gamma globulin (BGG) antibody (50μl, 1/50 dilution). After 20- 24h animals were injected intravenously with ^{111}In-eosinophils and ^{125}I-albumin for the measurement of cell accumulation and plasma leakage, over a 2h period, as described previously (Faccioli *et al.*, 1991). When injected into sensitised sites, antigen caused a dose-related increase in the accumulation of ^{111}In-eosinophils (0.04, 0.2, 1, 5 and 25 μg BGG/site causing 4.8 $\pm$ 0.9, 5.5 $\pm$ 1.6, 9.2 $\pm$ 2.3 10.8 $\pm$ 1.9 and 13.5 $\pm$ 1.8 x 10^3 cells at sensitised sites compared with 0.8 $\pm$ 0.2 x 10^3 cells at naive sites injected with 25μg BGG, n=6). Time-course experiments over 24h revealed that the maximal rate of ^{111}In-eosinophil accumulation occurred over the first 90 min, with little accumulation observed at later time points. Co-injection of antigen with the Paf antagonist WEB 2086 (100 nmol/site) or the 5 lipoxygenase inhibitor PF 5901 (100 nmol/site) did not significantly alter the accumulation of ^{111}In-eosinophils or plasma leakage, whereas these drug doses abolished responses to exogenous PAF (1 nmol/site) or arachidonic acid (30 nmol/site), respectively. The H_1 receptor antagonist chlorpheniramine (25 nmol/site) did not reduce antigen-induced ^{111}In-eosinophil accumulation. Drug combinations were also injected with antigen into sensitised sites but were unable to reduce ^{111}In-eosinophil accumulation. When injected into naive sites histamine (10 nmol/site) or the ECF-A tetrapeptides VGSE and AGSE (1-1000 nmol/site) stimulated a very weak ^{111}In-eosinophil accumulation.

These results indicate that anaphylactic eosinophil accumulation in this model involves mediators other than histamine, Paf or lipoxygenase products. This is in contrast with plasma leakage in this reaction, which can be abolished by a combination of antagonists blocking these mediators (Weg *et al.*, 1991).

We are grateful to Boehringer Ingelheim, Germany for the gift of WEB 2086 and Perdue Frederick, USA for PF 5901. This work was supported by The National Asthma Campaign, British Biotechnology Ltd and CAPES, Brazil.

Faccioli, L.H., Nourshargh, S., Moqbel, R., Williams, F.M., Sehmi, R., Kay, A.B. & Williams T.J. (1991) *Immunology* **73**,222-227
Weg, V.B., Watson, M.L., Cordeiro, R.S.B. & Williams, T.J. (1991) *Eur. J. Pharmacol.*, in press.

128P AN INTERLEUKIN-1 RECEPTOR ANTAGONIST INHIBITS ANTIGEN-INDUCED EOSINOPHIL ACCUMULATION AND BRONCHIAL HYPERREACTIVITY IN GUINEA-PIGS

D. Smith, M.L. Watson, A.D. Bourne, *R.C. Thompson & J. Westwick, Department of Pharmacology, University of Bath, Claverton Down, Bath, Avon, BA2 7AY, U.K. & * Synergen, Boulder, Colorado, U.S.A.

Eosinophil infiltration of the lung tissue and increased airway reactivity to spasmogens are both characteristic features of bronchial asthma. Antigen challenge by aerosol of actively sensitised guinea-pigs elicits these characteristic features and is a widely used model to assess compounds for the treatment of asthma (see Sanjar et al, 1990). To determine the role of the pro-inflammatory cytokine interleukin (IL) 1 in allergic inflammatory lung diseases we have examined the effect of a selective and potent IL-1 receptor antagonist (IL-1ra; Eisenberg et al, 1990) in this model. Male Dunkin Hartley guinea-pigs (400-500 g) were sensitised to ovalbumin (OA, 10 μg), exposed to aerosolised antigen (OA, 0.1% for 1 hr), airway reactivity to histamine examined, and bronchoalveolar lavage (BAL) performed essentially as previously described (Sanjar et al, 1990; Smith et al, 1991). Groups of sensitised guinea-pigs (n=5) were exposed for 30 mins to aerosol generated from a 5 ml solution of 0.25% BSA vehicle (Group A) or a similar solution containing 50 μg of IL-1ra per animal (Group B). Ten minutes later animals from Group A and Group B were exposed to an aerosol for one hour of OA (10 mls of 0.1% solution), while Group C were non-challenged animals. Twenty four hours later animals from each group were prepared for measurement of lung function (increase in airway resistance (R_L) and compliance) via a respiratory analyser (PMS, Mumed Ltd, UK). To assess airway reactivity the spasmogen histamine (1.0, 1.8 and 3.2 μg/kg) was administered at intervals of ten mins via a cannulated jugular vein. The R_Ls are shown in the table. Treatment of other groups of non challenged animals (n=5) revealed that IL-1ra was not an inhibitor of histamine induced bronchoconstriction.

Treatment	BAL (x10^6) eosinophils	R_L cms H_2O l^{-1} s^{-1} 1.0	1.8	3.2 μg hist. kg^{-1}
Group A (vehicle + OA)	8.7 ± 0.7	118 ± 45	500 ± 127	1909 ± 118
Group B (IL-1ra + OA)	3.9 ± 0.4	46 ± 18	227 ± 36	636 ± 55
Group C (non-challenged)	1.6 ± 0.6	20 ± 5	45 ± 12	120 ± 36

These results demonstrate that aerosolised IL-1ra produces a significant ($p<0.05$-0.001) inhibition of both aerosolised antigen induced eosinophil infiltration and histamine-induced bronchial hyperreactivity. Thus IL-1 is probably involved in allergic diseases and aerosolised IL-1ra may prove to be a useful therapy for the treatment of inflammatory lung disease, including asthma.

We are grateful to the Wellcome Trust for support.

Eisenberg, S.P., Evans, R.J., Arend, W.P., Verderber, E., Hannum, C.H. & Thompson, R.C. (1990) *Nature*, **343**, 341-346
Sanjar, S., Aoki, S., Kristersson, A., Smith, D. & Morley, J. (1990) *Br. J. Pharmacol.* **99**, 679-686.
Smith, D., Burrows, L. & Westwick, J. (1991) *Adv. Exp. Med. Biol.* **305**, 119-126.

129P CYCLIC NUCLEOTIDE PHOSPHODIESTERASE (PDE) ISOENZYME ACTIVITIES IN HUMAN PERIPHERAL BRONCHI

M. Shahid*, A.J. Philpott, J. de Boer[1], J. Zaagsma[1] & C.D. Nicholson,
Organon Laboratories Ltd., Newhouse, Lanarkshire, ML1 5SH and Department of Pharmacology and Therapeutics, University of Groningen, A Deusinglaan 2, 9713 AW Groningen, The Netherlands.

PDE isoenzyme selective inhibitors have therapeutic as potential anti-asthma drugs (Nicholson et al., 1991). These compounds, however, show species variation in their pharmacological activity which may be due to differences in tissue distribution and intracellular localisation of isoenzymes. Thus, it is important to characterise the PDE isoenzymes in human tissue. The aim of the present study was to determine the PDE isoenzymes present in human bronchial smooth muscle. Human lungs were obtained from lung cancer patients undergoing surgery. Diseased tissue was removed and peripheral bronchi (diameter 2-3 mm) were isolated as described by van Amsterdam et al (1990). Bronchi (9-10 g) from several lungs were pooled, homogenised and PDE isoenzymes in the soluble fraction separated on a Mono Q column and assayed (1 μM substrate) essentially as described by Shahid et al (1991). The cyclic AMP activity in the soluble extract was 4-5 fold greater than the cyclic GMP activity. Six peaks of PDE activity were observed in human bronchial smooth muscle, eluting at 0.13, 0.21, 0.24, 0.28, 0.35 and 0.65 M NaCl. The first peak showed higher activity for cyclic GMP than cyclic AMP, was not activated by Ca++/calmodulin, but was potently inhibited by zaprinast (IC_{50}: 0.49 μM). The second and third peaks were stimulated by Ca++/calmodulin but showed differential sensitivity to zaprinast (IC_{50}s: peak 2, 4.9 μM; peak 3, 84 μM). The cyclic AMP activity of peak 4 was stimulated 3 fold by 1 μM cyclic GMP. Peak 5 (representing 51% of total cyclic AMP activity) preferentially hydrolyzed cyclic AMP, it was not stimulated by Ca++/calmodulin but was weakly inhibited by cyclic GMP. Peak 6 (3.8% of total cyclic AMP activity) was essentially similar to peak 5. 3-isobutyl-1-methylxanthine inhibited the activity of peaks 1 to 5 with IC_{50}s of 19, 42, 30, 32 and 19 μM, respectively. According to current nomenclature peaks 1, 2/3, 4 and 5/6 can be classified as PDE V, I, II and IV, respectively. In conclusion, the present study shows human bronchial airway smooth muscle to contain PDE I, II, IV and V isoenzyme activities with PDE IV being the main cyclic AMP activity. Multiple PDE I and PDE IV activities, were observed. There was no clearly distinct cyclic GMP-inhibited, PDE III, isoenzyme activity, although the possibility of co-elution with other PDEs cannot be excluded.

We thank Mr K MacArthur, Royal Infirmary, Glasgow for the supply of human lungs.
Nicholson, C.D., Challiss, R.A.J. & Shahid, M. (1991). TIPS, 12, 19-27.
Shahid, M., van Amsterdam, R.G.M., de Boer, J., ten Berge, R.E.J., Nicholson, C.D. & Zaagsma, J. (1991). Br. J. Pharmacol., 104, 471-477.
van Amsterdam, R.G.M., Meurs, H., ten Berge, R.E.J., Veninga, C.C.M., Brouwer, F. & Zaagsma, J. (1990). Am. Rev. Respir. Dis., 42, 1124-1128.

130P THE INVOLVEMENT OF ACETYLCHOLINE ON ANTINOCICEPTION EVOKED BY ELECTRICAL STIMULATION OF THE ANTERIOR PRETECTAL NUCLEUS

H. Rees, Mariana G. Terenzi and M.H.T. Roberts, Department of Physiology, University of Wales College of Cardiff, PO Box 902, Cardiff, CF1 1SS.

Electrical stimulation of the anterior pretectal nucleus (APtN) causes long lasting antinociception which is not associated with aversive responses (Roberts & Rees, 1986). The APtN stains densely for acetylcholine esterase (Paxinos & Watson 1986). The cells of this region also display immunoreactivity for the enzyme choline acetyltransferase (Levey et al., 1987) and receive an input from the cholinergic cells of origin of the dorsal tegmental bundle (Wilson 1985). This study examines the involvement of acetylcholine in APtN evoked antinociception

Male Wistar rats (230-250g) were anaesthetised with sodium pentobarbitone (60 mg/kg i.p.) and steel guide cannulae implanted unilaterally above the APtN. Following one week recovery the APtN was stimulated (35 uA r.m.s., 50 Hz sine wave, 15 s). 15 min prior to stimulation animals were pretreated with either atropine (1 mg/kg i.p.), mecamylamine (1 mg/kg i.p.) or saline (1 ml/kg i.p.). Following saline pretreatment electrical stimulation of the APtN caused an immediate maximal increase in tail flick latency (6s cut off). The increase gradually declined to baseline 60 min later. Pretreatment with the nicotinic antagonist mecamylamine failed to attenuate the increase in tail flick latency caused by APtN stimulation. However, the muscarinic antagonist atropine severely attenuated the effects of APtN stimulation, the initial increase in tail flick latency was reduced by over 65% and baseline values were achieved 5 min later.

To test whether acetylcholine had effects at the level of the APtN, a variety of muscarinic agonists were microinjected into the APtN and the effects on the tail flick latency measured. The techniques for microinjection have been described previously (Azami et al., 1980), drugs were microinjected slowly over a 3 min period at the same concentration which was 5 ug in a volume of 0.5 ul . Three agonists were tested pilocarpine, McN A343 and arecoline. All caused an increase in tail flick latency, the greatest effects were seen 15-20 min following microinjection, in each case the increase never reached cut off.

In conclusion, we tentatively suggest that muscarinic receptors have a role to play in the neuronal pathways mediating APtN antinociception. The effectiveness of muscarinic agonists microinjected into the APtN would suggest the involvement of a cholinergic input.

This work was supported by the Wellcome Trust, MGT is a CAPES(Brazil) scholar.

Azami.J., Llewelyn,M.B. & Roberts,M.H.T. (1980) J.Physiol. 305, 18-19P.
Levey,A.I., Hallanger,A.E. & Wainer,B.H. (1987) J.Comp.Neurol. 257, 317-332.
Paxinos,G & Watson,C. (1986) The rat brain in stereotaxic coordinates. Academic Press.
Roberts,M.H.T. & Rees,H. (1986) Pain 25, 83-93.

129P CYCLIC NUCLEOTIDE PHOSPHODIESTERASE (PDE) ISOENZYME ACTIVITIES IN HUMAN PERIPHERAL BRONCHI

M. Shahid*, A.J. Philpott, J. de Boer[1], J. Zaagsma[1] & C.D. Nicholson,
Organon Laboratories Ltd., Newhouse, Lanarkshire, ML1 5SH and Department of Pharmacology and Therapeutics, University of Groningen, A Deusinglaan 2, 9713 AW Groningen, The Netherlands.

PDE isoenzyme selective inhibitors have therapeutic as potential anti-asthma drugs (Nicholson et al., 1991). These compounds, however, show species variation in their pharmacological activity which may be due to differences in tissue distribution and intracellular localisation of isoenzymes. Thus, it is important to characterise the PDE isoenzymes in human tissue. The aim of the present study was to determine the PDE isoenzymes present in human bronchial smooth muscle. Human lungs were obtained from lung cancer patients undergoing surgery. Diseased tissue was removed and peripheral bronchi (diameter 2-3 mm) were isolated as described by van Amsterdam et al (1990). Bronchi (9-10 g) from several lungs were pooled, homogenised and PDE isoenzymes in the soluble fraction separated on a Mono Q column and assayed (1 μM substrate) essentially as described by Shahid et al (1991). The cyclic AMP activity in the soluble extract was 4-5 fold greater than the cyclic GMP activity. Six peaks of PDE activity were observed in human bronchial smooth muscle, eluting at 0.13, 0.21, 0.24, 0.28, 0.35 and 0.65 M NaCl. The first peak showed higher activity for cyclic GMP than cyclic AMP, was not activated by Ca++/calmodulin, but was potently inhibited by zaprinast (IC_{50}: 0.49 μM). The second and third peaks were stimulated by Ca++/calmodulin but showed differential sensitivity to zaprinast (IC_{50}s: peak 2, 4.9 μM; peak 3, 84 μM). The cyclic AMP activity of peak 4 was stimulated 3 fold by 1 μM cyclic GMP. Peak 5 (representing 51% of total cyclic AMP activity) preferentially hydrolyzed cyclic AMP, it was not stimulated by Ca++/calmodulin but was weakly inhibited by cyclic GMP. Peak 6 (3.8% of total cyclic AMP activity) was essentially similar to peak 5. 3-isobutyl-1-methylxanthine inhibited the activity of peaks 1 to 5 with IC_{50}s of 19, 42, 30, 32 and 19 μM, respectively. According to current nomenclature peaks 1, 2/3, 4 and 5/6 can be classified as PDE V, I, II and IV, respectively. In conclusion, the present study shows human bronchial airway smooth muscle to contain PDE I, II, IV and V isoenzyme activities with PDE IV being the main cyclic AMP activity. Multiple PDE I and PDE IV activities, were observed. There was no clearly distinct cyclic GMP-inhibited, PDE III, isoenzyme activity, although the possibility of co-elution with other PDEs cannot be excluded.

We thank Mr K MacArthur, Royal Infirmary, Glasgow for the supply of human lungs.
Nicholson, C.D., Challiss, R.A.J. & Shahid, M. (1991). TIPS, 12, 19-27.
Shahid, M., van Amsterdam, R.G.M., de Boer, J., ten Berge, R.E.J., Nicholson, C.D. & Zaagsma, J. (1991). Br. J. Pharmacol., 104, 471-477.
van Amsterdam, R.G.M., Meurs, H., ten Berge, R.E.J., Veninga, C.C.M., Brouwer, F. & Zaagsma, J. (1990). Am. Rev. Respir. Dis., 42, 1124-1128.

130P THE INVOLVEMENT OF ACETYLCHOLINE ON ANTINOCICEPTION EVOKED BY ELECTRICAL STIMULATION OF THE ANTERIOR PRETECTAL NUCLEUS

H. Rees, Mariana G. Terenzi and M.H.T. Roberts, Department of Physiology, University of Wales College of Cardiff, PO Box 902, Cardiff, CF1 1SS.

Electrical stimulation of the anterior pretectal nucleus (APtN) causes long lasting antinociception which is not associated with aversive responses (Roberts & Rees, 1986). The APtN stains densely for acetylcholine esterase (Paxinos & Watson 1986). The cells of this region also display immunoreactivity for the enzyme choline acetyltransferase (Levey et al., 1987) and receive an input from the cholinergic cells of origin of the dorsal tegmental bundle (Wilson 1985). This study examines the involvement of acetylcholine in APtN evoked antinociception

Male Wistar rats (230-250g) were anaesthetised with sodium pentobarbitone (60 mg/kg i.p.) and steel guide cannulae implanted unilaterally above the APtN. Following one week recovery the APtN was stimulated (35 uA r.m.s., 50 Hz sine wave, 15 s). 15 min prior to stimulation animals were pretreated with either atropine (1 mg/kg i.p.), mecamylamine (1 mg/kg i.p.) or saline (1 ml/kg i.p.). Following saline pretreatment electrical stimulation of the APtN caused an immediate maximal increase in tail flick latency (6s cut off). The increase gradually declined to baseline 60 min later. Pretreatment with the nicotinic antagonist mecamylamine failed to attenuate the increase in tail flick latency caused by APtN stimulation. However, the muscarinic antagonist atropine severely attenuated the effects of APtN stimulation, the initial increase in tail flick latency was reduced by over 65% and baseline values were achieved 5 min later.

To test whether acetylcholine had effects at the level of the APtN, a variety of muscarinic agonists were microinjected into the APtN and the effects on the tail flick latency measured. The techniques for microinjection have been described previously (Azami et al., 1980), drugs were microinjected slowly over a 3 min period at the same concentration which was 5 ug in a volume of 0.5 ul . Three agonists were tested pilocarpine, McN A343 and arecoline. All caused an increase in tail flick latency, the greatest effects were seen 15-20 min following microinjection, in each case the increase never reached cut off.

In conclusion, we tentatively suggest that muscarinic receptors have a role to play in the neuronal pathways mediating APtN antinociception. The effectiveness of muscarinic agonists microinjected into the APtN would suggest the involvement of a cholinergic input.

This work was supported by the Wellcome Trust, MGT is a CAPES(Brazil) scholar.

Azami.J., Llewelyn,M.B. & Roberts,M.H.T. (1980) J.Physiol. 305, 18-19P.
Levey,A.I., Hallanger,A.E. & Wainer,B.H. (1987) J.Comp.Neurol. 257, 317-332.
Paxinos,G & Watson,C. (1986) The rat brain in stereotaxic coordinates. Academic Press.
Roberts,M.H.T. & Rees,H. (1986) Pain 25, 83-93.

131P ACTION OF TOPICAL CAPSAICIN ON BLOOD FLOW AND C-FIBRE NOCICEPTORS IN THE SKIN OF ANAESTHETIZED RATS

B. Lynn & B. Cotsell (introduced by J.P.Fry), Department of Physiology, University College London, Gower St., London WC1E 6BT

Capsaicin, applied topically to mammalian skin at high (30mM) concentration, causes vasodilatation, pain and nociceptor firing (Lynn, 1990). Repeated application leads to loss of these effects. It has been reported that repeated application of lower concentrations (1-2mM) can reduce post-herpetic pain (Bernstein, 1989). This study examines the dose dependence and time course of changes in nociceptor function after single applications.

Experiments were carried out on Wistar or SD rats anaesthetized with urethane (1.6g/Kg IP) or sodium pentobarbitone (60mg/kg IP, then 20mg/kg.hr IV). C-fibre recordings were made from saphenous nerve filaments. Skin blood flow was measured using a laser-Doppler flowmeter. Capsaicin was dissolved in a vehicle comprising 25% propylene glycol, 48% ethanol, 2% methyl laurate and 25% water. To examine recovery over 1-4 days, rats were anaesthetised with 2-4% halothane in 67% N_20/33% O_2 and capsaicin applied for 15 min.

Painting capsaicin on the skin increased blood flow in a dose dependent manner in the range 1-100mM; below 1mM no vasodilatation was seen. Excitation of C-polymodal nociceptors (C-PMNs) was seen in 9 out of 10 units with 33-100mM, 2 out of 5 with 0.3-3mM, 1 out of 4 at 0.03-0.1mM, but none of the 3 units at 0.003mM. Antidromic vasodilatation following electrical stimulation of the saphenous nerve was reduced after applying ≥3mM. Application of 0.3-30mM decreased mechanical, but increased heat sensitivity of most C-PMNs (Lynn et al., 1991).

Antidromic vasodilatation was 67% (±1%, S.E., n=2) compared with vehicle controls 1 day after treatment with 30mM, but recovered to 102% (±8%, n=4) at 2-4 days. Pressure thresholds of C-PMNs were elevated at 1 day (1.10log mN ±0.10, n=17) compared with vehicle controls (0.80log mN ±0.09, n=28). At 2 days pressure thresholds had recovered (0.92log mN ± 0.07, n=20). Heat thresholds were normal 1-2 days after treatment.

Thus topically applied capsaicin, at concentrations comparable to those used in clinical trials, has marked immediate effects on nociceptor function. These effects, at least with higher doses, last 24-48 hours, which is consistent with the clinical finding of cumulative effects of daily application.

This work was supported by an MRC project grant and a contract with the Proctor and Gamble Company.

Bernstein, J.E. (1989) Med. Times, 117, 113-116.
Lynn, B. (1990) Pain, 41, 61-69.
Lynn, B., Ye, W. & Cotsell, B. (1991) J.Physiol., 438, 165P.

132P DISTINCT EFFECTS OF SOMATOSTATIN-14 AND SOMATOSTATIN-28 ON NOCICEPTIVE RESPONSES OF SPINAL DORSAL HORN NEURONES

S. Fleetwood-Walker, P.J. Hope, V. Molony, R.M.C. Parker, F. Munro & *R. Mitchell. Department of Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, Edinburgh and *MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ

Both somatostatin-14 (SS-14) and its N-terminally extended form somatostatin-28 (SS-28) are present in dorsal root ganglia and superficial dorsal horn (Tessler *et al,* 1986). Whilst SS-14 is released into dorsal horn by noxious thermal stimuli (Morton *et al,* 1989), it is not known whether SS-28 is released by either the same or different stimuli. The two forms of SS, however, act with a reverse order of potency to elicit hormone secretion from certain endocrine cells and also at the two binding sites recognised by SS analogues (see Patel *et al* (1990) for review) and have quite distinct actions on K^+ currents of neocortical neurones (Wang *et al,* 1989). Intrathecal administration of SS can have either inhibitory or stimulatory effects on spinal nociceptive responses (Dirksen *et al,* 1990) but the possibility that multiple SS receptor types might be involved has not been addressed.

Experiments were carried out as described previously (Fleetwood-Walker *et al,* 1990) on cats anaesthetised with chloralose/urethane. Extracellular recordings of dorsal horn laminae IV/V neurones were made during responses to cycles of quantified sensory stimuli applied to the peripheral receptive field. Peptides (2 - 5 mM in water, pH 4.5 - 5.0) were applied by microionophoresis from separate micropipettes in the region of the substantia gelatinosa, immediately dorsal to the recording site. In 9 out of 11 neurones, SS-14 (350 - 400 nA for 5 - 14 min) selectively inhibited thermal nociceptive responses to 44 ± 6% of controls and in all 4 of these cells tested, responses to noxious pinch were similarly depressed to 36 ± 12% of controls. Similar results were obtained in 5 rat laminae IV/V neurones (applying peptides from side barrels of the recording electrode) where SS-14 (15 - 80 nA for 3 - 9 min) selectively inhibited nociceptive responses to an equivalent extent with no apparent distinction between thermal and mechanical stimuli. In contrast, SS-28 was never observed to exert antinociceptive effects. Instead, SS-28 selectively enhanced the thermal, but not mechanical nociceptive responses (of 4 out of 5 cat neurones tested) to 164 ± 19% of controls, after 350 - 400 nA for 6 - 15 min. One neurone showed no response at all to SS-28. It thus appears that SS-14 can act as an antinociceptive modulator of both thermal and mechanical inputs to these dorsal horn neurones. However, like neurokinin A (Fleetwood-Walker *et al,* 1990), it seems that SS-28 may act as a mediator or enhancer of the input specifically from thermal nociceptive afferents.

Dirksen, R., Lerou, J., Nijhuis, G.M.M., Booij, L.H.D.J. & Jurna, I. (1990) Life Sci. **47**, 1347-1354.
Fleetwood-Walker, S.M., Mitchell, R., Hope, P.J., El-Yassir, N., Molony, V. & Bladon, C.M. (1990) Brain Res. **519**, 169-182.
Morton, C.R., Hutchison, W.D., Hendry, I A. & Duggan, A.W. (1989) Brain Res. **488**, 89-96.
Patel, Y.C., Murthy, K.K., Escher, E.E., Banville, D., Spiess, J. & Srikant, C.B. (1990) Metabolism **39** (Suppl 2), 63-69.
Tessler, A., Himes, B.T., Gruber-Bollinger, J. & Reichlin, S. (1986) Brain Res. **370**, 232-240.
Wang, H.-L., Bogen, C., Reisine, T. & Dichter, M. (1989) Proc. Natl. Sci. USA **86**, 9616-9620.

133P EXCITATORY ACTION OF PROSTAGLANDIN E_2 ON RAT NEONATAL CULTURED DORSAL ROOT GANGLION CELLS

R.M Puttick (introduced by G. Henderson). Department of Pharmacology, Cambridge University, Tennis Court Road, Cambridge.

Nociceptive afferent neurones may be activated/sensitised by compounds such as prostaglandins, amines and bradykinin that are released locally in damaged tissue (Baccaglini & Hogan, 1983). These agents are thought to have a role in the pain associated with injury and inflammation. In this study, we have examined the electrophysiological effects of PGE_2 on cultured dorsal root ganglion (DRG) cells.

Whole-cell patch clamp recordings were made using DRG cells cultured from neonatal rats (McGuirk *et al.*, 1989) and used 1-2 days after plating. The pipette solution contained (mM): KCl, 120; NaCl, 5; $MgCl_2$, 2; Na_2ATP, 1.5; GTP, 0.1; EGTA, 1; HEPES, 5; pH 7.4. Cells were superfused at 18-24°C with (mM): NaCl, 120; KCl, 6; $MgCl_2$, 1; $CaCl_2$, 2; HEPES, 10; glucose, 6; pH 7.4.

Under current-clamp conditions PGE_2 (0.1-1μM) induced membrane depolarisation, the amplitude of the depolarisation induced by 0.1 μM PGE_2 being 10.8 ± 1.8 mV (n = 4). When cells were voltage-clamped at -60 mV, PGE_2 (1 μM) evoked an inward current (120 ± 20 pA; n = 19) associated with an increase (38 ± 4%; n = 19) in input conductance. These effects were observed in about 30% (23/74) of DRG cells. The response to PGE_2 desensitised, a second application of the drug inducing little or no response. To determine the reversal potential of the PGE_2-induced inward current, membrane potential was ramped between -100 and -20 mV at a rate of 50 mV s^{-1} before, during and after PGE_2application. The current induced by PGE_2 was linear in the voltage range -100 to -50 mV and extrapolation of this linear region to the zero current level revealed that the PGE_2-induced inward current reversed at -4 ± 4 mV (n = 9). When the KCl in the pipette was replaced with KAsp, PGE_2 still induced an inward current which reversed at 5 ± 5 mV (n = 6) indicating that an increase in chloride conductance does not underlie the PGE_2-induced inward current. The inward current was greatly reduced by replacement of extracellular Na^+ with N-methyl-D-glucamine showing that the inward current is carried, at least in part, by Na^+ (n = 7). The excitatory effects of PGE_2 on sensory neurones may result from the activation of a cation conductance which generates an inward current and thus membrane depolarisation. This is similar to the action of another inflammatory mediator, bradykinin, on sensory neurones (Burgess *et al.*, 1989). Studies are in progress to characterise this PGE_2-activated conductance and to examine the pharmacology of the response.

The financial support of ICI is gratefully acknowledged.

Baccaglini, P.I. & Hogan, P.G. (1983). *Proc. Natl. Acad. Sci. USA.* **80**, 594-598.
McGuirk,S.M.,Vallis, Y., Pasternak, C.A. & Dolphin, A. (1989). *Neurosci. Lett.* **99**; 85-89.
Burgess, G.M., Mullaney, I., McNeill, M., Dunn, P.M. & Rang, H.P. (1989). *J. Neurosci.* **9**, 3314-3325.

134P PROFILE OF ACTIVITY OF THE PEPTIDE NK_1 RECEPTOR ANTAGONIST, GR82334, IN ACUTE NOCICEPTIVE TESTS

P.J. Birch, I.J.M. Beresford, H. Rogers, R.M. Hagan, F. Bailey, A.G. Hayes, S.M. Harrison & S.J. Ireland, Department of Neuropharmacology, Glaxo Group Research, Ware, Herts, SG12 ODP.

The pharmacological actions of substance P are mediated predominantly through the NK_1 receptor subtype which is thought to play an important role in nociception. In the present study the ability of the selective, peptide NK_1 receptor antagonist, GR82334 (D-Pro^9[Spiro-γ-lactam]Leu^{10},Trp^{11}]Physalaemin(1-11); Hagan et al., 1991), to produce antinociceptive effects in a range of acute models has been assessed.

The following nociceptive tests were performed on male CRH mice (Glaxo-bred, 17-22g; see Tyers, 1980; Dubuisson & Dennis, 1977): abdominal constriction induced by acetylcholine (ACh, 3mg kg^{-1} i.p.; mice observed from 0-4min post-injection); paw-licking induced by intraplantar formalin (20μl of 5% phosphate-buffered formalin; animals observed from 0-5min (1st phase) and 15-30min (2nd phase)); 55°C hotplate (cut-off 60sec); tailflick (cut-off 10sec). Motor incapacitation was assessed using the rotarod test. GR82334 was administered via the intracerebroventricular (icv) or the intrathecal route (dose volume: 5μl) 5min prior to test. Each dose group consisted of 8-14 animals. Where appropriate MPE_{50} (dose producing 50% of the maximum possible effect) values (95% confidence limits) were calculated.

	MPE_{50} (nmol icv)	MPE_{50} (nmol Intrathecal)
ACh Abdominal Constriction	2.5 (1-5)	4.4 (2-23)
Formalin (1st Phase)	0.9 (0.6-2)	>10
Formalin (2nd Phase)	0.9 (0.2-4)	0.8 (0.3-1.8)
55°C Hotplate	>10	>10
Tailflick	low maximum ↑ latency at 1-10	4.9 (2-17)
Rotarod	>10	>10

The table shows that GR82334 produces antinociceptive effects in models employing chemical stimuli and in the tailflick test. Although not active in the rotarod test, GR82334 at 10nmol icv did induce a series of behaviours including barrel-rolling, tremor and mild depression. The results indicate that GR82334 is an effective antinociceptive agent in the mouse at doses which do not cause marked motor side-effects. As these doses block centrally-mediated NK_1 responses in this species (Birch et al., 1991; Elliott et al., 1991), the antinociceptive action of GR82334 is most probably mediated via blockade of NK_1 receptors and both spinal and supraspinal sites are implicated.

Birch, P.J., Rogers, H. & Harrison, S.M. (1991). Brit. J. Pharmacol., in press.
Dubuisson, D. & Dennis, S.G. (1977). Pain, 4, 161-174.
Elliott, P.J., Turpin, M.P., Mason, G.S. & Hagan, R.M. (1991). Brit. J. Pharmacol., **102**, 73P.
Hagan, R.M., Ireland, S.J., Bailey, F., McBride C., Jordan, C.C. & Ward, P. (1991). Br. J. Pharmacol., **102**, 168P.
Tyers, M.B. (1980). Brit. J. Pharmacol., **69**, 503-512.

135P ANTINOCICEPTIVE ACTIVITY OF THE BRADYKININ ANTAGONIST HOE-140 IN RAT AND MOUSE

I.J.M. Beresford and P.J. Birch, Department of Neuropharmacology, Glaxo Group Research, Ware, Herts, SG12 ODP.

HOE-140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) is a potent bradykinin (BK) B_2 receptor antagonist (Wirth et al., 1991). In the present study, HOE-140 has been evaluated for BK antagonist activity *in vivo* against BK-induced abdominal constriction in the mouse and BK-induced paw oedema in the rat. In addition, HOE-140 has been examined in acute nociceptive tests in the mouse (acetylcholine and acetic acid-induced abdominal constriction and formalin-induced licking) and in carrageenan-evoked inflammation in the rat.

Male CRH, Glaxo-bred mice (15-25g) were pretreated with PGE_2 (1mgkg^{-1} ip) followed 30min later by BK (1mgkg^{-1} ip) and the number of abdominal constrictions occurring within 90sec was determined. Control animals exhibited 6.5±0.4 abdominal constrictions per mouse (n=60). Pretreatment with HOE-140 (0.1-10nmolkg^{-1} sc, 30min prior to BK) abolished the constriction response with an ED_{50} value (95% confidence limits) of 0.16 (0.02-0.49) nmolkg^{-1} (n=12). Increasing the pretreatment time with HOE-140 to 60, 120 or 180min produced ED_{50} values of 0.41 (0.17-0.82), 0.48 (0.20-0.98) and 1.45 (0.59-4.16) nmolkg^{-1} respectively (n=12). HOE-140 (0.1-1000nmolkg^{-1} sc, 30min pretreatment) also antagonised abdominal constrictions elicited by either acetylcholine (3mgkg^{-1} ip) or acetic acid (0.6%, 10mlkg^{-1} ip). Maximum inhibitory effects following acetic acid and acetylcholine were 72% (ED_{50} 20 (6-98) nmolkg^{-1}) and 54% (at 1000nmolkg^{-1}). Intraplantar injection of formalin to mice (50µl, 5%) produced a biphasic licking response (0-5 and 15-30min). HOE-140 (100nmol intraplantar, 10min pretreatment and 800nmolkg^{-1} sc, 30min pretreatment) had no effect on the first phase but inhibited the time spent licking during the second phase by 54 and 45% respectively ($P<0.05$, n=8-11). Intraplantar injection of a higher dose of HOE-140 (1000nmol) suppressed first and second phases of the response by 43 and 84% respectively ($P<0.05$, n=11).

For measurement of BK-induced paw oedema, male RH, Glaxo-bred rats (80-150g) were injected (100µl intraplantar) with BK (0.1-30nmol) and paw oedema was measured 30min later using a plethysmometer. HOE-140 at 800nmolkg^{-1} sc (30min pretreatment; n=5) or iv (10min pretreatment; n=5) produced rightward shifts in the BK dose-response curve; dose-ratios were 7.1 (3.1-18.6) and 7.0 (3.2-17.4) respectively. Intraplantar injection of carrageenan (100µl, 2%) to rats produced a decrease in mechanical nociceptive threshold (hyperalgesia), determined using an algesymeter, and an increase in paw volume, which was maximal 3 hours following carrageenan. At 3 hours, HOE-140 (800nmolkg^{-1} iv 10min prior to carrageenan) antagonised hyperalgesia and paw oedema by 34% and 43% ($P<0.05$) respectively (n=10).

These results indicate that HOE-140 is a potent and long-lasting antagonist of BK-evoked abdominal constriction in the mouse but is a weaker antagonist of BK-induced paw oedema in the rat. In addition, HOE-140 is active against other forms of chemically-induced nociception in the mouse and carrageenan-induced inflammation in the rat, although the potency varies between tests.

Wirth, K., Hock, F.J., Albus, U., Linz, W., Alpermann, H.G., Anagnostopoulos, H., Henke, St., Breipohl, G., Konig, W., Knolle, J. & Scholkens, B.A. (1991). Br. J. Pharmacol., **102**, 774-777.

136P CONCANAVALIN-A PREVENTS CYCLIC-GMP-INDUCED DESENSITIZATION OF BRADYKININ ON PERIPHERAL NOCICEPTORS IN THE NEONATAL RAT *IN VITRO*

Patel, I.A., A. Rueff, L. Urban & A. Dray. Sandoz Institute for Medical Research, 5 Gower Place, London. WC1E 6BN.

Bradykinin (Bk), a proinflammatory and algesic nonapeptide, activates nociceptors via specific membrane receptors coupled to a number of second messenger systems. Prolonged or repeated applications of Bk will result in desensitization of the Bk receptors. It has been suggested that cyclic-GMP-dependant protein kinase may be involved in Bk signal transduction and in receptor desensitization (Burgess *et al*, 1989). In the present study, we have investigated the mechanisms of Bk-induced desensitization in nociceptors using concanavalin-A, a tetrameric lectin which has been shown to reduce desensitization of other mammalian neuronal receptors (Lin & Levitan, 1991), and agents interacting with the cyclic-GMP second messenger pathway.

The spinal cord with the functionally attached tail was removed from 1 to 2 day old rats following decapitation and the skin was stripped off the tail. The spinal cord and the tail were separately superfused (3 ml and 6 ml min^{-1} on the cord and tail respectively) with physiological solution and gassed with 95% O_2/ 5% CO_2 at 24±2°C. Nociceptors in the tail were activated by either thermal (50±2°C) or chemical (Bk 0.35µM, capsaicin 0.7µM) stimuli and the consequent spinal ventral root (VR) depolarization was recorded via an extracellular glass micropipette from either L3-L5 lumbar roots.

Brief administration of Bk (10s, 0.35µM) to the tail produced a VR depolarization which was reproducible over several hours with 1hr between applications. However, following prolonged application of Bk (5min. 1µM) there was a marked and selective desensitization to Bk such that subsequent administration of Bk produced no further response. Prior exposure of the tail to concanavalin-A (10min, 1µM,) prevented this Bk-induced desensitization. The effect of concanavalin-A was prevented by co-administration of the hapten sugar, α-methyl-D-mannoside (10mM), which interacts with concanavalin-A binding sites. In contrast, the monovalent sugar binding agent, succinyl concanavalin-A (1µM) did not prevent Bk-induced desensitization. Similarly, pretreatment with phenylarsine oxide (100µM), which has been shown to prevent desensitization of β-adrenoceptors by preventing receptor internalization (Hertel *et al*, 1985), was without effect on Bk-induced desensitization. Wheat germ agglutinin, a lectin which binds to N-acetyl-D-glucosamine and not to the mannose sugar, did not prevent desensitization of Bk receptors.

Pretreatment of the tail (10 min) with sodium nitroprusside (50µM) or dibutyryl cyclic-GMP (500nM) attenuated the response to a brief application of Bk (10s, 0.35µM), but was without effect on capsaicin- or heat-induced responses. However, in the presence of concanavalin-A (1µM) both sodium nitroprusside and dibutyryl cGMP were without effect on Bk-evoked depolarization. This protective effect of concanavalin-A persisted even after prolonged wash-out. Again, application of α-methyl-D-mannoside with the concanavalin-A prevented the action of concanavalin-A.

Our results suggests that cyclic-GMP sensitive mechanisms are involved in desensitzation of Bk receptors on peripheral nociceptors. Furthermore receptor desensitization may involve mannose sugar residues on the receptor binding site but does not appear to involve receptor internalization.

Burgess GM, Mullaney I, McNeill M, Coote PR, Minhas A &Wood JN. (1989), *J. Neurochem.* **53**, 1212-1218.
Hertel C, Coulter SJ & Perkins JP. (1985), *J. Biol. Chem.* **260**, 12547-12553.
Lin SS & Levitan IB. (1991), *Trends Neurosci.* **14**, 273-277.

137P NMDA AND NON-NMDA RECEPTOR-MEDIATED SLOW VENTRAL ROOT POTENTIALS RECORDED FROM THE NEONATAL RAT HEMISECTED SPINAL CORD *IN VITRO*

Thompson,S.W.N.,Gerber,G.[1],Sivilotti,L.& Woolf,C.J. Dept. of Anatomy & Dev.Biol. University College London, WC1E 6BT & [1] 2nd Dept. Anatomy, Semmelweis University of Medicine, Budapest, Hungary.

The generation of slow synaptic potentials in dorsal and ventral horn neurones may be an important step in the generation of excitability changes within the spinal cord (Thompson et.al.1990) which may underlie post-injury pain sensitivity states (central sensitization). In the present experiments we have examined the relative contributions of NMDA and non-NMDA receptor classes in the production and summation of slow synaptic potentials in the neonatal rat hemisected spinal cord *in vitro* following dorsal root stimulation.
Ventral root potentials (VRP's) were recorded with glass suction electrodes from cut lumbar (L4-L5) ventral roots following single shock stimulation of ipsilateral dorsal roots (Evans & Watkins 1978).
Stimulation of low threshold afferents evoked a short latency (time to peak 6.00 $\pm$0.5 ms) VRP which persisted in the presence of the NMDA receptor antagonist d-APV (40µM). Stimulation of high threshold, small calibre dorsal root afferents evoked a long duration slow VRP (>2.0s). This slow VRP was composed of two distinct components, i) A d-APV sensitive slow potential (time to peak 137 $\pm$5.1 ms), and ii) a d-APV resistant slow potential (time to peak 2.03 $\pm$0.2s) which was unmasked in the presence of the NMDA receptor antagonist. Low frequency (1-10Hz) repetitive stimulation of high threshold dorsal root afferent fibers for 20s evoked a temporal summation of synaptic activity, generating a progressively depolarizing VRP. Following cessation of the repetitive stimulation this depolarization of the VRP decayed to pre-stimulus levels over a period of several minutes. In the presence of 40µm d-APV the rate of rise of the cumulative VRP depolarization was markedly attenuated, however the ultimate peak level was unaffected. These experiments demonstrate that both NMDA and non-NMDA receptors are responsible for the generation and integration of prolonged synaptic potentials which may provide the basis for nociceptive afferent evoked central excitability changes.

References,
Evans,R.H. & Watkins,J.C. (1978). Eur.J.Pharmac.,*50*, 123-129
Thompson,S.W.N. et.al.(1990). Eur.J.Neurosci. *2*, 638-649

138P ANTINOCICEPTIVE EFFECTS IN THE SPINAL CORD OF MORPHINE AND NMDA ANTAGONISTS IN COMBINATION

V. Chapman & A.H. Dickenson, (introduced by D.H.Jenkinson) Dept. Pharmacology, University College, London WC1E 6BT

Nociceptive neurones in the dorsal horn which respond to C fibre inputs can exhibit a frequency-dependent potentiation of their responses (wind up) to C-fibre stimulation. Wind up occurs as a consequence of N-methyl - D-aspartate (NMDA) receptor activation and can be inhibited by competitive antagonists, channel blockers and antagonists at the allosteric glycine site as shown by electrophysiological studies in the intact halothane anaesthetized rat (Dickenson 1990). 7-Chlorokynurenate (7CK), an antagonist at the glycine site of the NMDA receptor significantly reduces wind-up without any marked change in the initial responses of the cells. In complete contrast, opiates acting at either the mu or delta receptors markedly reduce the inputs onto the cells but are considerably less effective against wind-up (Dickenson and Sullivan 1986).

This study investigates the effects of morphine and 7CK, alone and in combination applied intrathecally on groups of 10 nociceptive neurones recorded in the dorsal horn of the halothane anaesthetized rat. The responses of the cells to C-fibre stimulation applied to the receptive field were quantified. Co-application of 2.5µg 7CK (without effect alone) and 5µg morphine on to the spinal cord produces a 94±4% inhibition of wind-up compared to a 35±9% inhibition produced by 5µg morphine alone.The effect of 7CK and morphine on the C-fibre input onto the cells was a profound inhibition (91±4%,) compared to the 62±7% reduction produced by 5µg morphine alone and is similar to that produced by 50 µg of morphine alone (89±5% inhibition).

These results show that a combination of low doses of an NMDA antagonist and an opiate agonist can produce almost complete block of the nociceptive responses, an effect neither can elicit alone except at extremely high doses. This may have important clinical consequences for pain relief.

Supported by the MRC and a SERC-CASE studentship to V Chapman.
Dickenson, A.H. (1990) Trends in Pharmacological Sciences **11**.,307-309.
Dickenson, A.H. & Sullivan,A.F. (1986) Pain **24**., 211-222.

139P CROSS-TOLERANCE BETWEEN α-ADRENERGIC AND μ OPIOID BUT NOT δ OPIOID ANTINOCICEPTION IN THE RAT SPINAL CORD

A.H. Dickenson, E.A. Kalso[1], A.F. Sullivan and H.J. McQuay[1],(introduced by S.C. Stanford), Department of Pharmacology, University College London, Gower St, London WC1E 6BT, UK; [1]Nuffield Department of Anaesthetics, University of Oxford, Oxford, UK

Under halothane anaesthesia the spinal antinociceptive actions of a selective α_2-adrenergic agonist dexmedetomidine (Virtanen,1989), the μ opioid agonist morphine and the δ opioid agonist DSTBULET (Sullivan et al,1989) on convergent dorsal horn neuronal responses to peripheral electrical stimulation was examined in rats behaviourally tolerant (tail-flick test) to the intrathecal antinociceptive effects of one of the above agonists.

In rats behaviourally tolerant to morphine, inhibitions of C fibre-evoked responses by intrathecal morphine (5, 50μg in 50μl) were significantly reduced (-2±8%; 30±11%)(mean±SE) as were the inhibitions mediated by 2.5 and 10μg dexmedetomidine (-2±14%; 39±12%) compared to non-tolerant controls in which morphine produced inhibitions of 32±9% and 90±4% and dexmedetomidine inhibitions of 46±9% and 86±10%. The potency of these two agonists were also reduced, although less so, in rats behaviourally tolerant to dexmedetomidine; morphine; 16±14%, 60±20%, dexmedetomidine; 24±4%, 45±9%. The inhibitions of C fibre-evoked responses by DSTBULET were unchanged in rats tolerant to either morphine or dexmedetomidine. In rats behaviourally tolerant to DSTBULET the inhibitions of C fibre-evoked responses by 5 and 50 μg of this agonist were significantly reduced (-8±13%; 9±20%) compared to non-tolerant controls (30±6%; 67±8%) but inhibitions by dexmedetomidine and morphine were unaffected.

The results demonstrate the presence of tolerance to the inhibitory effects of either μ-, δ-opioid or α_2 adrenergic agonists on C fibre-evoked dorsal horn neuronal responses in rats behaviourally tolerant to the antinociceptive actions of the respective agonist. Furthermore cross-tolerance between α_2-adrenergic and μ-opioid mediated neuronal inhibitions was apparent. No cross-tolerance was found between δ-opioid and either μ- opioid or α_2 adrenergic mediated antinociceptive effects on dorsal horn neurones.

Supported by the Medical Research Council and the European Academy of Anaesthesiology.

Virtanen R. (1989) Acta vet.scand. **85**, 29-37.
Sullivan A.F., Dickenson A.H. and Roques B.P. (1989) Br.J.Pharmacol. **98**, 1039-1049.

140P FUNCTIONAL EVIDENCE FOR κ RECEPTOR HETEROGENEITY BETWEEN SPINAL AND SUPRASPINAL SITES

Juan F. Herrero & P. Max Headley, Department of Physiology, The Medical School, University Walk, Bristol BS8 1TD

Kappa opioids are effective analgesics and reduce the responses of spinal neurones to peripheral noxious stimuli (Parsons & Headley, 1989). The potency at supraspinal sites is greater than that at spinal sites (Herrero & Headley, 1991). There have been suggestions that there may be kappa receptor heterogeneity, although the evidence is equivocal (Traynor, 1989). We have now examined this issue in electrophysiological experiments. We have compared the relative potencies and naloxone reversibility of the selective kappa ligands U50,488, U69,593, PD117,302, CI977 (Hunter *et al*, 1990), and GR103,545A (Hayes *et al*, 1990), all given i.v. in a dose-doubling cumulative regime. Reflexes to 15s noxious pinch stimuli (repeated at 3 min intervals) were recorded as single motor unit responses in hindlimb flexor muscles of rats anaesthetized with alpha-chloralose (Herrero & Headley, 1991). The spinal cord was either sectioned (n=43) or was left intact with sham spinalization surgery (n=48).

ED_{50} values in spinalized animals were 6mg/kg for U50,488, 5mg/kg for U69,593, 3mg/kg for PD117,302, 0.3mg/kg for GR103,545A and 0.2mg/kg for CI977. With the cord intact the ED_{50} values were lower - 1.5 fold for PD117,302, 4-8 fold for U-50,488, U69,593, and CI977 and 60 fold for GR103,545A. Comparison of dose-response curves between spinalized and intact states showed that the curves were parallel for U50,488, U69,593 and PD117,302 but were shallower in the spinally intact state for CI977 and GR103,545A: the ratios of ED_{25} values for the latter two agents were 20 and 150 and the mean maximal effect of GR103,545A was only about 50% inhibition. All drug effects were reversed by naloxone at doses varying between 0.1 and 10mg/kg. A dose of 0.1mg/kg caused more than 50% reversal of all agents except GR103,545 (25%); there were no clear differences between the two preparations. Lastly the hypertensive effects varied between agents, U50,488 having the greatest effect and GR103,545A the least.

These differences, in spinal *vs* supraspinal potency ratios, in the slopes of the dose-response curves, in the effectiveness of naloxone and in the hypertensive effects, all suggest that these agents were acting at more than one receptor, the populations of which are different at spinal and supraspinal sites. Whether these different receptors represent kappa receptor subtypes awaits further study.

We wish to thank the Wellcome Trust for support and Glaxo, Parke-Davis and Upjohn for gifts of compounds.

Hayes, A.G. Birch P.J., Hayward, N.J., Sheehan, M.J., Rogers, H., Tyers, M.B. Judd, D.B., Scopes, D.I.C. & Naylor A. (1990) Br. J. Pharmac. **101**, 944-948.
Herrero, J.F. & Headley P.M. (1991) Br. J. Pharmac. **104**, 166-170.
Hunter, J.C., Leighton, G.E., Meecham, K.G., Boyle S., Horwell, D.C., Rees, D.C. & Hughes, J. (1990) Br. J. Pharmac. **101**, 183-189.
Parsons, C.G. & Headley, P.M. (1989) Br. J. Pharmac. **98**, 523-551.
Traynor, J.R. (1989) Trends Pharmac. Sci. **10**, 52-53.

141P [^{3}H]-DPDPE BINDING TO MOUSE BRAIN MEMBRANES: FURTHER EVIDENCE FOR δ OPIOID RECEPTOR BINDING SITE HETEROGENEITY

E. Bardaji & J.R. Traynor, Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire, LE11 3TU.

[DPen^2,DPen^5]enkephalin (DPDPE) is a highly selective ligand for the δ-type of opioid receptor. However we have previously shown that a fraction of the δ-binding of [^{3}H]DPDPE can be displaced with high affinity by μ-agonists such as morphine and DAMGO in homogenates of both brain and spinal tissue (Cotton *et al.*, 1985; Traynor *et al* 1990). Since evidence has recently been presented for the existence of delta receptor subtypes (Jiang *et al.*, 1991) we have re-investigated this phenomenon in homogenates of mouse brain tissue.

Binding of [^{3}H]DPDPE was studied in membranes (48,000 x g) prepared from brains (-cerebella) of male CSI mice (30-35g) at 25°C for 40min in Tris-HCl buffer (pH 7.4) by standard methods (Cotton *et al.*, 1985). In certain experiments membranes were washed by centrifugation and resuspension for up to six times. Saturation experiments demonstrated that [^{3}H]DPDPE bound to an apparently single class of receptor binding sites in mouse brain membranes, with an affinity (K_D) of 2.2±0.5 nM and a maximum binding capacity (Bmax) of 6.4±0.8 pmol g^{-1} tissue. However the μ-selective agonist DAMGO displaced [^{3}H]DPDPE (2nM) binding in a bi-phasic manner as previously reported, with an IC_{50} of 404±45nM and Hill coefficient of 0.79±0.05, significantly less than unity. The Bmax for [^{3}H]DPDPE, but not its affinity, was found to be reduced by 40% by washing the membranes a further four-times. The binding remaining was stable to further washing. In such washed preparations , in contrast to control preparations, DAMGO displaced [^{3}H]DPDPE (2 nM) with an IC_{50} of 624±100nM and a Hill coefficient of unity, with no evidence of a high affinity component.. Extensively washed preparations were also distinguished from control preparations by other μ- and δ -ligands.

The results show that [^{3}H]DPDPE binding sites may be differentiated on the basis of their stability towards washing procedures and provides further evidence that δ-opioid receptors may not be a homogeneous population.

E.B. was supported by a grant from the Ministerio de Educacion y Ciencia, Spain.

Cotton, R., Kosterlitz, H.W., Paterson, S.J., Rance, M.J. & Traynor, J.R. (1985) Br. J. Pharmac. **84**, 927-932.
Traynor, J.R., Hunter, J.C., Rodríguez, R.E., Hill, R.G. & Hughes, J. (1990) Br. J. Pharmac. **100**, 319-323.
Jiang, Q., Takemori, A,E., Sultana, M., Portoghese, P.S., Bowen, W.D., Mosberg, H.I. & Porreca, F. (1991) J. Pharmac. Exp. Ther. **257**, 1069-1075.

142P *IN VITRO* EVALUATION OF THE OPIOID ANTAGONIST 3-HYDROXYCYPRODIME

D. Patel, H.K. Jennewein[1], H. Schmidhammer[1], C.F.C. Smith[2] & J.R. Traynor, Department of Chemistry, Loughborough University of Technology, Loughborough, Leics. LE11 3TU, UK, [2]Pharmaceutical Division, Reckitt & Colman, Hull, HU8 7DS, UK and [1]Institute of Organic and Pharmaceutical Chemistry, University of Innsbruck, Austria.

Cyprodime, (-)-N-(Cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one, is a selective μ-opioid receptor antagonist (Schmidhammer *et al.*, 1989). Although useful because it is not a peptide, cyprodime suffers from the disadvantage of a relatively low affinity (approximately 50nM) for the μ-opioid receptor. In order to improve this affinity, whilst retaining selectivity, we have introduced a hydroxyl group into the 3-position. Here report the *in vitro* pharmacological profile of this compound.

Binding assays and *in vitro* bioassays were conducted by standard methods (Franklin & Traynor, 1991; Traynor *et al.*, 1987). In the guinea-pig ileum and mouse vas deferens 3-hydroxycyprodime behaved as a pure antagonist. In the mouse vas deferens against the selective μ-ligand DAMGO a Ke of 4.6±0.6nM was obtained, representing a ten-fold improvement on the parent compound cyprodime. Furthermore the compound was selective for μ–receptors with δ/μ and κ/μ selectivity ratios of 30 and 80. In the guinea-pig ileum however 3-hydroxycyprodime showed high μ-receptor affinity (Ke 1.3±0.2nM) but a similar affinity at κ-receptors (Ke 3.9±0.3nM). Also in binding assays in guinea-pig brain homogenates 3-hydroxycyprodime was nonselective with affinities at μ, δ and κ–binding sites of 6.0±1.6nM, 12.7±3.4nM and 4.3±0.6nM respectively.

The findings show that 3-hydroxycyprodime has improved affinity for all three opioid receptor sites compared with cyprodime and that in the mouse vas deferens, but not the guinea-pig ileum or guinea-pig brain, selectivity is retained. This further suggests that opioid receptors in different tissues may not be the same, supporting previous findings (Shimohigashi *et al.*, 1987; Kawasaki *et al.*, 1990; Franklin & Traynor, 1991).

DP is supported by an SERC studentship.

Franklin, T.G. & Traynor, J.R. (1991) Br. J. Pharmacol. **102**, 718-722.
Kawasaki, A.M., Knapp, R.J., Kramer, T.H., Wire, W.S., Vasquez, O.S., Yamamura, H.I., Burks, T.F. & Hruby, V.J. (1990) J. Med. Chem. **33**, 1874-1879.
Schmidhammer, H., Burkard, W.P., Eggstein-Aeppli, L. & Smith, C.F.C. (1989) J. Med. Chem. **32**, 418-421.
Shimohigashi, Y., Costa, T., Pfeiffer, A., Herz, A., Kimura, H. & Stammer. (1987) FEBS Lett. **22**, 71-74.

143P POTENTIATION OF AMITRYPTYLINE-INDUCED ANTINOCICEPTION BY ACETORPHAN

A.M. Gray. L.J. Kirkham and R.D.E. Sewell, Department of Pharmacology, Welsh School of Pharmacy, University of Wales, PO Box 13, Cardiff CF1 3XF

We have shown that certain antidepressants possess intrinsic antinociceptive activity in laboratory animal studies, and that the antinociception is subject to blockade by naloxone (Gray et al., 1990). Furthermore, the non-opioid analgesic nefopam, which resembles tricyclic antidepressants (TCA) in its monoamine uptake blockade profile, has been shown to possess a naloxone reversible component to its antinociceptive action in the abdominal constriction model (Nevinson et al., 1991).

To further our studies on the opioid-like characteristics of "TCA antinociception", the effect of acetorphan which yields inhibition of neutral endopeptidase - 24.11, (an opioid peptide degrading enzyme) (Lecomte et al., 1986) on amitriptyline antinociception in the mouse abdominal constriction assay was investigated. Male ICI GB1 mice (30-35g; n=8) were pretreated with acetorphan (10mg/kg; 0.5mg/100g; sc) at 45 min and either amitriptyline (AMI), or aspirin (ASP) 30 min prior to 1% acetic acid challenge (1ml/100g; ip). Abdominal constrictions were evaluated over 20 min and both amitriptyline and aspirin produced linear dose-related inhibitory actions in the assay. Acetorphan potentiated amitriptyline's inhibitory activity (see Table 1), but had no effect upon aspirin's action in this assay.

Table 1. Potentiation of amitriptyline activity in the abdominal constriction assay.

	ALONE	ACETORPHAN
AMI 2.5	10.75 ± 4.74	51.00 ± 3.42*
AMI 7.5	70.39 ± 0.60	86.57 ± 0.90*
ASP 22.5	43.17 ± 3.18	47.61 ± 4.89
ASP 100	72.19 ± 4.76	77.27 ± 8.14
ACETORPHAN	7.43 ± 5.32	

Results are expressed as % protection (±% sem). *P<0.05 versus antidepressant alone/aspirin - Kruskal Wallis

Our data provides further support for the enkephalin-induction hypothesis of Lindsay & Olsen (1985) which suggests that antidepressants increase enkephalin activity. This accords with our previous findings of the naloxone reversibility of AMI antinociception (Gray et al. 1990) and the possibility that AMI may in fact be releasing opioid peptides (Sacerdote et al., 1987).

Gray, A.M., Spencer, P.S.J. and Sewell, R.D.E. (1990) Br. J. Pharmacol. 101 554P
Nevinson, M.J., Gray, A.M. and Sewell, R.D.E. (1990) Br. J. Pharmacol. 104 (Suppl) 267P
Lindsay, P.G. and Olsen, R.B. (1985) J. Clin. Psychiat. 46 226-228
Lecomte, J., Costentin, J., Vlaiculescu, A., Chaillet, P., Marcais-Collado, H., Llorens-Cortes, C. and Schwartz, J.C. (1986) J. Pharmacol. Exp. Ther. 237 (3), 937-944
Sacerdote, P.A., Brini, A., Nantegazza, P. and Panerai, A. (1987) Pharmacol. Biochem. Behav. 26 153-158

144P OPIOID RECEPTOR INVOLVEMENT IN THE ANTICONVULSANT ACTION OF PHENYTOIN

Helen C. Jackson & D.J. Nutt, Reckitt & Colman Psychopharmacology Unit, Department of Pharmacology, School of Medical Sciences, University Walk, Bristol, BS1 8TD.

Phenytoin (P) is an established anticonvulsant agent whose mechanism of action is still not fully understood. This study explores the possible involvement of endogenous opioids in its protective effects against electroshock using the general opioid antagonist naloxone (N) and the selective μ-opioid antagonist cyprodime (C; Schmidhammer et al., 1989). Seizure thresholds were assessed in groups of 10 male TO mice (25-35g) using a constant current generator (0.5 s, unipolar, via earclip electrodes) by the up and down method and probit analysis (Finney, 1971). Drugs were dissolved in saline (acidified in the case of cyprodime) and injected i.p. 30 (phenytoin), 15 (morphine) or 5 min (naloxone and cyprodime) before electroshock. Phenytoin (3, 10 mg/kg) produced a dose-related increase in CC50 values (i.e. the current required to induce tonic hind-limb convulsions in 50% of the sample) which was significantly blocked by naloxone (0.3, 1 mg/kg). Cyprodime (3, 10 mg/kg) also attenuated the anticonvulsant effects of phenytoin (3 mg/kg). These doses of naloxone and cyprodime antagonised the small increase in seizure threshold induced by morphine (vehicle 11.0 (10.1-11.9); morphine 10 mg/kg 15.1 (13.9-16.4)*; morphine + naloxone 0.3 mg/kg 13.7 (12.9-14.4)*$; morphine + naloxone 1 mg/kg 11.0 (10.2-11.0)$; morphine + cyprodime 3 mg/kg 11.0 (10.1-12.1)$; morphine + cyprodime 10 mg/kg 13.7 (12.0-14.4)*$) but were not proconvulsant when given alone. Indeed, naloxone (1 mg/kg) produced a small increase in seizure threshold.

Table 1. Effect of the opioid antagonists naloxone and cyprodime on the anticonvulsant action of phenytoin

Treatment	CC50 (mA)	Treatment	CC50 (mA)	Treatment	CC50 (mA)
Vehicle	11.7(11.1-12.4)	Vehicle	11.7(11.1-12.4)	Vehicle	11.0(10.1-11.9)
P 3	16.4(15.4-17.4)*	P 10	82.5(63.2-107.5)*	P 3	16.3(15.3-17.4)*
+ N 0.3	15.0(13.8-16.4)*$	+ N 0.3	50.2(37.4-67.5)*$	+ C 3	11.9(11.3-12.6)$
+ N 1	13.8(13.1-14.5)*$	+ N 1	51.5(37.3-71.1)*$	+ C 10	13.8(13.1-14.5)*$

Doses are in mg/kg; numbers in brackets are the 95% fiducial limits; * P<0.05 vs vehicle; $ P<0.05 vs phenytoin.

These findings suggest that the anticonvulsant action of phenytoin may be mediated, in part, by the release of an endogenous opioid and subsequent activation of μ-opioid receptors. The fact that morphine has relatively small effects in this test, may mean that phenytoin produces a localised release of e.g. ß-endorphin, and that the anticonvulsant effects of morphine may be limited by proconvulsant activity in other brain areas (Frenk, 1983).

Finney D.J. (1971) Probit analysis. Cambridge University Press, Cambridge.
Frenk, H. (1983) Brain Res. 287, 197-210.
Schmidhammer, H., Burkard, W.P., Eggstein-Aeppli, L. & Smith, C.F.C. (1989) J.Med.Chem. 32, 418-421.

145P OPIOID BINDING SITES IN BOVINE RETINA

S.J. Paterson & J.R. Cunningham, Department of Pharmacology, UMDS, St Thomas's Hospital, London, SE1 7EH

Stereospecific opioid binding was first demonstrated in rat retinal membranes using [^{3}H]-etorphine (Medzihradsky, 1976). Subsequently, opioid binding sites were identified in homogenates prepared from the retina of the rat, cow, toad and skate using [^{3}H]-dihydromorphine, [^{3}H]-naloxone, [^{3}H]-[Met^5]enkephalin, [^{3}H]-[D-Ala^2, D-Leu^5]enkephalin, [^{3}H]-etorphine and [^{3}H]-diprenorphine (Howells et al., 1980; Osborne & Herz, 1980). However, these binding sites have not been characterised with highly selective opioid ligands.

Initially, total opioid binding was determined with 3nM [^{3}H]-bremazocine. The μ-sites were determined by displacement with 300nM unlabelled [D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin, the δ-sites by displacement with 450nM unlabelled [D-Pen^2,D-Pen^5]-enkephalin and the κ-sites by displacement with 450nM unlabelled U-69593. Subsequently, the μ-sites were labelled with [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin.

The total binding of 3nM [^{3}H]-bremazocine was 1.36 ± 0.05 pmol g^{-1} (n=4). Of this, 0.82 ± 0.09 pmol g^{-1} was displaced by [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin, 0.22 ± 0.06 pmol g^{-1} by [D-Pen^2,D-Pen^5]enkephalin and 0.23 ± 0.05 pmol g^{-1} by U-69593. In saturation experiments, [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin labelled a single population of binding sites with a capacity of 0.75 ± 0.13 pmol g^{-1} and a K_D of 0.66 ± 0.09nM (n=4). The concentration of opioid binding sites in the bovine retina was about 10% of that found in rabbit brain (Paterson, 1990). The binding of 1nM [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin was displaced by selective μ-, δ- and κ-ligands with K_i values similar to those found in homogenates of rabbit brain.

The present experiments confirm that the majority of the opioid binding sites in the bovine retina are of the μ-type, with a lesser number of δ- and κ-binding sites.

Howells, R.D., Groth, J., Hiller, L.M & Simon, E.J. (1980) J. Pharmac. Exp. Ther. 215, 60-64
Medzihradsky, F. (1976) Brain Res. 108, 212-219
Osborne, H. & Herz, A (1981) Neurochem. International 3, 143-148
Paterson, S.J. (1990) Brit. J. Pharmac. 101, 383P

146P APAMIN DIFFERENTIATES BETWEEN QUINPIROLE- AND BHT920-INDUCED INHIBITION OF DOPAMINE OVERFLOW *IN VITRO*

C. Patten & D.R. Bull. Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DP.

The activation of dopamine (DA) D_2 receptors induces an outward K^+-current which hyperpolarises DA neurones (Lacey et al, 1989). Recent evidence suggests the existence of two D_2 receptor subtypes in rat mammotrophs which activate separate potassium channel subtypes (Pizzi et al, 1990). One K^+-channel subtype was voltage dependent and opened by the D_2 receptor agonists BHT920 and quinpirole, while the other was calcium-dependent and only opened by quinpirole (Pizzi et al, 1990). We have used various K^+-channel blockers to try to determine whether the presynaptic D_2 receptors mediating inhibition of DA release in the nucleus accumbens exhibit a similar heterogeneity.

Nucleus accumbens slices were prepared from male Wistar-derived AH/A rats (100-180g). Endogenous DA overflow was evoked by single square wave electrical pulses of 0.1ms duration and 20V amplitude applied once every two minutes. The overflow was measured by fast cyclic voltammetry (Bull et al., 1990). Cumulative concentration response curves (CRC) for inhibition of DA overflow by quinpirole or BHT920 were constructed. Up to three further CRCs were performed in the presence of increasing concentrations of a K^+-channel blocker, 30mins equilibration being allowed for each concentration. Concentration ratios were calculated at the IC_{50} level. High concentrations of the K^+-channel blockers tetraethylammonium (TEA) ($3x10^{-5}$M - $3x10^{-3}$M), 4-aminopyridine (4-AP) (10^{-6}M - 10^{-5}M) and tolbutamide (10^{-4}M - $7x10^{-3}$M) all blocked, up to 10-fold, the BHT920- and quinpirole-induced inhibition of DA overflow equally and concentration-dependently. Lower concentrations were ineffective. At higher concentrations both TEA ($3x10^{-4}$M) and 4-AP (10^{-4}M) caused increases in electrically-evoked DA overflow of 400% and 280% respectively. This may be attributed to lack of selectivity of these compounds at these concentrations, probably due to blockade of delayed rectifier K^+-channels slowing repolarization. Apamin (10^{-6}M) did not block the response to BHT920 but caused a 3-fold rightward shift of the CRC to quinpirole ($p< 0.01$).

These results provide evidence that the presynaptic D_2 receptor-mediated inhibition of DA overflow from terminals involves the opening of K^+-channels, although the effective concentrations of channel blockers were high and do not allow the type of channel to be unequivocally identified. The small but statistically significant selective blockade of the response to quinpirole by apamin may indicate that BHT920 selectively activates a D_2 receptor subtype linked to a K^+-channel not blocked by apamin. This result is consistent with the D_2 classification proposed by Pizzi et al, (1990), but experiments with K^+-channel blockers of greater selectivity are required to confirm this.

Bull, D.R., Palij, P., Sheehan, M.J., Millar, J., Stamford, J.A., Kruk, Z.L. and Humphrey, P.P.A. (1990). J. Neurosci. Methods, 32: 37-44.
Lacey, M.G., Mercuri, N.B. and North, R.A. (1988). J. Physiol., 401:437-453.
Pizzi, M., Valerio, A., Benarese, M., Missale, C., Carruba, M.O., Memo, M. and Spano, P.F. (1990). Molecular Neuropharmacol., 1:37-42.

147P EFFECTS OF ACUTE AND CHRONIC BUPRENORPHINE TREATMENT ON DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS OF CONSCIOUS RATS

M.D. Lalies & R.B. Holman, Reckitt & Colman Psychopharmacology Unit, The Medical School, Bristol BS8 1TD

Buprenorphine (BUP) is a mixed partial mu agonist and kappa antagonist which has been reported to be successful in treatment of opioid addiction and possibly cocaine abuse (Jasinski et al., 1978; Mello et al., 1989). Dopamine (DA) release in the nucleus accumbens of the brain has been implicated in the rewarding properties of a number of drugs of abuse (Wise & Rompre, 1989). The present study was undertaken to determine the effects of both acute and chronic BUP on the release of endogenous DA in the nucleus accumbens of freely moving rats.

Male Wistar rats (270-300g) were anaesthetised with chloral hydrate (400 mg kg^{-1} i.p.) and a concentric dialysis probe stereotaxically implanted into the nucleus accumbens. After 48h recovery, the probe was connected via a liquid swivel to a syringe pump (flow = 2.0 µl min^{-1}) and, after 1 hour, samples of dialysate were collected every 20 min. Drugs or saline were administered only when the release of DA into three consecutive samples was stable. DA content of samples was measured using HPLC with electrochemical detection.

The basal release of DA was 12.3 ± 1.8 pg/sample (n=19). Administration of BUP (0.01, 0.05, 0.1, 0.5 and 1.0 mg kg^{-1} i.p.) caused significant increases in DA release which lasted for at least 3h. The values (% of basal release) at 3h were 174, 187, 267, 301 and 176 respectively. The corresponding value for saline controls was 91%. To determine if this effect was mediated via mu receptors, rats were treated with BUP (0.1 mg kg^{-1} i.p.) and either saline or naltrexone (1.0 mg kg^{-1} i.p.). Naltrexone (Nalt) inhibited the rise in DA release for 2h after administration (Sal + Bup = 276%, Nalt + Bup = 126% of basal release). The effect of repeated BUP treatment on DA release was also investigated. Rats were treated chronically with either BUP (0.5 mg kg^{-1} s.c.) or saline, twice a day, for 4 days. On day 5 basal DA release did not differ between the groups. However, the response to a BUP (0.1 mg kg^{-1} i.p.) challenge was markedly attenuated in the chronic BUP rats (122%) as compared with the controls (225%).

The increased release of DA following acute BUP administration agrees with the work of Brown et al. (1991). The naltrexone inhibition of BUP-induced DA release suggests that the mu receptor agonist activity of the drug is responsible for the enhanced extracellular DA content. The decreased response to a BUP challenge after chronic treatment suggests development of tolerance to the DA-releasing effects of BUP in rat nucleus accumbens.

Brown,E.E., Finley,J.M., Wong,J.T., Damsma,G. & Fibiger,H.C. (1991) J. Pharmacol. Exp. Therap., 256:119-126.
Jasinski,D.R., Pevnick,J.S. & Griffith,J.D. (1978) Arch. Gen. Psychiat. 35:501-516.
Mello,N.K., Mendelson,J.H., Bree,M.P. & Lukas,S.E. (1989) Science, 245:859-862.
Wise,R.A. & Rompre,P-P. (1989) Ann. Rev. Psychol. 40:191-225.

148P DOPAMINE D_2 RECEPTOR CHARACTERISATION IN THE CORPUS STRIATUM AND NUCLEUS ACCUMBENS USING ACETYLCHOLINE RELEASE

C. Cammack and M.J. Sheehan; Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DP.

The K^+ depolarisation-induced release of acetylcholine (ACh) has been proposed to provide an indication of postsynaptic dopamine (DA) D_2 receptor activity (Stoof et al, 1979; Starke et al, 1983; Hoffmann and Cubeddu, 1984). Neuroanatomical evidence suggests a link between DA and ACh neurones in the corpus striatum and nucleus accumbens (Hattori et al, 1976) where dopaminergic fibres exert a tonic inhibitory action upon cholinergic interneurones. Dopamine and dopamine agonists have been shown to decrease, and dopamine receptor antagonists increase, ACh release (Stadler et al, 1973). The release of radiolabelled ACh in striatal and accumbens slices was thus used to compare the potency of two D_2 receptor antagonists, clozapine and metoclopramide, in the nucleus accumbens and striatum. Clozapine is reported to be selective for mesolimbic dopamine pathways, whereas metoclopramide may be selective for striatal dopamine systems (Costall and Naylor, 1976).

Corpus striatum and nucleus accumbens slices (0.35 x 0.35mm prisms) prepared from Wistar-derived rats were labelled with [^{3}H]-choline for agonist-antagonist studies, using a method based on that of Stoof et al. (1979) modified for a non-superfused incubation system. Total tritium (which closely represents [^{3}H]-Ach release: Hadhazy and Szerb, 1977) was measured in the aliquots of the incubation medium. [^{3}H]-ACh release (induced by stimulation with submaximal 15mM K^+ in both the striatum and accumbens) was inhibited in a concentration-dependent manner by the D_2-selective agonist, quinpirole (IC_{50} values $2x10^{-7}$M and $1x10^{-7}$M respectively; n=4). The maximal inhibition of release was >90% in striatum and 60-70% in nucleus accumbens. This inhibitory effect could be antagonised by both clozapine (0.3-3µM) and metoclopramide (0.3-10µM) in both brain regions in a concentration-dependent manner. Clozapine shifted the quinpirole concentration-response curve to the right with a mean pK_B value of 7.2 in both brain areas (n=13-16). Similar potency values have been reported in D_2 receptor binding studies. Metoclopramide was tested in a different way, by using a range of concentrations to antagonise the inhibition produced by a single submaximal concentration of quinpirole. The IC_{50} values thus obtained were $3x10^{-7}$M and $4x10^{-7}$M in striatum and accumbens respectively; n=4.

The results of this study thus indicate that the release of [^{3}H]ACh is under the control of similar D_2 receptors in both the corpus striatum and nucleus accumbens.

Costall, B. and Naylor, R.J. (1976). Eur. J. Pharmacol. 40: 9-19.
Hadhazy, P. and Szerb, J.C. (1977) Brain Res. 123: 311-322.
Hattori, T., Singh, V.K., McGeer, E.G. and McGeer, P.L. (1976). Brain. Res. 102: 164-173.
Hoffman, I.S. and Cubeddu, L.X. (1984). J. Neurochem. 42: 278-282.
Stadler, H., Lloyd, K.G., Gadea-Cina, M. and Bartholini, G. (1973). Brain. Res. 55: 476-480.
Starke, K., Spath, L., Lang, J.D. and Acklung, C. (1983). Naunyn-Schmiedebergs Arch. Pharmacol. 323:298-306.
Stoof, J.C., Thienne, R.E., Vrijmoed-De Vines, M.C. and Malder, A.H. (1979). Naunyn-Schmiedeberg's Arch. Pharmacol. 309:119-124.

149P ACUTE COCAINE PRODUCES FOCAL UNCOUPLING OF BLOOD FLOW FROM METABOLIC DEMAND IN SUB-REGIONS OF THE RAT STRIATUM

R. Philip, J. Sharkey, & P.A.T. Kelly. Department of Clinical Neurosciences, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU.

Although cocaine abuse is known to increase the incidence of cerebrovascular accident, the underlying mechanisms are not fully understood. One possibility is that the inhibition of dopamine uptake by cocaine might mimic the acute effects of this monoamine upon the cerebral circulation (Tuor & McCulloch, 1986). In order to investigate this hypothesis, we have examined the effects of cocaine upon the relationship between local cerebral blood flow (LCBF) and glucose metabolism (LCGU) in the caudate nucleus. Blood flow in this area, a major target site for ascending dopaminergic neurones, has been shown to be particularly sensitive to pharmacological intervention aimed at dopaminergic systems (Carlsson et al., 1975).

Conscious rats were injected iv on a single occasion with either saline (n = 10), or cocaine-HCl (10mg/kg; n = 10), and measurements of LCBF (n = 10) and LCGU (n = 10) were performed using [^{14}C]iodoantipyrine or [^{14}C]2-deoxyglucose quantitative autoradiography respectively (Sharkey et al., 1991). This dose of cocaine was sufficient to produce the typical behavioural syndrome, but did not result in any significant change in mean arterial blood pressure.

There were no significant changes in striatal LCGU from control (+8% maximum). However, there were heterogeneously distributed increases in striatal LCBF. In dorsolateral striatum, LCBF was markedly increased (from 144 ± 6 to 235 ± 15 ml/100g/min; mean ± S.E.M.) whilst there was no significant change in ventromedial striatum (from 125 ± 11 to 132 ± 14 ml/100g/min). The resultant 78% lateral to medial difference within the caudate nucleus of cocaine-treated rats could be clearly discerned from the autoradiographic images. This distinctive pattern of altered LCBF, together with the focal uncoupling of flow from metabolic demand, were qualitatively similar to those previously described following amphetamine (Carlsson et al., 1975), and suggest that any cerebrovascular effects of cocaine could indeed be mediated via dopaminergic systems. If this is the case, then cerebrovascular accident associated with cocaine abuse may be as a result of a dopamine-induced lowering of the upper limit of cerebrovascular autoregulation (Tuor & McCulloch, 1986).

Supported by the Scottish Home & Health Department.

Carlsson, Ch., Hagerdal, M. & Siesjo, B.K. (1975) Acta Physiol. Scand. 94, 128-129.
Sharkey, J. McBean, D.E. & Kelly, P.A.T. (1991) Brain Res. 548, 310-314.
Tuor, U.I. & McCulloch, J. (1986) Neurosci. Lett. 63, 253-258.

150P ANTAGONISM OF COCAINE-INDUCED HYPERACTIVITY AND STEREOTYPY BY THE D_1 ANTAGONIST SCH 23390

A C McCreary and C A Marsden. Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, UK.

The psychostimulants cocaine and amphetamine both augment locomotor activity and induce stereotyped behaviours, effects which have been attributed to an increase in dopaminergic neurotransmission. In the present study we have examined the role of the dopamine D_1 receptor in cocaine induced hyperactivity and stereotypy (head shakes) and amphetamine induced hyperactivity using the selective D_1 antagonist (R(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol) (SCH 23390) (Iorio *et al.*, 1983).

Male Wistar rats (6 rats/treatment group) were administered with either cocaine (15.0 mg/kg s.c.) or vehicle (saline 0.9%, 1.0 ml/kg s.c.) 40 min after treatment with SCH 23390 (0.05 mg/kg s.c.) or vehicle. This treatment schedule was repeated 7, 14 and 21 days later. Locomotor activity (measured by photobeam interruption) and stereotypy (head shakes assessed on an arbritary rating scale of 0-3) were measured 20 min after injection with cocaine for a 120 min period. An initial study investigated the effect of pretreatment (40 min) with SCH 23390 (0.05 mg/kg^{-1} s.c.) on the behavioural effects of amphetamine (2.5 mg/kg i.p.); in these experiments spontaneous locomotor activity was monitored (photobeam interruption) for a period of 60 min following the injection of amphetamine.

Amphetamine (2.5 mg/kg^{-1}) induced hyperactivity was fully antagonised by SCH 23390 (0.05 mg/kg^{-1} s.c.). Cocaine (15 mg/kg^{-1}) significantly increased locomotor activity (+226%, day 1 and 275%, day 7, $P<0.01$). Sensitization of the locomotor effects of cocaine were observed after the third (+700%, day 14) and fourth (+547%, day 21) administrations of the drug. Cocaine administration also induced head shakes; a response that again was sensitized on subsequent administrations of the drug ($P\leq0.01$). Pretreatment with the D_1 antagonist SCH 23390 had no significant effect on the cocaine induced hyperactivity after administration on day 1 but significantly attenuated the sensitized cocaine response after administration on day 14 ($P<0.05$) and fully antagonised the response after the fourth injection of cocaine (day 21). The head shakes induced by cocaine were antagonised by SCH 23390 from the first injection of the psychostimulant.

The results demonstrate the involvement of D_1 receptors in the behavioural actions of cocaine and amphetamine particularly in cocaine induced sensitization; an effect associated with enhanced dopamine release (Kalivas & Duffy, 1990). The inhibition of the effects of cocaine by the D_1 antagonist contrasts with the increased response seen with haloperidol (McCreary & Marsden, 1991) which predominantly antagonises D_2 dopamine receptors.

Iorio, L. C., Barnett, A., Leitz, F., Houser, V. P. & Korduba, C. A. (1983). *J. Pharmacol. Exp. Ther.*, **226**, 462-268.
Kalivas, P. W. & Duffy, P. (1990). *Synapse*, **5**, 48-58.
McCreary, A. C. & Marsden, C. A. (1991). *Br. J. Pharmacol.*, **1045**, 357P.

151P NEUROLEPTIC EFFECTS ON APOMORPHINE-INDUCED INHIBITION OF STRIATAL NEURONAL FIRING AND PASSAGE ACROSS THE RAT BLOOD BRAIN BARRIER

J. W. Dalley & R. A. Webster, Department of Pharmacology, University College London, Gower St, London, WC1E 6BT.

Haloperidol and other neuroleptic (NL) drugs have been reported to reduce the concentration of apomorphine hydrochloride (APO) in various areas of the rat brain following their systemic administration (Westerink & Horn, 1979). If this results from NLs reducing the penetration of APO across the blood brain barrier it would contribute to their frequently reported ability to reduce the central actions of APO and other DA agonists. We have therefore measured the appearance of APO in the rat caudate nucleus using microdialysis and compared this with its effects on striatal neuronal firing before and after treatment with NLs.

Sprague-Dawley rats (250-300g) were anaesthetised with halothane and glass-coated tungsten microelectrodes employed to extracellularly monitor the spontaneous activity of rostral striatal neurons (A+1.0-1.5, L2.5-3.0, V3-6 mm relative to bregma). APO was administered in increasing doses every 40 minutes (25, 50, 100, 200 and 500µg/kg i.v.) and the maximum inhibition from baseline firing recorded. In separate studies animals were pretreated with either clozapine (20 mg/kg i.p.) or haloperidol (0.5 mg/kg i.p.) 40 minutes before the administration of APO and subsequent evaluation of its effects on neuronal activity. In further studies microdialysis probes (perfused at 5.0 µl/min with artificial CSF (mM) Na^+ 147, Ca^{2+} 2.30, K^+ 4.00, Cl^- 155.6 pH 6.5) were used to monitor the appearance of APO in the rostral striatum following its i.v. administration (0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg/kg) before and after either clozapine (20 mg/kg i.p.) or haloperidol (0.5 mg/kg i.p.). APO was analysed using HPLC coupled with electrochemical detection (lower detection limit 0.5 pmoles).

APO produced a progressive dose dependent inhibition of the firing of striatal neurons (IC_{50} 0.102 ± 0.007 mg/kg (n=5., s.e.mean). This was effectively blocked by haloperidol (dose ratio 8.43) but less so by clozapine (dose ratio 1.76). Alone, neither NL showed any consistent actions on neuronal firing. APO was detected in striatal dialysates after each injection and the peak concentration occurred 10-20 minutes post administration although the actual concentration was only directly related to the dose of APO above 0.5 mg/kg. Clozapine failed to modify the time course of appearance of APO at all doses employed and haloperidol only reduced the appearance of APO at one dose (2.0 mg/kg)($p<0.01$).

These results show that NLs antagonise APO induced inhibition of cell firing in the rat striatum and that this is a direct effect and does not appear to depend on a reduction in the passage of APO across the blood brain barrier.

Westerink, B. & Horn, A. S. (1979) Eur. J. Pharmacol. 58.,39-48.

152P LOCOMOTOR ACTIVITY IN BEHAVIOURALLY DISABLED AND RECOVERED MPTP-TREATED MARMOSETS AFTER ADMINISTRATION OF THE DOPAMINE PARTIAL AGONIST TERGURIDE

K. W. Lange (1), P.-A. Löschmann (2), R. Horowski (2), H. Wachtel (2), P. Jenner (1) & C. D. Marsden (1)
(1) Pharmacology Group, King's College London, U.K. (2) Research Laboratories, Schering AG, Berlin, Germany

The dopamine partial agonist terguride acts as a dopamine antagonist on normosensitive receptors but shows dopamine agonistic properties at supersensitive dopamine receptors (Wachtel & Dorow, 1983). Such a compound could offer an alternative to the treatment of Parkinson's disease with direct or indirect dopamine agonists. We have compared the effects of terguride in naive and parkinsonian common marmosets.

Six marmosets were rendered parkinsonian by subcutaneous administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The animals were tested either 2 months or 10 months following MPTP treatment with terguride (4.0, 8.0, 12.0 mg/kg i.p.) or vehicle. Four marmosets without MPTP treatment were also tested.

Table 1. Effects of terguride on locomotor activity accumulated over 180 min, mean counts ± s.e.mean

	N	Vehicle	4.0 mg/kg	8.0 mg/kg	12.0 mg/kg
Naive animals	4	364 ± 49	127 ± 37	116 ± 42	56 ± 8
		($p < 0.01$, Page test, ordered alternative: veh > 4.0 > 8.0 > 12.0 mg/kg)			
MPTP treatment 2 months before testing	3	76 ± 55	862 ± 187	277 ± 35	268 ± 55
		($p < 0.05$, Page test, ordered alternative: veh < 4.0 and 8.0 < 12.0 mg/kg)			
MPTP treatment 10 months before testing	3	539 ± 290	91 ± 38	161 ± 112	240 ± 138

Terguride reduced locomotor activity in naive marmosets in line with the dopamine antagonistic activity of the compound. In marmosets treated with MPTP 2 months previously and exhibiting pronounced behavioural deficits, terguride stimulated locomotor activity in accord with its agonistic properties at supersensitive receptors. By contrast, locomotor activity of animals recovered from previous MPTP treatment remained unaltered by terguride. These results suggest that terguride has anti-akinetic efficacy in this primate model of Parkinson's disease. Terguride offers the opportunity to differentiate pharmacologically degrees of recovery from MPTP treatment in the marmoset.

K.W.L. was supported by the European Science Foundation and the European Medical Research Councils.

Wachtel, H. & Dorow, R. (1983) *Life Sci.* **32**, 421.

153P REVERSAL OF MPTP- INDUCED MOTOR DEFICITS IN COMMON MARMOSETS FOLLOWING ORAL OR TRANSDERMAL ADMINISTRATION OF PIRIBEDIL

L.A. Smith, P. Jenner, C.D. Marsden, Parkinson's Disease Society Experimental Research Laboratories, Pharmacology Group, Biomedical Sciences Division, King's College, London, U.K.

The D-2 agonist piribedil is used mainly in conjunction with L-DOPA in the treatment of Parkinson's disease (Rondot, et al., 1975). However, its clinical effectiveness as a monotherapy remains to be established. We have looked at the ability of piribedil to reverse motor deficits in MPTP-treated common marmosets following oral administration in the presence or absence of domperidone pretreatment and following topical application to the skin.

Piribedil (1.25-12.5 mg/kg) alone was effective following oral administration in reversing all components of the motor deficits induced by MPTP-treatment. However, since piribedil also induced nausea and retching, its effectiveness in producing locomotion was limited. Following domperidone (2 mg/kg, p.o. 30 min previously) pretreatment, piribedil was effective in producing controlled locomotion and reversed other motor abnormalities, an effect which lasted for at least two hours (Table 1). The topical application of piribedil (2.5-10 mg) to a skin area of 9 cm^2 produced a reversal of motor deficits and the induction of controlled locomotion within 20 minutes, and these effects lasted for at least 10 hours. No retching or nausea was observed following topical application.

Table 1 : Total locomotor counts ± s.e.mean (N=4) for piribedil, in 2hr (orally, mg/kg) or in 11hr (topically, mg). [*p<0.05, compared to controls, Student's t-test].

Pretreatment	Piribedil			
Oral (mg/kg)	**Vehicle**	**1.25**	**5.0**	**12.5**
None	967 ± 510	1,578 ± 202	3,292 ± 1,261	4,090 ± 1,908
Domperidone	1,702 ± 321	3,523 ± 1173	5,911 ± 1,165*	7,938 ± 1,946*
Topical (mg)	**Vehicle**	**2.5**	**5.0**	**10.0**
None	4,989 ± 1,040	12,605 ± 2,202*	26,621 ± 4,609*	32,942 ± 3,018*

These results suggest that piribedil could be an effective monotherapy for Parkinson's disease following oral administration or by the transdermal route.

Rondot, P., Bathien, N. and Ribadeau Dumas, J.L., (1975) Advances in Neurol., 9, 373-381.

154P EFFECTS OF VARIOUS TREATMENTS WHICH MODIFY CENTRAL DOPAMINERGIC FUNCTION ON STRIATAL D_1 RECEPTOR BINDING

S.C. Cheetham, C.J. Kettle, K.F. Martin & D.J. Heal, Boots Pharmaceuticals Research Department, Nottingham NG2 3AA.

The up-regulation of D1 receptors in rat striatum by chronic administration of the D1-selective antagonist, SCH 23390 [(R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine] is well documented, whereas D2-selective antagonists have no effect (Hyttel & Arnt, 1986; McGonigle et al., 1989). We have now examined the effect of repeated administration of a variety of treatments which alter dopaminergic function, including electroconvulsive shock (ECS), on the number of D1 receptors, labelled by [3H]SCH 23390, in rat striatal membranes. In addition, we have determined the effect of repeated ECS on the displacement of [3H]SCH 23390 binding by the D1 agonist, SKF 82958 (O'Boyle et al., 1989), and D1 antagonist, SCH 39166 (Chipkin et al., 1988).

Male CD rats (70-90g; Charles River) were administered drugs (Table 1) or saline i.p. once daily for 14 days. Halothane anaesthetised rats were given ECS (200V, 2sec) 5 times over a 10 day period. Striata were removed 24 h after the final treatment. [3H]SCH 23390 binding was performed on freshly prepared membranes (1mg wet weight of tissue/ tube) at 8 concentrations (0.1-8nM) for saturation analysis or a single concentration (0.42nM) for drug displacements. Non-specific binding was defined by SCH 23390 (100nM). Binding parameters were determined using weighted non-linear least-squares curve fitting. Comparisons between groups were made using Student's t-test.

Table 1 Effect of repeated administration of drugs and ECS on [3H]SCH 23390 binding

	Bmax		Bmax		Bmax
Pooled Control [n=48]	1200 ± 27	Nomifensine (10)	1380 ± 32	D-Amphetamine (5)	1195 ± 25
Haloperidol (1)	980 ± 70	GBR 12909 (10)	1227 ± 51	Halothane Control	976 ± 36
Chlorpromazine (10)	1210 ± 44	Bupropion (10)	1120 ± 43	ECS	942 ± 33

Bmax values (fmol/mg protein) ± s.e. mean (n=8-10). Dose mg/kg in parentheses.

[3H]SCH 23390 binding was of high affinity and fitted well to a single site binding model. In agreement with previous reports, the D2 antagonist, haloperidol, had no effect on the number of D1 receptors in rat striatum, as did the D1/D2 antagonist, chlorpromazine (Table 1). Klimek and Nielsen (1987) reported that repeated bupropion and ECS administration decreased the number of D1 receptors. In contrast, we found that the dopamine uptake inhibitors, nomifensine, GBR 12909 [1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl] piperazine] and bupropion, the dopamine releasing agent, d-amphetamine, and ECS had no effect on the rat striatal D1 receptor number (Table 1). Furthermore, repeated ECS had no effect on the displacement of [3H]SCH 23390 binding by (±)SKF 82958 and SCH 39166 [Ki values (nM) ± s.e. mean; SKF 82958, halothane control 29.9 ± 1.1, ECS 30.2 ± 0.7 (n=10); SCH 39166, halothane control 0.94 ± 0.06, ECS 1.02 ± 0.04 (n=9-10)]. Overall the results presented in this communication suggest that adaptive changes in dopaminergic function occurring after repeated drug or ECS administration are not necessarily associated with changes in the number of D1 receptors.

Chipkin, R.E., Iorio, L.C., Coffin, V.L., McQuade, R.D., Berger, J.G. & Barnett, A. (1988) J. Pharmacol. Exp. Ther. 247, 1093-1102.
Hyttel, J. & Arnt, J. (1986) in Neurobiology of central D1-dopamine receptors. Ed. Breese and Creese, pp 15-28. Plenum Press: N.Y.
Klimek, V. & Nielsen, M. (1987) Eur. J. Pharmacol. 139, 163-169.
McGonigle, P., Boyson, S.J., Reuter, S. & Molinoff, P.B. (1989) Synapse 3, 74-82.
O'Boyle, K.M., Gaitanopoulos, D.E., Brenner, M. & Waddington, J.L. (1989) Neuropharmacology 28, 401-405.

155P EVIDENCE FOR HISTAMINE H_3 RECEPTOR REGULATION OF DRINKING IN THE RAT

J. Clapham and G.J. Kilpatrick; Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DP.

Histamine is recognized as being a neurotransmitter in the mammalian central nervous system (CNS); one of its functions is thought to be the regulation of water balance. Thus, peripheral or intracerebral injection of histamine to hydrated rats increases drinking and this effect is partially blocked by H_1 and H_2 receptor antagonists (Hough, 1988). The more recently characterized H_3 receptor is a presynaptic receptor, stimulation of which inhibits the release of histamine in the CNS and several other neurotransmitters in the CNS and periphery (see Timmerman, 1990). In order to assess the role of this receptor in drinking, we have examined the effect of administration of the selective H_3 receptor agonist (R)α-methylhistamine (RAMH) and antagonist thioperamide on drinking in hydrated rats.

Male Lister Hooded rats (280-330g) were allowed free access to water. Animals were pretreated with RAMH and/or thioperamide i.p. and water was removed at this point. Thirty minutes later rats were placed in cages individually and water consumption was measured after 10mins (40mins post-dose). The results are expressed as mean volume of water consumed (mls)±standard error (n=5/6 animals/drug treatment in each experiment).

RAMH (1-10mg/kg i.p.) evoked a dose-dependent increase in water consumption (Table 1). The maximum response observed was an increase above control of approximately 250% at 10mg/kg RAMH. Thioperamide (0.2, 2 and 10mg/kg i.p.) antagonised the responses to RAMH (1, 3 and 10mg/kg i.p.) in an apparently dose-dependent manner - see Table 1. Thioperamide alone caused a small decrease in drinking in some experiments, but it did not have a significant dose-dependent effect.

Table 1 The effect of thioperamide on RAMH-induced drinking

Drug mg/kg i.p.	Volume of Water consumed (mls)			
	+ Thioperamide (mg/kg i.p.)			
	0	0.2	2	10
Vehicle	0.72 ± 0.13 (36)	0.75 ± 0.29 (11)	0.32 ± 0.10 (12)	0.49 ± 0.30 (12)
RAMH 1	1.58 ± 0.17 (36)*	2.53 ± 0.37 (12)	0.76 ± 0.36 (12)	0.35 ± 0.16 (12)•
3	1.97 ± 0.24 (35)*	1.49 ± 0.33 (11)	1.14 ± 0.30 (12)	0.35 ± 0.11 (12)•
10	2.49 ± 0.22 (36)*	2.13 ± 0.39 (12)	1.88 ± 0.29 (12)	0.75 ± 0.27 (12)•

Data from 6 experiments (number of rats in brackets)

Dunnetts T test:-
* $p<0.05$ vs vehicle
• $p<0.05$ vs RAMH matched control

In summary, the H_3 receptor agonist RAMH evoked a dose-dependent increase in drinking in the hydrated rat. The H_3 receptor antagonist thioperamide inhibited the response to RAMH, also in a dose-dependent manner. We therefore conclude that H_3 receptors may have a role in the water consumption of hydrated rats, but at present we do not know if this effect is mediated centrally or peripherally.

Hough, L.B. (1988) Prog. in Neurobiol., 30, 469-505.
Timmerman, H. (1990) J. Med. Chem., 33, 4-11.

We wish to thank Joanne England for technical assistance.

156P INHIBITION OF VOLTAGE-ACTIVATED CALCIUM CURRENT IN NG108-15 CELLS BY SELECTIVE α_2 ADRENOCEPTOR AGONISTS

J.B. Arbuckle, R.J. Docherty & I.F. James, Sandoz Institute for Medical Research, 5 Gower Pl., London WC1E 6BN.

Noradrenaline (NA) inhibits voltage-activated calcium currents in voltage-clamped differentiated NG108-15 cells (Docherty & McFadzean, 1989). We have measured the relative potency and intrinsic activity of a range of ligands, which are selective for α_2-receptors, as inhibitors of calcium currents in NG108-15 cells in an attempt to characterize the receptor mediating this response.

Experimental methods are as described in detail elsewhere (Docherty, Robbins and Brown, 1991) with the modification that all solutions contained ICS 205-930 (1µM). Calcium currents were evoked at a test potential of -10mV from a holding potential of -90mV. Agonist responses were measured as the proportion of calcium current inhibited during drug exposure. Responses were normalized to the effect of 10µM NA (supramaximal concentration) in each cell. Averaged concentration-effect data were fitted (least squares) to the following equation: $R = (\text{int. activity} * [\text{agonist}])/(EC_{50} + [\text{agonist}])$. Table 1 summarizes the data obtained.

Table 1.

agonist:	guanfacine	noradrenaline	dexmedetomidine	tizanidine	ST91	UK14304	clonidine
EC50:	13nM	171nM	256nM	274nM	304nM	inactive @ 5µM	inactive @ 5µM
intrinsic activity:	0.31	0.90	1.00	0.66	0.45	0	0

Data obtained for UK14304 were difficult to interpret since low doses (0.02-0.5 µM) of UK14304 produced responses in some cells but not others even though NA was known to be effective. This means that the concentration-effect curve derived from averaged data for UK14304 is bell-shaped rather than sigmoidal. Yohimbine (10 µM) reduced the effect of NA as shown previously but idazoxan (0.2-10µM), which is also an α_2-selective antagonist, produced an agonist effect and did not block the action of NA. These data suggest that the receptor mediating inhibition of calcium currents by NA in NG108-15 cells displays a pharmacology which is unlike that of known sub-types of α_2-receptor (Bylund, 1990) and may represent a novel sub-type of α-receptor.

Docherty, R.J. & McFadzean, I. (1989) Eur. J. Neuroscience 1, 132-140.
Docherty, R.J., Robbins, J. & Brown, D.A. (1991) In *Cellular Neurobiology - A Practical Approach* eds. Chad, J. & Wheal, H., pp 75-95. Oxford: IRL Press.
Bylund, D.B. (1988) Trends Pharmacol. Sci. 9, 356-361.

157P α_2-ADRENOCEPTORS IN THE HUMAN BRAIN POSTMORTEM: RATIO OF HIGH AFFINITY AGONIST SITES TO ANTAGONIST SITES AND EFFECT OF AGE

M. Sastre & J.A. García-Sevilla, Laboratory of Neuropharmacology, Department of Fundamental Biology and Health Sciences, University of the Balearic Islands, 07071 Palma de Mallorca, Spain.

α_2-Adrenoceptors (α_2R) express two conformational states of high (R_H) and low (R_L) affinity for agonists but only the R_H fraction mediates agonist response. Since various neuropsychiatric disorders (e.g. endogenous depression) are related to altered α_2R, it appeared relevant to quantitate the fraction of R_H in the normal human brain. Postmortem frontal cortex specimens from 21 control brains (13 men, 8 women, 43±5 years, range 17-79 years) were used. In each brain the specific binding (defined with 100 µM adrenaline) of the agonists [^{3}H]clonidine and [^{3}H]bromoxidine (R_H) and of the antagonist [^{3}H]RX821002 (methoxy-idazoxan) (R_{H+L}), as well as the competition parameters of adrenaline against [^{3}H]RX (K_{iH}, K_{iL}, R_H and R_L) were determined. The density of R_H with [^{3}H]clonidine (Bmax=52±4 fmol/mgP, Kd=2.81±0.42 nM) was not different of that with [^{3}H]bromoxidine (Bmax=57±5 fmol/mgP, Kd=0.65±0.10 nM) and the ratio of Bmax was 0.94±0.07. The density of R_{H+L} with [^{3}H]RX (Bmax=87±9 fmol/mgP, Kd=0.80±0.05 nM) was greater than that quantitated with [^{3}H]agonists ($P<0.01$) and the ratios of Bmax for [^{3}H]clonidine/[^{3}H]RX (0.68±0.07) and [^{3}H]bromoxidine/[^{3}H]RX (0.72±0.07) indicated that in the frontal cortex the proportion of R_H is 68-72%. Inhibition of [^{3}H]RX binding by adrenaline revealed the high and low affinity states of the α_2R (K_{iH}=8 nM , K_{iL}=498 nM, n_H=0.41; but in the presence of 100 µM GppNHp only one site with K_i=111 nM and n_H=0.72) with densities of R_H (Bmax=64±7 fmol/mgP) and R_{H+L} (Bmax=77±8 fmol/mgP) similar to those quantitated with [^{3}H]agonists and [^{3}H]antagonists, although the ratio of Bmax (R_H/R_{H+L}) detected in competition experiments was slightly greater (0.85±0.01, R_H=85%). The density of R_{H+L} quantitated in saturation experiments with [^{3}H]RX correlated ($r=0.79$, $p<0.001$) with the sum of densities R_H+R_L obtained in competition experiments with adrenaline/[^{3}H]RX. Also there were significant correlations between the densities of R_H quantitated with [^{3}H]clonidine ($r=0.53$, $P<0.05$) and [^{3}H]bromoxidine ($r=0.74$, $P<0.001$) and the fraction of R_H obtained in competition experiments. Furthermore, there was a negative correlation between the density of R_H or R_{H+L} and age both in saturation experiments with [^{3}H]clonidine ($r=-0.34$), [^{3}H]bromoxidine ($r=-0.61$, $P<0.005$) and [^{3}H]RX ($r=-0.33$), and in competition experiments with adrenaline/[^{3}H]RX ($r=-0.54$, $P<0.05$). This study shows that a high proportion (68-85%) of α_2R in the human brain exist in the high affinity conformation (α_2R_H) and that this relevant fraction of α_2R declines with aging.

Supported by DGICYT (Project PM 88-0134) and MEC (PFPI fellowship to M.S.)

158P AFFINITY AND SELECTIVITY OF 6 AND/OR 7 AROMATIC SUBSTITUTED ANALOGUES OF IDAZOXAN FOR RABBIT BRAIN NON-ADRENOCEPTOR IDAZOXAN BINDING SITES

A.L.Hudson, N.J.Mallard, J.Wilson, R.Tyacke & D.J.Nutt, Reckitt & Colman Psychopharmacology Unit, Dept. of Pharmacology, School of Medical Sciences, University Walk, Bristol, BS8 1TD.

The α2-adrenoceptor antagonist idazoxan (IDX) also binds to non-adrenoceptor idazoxan binding sites (NAIBS, Michel & Insel, 1989). NAIBS have been observed in a variety of tissues and autoradiographically localised in distinct rat brain nuclei (Hudson *et al.*, 1991). Unfortunately, lack of selective ligands has hindered the search for a function of NAIBS. Inclusion of fluorine at position 6 and/or 7 in the aromatic ring of IDX has been noted to decrease affinity for α2-adrenoceptors whilst that for NAIBS remains high (Vauquelin *et al.*, 1990; Mallard *et al.*, 1991). Using radioligand binding, we have thus investigated the affinities of several more structural analogues of IDX, with substitutions in the aromatic ring, for α2-adrenoceptors and NAIBS in rabbit brain membranes. [3H]-RX821002 (2nM) and [3H]-IDX (2nM, in the presence of 10µM yohimbine) were used to label α2-adrenoceptors and NAIBS respectively. Membranes together with [3H]-ligand and displacing drug were incubated (50mM Tris/HCl buffer, pH 7.4) in triplicate to equilibrium (30min) in a final volume of 1ml at 25°C. Bound and free radioactivity were then separated by filtration. Specific binding of [3H]-RX821002 and [3H]-IDX was defined in the presence of 10µM and 1mM phentolamine respectively.

Table 1

	Ki (nM)		
	NAIBS	α2-receptors	α2 / NAIBS
Idazoxan	10.6 ± 1.4	55.4 ± 4.9	5.2
RX821002	> 30,000	6.9 ± 0.2	>0.0001
6- or 7-fluoro-	4.9 ± 0.5	142.8 ± 8.7	29.1
6,7-dibromo-	21.0 (n=2)	220.0 (n=2)	10.5
6- or 7-bromo-	28.0 (n=2)	389.8 (n=2)	13.9
6,7-dichloro-	8.7 ± 0.7	359.0 ± 16.4	41.3
6- or 7-chloro-	11.5 ± 2.3	158.1 ± 50	13.7
6-methyl-	26.7 (n=2)	367.0 (n=2)	13.7
7-methyl-	64.5 (n=2)	336.6 (n=2)	5.2
6-methoxy-	103.1 ± 4.9	939.3 ± 180.9	9.1
7-methoxy-	65.7 ± 6.3	356.6 ± 78.7	5.4
6,7-benzo-	81.1 ± 0.7	177.5 ± 18.6	2.2

Table 1 displays mean (± s.e.mean, n=3) Ki values for IDX and its analogues at NAIBS and α2-adrenoceptors. Overall, substitution at positions 6 and/or 7 resulted in compounds more selective for NAIBS compared with α2-adrenoceptors than IDX. In general this improved selectivity reflects a decrease in α2-adrenoceptor affinity, whilst that for NAIBS is improved, similar or attenuated depending on the substitution. In particular, halogen substitution at position 6 and/or 7 was preferable compared with either the 6- or 7-methyl, 6- or 7-methoxy and 6,7-benzo analogues. Of the halogenated compounds the 6- or 7-fluoro analogue displayed the highest affinity for NAIBS, although improved selectivity was evident with the 6,7-dichloro derivative.

Michel, M.C. & Insel, P.A. (1989). Trends in Pharmacol. Sci., 10, 342-344.
Hudson, A.L., Mallard, N.J. & Nutt, D.J. (1991). Br.J. Pharmacol., 102, 4P.
Vauquelin, G., De Vos, H., De Backer, J.P. & Ebinger, G. (1990). Neurochem. Int., 17, 537-546.
Mallard, N.J., Tyacke. R., Hudson, A.L. & Nutt, D.J. (1991). Br.J. Pharmacol., 102, 221P.

159P AN ETHOPHARMACOLOGICAL STUDY OF THE EFFECTS IN MICE OF THE β-ADRENOCEPTOR ANTAGONIST, PROPRANOLOL

B Gao and M G Cutler, Department of Biological Sciences, Glasgow Polytechnic, Cowcaddens Road, Glasgow, G4 0BA.

Propranolol has been known for more than 20 years to be effective as an anxiolytic to humans (Tyrer 1988). There have, however, been comparatively few studies of its effects on the behaviour of laboratory animals when given subchronically by the oral route which represents the normal method of human intake. After acute administration to rodents, propranolol is known to reduce defensive aggressive behaviour (Yoshimura et al 1987) and it also has been shown to antagonize the conditioned fear produced in rats by corticotropin release hormone (Cole & Koob 1988). The present studies use ethopharmacological methods to examine the effects of subchronic oral administration of propranolol on the behavioural responsiveness of mice when encountering an unfamiliar partner in their home cage and in an unfamiliar neutral cage.

The drug, dl-propranolol, was administered at two dose levels, 12.4 mg/l (1.9 mg/kg daily) and 24.9 mg/l (4.6 mg/kg daily) in drinking fluid to adult pair-housed male CD1 mice (n=16 in each group) for 12-15 days. Control animals (n=20) received tap water. Behaviour shown by each mouse during social interactions was recorded for a 5 min period, using the ethological procedures described by Dixon (1986). Encounters were firstly in the home cage with an untreated group-housed male and then in an unfamiliar neutral cage.

Propranolol, at both dose levels (1.9 mg/kg = LD; 4.6 mg/kg=HD) significantly increased social investigation by the mice in both their home cage (Control 70.1 ± 6.2; LD 128.9 ± 7.0; HD 99.8 ± 9.1; $P<0.01$) and the neutral cage (Control 38.2±6.9; LD 55.3±6.4; HD 51.6±6.6; $P<0.05$). Propranolol significantly increased aggressive behaviour (Control 11.0±3.9; LD 48.4±8.2; HD 32.0±8.6; $P<0.05$) in the unfamiliar circumstances of the neutral cage, although not in the home cage. The increased time spent by drug-treated mice in social investigation and aggression was associated with reductions of overall non-social activity (In the home cage, Control 220.0±8.0; LD 167.0±6.3, HD 174.0±11.3; $P<0.01$; in the neutral cage; Control 243.0±8.3; LD 190.0±7.3; HD 204.0±9.3; $P<0.01$). However, the time spent in digging was significantly increased in the neutral cage among propranolol-treated mice (Control 9.2±2.5; LD 24.4±3.9; HD 23.6±3.9; $P<0.01$). Effects of the drug treatments influenced behaviour of the partner animals. Partners to propranolol-treated mice showed more social investigation than partners to the controls, and in the neutral cage showed an increase of flight behaviour.

The present results showing an enhancement of social investigation in the home cage and an increase of social investigation, aggressiveness and of digging activity in the neutral cage indicate that propranolol after subchronic administration has increased reactivity of the mice to social and environmental stimuli. This enhancement of normal behavioural responsiveness suggests that effects on behaviour are not confined to anxiolytic action.

Results expressed as mean duration (s.) ± S.E.M.. Statistics; Mann-Whitney U and Kruskal-Wallis Tests.

Cole B.J. & Koob G.F. (1988) J. Pharmacol. Exp. Ther. 247: 902-910.
Dixon A. K. (1986) Arch. Suisses Neurol. Psychiatry 137, 151-163.
Tyrer P. (1988) Drugs 36: 773-783.
Yoshimura H., Kihara Y. & Ogawa N. (1987) Psychopharmacology 91: 445-450.

160P FELODIPINE ANTAGONISES CEREBRAL [^{3}H]-PN 200-110 BINDING, BUT NOT THE ETHANOL WITHDRAWAL SYNDROME

W. P. Watson[1], A. Misra[2], H. J. Little[1] and A. R. Green[2] (1) Department of Pharmacology, The Medical School, University Walk, Bristol BS81TD and (2) Astra Neuroscience Research Unit, 1, Wakefield Street, London WC1N 1PJ

Several dihydropyridine calcium channel antagonists effectively protect mice against the ethanol withdrawal syndrome (Littleton et al., 1990). The dihydropyridine PN 200-110 also blocked withdrawal hyperexcitability in hippocampal slices (Whittington and Little, 1991). We have now compared felodipine and nitrendipine on (i) the ethanol withdrawal syndrome (ii) the convulsive action of the calcium channel agonist, Bay K 8644, in naive mice and (iii) displacement of [^{3}H]-PN 200-110 binding in the CNS.

Male mice, TO strain, 32-38g, were used. The effects of ethanol withdrawal, and of Bay K 8644, were measured by ratings of convulsive behaviour on handling. Physical dependence on ethanol was produced by inhalation for 2 weeks; ratings were made hourly after removal from ethanol (n=10 per group). In separate groups of mice (n=8 per group), Bay K 8644 60 µg i.c.v. was given 1h before the tests. Either felodipine, 10 mg/kg, nitrendipine, 50 mg/kg, or Tween vehicle, were injected 3h into ethanol withdrawal and 30 min or 1h before Bay K 8644. Controls received vehicle injections but no ethanol or Bay K 8644. Times below are from administration of felodipine or nitrendipine. Results are expressed as medians, with the interquartile ranges.

Ethanol withdrawal

Time	Controls	Veh.*[a]	Felo.	Nitr.*
1h	1 (0-1)	2 (2-2)	1.5 (0-2)	0 (0-1)
2h	0 (0-1)	1.5 (1-2)	1 (0-1)	0 (0-0)
3h	1 (0-1)	2 (1-2)	1.5 (1-2)	0 (0-0)

Effects of Bay K 8644

Time	Controls	Vehicle	Felo.+	Nitr.
30 min	1 (0-2)	2 (2-2)	0.5 (0-1)	2 (2-2)
1h		2.5 (2-3)	1 (0.5-2)	2 (1-2.5)

+ $P<0.01$, Mann Whitney U test, compared with vehicle

*$P<0.05$ Nonparametric analysis of variance, [a]compared with controls, [b]compared with vehicle injections

Ex-vivo binding of [^{3}H]-PN 200-110 to homogenates of whole brain from mice (n=10) pretreated 3h earlier with felodipine, 10 mg/kg, or nitrendipine, 50 mg/kg, revealed that both drugs inhibited binding similarly in control (felodipine: 77±7%; nitrendipine 84±6%) and ethanol dependent (felodipine: 77±7%; nitrendipine: 85±5%) mice.

Therefore, while both felodipine and nitrendipine, at these doses, caused similar inhibition of dihydropyridine binding in brain, felodipine was ineffective against the ethanol withdrawal syndrome and nitrendipine did not protect significantly against the effects of Bay K 8644. It is possible therefore that these drugs do not act at identical loci in the brain and that their differential action on the behaviours described above reflects this.

Littleton, H. J, Whittington, M. A. & Little, H. J. (1990) Psychopharmacology, 100, 691-697
Whittington, M. A. & Little, H. J. (1991) Br. J. Pharmacol., 103, 1313-1320

161P PHOTOTHROMBIC LESIONS OF THE FRONTAL CORTEX IMPAIR THE ACQUISITION OF A SPATIAL MEMORY TASK IN RATS

P.W. Wright, D.C. Rogers, J.C. Roberts, A.L. Rothaul & A.J. Hunter. SmithKline Beecham Pharmaceuticals, Harlow, Essex, CM19 5AD.

In the assessment of potential therapeutic agents for stroke both histopathological and functional end points are important. Deficits in learning have been reported in rats with global forebrain ischaemia (Block et al., 1990; Davis et al., 1987). Few studies have investigated the effects of focal lesions on cognitive function. The aim of the present study was to investigate the effect of focal photothrombotic lesions on spatial learning in the Morris water maze (Morris, 1982).

Naive male Hooded Lister rats (200-250g) were anaesthetised with halothane in oxygen and placed in a stereotaxic frame. Bilateral lesions were generated in the frontal cortex (2mm anterior to Bregma and 2.2mm left then right of the midline) by the interaction of high intensity light (300W Xenon arc, 5 mins.) and rose bengal 20 $mgKg^{-1}$ delivered into the lateral tail vein (n=8). Sham animals did not receive rose bengal (n=10).

Four days post surgery the rats began training to find a hidden platform in the water maze. Each animal received 22 acquisition trials over a period of 4 days training, followed by a 23rd "probe trial" during which the platform was removed. The latency to find the platform during acquisition trials and the percentage time spent in the platform quadrant during the probe trial were recorded. This experiment was conducted blind and randomised.

After behavioural assessment the animals were perfusion fixed, coronal sections were taken through the lesion and stained with H&E. The total area of each lesion was determined by digital planimetry and lesion volumes calculated by Simpsons Rule.

Histological assessment revealed that in all lesioned animals the medial precentral cortex was the area most damaged. Variable damage was observed in the dorsal anterior cingulate cortex and there was some encroachment into the lateral precentral cortex. Mean volumes for left and right hemispheres were 5.7 ± 0.7 and 3.4 ± 0.6 mm^3 respectively. Sham animals failed to show any lesion.

The control animals over the four day assessment period demonstrated a characteristic acquisition curve. In contrast, the failure of lesioned animals to learn the task is evidenced by the fact that the latency to find the platform on day four is not significantly different to day one. The data from the probe trial shows a significant difference between lesioned and control groups ($p<0.05$) and provides further evidence that prefrontal lesions result in deficits in acquisition of spatial tasks.

This technique may provide a means of assessing the functional and histopathological efficacy of putative neuroprotective agents.

Block, F. et al. (1990) Life Sci. 47:1511-1518
Davis, H.P. et al. (1987) Physiol. Behav. 39:783-786
Morris, R.G.M. (1981) Learn Motiv. 12:239-260

162P THE CYTOPROTECTIVE PROPERTIES OF IFENPRODIL IN NEURONAL CELL CULTURE INJURY MODELS

D. Graham, G. Darles and S.Z. Langer, Department of Biology, Synthélabo Recherche (L.E.R.S.), 31, av. Paul Vaillant Couturier, F-92220 Bagneux, France

The phenyethanolamine, ifenprodil, has been shown to exhibit cytoprotective activity in animal models of focal ischaemia (Gotti *et al.*, 1990). In the present study, we have further examined the effects of ifenprodil in cell culture models of neurotoxicity induced either by excitatory amino acid treatment or by hypoxia.

Dispersed cocultures of neurones and glia were prepared from cerebral cortices of foetal mice at 15-17 d of gestation and maintained in a humidified CO_2 atmosphere at 37°C for 15-19 d. The neurotoxicity paradigms chosen were acute exposure to either (i) glutaminergic agonists or (ii) NaCN. Subsequent to these treatments, neuronal cell-death was assessed by measurement of the amount of the cytosolic enzyme, lactate dehydrogenase (LDH), released into the bathing medium after 20 h.

The glutaminergic agonists, NMDA, L-glutamate, kainate and quisqualate produced excitotoxic neuronal cell-death. However, the neurotoxicity induced by NMDA (100 µM) or L-glutamate (500 µM), in contrast to that induced by kainate (100 µM) or quisqualate (100 µM), was antagonized in the presence of each of the following agents, MK 801 (IC_{50} = 30 nM), ifenprodil (IC_{50} = 200 nM) and the ifenprodil analogue (Gotti *et al.*, 1990), SL 82.0715 (IC_{50} = 4 µM). The neurotoxicity induced by submaximal concentrations of either L-glutamate (100 µM) or NMDA (30 µM) was potentiated in the presence of 50 µM glycine. This effect of glycine was attenuated by 7-chlorokynurenate.

Exposure of the cultures to NaCN (10 mM) for 20 min also produced extensive neuronal cell-death. This NaCN-induced neurotoxicity was attenuated in the presence of 2 mM EGTA, 1 µM MK 801 and 10 µM ifenprodil.

Thus, ifenprodil exhibited cytoprotective effects in hypoxic and excitotoxic neuronal cell culture models. The pharmacological profile of ifenprodil in the excitatory amino acid neurotoxicity model supports the view that the neuroprotective property of this drug is exerted through an interaction with the NMDA receptor.

Gotti, B., Benavides, J. , MacKenzie, E.T. & Scatton, B. (1990) *Brain Res.* **522**: 290-307.

163P PHARMACOLOGICAL PROFILE OF THE MUSCARINIC RECEPTOR IN A MOUSE NEUROBLASTOMA (N18TG2) WHICH EXPRESSES THE m_4 MUSCARINIC RECEPTOR GENE

I.C.Wood, M.P.Caulfield, N.J.Buckley* and D.A.Brown. Department of Pharmacology, University College London, London WC1E 6BT. *National Institute for Medical Research, Mill Hill, London NW7 1AA.

Gene cloning studies have revealed the existence of at least five subtypes of muscarinic receptor genes, each of which has been shown to have a unique expression pattern. Although no single antagonist is available that is selective for any one subtype of receptor protein, each receptor subtype has a characteristic pharmacological profile defined by a range of antagonist potencies. It has been proposed that the pharmacologically-defined subtypes M_1, M_2 and M_3 are encoded by the genes m1, m2 and m3 respectively. Recently, studies of the receptor encoded by the m4 gene revealed a pharmacological profile which is different from those previously defined and has been putatively termed M_4 (Lazareno *et al* 1990).

In the present study, radioligand binding displacement experiments were performed to determine if the pharmacological profile of the muscarinic receptor encoded by the mouse m4 gene was similar to that already described for the putative M_4 receptor in the mouse neuroblastoma/rat glioma hybrid NG108-15 cell line. Binding studies were carried out as described by Lazareno *et al* (1990), on NG108-15 cells and their parental mouse neuroblastoma cell line, N18TG2.

Northern blot analysis of total RNA from both N18TG2 and NG108-15 cell lines showed hybridisation signals with m4-specific radiolabelled oligonucleotides. Log dissociation constants, (pK_D; mean ± s.e.m.), for pirenzepine, himbacine and methoctramine were: 6.94 ± 0.17 (n=3), 8.43, 8.02 (n=2) and 7.18 ± 0.18 (n=4), respectively for the N18TG2 cell line. The same antagonists gave pK_D values of 7.19 ± 0.18 (n=4), 8.11 ± 0.10 (n=3) and 7.21 ± 0.15 (n=4), respectively for the NG108-15 cell line. Thus these antagonists do not discriminate between the muscarinic receptors expressed in N18TG2 cells and those expressed in NG108-15 cells.

Two possible interpretations of these data are:

1) The mouse and rat m4 genes encode receptors that are pharmacologically similar.
2) The pharmacology of the mouse and rat m4 receptors is different but the NG108-15 cell line expresses predominantly the mouse m4 receptor gene.

Further molecular analysis of the NG108-15 cell line is being carried out to determine whether the expressed m4 gene is of mouse or rat origin.

Lazareno,S., Buckley,N.J. & Roberts.F.F. (1990) Mol. Pharmacol. 38, 805-815.

164P SDZ-ENS 163, A SELECTIVE M_1 MUSCARINIC RECEPTOR AGONIST, FACILITATES LTP IN RAT HIPPOCAMPAL SLICES

H.W.G.M. Boddeke & G. Shapiro (Introduced by J. Fozard), Preclinical Research, Sandoz Pharma Ltd., CH-4002 Basle, Switzerland.

In a previous study we have demonstrated that the pilocarpine derivative SDZ-ENS 163 ((+)-(3S, cis)-3-ethyldihydro-4-[(1-methyl-1H-imidazol-5-yl) methyl-2(3H)-thiophenone dihydrogenphosphate]), is a selective M1 muscarine receptor agonist which in addition displays presynaptic M2 receptor antagonist activity (Boddeke & Shapiro, 1991). Here we show the effect of SDZ-ENS 163 upon long-term potentiation of synaptic transmission (LTP), a correlate for information storage and processing in the brain.

Hippocampal slices were prepared from 5-8 week old Wistar rats and maintained in a perfusion chamber. A stimulation electrode was placed in the stratum radiatum of area CA2 and an extracellular recording pipette was placed in the dendritic layer of the CA1 area. Excitatory post synaptic potentials (epsp's) were recorded at a stimulus frequency of 0.02 Hz. LTP was induced by theta burst stimulation (6-12 trains, each composed of 4 pulses at 100 Hz, at 5Hz). Potentiation was quantified as the change in epsp amplitude determined before and 60 min after induction of LTP. The data are expressed as means ± s.e. mean

The degree of LTP was dependent upon the number of trains applied and was maximal after application of 10 trains yielding a maximal increase of the epsp amplitude of 54 ± 7.2 % (n = 24). Incubation (15 min) with SDZ-ENS 163 ($2x10^{-6}$M) induced a small increase of the epsp amplitude (12 ± 4.3 %) per se and markedly facilitated the induction of LTP such that the maximum occurred after application of 8 trains. The maximal increase in epsp amplitude after induction of LTP in the presence of SDZ-ENS 163 was 62 ± 8.4 % which was not significantly different from control LTP experiments ($P < 0.05$). The effect of SDZ-ENS 163 was completely inhibited after pretreatment with the selective M1 receptor antagonist pirenzepine ($6x10^{-8}$M) but unaffected by pretreatment with the M2 antagonist AF-DX 116 (10^{-6}M).

We conclude that the facilitation of LTP by SDZ-ENS 163 is a consequence of its M1 receptor agonist activity.

Boddeke H.W.G.M. & Enz A. (1991) Naunyn Schmiedeberg's Arch. Pharmacol., **Suppl. 343, R 89**

165P CHOLINERGIC VAGAL AFFERENT FIBRES AND PRESYNAPTIC MUSCARINIC RECEPTOR BINDING SITES ARE PRESENT IN THE DORSAL VAGAL COMPLEX OF THE FERRET

D.J.M. Reynolds, P.R. Lowenstein[1], J.M. Harvey and R.A. Leslie, Oxford University SmithKline Beecham Centre for Applied Neuropsychobiology, Department of Clinical Pharmacology, Oxford, OX2 6HE, and [1]Department of Anatomy and Physiology, University of Dundee, Dundee, DD1 4HN.

The dorsal vagal complex (DVC) comprises the area postrema (AP), the solitary tract nucleus (NTS) and the vagal dorsal motor nucleus. Lesioning studies suggest that presynaptic terminals of vagal fibres in the NTS contain many receptor binding sites including muscarinic cholinergic receptors. These fibres are the main route of visceral sensory input to the brain and their presynaptic receptors may modulate autonomic reflexes. The present study was performed to identify which subtype of muscarinic receptor may be presynaptic on vagal fibres in the DVC.

Adult ferrets of either sex (0.7-1.5kg) were used in this study. Under general anaesthesia animals underwent unilateral cervical vagotomy and nodose ganglion excision or sham operation. After 11 days they were reanaesthetized and perfused transcardially with 0.1M phosphate buffer. The brainstems were removed and serial frozen sections (12μm) were mounted on gelatinized slides. Sections were incubated with 5.0nM [^{3}H]QNB or 4.6nM [^{3}H]pirenzipine, with or without unlabelled 10μM atropine to define non-specific binding, or with 10nM [^{3}H]hemicholinium-3 (HC-3) with or without 10μM unlabelled HC-3. After washing and air drying, sections were apposed to tritium sensitive film for 3-4 weeks along with tritium standards. Densitometric analysis was performed on a minimum of 12 sections per animal.

Displaceable binding of [^{3}H]QNB was detected throughout the DVC with the heaviest binding in the subnucleus gelatinosus (SNG) of the NTS. In lesioned animals [^{3}H]QNB binding was attenuated by 40.1% in the NTS ipsilateral to the lesion; binding elsewhere was unaffected. Within the DVC, [^{3}H]pirenzipine binding was confined to the SNG and after vagotomy, binding ipsilateral to the lesion was reduced by 52%. Binding on the contra-lateral side was unaffected. [^{3}H]HC-3 binding in the brainstem was widely distributed with heavy binding in the NTS and AP. [^{3}H]HC-3 binding in vagotomized animals was attenuated by 39.8% in the SNG of the NTS ipsilateral to the lesion but not in other regions of the brainstem.

The attenuation of [^{3}H]HC-3 binding in the DVC following vagotomy is indicative of the presence of cholinergic vagal afferent terminals in the NTS. Similar attenuation of [^{3}H]pirenzipine binding in these conditions suggests that muscarinic receptors are presynaptic on these vagal afferent terminals. Pirenzipine shows selectivity for M_1 versus M_2 muscarinic receptors, but binds with a similar affinity to M_1, M_3 and M_4 receptors. Together, these observations indicate that the NTS may be a site of action of muscarinic receptor antagonists such as atropine which modulate gastrointestinal reflexes and emesis.

166P EFFECTS OF CHRONIC ADMINISTRATION OF ATROPINE AND SCOPOLAMINE ON ELEVATED PLUS-MAZE BEHAVIOUR IN THE RAT

C.P.S. Smith, A. Chape, A.J. Hunter* & G.W. Bennett. Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, *SB Pharmaceuticals, Coldharbour road, The Pinnacles, Harlow, Essex, U.K.

The elevated plus-maze (EPM) is well established as a behavioural model of anxiety (Pellow et al., 1985). Recently, the EPM has been used as a tool for the evaluation of learning and memory in mice (Itoh et al., 1990, 1991). The present study assessed the mnemonic effects of two muscarinic cholinergic antagonists, atropine and scopolamine, on EPM performance in the rat.

Male, Hooded Lister rats (250-450g) were tested, one trial per day, for three consecutive days on a black elevated plus-maze (raised 70 cm above the floor; open/closed arms, 45x15x10 cm) set in a room containing a variety of extramaze spatial cues. For each trial, animals were placed at the end of one open arm facing away from the central platform, and the time taken for all four paws to cross the centre of either of the enclosed arms (transfer latency, TL) was recorded. The time spent within the enclosed arm (residence latency, RL) was noted when all four paws of each individual animal crossed the entrance to the central square. Three groups of animals received single, daily injections of either saline (SAL; 1 ml/kg i.p. n=8 and 10), SCOP (0.15 mg/kg i.p. n=8) or ATR (2.0 mg/kg i.p. n=9) 30 min prior to testing. Inter-group data analysis was performed using the 2-tailed Mann-Whitney U test while intra-group data over time was analysed using the Spearman rank correlation coefficient test.

On day 1 of testing on the EPM, both ATR and SCOP treated animals exhibited a significantly reduced TL ($p<0.01$-0.05) and increased RL ($p<0.001$-0.05) compared to SAL controls (Mann-Whitney U test). On days 2 and 3 of EPM testing, SAL TL scores were highly significantly reduced (Spearman rank correlation coefficient; $p<0.001$) compared to day 1, whereas the TL scores for the ATR and SCOP treated animals were not significantly different between days 1, 2 and 3. In addition, on these days no significant differences for TL and RL between either of the treatment groups and their respective SAL controls were observed.

This study shows that a), rats on the EPM show apparent learning on days 2 and 3, consistent with previous reports in mice (Itoh et al., 1990, 1991) and b), atropine and scopolamine treatment produce anxiogenic effects suggesting a role for central cholinergic neurones in regulating anxiety states. The lack of any possible learning impairment following anticholinergic drug treatment on the EPM may result from the masking effect of an anxiogenic response thus limiting the use of the EPM as a test for learning and memory.

C.P.S. Smith is a SERC CASE student in collaboration with SB.

Itoh, J., Nabeshima, T. & Kameyama, T. (1990). Psychopharmacol. **101**, 27-33.
Itoh, J., Nabeshima, T. & Kameyama, T. (1991). Eur. J. Pharmacol. **194**, no.1, 71-77.
Pellow, S., Chopin, P., File, S.E. & Briley, M. (1985). J. Neurosci. Meth. **14**, 149-167.

167P LOCOMOTOR ACTIVITY AFTER NICOTINE INFUSIONS INTO THE FOURTH VENTRICLE OF RATS

M. Shoaib & I.P. Stolerman, Department of Psychiatry, Institute of Psychiatry, De Crespigny Park, London SE5 8AF

Abood and co-workers have reported a characteristic prostration syndrome (PS) when a small dose of nicotine is infused into the fourth ventricle of rats (Abood et al 1981). The present experiments aim to extend these findings by assessing the effects on locomotor activity. Tests were carried out to determine whether tolerance developed to the effects seen.

Male hooded rats (n=6-8) were implanted with a single cannula aiming towards the fourth ventricle. Following recovery, nicotine (0-8µg) was administered into the fourth ventricle and the rats were immediately placed into photocell activity cages (Clarke and Kumar 1983). The magnitude of prostration was rated (0 for no response to 4, complete immobilisation), and locomotor activity was recorded over 60 min sessions. Each rat was tested with vehicle and nicotine in a randomised sequence of doses.

Increasing doses of nicotine into the fourth ventricle produced increasing magnitudes of PS (figure 1). Maximal effects were observed with 8µg nicotine. Subsequent locomotor measurements (figure 1) found consistent dose-related decreases in locomotor activity [$F(4,20)=7.6$, $P<0.001$] with maximal effects occurring at 4µg. In rats tolerant to effects of nicotine (0.4 mg/kg) administered subcutaneously for 10 days, no tolerance was seen to either the PS or locomotor depression across increasing doses of nicotine. Mecamylamine (1.0 mg/kg SC) completely antagonised the PS evoked with 4 µg nicotine (100% block), but the locomotor depression was still evident (29% block, not significant).

Our observations of the PS confirm previous reports (Abood et al 1981) on tolerance and blockade of PS by mecamylamine. However the subsequent locomotor depression observed with nicotine differed from the PS in that it was relatively insensitive to mecamylamine. Furthermore these locomotor changes appeared to be different from the locomotor depression seen following systemic administration, which showed tolerance with repeated exposures of nicotine and was blocked by mecamylamine (Clarke and Kumar 1983).

We thank MRC for financial support.

Abood, L.G.,Reynolds, D.T.,Booth,H. & Bidlack, J.M. (1981) Neurosci. Biobehav. Rev.5, 479-486
Clarke, P.B.S. & Kumar R. (1983) Br. J. Pharmac.78, 329-337

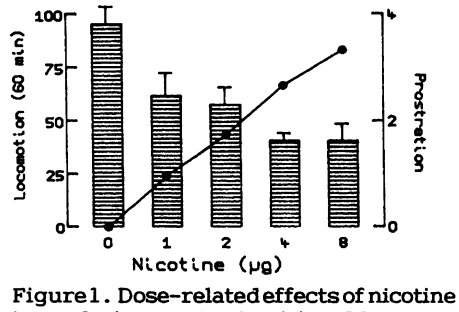


Figure1. Dose-related effects of nicotine in producing prostration (●) and locomotor depression (bars).

168P ARE CHANGES IN NEURAL METABOLISM FOLLOWING EXPOSURE TO ALUMINIUM CHLORIDE IN CELL CULTURE ASSOCIATED WITH FREE RADICAL PRODUCTION?

H B Johnston, S M Thomas & C K Atterwill
CellTox Centre, Division of Biosciences, Hatfield Polytechnic, Hatfield, Herts AL10 9AB, UK

In neural tissue culture the cytotoxic effects of high concentrations of Aluminium (Al) are neurone-specific, with effects on cholinergic function and neurofibrillary tangle production (Atterwill et al, 1990; Langui eta al, 1988). Al salts also enhance brain lipid peroxidation possibly mediated by iron-stimulated free radical production (Gutteridge et al, 1985). The time-course of cellular changes following aluminium exposure to a variety of different cultured neural cell types has now been further investigated at low metal concentrations and the effects of a free-radical scavenger, α–tocopherol, examined.

Human (IMR 32) and rat-derived (C1300-N2A) differentiated neuroblastoma cells, the SH-SY-5Y cholinergic cell line, and rat primary midbrain cultures were cultivated in 96 well plates and exposed to aluminium chloride at a range of concentrations from 0 - 1000µg/ml. Cell function and viability were assessed at different timepoints (0 - 7 days) using colorimetic assays for mitochondrial activity (MTT test) and lysozomal activity (neutral red uptake). In all the culture systems low concentrations of alum inium (0.01 - 0.1 µg/ml) produced large increases in both mitochondrial and lysozomal activities without causing cytotoxicity as determined by LDH release. For example, mitochondrial activity was up to 137% of control values for the cholinergic (SH-SY-5Y) cells, 145% (IMR32), 130% (C1300-N2A) and 170% (rat primary midbrain cells) following 18 hours exposure. Preincubation of the primary midbrain cultures with α–tocopherol (200 µM; 24h) prior to aluminium exposure for three hours completely prevented this early increase in cellular function. Since α–tocopherol scavenges oxygen-centred free radicals these data suggest, therefore, some link between free radical generation in the cultured neural cells and the early metabolic changes induced by aluminium. These early increases in neural cellular metabolism occurring during aluminium exposure may provide useful markers for neurotoxicity in vitro.

Atterwill C K, Davies W J, and Kiriakides M A (1990) ATLA. **18,** 181-190.
Gutteridge J M C, Quinlan G J, Clarke I and Halliwell B (1985) Biochimica et Biophysica Acta **835**, 441-447.
Langui D, Anderton B H, Brion J P and Ulrich J (1988) Brain Res. **438**, 67-76.

169P NALOXONE ATTENUATES CAFFEINE-INDUCED HYPOTHERMIA

M.J. Durcan* & P.F. Morgan, Laboratory of Clinical Studies, Bldg. 10 Rm. 3C218, National Institute on Alcohol Abuse and Alcoholism, 9000 Rockville Pike, Bethesda, MD 20892, USA.

Caffeine and other methylxanthines induce a dose dependent reduction in core body temperature in mice (Durcan and Morgan, 1991). The precise mechanism of this methylxanthine-induced hypothermia is at present unclear, however, the efficacy of compounds to induce a 2^0 C drop in core body temperature correlates highly with their calcium independent phosphodiesterase inhibiting effects (Durcan and Morgan, 1991). In the experiments reported here the effects of pretreatment with the opiate antagonist naloxone on caffeine induced hypothermia was investigated.

Naive male NIH Swiss mice weighing between 21 and 25 g were used. Core body temperature was measured using a rectal probe and digital thermometer immediately prior to treatment with 3 mg/kg naloxone (i.p.) or distilled water vehicle. After 20 min, core body temperature was again measured immediately prior to administration of caffeine (30-140 mg/kg, i.p.) or distilled water vehicle and further remeasured 20 min later. The administration of naloxone had no significant intrinsic effect on core body temperature whereas caffeine administration dose dependently reduced it; however, the caffeine-induced reduction in core body temperature was significantly attenuated in mice pretreated with naloxone. In a second experiment mice were treated in an identical fashion except that varying doses of naloxone (0.3-30 mg/kg) were administered to animals subsequently treated with caffeine (100 mg/kg) or vehicle. In this experiment only the highest dose of naloxone (30 mg/kg) had any intrinsic effects on core body temperature (a reduction seen 20 minutes after administration) and the caffeine treatment reduced body temperature. Pretreatment with all doses of naloxone except the lowest dose used (0.3 mg/kg) significantly attenuated the hypothermic effect of caffeine.

The results suggest that opiate receptors, but not alpha or beta adrenoceptors, serotonin, dopamine or benzodiazepine receptors (antagonists of which were also investigated), play a role in attenuating the hypothermic action of caffeine and possibly other methylxanthines.

Durcan M.J. and Morgan P.F. (1991) *Eur. J. Pharmacol.*, In press.

170P CHRONIC TREATMENT WITH THE GABA-TRANSAMINASE INHIBITORS EOS AND GVG ENHANCE BASAL GABA RELEASE FROM CROSS-CHOPPED HIPPOCAMPAL SLICES

Qume, M., Davies, J. & Fowler, L.J. (Introduced by M.A. Simmonds), Department of Pharmacology, School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX.

Neal and Shah (1990) have reported effects of the irreversible GABA-transaminase (GABA-T) inhibitor vigabatrin (GVG) on GABA release from cerebral cortex and spinal cord slices and from retina. We have examined the effect of 8 day oral GVG and a similar drug ethanolamine-O-sulphate (EOS) administration on the basal and potassium (K^+) stimulated release of GABA from cross-chopped hippocampal slices.

3 Groups of 6 male Wistar rats (263 ± 8g) were treated for 8 days with: 1g/l sucrose (vehicle); 3g/l GVG in vehicle or 3g/l EOS in vehicle in the drinking water. Body weights and fluid consumption were monitored regularly. After 8 days the animals were sacrificed, the brain removed onto ice. Both hippocamppi were removed, weighed and cross-chopped (250µm) using a McIlwain tissue chopper. The remaining brain tissue was homogenised in ice cold water for subsequent analysis of GABA-T and GABA levels. The hippocampii were suspended in 9ml warmed, gassed, low Ca^{++} (0.1mM) Krebs for 10 minutes after which time the fluid was aspirated off and the tissue was resuspended in 6ml Krebs which was evenly distributed between the 12 wells of a Millipore multiwell fliter unit. After washing (10 x 2ml) and preincubation (2ml x 5min) the tissue was incubated in 500µl of Krebs (Normal/low Ca^{++}) for 5 min, this was then drained off under vacuum and retained and the fluid replaced for a further 5 min with 500µl: 50mMK^+/low Ca^{++}, 20, 50 or 100mMK^+ normal Ca^{++}.

After the 8 day treatment period animals treated with GVG weighed significantly less than either controls or EOS treated. Assuming all fluid lost from the drinking bottles was consumed, GVG treated animals received approximately 226mg/kg/day whereas EOS, 423mg/kg/day. GABA-T activity was significantly reduced to 33 and 44% (GVG and EOS respectively) of controls. Tissue GABA levels were significantly enhanced on treatment to: GVG 163% and EOS 130% of controls. Incubation medium and protein-precipitated tissue samples were analysed for amino acid content by fluorescence HPLC of ortho-pthaldialdehyde/2-mercaptoethanol derivatised amino acids. Basal GABA release was significantly elevated from 86.98 ± 18.53 to GVG: 396.55 ± 53.75 and EOS: 242.91 ± 32.59nm/500µl/5min/mg, basal levels of other amino acids were unchanged. Potassium stimulation caused an increased efflux of aspartate, glutamate and GABA. Stimulated release of GABA by 20, 50 and 100mMK^+ was significantly elevated from that of controls (GVG treated) whereas for EOS only 50mMK^+ was increased above controls, these increases were not as marked as those of basal release. Control GABA release was found to possess significant Ca^{++} dependency (>40%) of 50mMK^+ stimulated release, however in treated tissues the release obtained with low Ca^{++} was not significantly different from controls.

We are grateful for support from the MRC and the gift of GVG from Merrell Dow.

Neal, M. J. & Shah, M. A.(1990). *Br. J. Pharmac.* **100,** 324-328.

171P SOMATIC AND AFFECTIVE BENZODIAZEPINE WITHDRAWAL SIGNS AFTER SHORT TERM CHLORDIAZEPOXIDE TREATMENT IN RATS

A.J. Goudie, A.A. Harrison, M.J. Leathley & M.A.I. Taylor, Department of Psychology, Liverpool University, P O Box 147, Liverpool. L69 3BX.

Chlordiazepoxide (CDP) withdrawal in rats causes weight loss and anorexia after 21 days treatment with doses up to 30 mg/kg b.i.d. (Goudie & Leathley, 1990; 1991). In this study, we attempted to observe such effects after a shorter period (10 days) of treatment. In addition, we examined the relationship between a somatic sign of withdrawal (weight loss) and an affective sign (conditioned taste aversion - C.T.A.). Rats (n=37) were treated for 10 days with CDP on an escalating regime (10 mg/kg/day b.i.d. up to 28 mg/kg/day b.i.d.) or with vehicle (n=39). Significant withdrawal-induced weight loss of c. 3% was observed [$F(1,74) = 40.94$, $p < 0.001$ relative to controls]. The extent of weight loss was much smaller than that in prior studies. After 21 days treatment with CDP we typically observed weight loss of c. 6-7% (Goudie & Leathley, 1990; 1991). Thus the duration of CDP treatment determines the severity of withdrawal. On Day 11, after 10 days of CDP or vehicle treatment, all animals received an infusion into the oral cavity of saccharin to try to establish a C.T.A. as a result of CDP withdrawal. In a saccharin versus water choice test CDP withdrawn animals showed a smaller (mean ± S.D.) preference score [46.3 ± 30.6%] than controls [63.8 ± 25.3%]. This difference was significant [$t (70) = 2.681$ $p < 0.005$]. Thus CDP withdrawal induced C.T.A. Analysis of the somatic (weight loss) and the affective (C.T.A.) withdrawal signs revealed that they did not correlate [$r = + 0.05$, $df = 31$, $p > 0.7$] even with the large sample of subjects used. These findings suggest that CDP withdrawal is a heterogeneous phenomenon and that somatic and affective withdrawal signs may be dissociated. Similar conclusions have been reached based on studies of opiate withdrawal (Mucha, 1987). Thus studies attempting to demonstrate that drug treatments can alleviate benzodiazepine (BZ) withdrawal need to utilise a number of withdrawal signs, incorporating both somatic and affective components. To develop clinically useful treatments for BZ withdrawal, it also seems important to examine such treatments after relatively prolonged BZ administration, as duration of treatment determines severity of withdrawal.

Goudie, A.J. & Leathley, M.J. 1990, Eur. J. Pharmacol. 185, 179-186.
Goudie, A.J. & Leathley, M.J. 1991, Psychopharmacology 103, 529-537.
Mucha, R.F. 1987, Br. Res. 418, 214-220.

172P AUTORADIOGRAPHIC ANALYSIS OF [^{3}H]-FLUNITRAZEPAM BINDING AFTER CHRONIC LOW DOSE DIAZEPAM TREATMENT IN RATS

R.R. Brett & J.A. Pratt, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

There are conflicting reports as to whether tolerance to the effects of benzodiazepines (BZs) is accompanied by BZ receptor downregulation. In general, such downregulation cannot be shown by radioligand binding after chronic low dose treatment. This technique, however, may not reveal alterations in discrete brain structures. Regional changes have been shown autoradiographically after high dose BZ treatment (Tietz *et al.*, 1986). The present experiment investigated BZ binding by receptor autoradiography in rats treated with a low dose of diazepam (DZP) intermittently and by continuous administration.

3 groups of male Long-Evans rats received daily i.p. injections, either 28 days vehicle (control), 25 days vehicle and 3 days DZP 5 mg/kg (subacute), or 28 days DZP (chronic). 4 groups of rats were implanted with 2 silastic capsules on day 1 and a further capsule on day 15 (Gallager *et al.*, 1985, adapted), either empty (control and subacute) or filled with DZP (chronic, and chronic plus i.p.). The 'chronic plus i.p.' group received concurrent daily i.p. vehicle for 28 days. On days 26-28, the other 3 implanted groups received i.p. vehicle (control and chronic) or DZP 5 mg/kg (subacute). All capsules were removed on day 28. Animals were tested on the elevated plus-maze on day 29 after challenge with vehicle (control groups) or DZP (all other groups), and sacrificed 24 h later. Slide-mounted 20 µm cryostat-cut brain sections were incubated with 1 nM flunitrazepam (FNZP), rinsed in buffer and distilled water, dried and apposed to ^{3}H-sensitive film together with ^{3}H standards to facilitate quantitative image analysis of the 47 structures measured.

Table 1 Effect of intermittent or continuous BZ receptor occupation on 3H-FNZP binding (pmol/g tissue)

	i.p. injection			implant		
	control	subacute	chronic	control	subacute	chronic
dentate gyrus	138±12	130±9	132±9	136±10	127±10	132±10
CA3 molecular layer	146±12	135±8	135±10	142±12	138±11	131±15
auditory cortex layer IV	117±13	101±9	114±12	104±9	109±10	103±15
substantia nigra, pars reticulata	89±10	88±8	85±7	90±10	89±12	87±8

Data are expressed as mean ± s.e. mean of 6 rats per group.

No differences were found between groups in any of the structures measured. In particular, no changes were found in regions in which Tietz *et al.* (1986) found significant reductions in BZ binding after chronic treatment with high doses of flurazepam (Table 1). Thus it seems that, with low doses, intermittent or continuous BZ receptor occupation does not lead to BZ receptor downregulation. We are currently investigating changes in other components of the $GABA_A$ complex.

We thank the Wellcome Trust and the Mental Health Foundation for financial support.

Gallager, D.W., Malcolm, A.B., Anderson, S.A. & Gonsalves, S.F. (1985) Brain Res. **342**, 26-36
Tietz, E.I., Rosenberg, H.C. & Chiu, T.H. (1986) J Pharmacol Exp. Therap. **236**, 284-292.

173P REVERSAL OF DIAZEPAM WITHDRAWAL ANXIETY BY BRL 46470A, A NOVEL 5-HT_3 RECEPTOR ANTAGONIST

Kennett, G.A., Grewal, S.S., Upton, N., and Blackburn, T.P., SmithKline Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex, CM19 5AD.

A major drawback to the use of benzodiazepine anxiolytics is the induction of withdrawal anxiety. In the present study the effects of withdrawal from the novel 5-HT_3 receptor antagonist, BRL 46470A (Kennett and Blackburn 1990), was studied, as was the effect of the drug on withdrawal from diazepam using the rat social interaction (SI) anxiety model.
Male SD rats were given BRL 46470A (at doses previously found to be active in the SI test (Kennett & Blackburn 1990) (0.01, 0.1 mg/kg) or diazepam (40 mg/kg) p.o. b.i.d. x14 days. On day 11 they were housed singly and on days 13 and 14 placed alone in an SI test box (black perspex 54 x 37 x 26 cm) under red light for 10 min. On day 15 they were given BRL 46470A 0.01 or 0.1 mg/kg p.o. or vehicle 30 min pretest and placed with a similarly treated, weight matched pair mate in the SI box, under red light, for 15 min. Active SI (sniffing, grooming, following, mounting, biting, boxing, crawling over or under) and locomotion was scored blind for each rat by video.

Table 1: Effect of withdrawal from chronic BRL 46470A and diazepam on rat social interaction

Pretreatment P.O. B.i.d x 14		Treatment P.O. Tested Day 15	Total Interaction(s)	Locomotion (Squares Crossed)
Vehicle		Vehicle	162 ± 10	438 ± 37
BRL 46470A	0.01 mg/kg	''	179 ± 14	430 ± 28
''	0.1 mg/kg	''	163 ± 10	456 ± 30
Diazepam	40 mg/kg	''	92 ± 8**	447 ± 17
''	40 mg/kg	BRL 46470A 0.01 mg/kg	149 ± 11++	447 ± 23
''	40 mg/kg	BRL 46470A 0.1 mg/kg	134 ± 12+	408 ± 17

Significantly different from vehicle + vehicle group ** $P < 0.01$, from Diazepam + Vehicle treated group + $P < 0.05$, ++ $P < 0.01$ by Newman-Keuls test following significant 2 way ANOVA. All points means and SEM n=10-14.

Withdrawal from chronic BRL 46470A did not affect SI; unlike withdrawal from diazepam, which reduced SI but not locomotion at 40 mg/kg p.o. (table 1). This anxiogenic-like effect was reversed by treatment with BRL 46470A 0.01 or 0.1 mg/kg given 30 min pretest (table 1). Thus, BRL 46470A might be of use in the treatment of anxiety, particularly after withdrawal from benzodiazepine treatment.

Kennett, G.A., and Blackburn, T.P. (1990) J. Psychopharmacol 4,4.

174P DIAZEPAM CAUSES A VOLTAGE-DEPENDENT BLOCK OF ACTION POTENTIALS IN ISOLATED FROG MUSCLES

J.J. Eigenhuis, R.S. Leeuwin, T.Piek and H. van Wilgenburg
Department of Pharmacology, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

Twitch and tetanic contraction of the isolated rat diaphragm in vitro can be blocked by benzodiazepines, both at indirect stimulation and at direct stimulation of curarized muscles (Bokkinga et al., 1991). The present work deals with the effects of diazepam on the excitability of the muscle fibre isolated from the frog semitendinosus muscle.

Experiments were performed on single muscle fibres, isolated from the semitendinosus muscle of the frog Rana esculenta in a dissecting dish containing physiological solution (95% O_2;5% CO_2) and transferred into a mannitol gap chamber. The electrical activity of the artificial node was studied under current clamp or voltage clamp conditions in a double mannitol gap device. Before generating an ionic current by means of a step depolarization from the holding potential of -100mV to -30 mV (the first pulse), the muscle fibre was depolarized by blocks of varying amplitudes (0, 30, 50, 70 mV) and a duration of 500 ms or by blocks of varying duration (50, 200, 500 ms) and an amplitude of 50 mV. Between this prepulse and the test pulse the de-activation period was 12 ms.

Current clamp experiments show that diazepam (0.05 mmol/l) causes a marginal decrease in amplitude and a significant increase in duration of the action potential within 60 sec. A quick and complete restoration of the action potential is seen during washings. At a ten times higher concentration (diazepam, 0.5 mmol/l) the excitability of the fibre is completely blocked. Voltage clamp experiments show that at a concentration of 0.02 mmol/l no significant effect on the Na^+ current but a significant effect on the K^+ current is observed within 40 sec. Diazepam 0.05 mmol/l causes a full block of both the Na^+ and the K^+ currents. These effects are reversible after washing. In the presence of diazepam, 0.03 mmol/l, the decrease in sodium current, following the testpulse step depolarization from the holding potential of -100 mV to a level of -30 mV, depends on the amplitude of the prepulse, as well as on the duration of the prepulse. Stimulation of the preparation with pulse trains show that the effect is not use-dependent.

In conclusion the results indicate that diazepam affects the voltage-dependent excitability of the muscle fibre isolated from the frog semitendinosus muscle.

Bokkinga, A., Cheung Tam Hé, M.A.K.K., Eigenhuis, J.J., Leeuwin, R.S., Slagmaat, R., van Wilgenburg, H. (1991) Br.J.Pharm. suppl.proc. 102, 281

175P ROLE OF ECTO-ADENYLATE KINASE ON AMP FORMATION FROM ADP AT THE INNERVATED SARTORIUS MUSCLE OF THE FROG

J.F. Cascalheira & A.M. Sebastião, Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781 Oeiras, Portugal.

Adenine nucleotides are released from motor nerve endings and skeletal muscle cells, and dephosphorylation of AMP contributes to the formation of extracellular endogenous adenosine that inhibits neuromuscular transmission (Ribeiro & Sebastião, 1987). Either dismutation of ADP into ATP and AMP by ecto-adenylate kinase (Dunkley et al., 1966) or dephosphorylation of ADP (Cunha & Sebastião, 1991) have been proposed as mechanisms of AMP formation from extracellular ADP at the frog skeletal muscle. The present work was undertaken to investigate the role of ecto-adenylate kinase on AMP formation from ADP at the innervated frog sartorius muscle, by using the ecto-adenylate kinase inhibitor, P^1,P^5-di(adenosine-5')pentaphosphate (AP_5A).

Innervated frog sartorius muscles were mounted in a 2 ml perspex chamber at room temperature (22-25°C). The bathing solution (pH=6.8) contained (mM): NaCl 117, KCl 2.5, $MgCl_2$ 1.2, $CaCl_2$ 1.8, Na_2HPO_4 1.0, NaH_2PO_4 1.0 and dipyridamole 0.0005. Before starting the experiments, the preparation was previously superfused for 15 min at a rate of 20 ml/min and then for 45 min at a rate of 5 ml/min. Throughout the assays, air was bubbled into the bath. Kinetic assays were done by incubating the preparation, at zero time, with ADP (30 µM). Samples of 75 µl were collected from the bath at different times from zero to 90 min of incubation, and analysed by ion-pair reverse-phase HPLC, with U.V. (254 nm) detection.

ADP (30 µM) was transformed into ATP, AMP, IMP, adenosine and inosine. The $t_{50\%}$ for ADP (30 µM) metabolism was 38.7 ± 9.1 min (n=3). The concentration of ATP in the bath reached a maximum (2.7 ± 0.2 µM) after 14 ± 2 min of incubation with ADP (30 µM, n=3) and then decreased. Formation of extracellular ATP from ADP was also observed when the phosphate buffer in the bath was substituted by HEPES (10 mM) (n=2). In the presence of AP_5A (100 µM) a nearly complete inhibition of ATP formation from ADP (30 µM) was observed, while only a 29 ± 8% reduction of ADP metabolism and a 21 ± 6% (n=3) reduction of AMP formation, at 30 min of incubation, were observed. There was no measurable release, from the preparation to the bath, of adenine nucleotides or of enzymatic activity responsible for ADP transformation during the period of incubation.

The results suggest that both 1) ecto-adenylate kinase mediated dismutation of ADP and 2) dephosphorylation of ADP contribute to the formation of extracellular AMP at the innervated frog sartorius muscle, but dephosphorylation of ADP can account for most of the extracellular AMP formed from ADP.

J.F.C. was supported by INIC and is now supported by JNICT.

Cunha, R.A. & Sebastião, A.M. (1991) Eur. J. Pharmacol. **197**, 83-92
Dunkley, C.R., Manery, J.F. & Dryden, E.E. (1966) J. Cell Physiol. **68**, 241-248
Ribeiro, J.A. & Sebastião, A.M. (1987) J. Physiol. **384**, 571-585

176P THE FROG BRAIN POSSESSES ONE BINDING SITE FOR [^{3}H]-DPCPX DIFFERENT FROM THE A_1 OR THE A_2 ADENOSINE RECEPTORS

J.C. Oliveira, A.M. Sebastião & J.A. Ribeiro
Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781 Oeiras, Portugal.

It has been proposed that the frog motor nerve terminals possess a third (A_3) adenosine receptor (Sebastião & Ribeiro, 1989). In the present work we investigated the presence of this receptor (A_3) in the frog brain. Frog brains were homogenized with Buffer A (50 mM-Tris/HCl, pH7.4, 100 mM-NaCl, 10 mM-$MgCl_2$, 1 mM-EDTA, 0.1 mM-DTPA, 1 mM-dithiothreitol, 0.1 mM-PMSF and 1 µg/ml of pepstatin A, leupeptin, chymostatin, antipain and aprotinin), centrifuged at 2000 rpm (10 min, 4°C). The supernatant was centrifuged at 15000 rpm (20 min, 4°C) and washed 3 times. Membranes were incubated (25°C, 2 h) with increasing concentrations of [^{3}H]-DPCPX (1,3-dipropyl-8-cyclopentylxanthine) (control), and in presence of GTP (100 µM) or Mg^{2+} (8 mM). We also studied the effects of GTP (100 µM) and Mg^{2+} (8 mM) on the specific binding of the agonist [^{3}H]-L-PIA (L-phenylisopropyladenosine) (10 nM), and we used the putative affinity probe m-DITC-[^{3}H]-XAC (50 nM) to determine of the apparent molecular weight of this receptor.

Table 1. Binding parameters of [^{3}H]-DPCPX to frog brain membranes.

	n	K_d (nM)	B_{max} (pmol/mg prot)
Control	4	44±7.2	0.238±0.016
Mg^{2+}	3	14±1.6*	0.261±0.018[a]
GTP	3	67±5.1*	0.266±0.026[a]

* $p<0.05$ vs control; [a] $p>0.05$ vs control.

Table 2. [^{3}H]-L-PIA binding to frog brain membranes.

	n	% of Control
GTP	3	52.4±8.1 *
Mg^{2+}	4	117.6±1.6 *
GTP+Mg^{2+}	3	84.6±4.3
GTP+Na^+(a)	3	59.2±15 *

* $p<0.05$ vs control; [a] $[Na^+]=125$ mM.

The [^{3}H]-DPCPX saturation binding experiments showed that frog brain membranes possess a binding site for DPCPX with a K_d value different from the A_1 or the A_2 adenosine receptors and correlates well with the K_i of DPCPX as antagonist of adenosine analogues inhibition of neuromuscular transmission in frog. DPCPX could discriminate two affinity conformations of the receptor molecule, since two distinct K_d values for [^{3}H]-DPCPX were obtained when the binding was performed in the presence of Mg^{2+}, or in the presence of GTP (Table 1). The agonist binding studies confirmed that the receptor present in the frog brain is like a receptor-G protein complex. The incorporation of m-DITC-[^{3}H]-XAC followed by SDS-PAGE electrophoresis showed that the frog brain adenosine receptor has an apparent m.w. of 50-55 KDa, which is different from the m.w. of the A_1 or A_2 adenosine receptors.

The apparent m.w. found for this adenosine receptor and its affinity value for [^{3}H]-DPCPX, together with the fact that the antagonist discriminated between two affinity conformations of this receptor suggest that this entity is different from the A_1 or the A_2 adenosine receptors, and might represent the third (A_3) adenosine receptor subtype.

J.C.O. is supported by JNICT. m-DITC was a gift from Dr. K.A. Jacobson.
Sebastião, A.M., Ribeiro, J.A. (1989), Br. J. Pharmacol. **96**, 211-219.

177P ANAESTHETIC SENSITIVITY AFFECTS THE CARDIOVASCULAR DEFENCE RESPONSE IN DIFFERENT BREEDING STRAINS OF RAT

Simon R.G. Beckett, John O. Curwen*, Paul W. Marshall* and Charles A. Marsden. Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, *ICI Pharmaceuticals, Bioscience II, Mereside, Alderley Park, Macclesfield, Cheshire, U.K.

Saffan anaesthesia is the anaesthetic of choice when studying centrally mediated cardiovascular changes such as the defence response (Timms 1981). It has been shown not to interfere with the integrative activities of the forebrain in the way that barbiturate anaesthetics do, inasmuch as the visceral changes characteristic of the defence response can be evoked as a reflex, or by amygdala stimulation, as well as by stimulation of the brain stem defence areas such as the midbrain periaqueductal grey. In the present study the effect of anaesthetic sensitivity on a chemically induced cardiovascular defence response was examined in two different independent breeding lines of hooded Lister rat.

Male hooded Lister rats from colonies 1 (ICI) and 2 (QMC Nottingham) were anaesthetised via a tail vein injection of Saffan and the jugular vein and carotid artery cannulated for infusion of anaesthetic and measurement of blood pressure and heart rate. Maintenance levels of anaesthesia were obtained by infusing Saffan at between 10 and 25 mg/kg/hr according to individual requirements. Stainless steel guide cannulae were then stereotaxically implanted 2mm above the dorsal periaqueductal grey (DPAG) (AP -7.0, ML +0.2, DV -3.1mm relative to bregma). Skeletal muscle blood flow was determined by means of a laser doppler blood flow probe placed on the gastrocnemius muscle. The response to D,L-Homocysteic acid (DLH 8nmols in 250nl) microinjected into the dorsal PAG was subsequently recorded. In a further experiment eight animals from the second group were anaesthetised with urethane (1.4g/kg) rather than Saffan and the above procedures carried out. Histological verification was performed on all microinjection sites.

Rats from group 1 (n=15) needed 9±1.4mg/kg/hr saffan to maintain anaesthesia, whilst animals from group 2 (n=12) required 25±2.1mg/kg/hr. Basal blood pressure, heart rate and skeletal muscle blood flow were comparable between the two groups. Animals in group 1 exhibited a cardiovascular defence response following DLH administration into the DPAG, characterised by an increase in blood pressure (+26±1.4mmHg^{-1}), an increase in heart rate (+34±2bpm) and an increase in skeletal muscle blood flow (+89% from the basal level), whilst animals in group 2 showed no significant change from basal values. Urethane anaesthetised animals from the second group all exhibited the full cardiovascular defence response (BP+ 35±3mmHg^{-1}, HR +60±8bpm; n=7). These results suggest that sensitivity to steroid anaesthesia can vary significantly between different breeding lines of the same strain of rat. Furthermore it appears that Saffan used in high concentrations (up to 25mg/kg/hr) to maintain anaesthesia in rats with low sensitivity can interfere with a centrally mediated haemodynamic response such as the defence response.

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

Timms R. J. (1981). Pflugers Archiv. **391,** 49-56.

178P COMPARISON OF HPLC AND ENZYMATIC RECYCLING ASSAYS FOR THE MEASUREMENT OF OXIDISED GLUTATHIONE LEVELS IN BRAIN

[1]J. Sian, [1]D.T. Dexter, [1]P. Jenner, [1]C.D. Marsden and [2]G. Cohen. [1]Parkinson's Disease Society Experimental Research Laboratories, Pharmacology Group, Biomedical Sciences Division, King's College, Manresa Road, London, U.K. [2]Mount Sinai School of Medicine,City University of New York, New York, U.S.A.

Previously, Perry and colleagues (1986) using high pressure liquid chromatography (HPLC) found a high proportion of oxidised (GSSG) compared to reduced (GSH) glutathione present in human brain, but others (Slivka et al,1987) using an enzymatic recycling assay reported much lower levels. We have compared the levels of GSSG obtained in rat brain using both techniques and attempted to discover the reason for this difference.

In rat cerebellar homogenates the levels of GSSG found using an HPLC-UV (Reed et al,1980) assay (0.452 ± 0.09 µmoles/g) were much higher then those found using the enzymatic reduction with glutathione reductase (0.00208 ± 0.00011 µmoles/g). Reduction of synthetic GSSG by glutathione reductase showed total conversion to GSH as assessed by HPLC-UV analysis. However in rat cerebellar homogenates only approximately 50% of the HPLC peak for GSSG could be reduced by glutathione reductase (Table.1).

Table. 1 GSSG reduction in cerebellar homogenates after incubation with glutathione reductase

Group	n	GSSG content (µmoles/g wet weight).
Control	6	0.443 ± 0.09
Glutathione reductase (16µg/ml)	3	0.246 ± 0.01*
Glutathione reductase (32µg/ml)	3	0.211 ± 0.11*

Values are mean ± SEM. *p< 0.05 as compared to control values (ANOVA).

These results suggest that another substance present in brain tissue is derivatised and eluted at the same retention time as GSSG and contributes to the high GSSG levels found using the HPLC assay.

Perry,T.L., Yong,V.W. (1986). Neurosci.Lett. **67**, 269-274.
Reed,D.J., Babson,J.R., Beatty,P., Brodie,A., Ellis,W., Potter,D. (1980). Anal.Biochem. **106**, 55-62.
Slivka,A., Spina,M.B., Cohen,G. (1987). Neurosci.Lett. **74**, 112-118.

179P GLUTAMATE MODULATES ENDOGENOUS DOPAMINE RELEASE FROM SUPERFUSED MINISLICES OF BOTH OLFACTORY TUBERCLE AND STRIATUM OF RAT

É. Zempléni, C.A. Jones, S. Pearson and G.P. Reynolds, Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN.

It is known that glutamate (Glu) can modulate release of endogenous dopamine (DA) from striatum both *in vivo* (Moghaddam *et al.*, 1990) and *in vitro* (Clow and Jhamandas, 1989). Evidence also suggests glutamatergic modulation of DA release from the olfactory tubercle (OT) (Marien *et al.*, 1983). This preliminary study compared the receptor subtypes involved in the two areas.

Minislices (350 μm x 350 μm) of tissue were superfused with Krebs-phosphate buffer (containing mazindol 10 μM, pargyline 10 μM and tyrosine 50 μM) at 500 μl/min. After a 30 min equilibration period 2 min fractions of the superfusate were collected for 30 min. Release of DA was stimulated by 2 min pulses of Glu, N-methyl -D-aspartic acid (NMDA), kainic acid (KA) or K+ solution at 6 and 20 min. The samples were collected into vials containing 100 μl perchloric acid 1M, and stored on ice (for up to 1 hour) or at -20°C until assayed for endogenous catecholamine content by HPLC- electrochemical detection.

Expt 1. DA release stimulated by 1mM Glu (S1) was compared to that stimulated by 25 mM K+ (S2) in Mg2+-free and Mg2+-containing medium. Results are summarised in Table 1 as the S2/S1 ratio (where S1 and S2 are the DA released (ng) during the 8 min period after drug stimulus). All results are mean ± s.e.mean (n). The Glu stimulated DA release was much more marked in Mg2+-free medium ($p<0.05$ Student t-test), suggesting a NMDA-receptor mediated component.
Expt 2. Effects of Glu, KA and NMDA on DA release (S1 only) were compared using Mg2+-free medium. Maximal release of DA was achieved with 3 mM Glu. NMDA was found to increase DA release much more than KA.

Table 1 DA released by Glu/K+ (S2/S1 ratio)

	+Mg^{++}	Mg^{++}-free
Striatum	2.55 ± 0.32 (6)	1.51 ± 0.22 (8)
OT	2.47 ± 0.33 (4)	1.35 ± 0.14 (4)

Table 2 DA released by Glu agonist (ng/8 min)

Agonist	Striatum	OT
0.1 mM Glu	1.52 ± 0.2 (8)	0.76 ± 0.13 (4)
1 mM Glu	2.62 ± 0.23 (13)	1.50 ± 0.12 (7)
3 mM Glu	4.83 ± 1.25 (6)	3.48 ± 0.63 (4)
0.3 mM KA	1.95 ± 0.18 (11)	1.13 ± 0.42 (3)
3 mM KA	2.15 ± 0.26 (6)	0.86 ± 0.21 (4)
0.3 mM NMDA	4.06 ± 0.32 (8)	1.94 ± 0.4 (4)

These initial experiments confirm that endogenous DA release from both striatum and OT can be modulated by Glu, involving NMDA and non-NMDA receptor mechanisms. Further studies are needed to confirm these findings using selective antagonists and to determine quantitatively the contributions of each receptor subtype in the two tissues.

Moghaddam, B., Gruen, R.J., Roth, R.H., Bunney, B.S. and Adams, R.N. (1990) Brain Res. 518, 55-60.
Clow, D.W. and Jhamandas, K. (1989) J. Pharmacol. Exp. Ther. 248, 722-728.
Marien, M., Brien, J. and Jhamandas, K. (1983) Can. J. Physiol. Pharmacol. 61, 43-58.

180P GLUTAMATE-STIMULATED RELEASE OF [^{3}H]-DOPAMINE FROM MINISLICES OF RAT FRONTAL CORTEX INVOLVES MORE THAN ONE RECEPTOR SUBTYPE

B. Davis, C.A. Jones & G.P. Reynolds, Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN.

Recent *in vivo* experiments (Hata *et al.* 1990; Rao *et al.* 1990) suggested an inhibitory effect of glutamate on dopaminergic systems in rat frontal cortex by showing increased dopamine (DA) metabolism after N-methyl-D-aspartate receptor (NMDA) blockade. This study investigated effects of glutamate agonists on rat frontal cortex minislices *in vitro*.

Minislices (350 μm x 350 μm) of rat frontal cortex, pre-incubated with [^{3}H]DA in the presence of desipramine (0.1 μM) and pargyline (10 μM) were superfused with Mg^{++}-free Krebs solution containing desipramine and pargyline as above and mazindol (10 μM) at 400 μL/min. After 30 min of equilibration, fractions of superfusate were collected at 2 minute intervals. Release of [^{3}H]DA was stimulated by 2 min pulses of glutamate, NMDA and kainate solutions at 6 and 24 min. In some experiments, to determine specific receptor involvement, 2-amino-5-phosphonopentanoic acid (AP-5; NMDA receptor antagonist) and 6,7-dinitroquinoxaline-2,3-dione (DNQX; non-NMDA receptor antagonist) were administered at 18 min onwards. [^{3}H]DA release was estimated by liquid scintillation counting. For agonists alone peak sizes above basal release were measured. Peaks (S1 and S2) are the increase in release over basal observed during the 10 min period after each stimulus, and are expressed as a "cumulative percentage" of the total tissue [^{3}H]DA content. For antagonists the S2/S1 ratios were compared with controls.

No response to 1 mM glutamate was observed in the presence of 1.2 mM Mg^{++}, suggesting the involvement of NMDA receptors. Effect of glutamate agonists on DA release (in Mg^{++}-free Krebs) are summarised in Table 1. The mean S2/S1 ratio of 300 μM NMDA was 0.62 ± 0.10, reduced to 0.29 by 50 μM AP-5 ($p<0.005$ (n=5)). Due to poor second response when stimulating release using kainate (all concentrations), 60 μM DNQX could not be shown to significantly reduce the S2/S1 ratio. The S2/S1 ratio of 1 mM glutamate was 0.65 ± 0.12 and was reduced to 0.16 by 200 μM AP-5 ($p<0.05$ (n=6)). The response to 1 mM glutamate was completely blocked by a combination of 200 μM AP-5 with 60 μM DNQX ($p<0.005$ (n=4)).

Table 1 **Effect of glutamate agonists on DA release**

Agonist	100 μM	300 μM	1 mM	3 mM	
Glutamate	0.6 ± 0.2	1.3 ± 0.4	2.8 ± 0.3	3.9 ± 1.8	S1 values,
Kainate	-	1.6 ± 0.5	18.2 ± 3.5	5.3 ± 1.8	mean ± s.e. mean,
NMDA	-	6.4 ± 0.9	27.1 ± 2.5	8.8 ± 1.1	n = 3-14

The results show that *in vitro*, using Mg^{++}-free medium, glutamate stimulates the release of dopamine from nerve terminals in the frontal cortex in a concentration-dependent manner. The mechanism involves both NMDA and non-NMDA receptor subtypes. The contrast between the excitatory effect of glutamate observed *in vitro* and the inhibitory effect *in vivo* may illustrate the role of tonic neural activity in modulating local control of neurotransmitter release.

This work was funded by the Nuffield Foundation.

Hata, N., Nishikawa, T., Umino, A., Takahashi, K.. (1990) Neurosci. Lett. 120:101-104.
Rao, T.S., Kim, H.S., Lehmann, J., Martin, L.L., & Wood, P.L. (1990) J. Neurochem. 54:1157-1162.

181P AGONISTS OF METABOTROPIC GLUTAMATE RECEPTORS ENHANCE NMDA RECEPTOR-MEDIATED COMPONENTS OF POTENTIALS IN OLFACTORY CORTEX

G G S Collins, University Department of Medicine and Pharmacology, Royal Hallamshire Hospital, Sheffield S10 2JF

Mono and di-synaptic excitations in the olfactory cortex are largely mediated by AMPA-(amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) sensitive glutamate receptors, with NMDA (N-methyl-D-aspartate) receptors making a small but significant contribution (Collins, 1991). The possible role of glutamate receptors linked to phosphoinositide turnover, the metabotropic receptor subtype, is unknown. Evoked potentials mediated by NMDA receptors were recorded in slices of mouse olfactory cortex perfused with Mg^{2+}-free solution containing 10 μM (DNQX; (6,7-dinitroquinoxaline-2,3-dione) to block AMPA receptors, using procedures reported previously (Collins, 1991). Responses were little affected by the presence of 25-500 μM trans-ACPD (1RS,3RS-cis-1-aminocyclopentyl-1,3-dicarboxylic acid), a selective agonist of metabotropic glutamate receptors (Schoepp et al, 1990). However, following washout there was a progressive, concentration-dependent increase in the area of the potential which required 15 min fully to develop and persisted for at least 1 hr. The maximum percentage increases caused by trans-ACPD, quisqualate and L-glutamate (mean ± s.e. mean, n = 4-7) were 44.2 ± 3.7, 38.2 ± 3.8 and 26.9 ± 1.9, respectively, the corresponding μM concentrations which increased the area of the potential by 25% (EC_{25} values) being 82.3 ± 7.5, 11.2 ± 2.7 and 1120 ± 207, respectively. The EC_{25} value for 1S,3R-trans-ACPD, the active isomer of trans-ACPD, was 8.0 ± 1.6 μM (n = 4), whereas kainate, AMPA, NMDA and L-aspartate were inactive. Racemic 2-amino-3-phosphonopropionate (AP3) and 2-amino-4-phosphononbutyrate (AP4), 0.5 - 2 mM, partially antagonised the actions of trans-ACPD and quisqualate (glutamate not studied). At 0.125 mM, S-AP3 antagonized the effects of 1S,3R-trans-ACPD whereas R-AP3 was inactive : in contrast, 0.25 mM D-(-)- and L-(+)-AP4 were equipotent. Antagonism was characterised by a shift in agonist concentration-effect curves to the right with a reduction in the maximum responses. Other experiments showed that 1S,3R-trans-ACPD potentiated the short and long latency components of potentials equally, but had no effect on AMPA receptor-mediated potentials. These results show that metabotropic glutamate receptors selectively regulate NMDA receptor-mediated excitations in olfactory cortex.

Supported by the Wellcome Trust.

Collins, G.G.S. (1991) Neuropharmacol. 30, 547-555
Schoepp, D., Bockaert, J. & Sladeczek, F. (1990) Trends Pharmacol. Sci. 11, 508-515.

182P POLYAMINES MODULATE THE BINDING OF THE NMDA ANTAGONIST [^{3}H]CGP 39653 VIA THE GLYCINE SITE

A. Oblin, S. Bidet & H. Schoemaker (Introduced by B. Scatton), Department of Biology, Synthélabo Recherche (L.E.R.S.), 31 av. Paul-Vaillant Couturier, 92220 Bagneux, France

The activation of the N-methyl-D-aspartate (NMDA) receptor/ionophore complex is believed to be controlled by several regulatory sites. In particular, a strychnine-insensitive glycine binding site located on the NMDA receptor complex modulates the action of glutamate agonists (Johnson *et al.*, 1987). Recent studies have demonstrated that polyamines may also modulate radioligand binding to the NMDA receptor as well as NMDA-induced ion channel opening (Williams *et al.*, 1991). We have characterised the effects of polyamines on the binding of the competitive NMDA antagonist [^{3}H]CGP 39653.

Binding experiments were carried out as previously described using whole rat forebrain membranes (Stills *et al.*, 1991). Well washed membranes (60-100 μg of protein) were incubated for 1 h at 4° C in 1 ml of 50 mM Tris-HCl buffer (pH 7.7 at 25° C) with 1-2 nM of [^{3}H]CGP 39653 (spec. act. 51.3 Ci/mmol ; Dupont de Nemours/NEN products). The incubation was terminated by filtration over 0.3 % polyethylenimine-pretreated Whatman GF/B filters. Nonspecific binding was determined in the presence of 100 μM of L-glutamate.

Specific [^{3}H]CGP 39653 binding is potently inhibited by glutamate (IC_{50} = 52 nM) and the competitive NMDA antagonists CGS 19755, CPP and D-AP5 with IC_{50} values of 65, 95 and 100 nM respectively, but not significantly altered by spermine, and the putative polyamine antagonists arcaine and ifenprodil. Approximately 60 % of specific [^{3}H]CGP 39653 binding is inhibited by glycine at low concentrations (IC_{50} = 332 nM) while the remainder is only affected by high concentrations of glycine ($IC_{50} \geq 1$ mM). In the presence of 7-Cl-kynurenate (10 μM) the potency of glycine is significantly decreased (IC_{50} = 9 μM). Spermine (100 μM) fails to affect the inhibitory effects of glutamate but produces a leftward shift in the concentration-effect curve to glycine (IC_{50} = 33 nM). Ifenprodil and arcaine (10 μM) partially but significantly antagonize the potentiation of the inhibitory effects of glycine by 100 μM spermine (IC_{50} of glycine = 101 and 190 nM, respectively).

The present data show that glycine exerts an allosteric inhibition of the binding of the competitive NMDA antagonist [^{3}H]CGP 39653. Spermine fails to directly affect the binding of [^{3}H]CGP 39653 or its competitive inhibition by glutamate, but potentiates the allosteric inhibition of [^{3}H]CGP 39653 binding by glycine. Both ifenprodil and arcaine antagonize the effects of spermine in this experimental model.

Williams, K., Romano, C., Dichter, M.C. & Molinoff, P.B. (1991) Life Sci. 48, 469-498
Stills, M.A., Fagg., G., Pozza, M., Angst, C., Brundish, D.E., Hurt, S.D., Wilusz, E.J. & Williams, M. (1991) Eur. J. Pharamacol. 192, 19-24
Johnson, J.W. & Ascher, P. (1987) Nature 325, 529-531

183P CHARACTERIZATION OF ANGIOTENSIN II STIMULATION OF PHOSPHOINOSITIDE METABOLISM IN RAT HEPATOCYTES

F.H. Marshall, J.C. Barnes, A.D. Michel. Department of Neuropharmacology, Glaxo Group Research, Ware, Herts, SG12 ODP.

Angiotensin II (AII) has been shown to activate phospholipase C leading to the production of inositol phosphates in a variety of tissues (see Garcia-Sainz, 1987). In this study, we have investigated the influence of extracellular calcium concentration $[Ca^{2+}]_e$ on the AII-induced stimulation of phosphoinositide (PI) turnover in rat hepatocytes, since major differences have been shown to exist in the calcium dependency of other neurotransmitters coupled to PI metabolism (Baird et al., 1989). In addition we have determined the potency of the AT_1 selective non-peptide antagonists GR117289 (Robertson et al, 1991) and losartan (DuP 753), and the AT_2 antagonist PD123177 (Chiu et al, 1989) to inhibit the AII response.

Hepatocytes were prepared, according to a modification of the method of Seddon et al. (1989). These were incubated at 37°C with [^{3}H]-myoinositol in Krebs buffer containing 0-2.5mM $[Ca^{2+}]_e$ for 1hr. The cells were then centrifuged and resuspended in buffer containing 5mM LiCl and a range of $[Ca^{2+}]_e$ (0-2.5mM). The cells were equilibrated at 37°C for 15min prior to the addition of varying doses of AII for 30min. Antagonists were added 15min before the AII. The reaction was terminated and inositol phosphates separated according to the method of Johnson & Minneman (1987).

AII potently stimulated the production of inositol phosphates. The magnitude of the response was sensitive to changes in $[Ca^{2+}]_e$. Maximal incorporation of [^{3}H]-inositol into the total PI pool was achieved in 'calcium free' conditions i.e. no added calcium. Increasing $[Ca^{2+}]_e$ to 2.5mM resulted in a reduction in the basal incorporation of [^{3}H]-inositol by approximately 60%. Maximal stimulation of PI turnover by AII occurred in the presence of 0.6mM $[Ca^{2+}]_e$. Under these conditions AII produced a concentration-dependent increase in inositol phosphate production (EC_{50}=77±23nM, n=6) reaching a maximum of 277% of basal at 10μM. A maximum of only 120% was reached in calcium free conditions and of only 173% in 2.5mM $[Ca^{2+}]_e$. Losartan (30-300nM) caused a rightward parallel shift in the AII dose response curve. The mean pK_B was 8.1±0.16 (n=8); Schild slope=1.2±0.4 (not significantly different to 1). GR117289 (0.3-3nM) caused a dose dependent suppression of the maximum response, similar to that previously reported in the rabbit aorta (Robertson et al, 1991). PD123177 (3μM), had no effect on the AII response. In conclusion AII stimulation of PI turnover in hepatocytes is sensitive to changes in $[Ca^{2+}]_e$ and is mediated by an AT_1 receptor.

Baird, J.G., Chilvers, E.R., Kennedy, E.D., Nahorski, S.R. (1989) Naunyn-Schmiedberg's Arch.Pharmacol. **339**, 247-251.
Chiu, A.T., Herblin, W.F., McCall,D.E., Ardecky,R.J., Carni,D.J., Duncia,J.V., Pease,L.J., Wong,P.C., Wexler,R.R., Johnson,A.L. & Timmermans,P.B.M.W.M. (1989).Biochem. Biophys. Res. Commun. **165**, 196-203.
Johnson,R.D. & Minneman,K.P.(1987). Mol.Pharmacol. **31**, 239-246.
Garcia-Sainz, J.A. (1987). TIPS. **8**, 48-49.
Robertson,M.J., Middlemiss,D., Ross,B.C., Drew,G.M., Scopes,D.I.C., & Dowle, M.D. (1991). Br.J.Pharmacol.**104**,300P.
Seddon,T., Michelle,I. & Chenery,R. (1989). Biochem. Pharmacol. **38**, 1657-1665.

184P $\alpha 1_A$- AND $\alpha 1_B$-ADRENOCEPTORS STIMULATE INOSITOL PHOSPHATE GENERATION IN RAT KIDNEY

R. Büscher, F. Siepmann, T. Philipp, M.C. Michel & O.-E. Brodde, Dept. Medicine, University of Essen, Hufelandstr. 55, D-4300 Essen, Germany

We have previously demonstrated that α_{1B}- but not α_{1A}-adrenoceptors mediate inositol phosphate (IP) generation in slices from rat cerebral cortex (Michel et al. 1990). We have now studied the presence of these subtypes and their coupling to IP production in rat renal slices. In radioligand binding experiments with [^{3}H]prazosin 50±1% and 66±3% of the total sites in renal membranes had high affinity for the α_{1A}-selective antagonists 5-methyl-urapidil and (+)-niguldipine, respectively. Pre-treatment with the irreversible α_{1B}-selective chloroethylclonidine (10 μM, 30 min at 37°) reduced the density of [^{3}H]prazosin binding sites by 48%. Thus, α_{1A}- and α_{1B}-adrenoceptors co-exist in rat kidney in similar amounts with a slight dominance of the α_{1A}-subtype. In the presence of 100 μM GTP noradrenaline and methoxamine competed for [^{3}H]prazosin binding with shallow and biphasic curves with 48±9% and 59±4% of the sites being in the high-affinity state. Analysis of these data indicated that noradrenaline and methoxamine are 30- and 45-fold α_{1A}-selective which is in agreement with recently published affinities at cloned rat α_{1A}- and α_{1B}-adrenoceptors (Lomasney et al. 1991).

Noradrenaline and methoxamine concentration-dependently stimulated IP generation in rat renal slices (assessed as accumulation of total [^{3}H]IP in [^{3}H]myo-inositol labelled slices during a 45 min incubation at 37° in the presence of 10 mM LiCl). 5-Methyl-urapidil and (+)-niguldipine inhibited noradrenaline- and methoxamine-stimulated IP accumulation with shallow biphasic curves; only 15-40% of IP accumulation by either agonist was inhibited by the high affinity component of either α_{1A}-selective antagonist. Similarly, pre-treatment with the α_{1B}-selective chloroethylclonidine (see above) reduced noradrenaline- and methoxamine-stimulated IP generation by 57% and 54%, respectively. Taken together our data demonstrate that both α_{1A}- and α_{1B}-adrenoceptors couple to IP generation in rat renal slices. The coupling of α_{1B}-adrenoceptors, however, appears to be more efficient since α_{1A}-selective agonists stimulated IP accumulation pre-dominantly via α_{1B}-adrenoceptors.

Lomasney, J.W., Cotecchia, S., Lorenz, W., Leung, W.-Y., Schwinn, D.A., Yang-Feng, T.L., Brownstein, M., Lefkowitz, R.J. & Caron, M.G. (1991) J. Biol. Chem. **266**, 6365-6369.
Michel, M.C., Hanft, G. & Gross, G. (1990) Naunyn-Schmiedeberg's Arch. Pharmacol. **341**, 385-387.

185P DIFFERENTIAL EFFECTS OF PHOSPHODIESTERASE ISOENZYME INHIBITORS (PDEIs) IN REVERSING INDUCED TONE IN GUINEA-PIG TRACHEA

B. Hughes, N. Gozzard & G. Higgs., Celltech Ltd, 216 Bath Road, Slough, Berkshire.

Harris et al., (1989) have shown that the PDEIs have differential effects in reversing spasmogen induced tone in guinea pig trachea. To determine whether the differential selectivity was dependent on the spasmogen employed and the possible mechanism underlying it, we have investigated the ability of the PDEIs rolipram and Ro20-1724 (PDE IV inhibitors) SKF94120 (PDE III inhibitor) and isobutylmethylxanthine, (IBMX non-selective PDE inhibitor) and zaprinast (PDE V inhibitor) to reverse tone induced by histamine or carbachol.

Six ring tracheal segments were removed from male guinea pigs and mounted in organ baths containing Krebs-Henseleit solution and gassed with 95% O_2 and 5% CO_2 under a resting tension of 2g. Tone was induced with either carbachol (0.3μM) or histamine (10μM). Cumulative concentration response curves to the PDEIs were constructed and isometric recordings of changes in tension were made. Concentration response curves were also constructed in the presence of indomethacin (2.8μM) and for rolipram in the presence of ranitidine (10μM). A single curve was performed on each tissue and results were expressed as a % of the maximal relaxation induced by 300μM papavarine. The effects of the PDEIs in reversing tone is shown in Table I (below).

	CONTROL		INDOMETHACIN	
PDEI	HISTAMINE(H)	CARBACHOL (C)	HISTAMINE (H)	CARBACHOL (C)
IBMX	2.0±1.1	1.8±0.3	2.8±0.5	16.3±1.3
SKF94120	2.7±1.8	6.6±1.8	8.3±3.3	17.6±3.5
Rolipram	0.1±0.1	12.3±2.7	13.2±3.8#	33.0±2.8
Ro20-1724	0.2±0·1†	25.2±2.5	11.9±4·7#†	36.3±4.8
Zaprinast	10.2±1.4	27.5±0.9	20.0±2.0	20.0±2.3

Values are EC50 (μM) ± s.e.mean (n=6) †EC25 #P<0.05 compared to control values

The PDE IV inhibitors exhibited an increased potency in reversing histamine induced tone which was not affected by 10μM ranitidine (EC50 for Rolipram 0.30±0.2μM). Indomethacin reduced the potency of all the PDEIs in reversing C induced contraction with the exception of the weakly acting zaprinast but caused significant reduction in the potency of the PDE IV inhibitors in reversing H induced tone.

These data support those of Berry et al (1991), in suggesting that PDE IV inhibitors play an important role in controlling guinea pig tracheal tone by modulating cAMP turnover in a specific pool. Elevation of cAMP within this pool can be mediated by prostanoids.

1) Berry, J.L., Elliot, K.R.F., Foster, R.W. & Small, R.C. (1991) Br.J.Pharmac. Glasgow Meeting P110
2) Harris, A.L., Connell M.J., Ferguson, G.W., Wallace, A.M., Gordon, R.J., Pagani, E.D., & Silver, P.J., (1989) J.Pharmac.Exp Therap. 251, 199-206.

186P STIMULATION OF EOSINOPHIL CYCLIC AMP PHOSPHODIESTERASE (PDE) BY VANADATE/GLUTATHIONE

John E. Souness & Lisa C. Scott (Introduced by David Riddell) Dagenham Research Centre, Rhône-Poulenc Rorer Ltd, Dagenham, Essex RM10 7XS.

Cyclic AMP PDE (Type III) in rat adipocytes is activated by a complex of sodium vanadate and glutathione (V/GSH) (Souness et al, 1985). We have now undertaken studies to examine the action of V/GSH on Type IV cyclic AMP PDE from guinea pig eosinophils.

Cells were harvested from the peritoneal cavity of horse-serum treated (0.5ml i.p, bi-weekly) guinea pigs and centrifuged on discontinuous metrazamide gradients. Eosinophils were homogenized in isotonic buffer containing a cocktail of protease inhibitors. The homogenate was centrifuged at 105,000 x g for 60 min and the pellet resuspended in homogenization buffer. Particulate PDE activity was solubilized with homogenization buffer containing deoxycholate (0.5%) and NaCl (100mM). Solubilized cyclic AMP PDE was partially purified by DEAE - trisacryl anion-exchange chromatography. V/GSH was prepared as described by Souness et al (1985) and PDE activity was measured according to Thompson et al (1979).

V/GSH reversibly activated bound, particulate Type IV PDE by increasing Vmax 4-fold without influencing the Km. The sensitivity of Type IV PDE to inhibition by rolipram and denbufylline, but not trequinsin, was increased 12- and 15- fold, respectively, by V/GSH. Solubilized Type IV PDE was activated only 1.7-fold by V/GSH, an effect which was again due to an increase in Vmax with little change in Km. Solubilization increased the inhibitory potencies of rolipram (IC_{50}'s = 0.2µM bound, 0.02µM solubilized) and denbufylline (IC_{50}'s = 0.4µM bound, 0.04µM solubilized), but not trequinsin (IC_{50}'s = 0.3µM bound, 0.8µM solubilized). In contrast to its effect on the bound, particulate enzyme, V/GSH did not influence the inhibitory potencies of rolipram and denbufylline against the solubilized PDE. Partially purified Type IV PDE was not influenced by V/GSH.

The results suggest that V/GSH activates eosinophil Type IV PDE by an indirect mechanism involving predominantly membrane - bound components that may be biologically important enzyme regulatory elements. This activation is associated with an increase in the inhibitory potencies of some, but not all, PDE inhibitors against the bound, particulate enzyme, suggesting different sites of action.

Souness, J.E., Thompson, W.J. & Strada, S.J. (1985) J. Cyclic Nucleotide and Protein Phosphorylation Res. 10 69-92.
Thompson, W.J., Terasaki, W., Epstein, P.M. & Strada, S.J. (1979) Adv. Cyclic Nucleotide Res. 10 69-92.

187P ANAESTHETIC POTENCY OF PROPOFOL IS REDUCED BY HYDROSTATIC PRESSURE

Peter H. Tonner, David M. Poppers & Keith W. Miller, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114, USA

The reversal of anaesthesia by the application of hydrostatic pressure has been reported for all classes of general anaesthetics. Propofol (2,6-diisopropylphenol) is an alkyl substituted phenol that defines a new class of clinically available intravenous anaesthetics. The general anaesthetic potency of propofol has been reported recently (Tonner et al., 1991). Here we determine if and to what degree the anaesthetic potency of propofol is dependent upon hydrostatic pressure, since it has been shown that some i.v.-anaesthetics exhibit different degrees of pressure reversal (Halsey et al., 1986).

Rana pipiens tadpoles were used to determine the anaesthetic potency of propofol at different hydrostatic pressures. Tadpoles were placed in a 0.34 l steel cylindrical pressure chamber. The pressure chamber was completely filled with water saturated with oxygen and containing different concentrations of pure propofol. Hydrostatic pressure was applied manually by a hydraulic pump in steps of 30 atm. At each pressure, the pressure chamber was disconnected from the hydraulic pump and rotated manually. The tadpoles (5 tadpoles/concentration) were viewed through a 2.5 cm thick plexiglass window. Each experiment was conducted twice. Control groups with non-anaesthetized and with urethane-anaesthetized animals were treated in the same way. Anaesthesia was defined as loss of rolling response (LRR). The results were fitted to a logistic function according to the method of Waud (1972) for quantal biological responses.

At pressures up to 120 atm the propofol concentration vs. LRR curves were shifted to the right with increasing pressure. The half maximal effects (EC_{50}) increased linearly with pressure from 2.55 ± 0.21 μM at 1 atm to 4.06 ± 0.41 μM at 120 atm. At pressures higher than 120 atm the increased excitability of the tadpoles in the presence of propofol made it difficult to distinguish purposeful movements from involuntary movements.

The anaesthetic potency of propofol is reduced by hydrostatic pressure, indicating its mechanism of action is similar to that of other general anaesthetics. This is consistent with the fact that propofol, like most general anaesthetics, does obey the Meyer-Overton rule (Tonner et al., 1991). Although the phenomenon of pressure reversal of anaesthesia has been described for a wide variety of substances, the characteristics of the reduction of anaesthetic potency for i.v.-anaesthetics are still unresolved. Halsey et al. (1986) reported that Althesin and methohexitone pressure reversed nonlinearly to 70 atm with Althesin's EC_{50} increasing 4.1 fold and methohexitone's EC_{50} increasing 6.9 fold at 70 atm (above this pressure the EC_{50}s exhibited a plateau). Propofol's and urethane's EC_{50} increased linearly up to 120 atm with values of 1.3 and 2.0 at 70 atm, respectively. Thus propofol is less antagonized by pressure than other i.v.-anaesthetics.

This research was supported by GM15904. P.H.T. is a NATO research fellow (#300/402/501/1).

Halsey, M.J., Wardley-Smith, B. & Wood, S. (1986) Br. J. Pharmac. **89**, 299-305
Tonner, P.H., Poppers, D., Alifimoff, J. & Miller, K.W. (1991) Anesthesiology **75**, 3a, A 586
Waud, D.R. (1972) J. Pharmacol. Exp. Ther. **183**, 577-607

188P EFFECTS OF PERFLUOROCHEMICALS ON LIVER MICROSOMAL ENZYMES IN RATS

K.C. Lowe & F.H. Armstrong, Department of Life Science, University of Nottingham, Nottingham NG7 2RD.

Emulsified perfluorochemicals (PFCs) are being evaluated for therapeutic tissue oxygenation (Lowe, 1991). A commercial formulation, *Fluosol* (Alpha Therapeutic, U.K.) has recently been approved as an oxygen-carrying perfusate during clinical coronary angioplasty. While previous work has examined the effects of complex emulsions on liver microsomal enzymes (Armstrong & Lowe, 1989, 1991), there have been relatively few studies with their component PFC oils. We have therefore investigated the effects of injecting low doses of different PFCs on rat liver cytochromes P-450 (P-450) concentrations and aryl esterase (LAE) activity.

Male or female rats (body weight (b.w.): 220-270 g; n = 54) were injected intraperitoneally with 20 ml/kg b.w. of either (i) saline (0.9% w/v NaCl); (ii) corn oil (Sigma, U.K.); (iii) perfluorodecalin (FDC); (iv) perfluorotripropylamine (FTPA); (v) perfluorotributylamine (FTBA); (vi) a C-16 oil, perfluoroperhydrofluoranthrene (Rhône-Poulenc, U.K.) or (vii) perfluorooctylbromide (PFOB; I.C.I., U.K.). 72 h after injection, animals were killed and their livers removed, weighed and homogenized in 1.15% (w/v) KCl solution buffered with Tris-HCl. Hepatic microsomal protein and P-450 were measured by spectrophotometric assays (Armstrong & Lowe, 1991); LAE was measured by a modification of the method of Bosmann (1972) with 25 mM indoxylacetate as substrate (Armstrong & Lowe, 1991).

Mean liver weight increased by 12% ($P < 0.05$) following injection of FDC in female rats, but was otherwise unchanged in both male and female rats injected with PFCs. Mean liver P-450 in male rats injected with FDC (0.56 ± 0.06 nmol/mg protein) was significantly ($P < 0.05$) greater than in saline-injected controls (0.26 ± 0.04 nmol/mg protein), but was unaffected by other treatments. P-450 concentrations in female rats were unchanged throughout. Mean LAE activity in male rats increased by 63% ($P < 0.01$) and 45% ($P < 0.05$) following injection of C-16 oil and FDC respectively; LAE in female rats was unaltered throughout. These results show that FDC is an effective inducer of hepatic P-450 in male rats as suggested in previous studies with its emulsions (Armstrong & Lowe, 1989, 1991). The LAE response to injection of FDC and the C-16 oil in male rats was more rapid than when a mixed emulsion of these oils was administered (Armstrong & Lowe, 1991), but this probably reflects the correspondingly greater doses of PFCs used here. The absence of changes in liver enzymes following PFOB injection suggests that it may be preferable over FDC as the primary component of concentrated, "second generation" emulsions currently being developed (Lowe, 1991). A further speculation is that emulsions based on FDC, which may induce hepatic microsomal enzymes leading to enhanced metabolism of xenobiotics, could have value in the treatment of drug overdose and poisoning.

Armstrong, F.H. & Lowe, K.C. (1989) Comp. Biochem. Physiol. 94C, 345-349.
Armstrong, F.H. & Lowe, K.C. (1991) Comp. Biochem. Physiol. 99C, 561-565.
Bosmann, H.B. (1972) Biochem. Biophys. Acta 276, 180-191.
Lowe, K.C. (1991) Vox Sang. 60, 129-140.

189P THE EFFECT OF MALARIA INFECTION ON THE GLUCURONIDATION OF AZT AND PARACETAMOL *IN VITRO*

S. Ismail, D.J. Back & G. Edwards. [1]Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L69 3BX and Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool L3 5QA.

While malaria infection (MI) is known to adversely affect Phase I drug metabolism, (Alvares *et al.* 1984) little is known of the effects of MI on Phase II conjugation reactions (Mansor *et al.* 1991). We have investigated the effect of MI on UDP-glucuronyltransferase activity in rat liver microsomes using two model substrates, azidothymidine (AZT) and paracetamol (P). MI was induced in two group of male Wistar rats (n = 6) as described by Mansor *et al.* 1991 to parasitaemias of 10-20% and 25-35% respectively. Controls (n = 6) were uninfected and matched for weight and age. Liver microsomes were prepared by standard methods. Glucuronidation of AZT and P was achieved in microsomal incubations (37°C; 1 hr), containing UDP glucuronic acid (AZT - 10 mM; P - 20 mM), microsomal protein (AZT - 0.8 mg; P - 0.5 mg), $MgCl_2$ (AZT - 25 mM, P - 20 mM), Brij 58 (detergent:protein ratio (AZT - 0.1; P - 0.075), substrate (AZT - 1-16 mM, P - 1-20 mM) and tris-HCl buffer pH 7.4. Reactions were started by addition of UDP glucuronic acid and stopped by addition of acetonitrile (AZT) and $Ba(OH)_2$ and $ZnSO_4$ (P). The glucuronides of P and AZT were determined by HPLC. K_m and V_{max} values were obtained by graphical methods (Table 1).

Table 1. Kinetic parameters for AZT and paracetamol glucuronidation in liver microsomes from control and MI rats

	AZT			Paracetamol		
Parasitaemia	Controls	10-20%	25-35%	Controls	10-20%	25-35%
Km (mM)	13.2 ± 8	16.1 ± 8	18.5 ± 9	18.2 ± 4	9.0 ± 5**	7.6 ± 2**
V_{max} (nmol/min/mg)	1.83 ± 0.9	0.98 ± 0.6	0.93 ± 0.4*	6.7 ± 2	4.0 ± 0.8**	3.5 ± 1**

Values are means ± SD for 6 livers. * $P < 0.05$; ** $P < 0.005$ compared with controls (one-way ANOVA)

We conclude that MI significantly impairs glucuronyl transferase activity but that the effect appears to be dependent upon the substrate used.

Alvares, A.P., Yeng, T.H., Scheibel, L.W. & Hollingdale, M.R. (1984). Mol. Biochem. Parasitol. 13, 277-282.
Mansor, S.M., Edwards, G., Roberts, P.J. & Ward, S.A. (1991). Biochem. Pharmacol. 41, 1707-1711.

190P FEASIBILITY OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS FOR TRANSDERMAL DELIVERY: EFFECT OF VEHICLES *IN VITRO*

M.B. Lambert, A.A.M. Omara, & P.B. Deasy[1], Department of Pharmacology and Therapeutics, Trinity College and [1]Department of Pharmaceutics, School of Pharmacy, Trinity College, Dublin , Ireland.

Gastro-intestinal complications are among the most frequent side-effects associated with orally administred non-steroidal anti-inflammatory drugs (NSAIDs) (Rainsford,1984). This evidence suggests that an effort to deliver NSAIDs through the skin might be of value. Many feasibility studies, first *in vitro*, must be conducted to investigate the release characteristcs of a drug from its formulation before it can be developed in a transdermal therapeutic system (TTS) for systemic delivery. The selection of vehicle has long been recognized to affect bioavilability and partitioning of drug into skin layers (Poulsen *et al.*, 1968). The aim of this work is to carry out preliminary studies on the feasibility of a number of NSAIDs for transdermal delivery. The effects of various vehicles on the release of the selected NSAIDs through hairless mouse skin were investigated *in vitro*. Yellow soft paraffin (YSP), macrogol ointment BP (MO), 3% sodium carboxymethylcellulose (NaCMC) and plastibase (PB) were used, each containing 5% (w/w) of drug. The excised skin sample was assembled on the diffusion cell (Keshary & Chien,1984). The receiver solution was phosphate buffer pH7.4. Samples were assayed by U.V. spectrophotometry.

Release rates of NSAIDs from various vehicles are presented Table1. NaCMC gave much higher release rates over other vehicles and, therefore, was tested at different pH values (5.2-8.2). The faster release rate for each drug was obtained at pH 5.2. No significant differences in the release rate were obtained from YSP, MO, and PB ($p>0.05$,ANOVA). The present findings show that all NSAIDs studied penetrate through the skin at constant-order kinetics and seem to be feasible for further transdermal delivery studies in order to select one or more drug candidates for the development of TTS. To increase drug release rates from 3%NaCMC (pH5.2) system, further studies will address the use of penetration enhancers .

Table1: Release rates (mg cm^{-2} h^{-1}) of NSAIDs from various systems employed

Drug	YSP	MO	PB	NaCMC			
				(5.2)	(6.2)	(7.2)	(8.2)
Biphenylacetic acid	0.236	0.275	0.245	0.347	0.337	0.329	0.319
Diclofenac sodium	0.018	0.027	0.017	0.034	0.033	0.031	0.042
Diclofenac	0.028	0.028	0.020	0.040	0.037	0.035	0.032
Indomethacin	0.021	0.018	0.021	0.024	0.023	0.021	0.019
Piroxicam	0.011	0.013	0.009	0.023	0.022	0.020	0.020

Keshary, P. R., & Chien, Y. W. (1984) *Drug Develop. Ind. Pharm.* **10**, 883
Poulsen, B.J., Young, E., Coquilla, V. , & Katz, M. (1968) *J. Pharm. Sci.* **57**, 928
Rainsford, K. D. (1984) *Trends Pharmacol. Sci.* **5**, 205

191P EFFECTS OF NAFTIDROFURYL ON THE RENAL FUNCTION AND THE SYNTHESIS OF PROSTANOIDS IN ISOLATED PERFUSED KIDNEYS OF SPONTANEOUSLY HYPERTENSIVE RATS

Bei-Li Zhang, Kiao-Ling Liu, Joëlle Sacquet, Daniel Benzoni, *Nicolas Wiernsperger & Jean Sassard. Department of Physiology and Clinical Pharmacology, URA CNRS 606, Faculty of Pharmacy, 8 avenue Rockefeller, 69008 Lyon and *Department of Pharmacology, International Division, Lipha, Lyon, France.

Naftidrofuryl is a vasodilator which blocks 5-HT2 receptors. In addition, it inhibits the release of noradrenaline (NA) and stimulates the synthesis of prostacyclin (PGI2) (Zander et al., 1986). The purpose of the present study was to determine the effects of naftidrofuryl on the renal function and on the synthesis of prostanoids in the isolated perfused kidney of spontaneously hypertensive rat (SHR). Kidneys of 8-week-old male SHR were isolated and perfused at a constant perfusion rate in a single pass system with a Krebs-Henseleit solution containing Haemaccel® (35g/l) as colloid osmotic agent. Three concentrations of naftidrofuryl (5×10^{-9}, 5×10^{-8} and 5×10^{-7} M) were used randomly and their effects were studied before (basal conditions) and after administration of 2 concentrations (3.76 and 6.26×10^{-9} M) of NA.

Under basal conditions, naftidrofuryl did not modify the renal vascular resistances (RVR) and the glomerular filtration rate (GFR), but significantly decreased the fractional sodium reabsorption (RNa) at concentrations of 5×10^{-8} and 5×10^{-7} M (from 94.7 ± 0.6 to 91.4 ± 1 and 89.9 ± 1 % respectively). In addition, it elicited a significant increase in the venous excretion of 6-keto-prostaglandin (PG) F1alpha, the stable breakdown product of PGI2, at the 3 concentrations used (from 0.8 ± 0.1 to 2.0 ± 0.5, 1.6 ± 0.3 and 3.1 ± 0.3 ng/min/g respectively) and of tromboxane (Tx)B2, the stable end product of TxA2, at 5×10^{-7} M only (from 0.25 ± 0.04 to 0.40 ± 0.04 ng/min/g). The 6-keto-PGF1alpha/TxB2 ratio was enhanced by naftidrofuryl. NA dose-dependently increased RVR and GFR, and stimulated the venous excretion of 6-keto-PGF1alpha and TxB2. Naftidrofuryl at the concentrations of 5×10^{-8} and 5×10^{-7} significantly attenuated the effects of NA on RVR and abolished those on GFR. In addition, naftidrofuryl enhanced the stimulation of prostanoid synthesis by NA and markedly increased the 6-keto-PGF1alpha/TxB2 ratio.

In conclusion, this study demonstrates that, on the isolated kidney of SHR, naftidrofuryl 1) decreases the sodium tubular reabsorption; 2) prevents the vasoconstrictor action of NA mostly at the postglomerular site as indicated by the changes in RVR and GFR and 3) stimulates the renal production of PGI2 more than that of TxA2.

Zander J.F., Aarhus L.L., Katusic Z.S., Rubanyi G.M. & Vanhoutte P.M. (1986) *J. Pharmacol. Exp. Ther.* 239 : 760-767.

192P COMPARISON OF THE VASCULAR RESPONSES TO DOPEXAMINE AND FENOLDOPAM IN NORADRENALINE- AND U46619-CONSTRICTED RAT KIDNEY

S.W. Martin & K.J. Broadley, Department of Pharmacology, Welsh School of Pharmacy, University of Wales, PO Box 13, Cardiff CF1 3XF

Dopexamine (DPX) (Brown et al., 1985) and fenoldopam (FEN) (Hahn et al., 1982) are selective agonists for dopamine D_1-receptors. Renal arteriolar vasodilatation by dopamine is mediated via D_1-receptors (Schmidt et al., 1987). In the present study we evaluated the renal vasodilator responses to DPX and FEN in a perfused rat kidney preparation.

Male Wistar rats (150-250g) were anaesthetized with pentobarbitone (60mgkg^{-1}i.p.). The abdominal aorta was cannulated retrogradely with the cannula tip opposite the left renal artery. The upper aorta and mesenteric artery were ligated and the vena cava cut for perfusate escape. Both kidneys were thus perfused at 10ml min^{-1} with Krebs solution (37.5°C) gassed with CO_2 (5%) in O_2 and perfusion pressure recorded. The thorax was opened. Perfusion pressure was raised with either noradrenaline (1µg ml^{-1}) or the thromboxane analogue, U46619 (50ng ml^{-1}) to 205.9±2.7 and 161±5.8mmHg respectively.

In noradrenaline-constricted preparations, bolus doses of DPX and FEN (in the presence of propranolol, 10^{-6}M) caused dose-related falls in renal perfusion pressure with ED50 values of 11.6 (9.3-14.6) and 22.5 (17.0-29.8)µg and maxima of 117±3.4 and 97±7.1mmHg respectively. These compared with a maximum β-adrenoceptor-mediated vasodilatation to isoprenaline of 18.4±2.7mmHg. The responses to DPX and FEN were not antagonized by the D_1-antagonist SCH23390 (10^{-6}M). The ED50 values (12.0 (9.9-14.6) and 19.1 (13.9-26)µg) and maxima (114±5 and 112±4mmHg) for DPX and FEN in its presence were not significantly different from those in its absence.

In U46619-constricted kidneys, DPX and FEN (in the presence of propranolol, 10^{-6}M and prazosin, 10^{-6}M) also caused dose-related falls in perfusion pressure. However, the maxima (48.0±6.0 and 42±5.0mmHg) andED50 values (2.5 (1.8-3.5)µg and 12.1 (7.7-18.8)ng) were significantly less than in noradrenaline-constricted kidneys. Furthermore, the responses were now antagonized by SCH23390 (10^{-9}M). The curves were displaced in non-parallel fashion with the maxima for DPX and FEN being depressed to 27.1±5.4 and 28±4.6% respectively of the pre-antagonist maximum (corrected for time-dependent changes from controls).

These results suggest that the renal vasodilation by DPX and FEN is via D_1-receptors when constricted by U46619. When constricted by noradrenaline, it is not D_1-receptor-mediated but probably due to α-receptor blockade since prazosin similarly dilated noradrenaline- but not U46619-constricted tissues.

This work is supported by Fisons Pharmaceuticals.

Brown, R.A., Dixon, J., Farmer, J.B., Hall, J.C., Humphries, R.G., Ince, F., O'Connor, S.E., Simpson, W.T. & Smith, G.W. (1985) Br. J. Pharmacol., 85, 599-608.
Hahn, R.A., Wardell, J.R., Sarau, H.M. & Ridley, P.T. (1982) J. Pharmacol. Exp. Ther., 223, 305-313.
Schmidt, M., Krieger, J.P., Giesen-Crouse, E.M. & Imbs, J.L. (1987) Arch. Int. Pharmacodyn. Ther., 286, 195-205.

193P CHLORIDE ANION CONCENTRATION AS A DETERMINANT OF RENAL VASCULAR RESPONSIVENESS TO VASOCONSTRICTOR AGENTS

C.P Quilley, Y-S R. Lin & J.C. McGiff. Dept Pharmacology, New York Medical College, Valhalla, NY 10595. U.S.A.

Increased chloride (Cl^-) concentration elevates renal vascular resistance (RVR) and lowers GFR (Wilcox, 1983). In salt-sensitive hypertension there is a requirement for Cl^- (Boegehold and Kotchen, 1989) which contributes to the hypertension by increasing RVR (Passmore and Jimenez, 1990). One possible mechanism, is that Cl^- modulates responsiveness of the kidney to pressor agents. We therefore studied the effect of high (117mM) and low (87 mM) Cl^- concentrations on vascular responses of the rat kidney to angiotensin II (AII), phenylephrine, (PE), and arginine vasopressin (AVP).

The right kidney was perfused in situ with oxygenated Krebs'-Henseleit buffer at 37°C. Basal perfusion pressure (PP) was maintained at ~ 90 mmHg by adjustments in flow rate. After equilibration for 10-15 min with 117mM Cl^-, a dose-response curve to either AII (2-100 ng), PE (0.1-100 ng) or AVP (1-150 ng) was constructed. One group of kidneys was then switched to low Cl^- buffer, (NaCl was substituted with a mixture of Na salts acetate, propionate and methylsulphonate), and the dose response curve repeated. For AII, injections were repeated during reperfusion with high Cl^- to evaluate recovery from the effect of low Cl^-. The second group of kidneys was perfused throughout with 117mM Cl^- to serve as a time-control.

Renal vasoconstrictor responses to all three agonists were lower in the presence of 87 mM Cl^-. Responses were not different between the two groups during perfusion with 117mM Cl^-. For AII, reduced vasoactivity with low Cl^- was evident both in terms of the threshold dose and on the linear part of the dose response curve but not for maximum responses and was completely reversed upon reperfusion at 117mM Cl^-. With 20 ng AII, the mmHg increase in PP of 105 ± 11 was reduced to 65 ± 13 mmHg with 87mM Cl^- and reversed to 126 ± 17 upon reperfusion with 117mM Cl^- compared to 92 ± 8, 102 ± 11 and 122 ± 10, respectively, in the 117mM Cl^- time-control. High Cl^- progressively increased the vasoconstrictor effect of lower doses of AII; i.e. 5 ng increased PP by 19 ± 3, 40 ± 10 and 66 ± 14 mmHg for consecutive curves. In contrast to the increases in AII responses at 117mM Cl^-, tachyphylaxis occurred with AVP. However, low Cl^- caused a greater reduction in responsiveness compared to high Cl^-. Similar to AII, this diminished response was evident at the lower doses but not for the maximum response e.g. 37 and 101% of the time-control at 3 and 150 ng, respectively. Low Cl^- had less of an attenuating effect on PE vasoactivity. Compared to the PE time-control, which like AVP showed tachyphylaxis, there was a 10-35% reduction to all doses of PE. Thus, sensitivity of the renal vasculature to vasoconstrictors, in particular sensitivity to AII, can be modulated by Cl^- concentration. This altered vascular responsiveness may play a role in the effect of Cl^- on renal hemodynamics and contribute to salt-sensitive hypertension.

Boegehold, M.A. & Kotchen, T.A. (1989) Hypertension **14**,579-583.
Passmore, J.C. & Jimenez, A.E. (1990) Proc.Soc. Exp. Biol. Med. **194**,283-288.
Wilcox, C.S. (1983) J. Clin. Invest **71**,726-735.

194P CHLORIDE ANION CONCENTRATION AS A DETERMINANT OF RENAL VASCULAR RESPONSIVENES VASOCONSTRICTOR AGENTS

M.A. Carroll, M. Balazy, P. Margiotta and J.C. McGiff. Department of Pharmacology, New York Medical College, Valhalla, New York 10595. USA.

We have shown that the vasoactive cytochrome P450-arachidonate metabolites are those that are substrates for cyclooxygenase (Carroll *et al.*, in press). Thus, in rabbit isolated kidneys preconstricted with phenylephrine, the vasodilator responses to 5,6-EET and 19- and 20- hydroxyeicosatetraenoic acid (HETE) are abolished with inhibition of cyclooxygenase. In particular, 5,6-EET, the most potent vasodilator and an inhibitor of AA-induced platelet aggregation (Balazy, in press), can be metabolized by both cyclooxygenase and lipoxygenases to epoxy analogs of prostaglandin (PG) I_1, E_1 (Oliw, 1984) and TxB_1, and HETEs (Balazy, in press), respectively. We have now investigated the metabolism of 5,6-EET by rabbit kidneys in order to characterize those metabolites that may account for its vasoactivity.

After anaesthesia, kidneys were excised from male NZW rabbits and perfused with oxygenated Krebs'-Henseleit buffer (37°C) at a constant flow of 1 ml/g/min. Perfusion pressure was raised from 40 mmHg, to 90-100 mmHg with phenylephrine (2-3 μM). The 5,6-EET (1, 5 and 10μg) was injected close-arterially (n=4) and the effluent collected throughout the response period. Basal collections following injection of 100μl vehicle were made at 20 min intervals after each 5,6-EET injection. Prior to acidic extraction with ethyl acetate, 1 ng/ml of deuterated 6-keto-$PGF_{1\alpha}$ and PGE_2 were added as internal standards. The extracts were separated by TLC (A9 system) and zones corresponding to 6-keto-$PGF_{1\alpha}$ and PGE_2 were extracted and derivatized for gas chromatographic-mass spec- trometric analysis using a negative ion chemical ionization mode. Pentafluorobenzyl esters of trimethylsilyl ethers were prepared and PGI metabolites were monitored at 585 and 589 atomic mass units (amu) and PGE metabolites at 511, 495 and 499 amu.

Injection of 1,5 and 10μg of 5,6-EET resulted in dose-related decreases in perfusion pressure of 12 ± 6, 21 ± 4 and 26 ± 4 mmHg, respectively. Basal perfusates contained 6-keto-$PGF_{1\alpha}$ and PGE_2, the levels remaining constant throughout the experiment, however, 5,6-EET stimulated the release of 6-keto-$PGF_{1\alpha}$ and PGE_2 by twofold or more. In addition, perfusates contained 5-hydroxy-PGI_1 and 5,6-epoxy-PGE_1, cyclooxygenase metabolites of 5,6-EET, in response to 5,6-EET injection.

Thus, injection of 5,6-EET into the rabbit isolated preconstricted kidney results in formation of cyclooxygenase metabolites of 5,6-EET and promoted release of vasodilator prostaglandins.

Carroll, M.A. Garcia, M.P., Falck, J.R., and McGiff, J.C. J. Pharmacol. Exp. Ther. *in press.*
Balazy J. Biol. Chem. *in press*
Oliw, E.H (1984) J. Biol. Chem. **259**,2716-2721.

195P LUZINDOLE (2-BENZYL N-ACETYLTRYPTAMINE) IS A COMPETITIVE MELATONIN RECEPTOR ANTAGONIST IN *XENOPUS LAEVIS* MELANOPHORES

D.Sugden, Physiology Group, Biomedical Sciences Division, King's College London, London W8 7AH.

Melatonin (5-methoxy N-acetyltryptamine) regulates seasonal changes in reproduction in photoperiodic species, synchronizes circadian rhythms and acts as a local hormone in the eye. In addition, in amphibians such as Xenopus laevis pineal melatonin adapts skin colour to background illumination by condensing the melanin-containing pigment granules in dermal melanophores. This model has been used to study structure-activity relationships at the melatonin receptor (Sugden, 1991) and is a useful technique for screening putative antagonists.

Several compounds reported to be melatonin antagonists either in vivo or in vitro were tested. Compounds were added to cultures of melanophores which had differentiated from fragments of Xenopus embryo neural crest (Messenger & Warner, 1977). Responses were quantitated in single cells by measuring the area occupied by pigment. Antagonists were tested for their ability to reverse pigment aggregation induced by a maximal concentration of melatonin (10 nM). Neither 6-methoxy-2-benzoxazolinone (Sanders et al., 1981) nor N-(2,4-dinitrophenyl)-5-methoxytryptamine (Zisapel & Laudon, 1987) had agonist or antagonist activity upto 10 μM. N-acetyl- and N-butanoyltryptamine (10 and 1 μM respectively) appeared to be partial agonists in that aggregation was clearly induced but only in a fraction of the cells tested (2/12 and 3/12 respectively); similarly these compounds reversed melatonin-induced aggregation in some cells (4/12 and 3/8 respectively). Luzindole (2-benzyl N-acetyltryptamine; Dubocovich, 1988) did not have agonist activity upto 10 μM but did produce a concentration-dependent reversal of melatonin-induced aggregation. Luzindole (1 and 10 μM) produced a rightward shift in the concentration-response curve to melatonin when added to the cultures prior to melatonin suggesting that this compound is a competitive melatonin receptor antagonist.

Dubocovich,M.L. (1988) J.Pharm.Exp.Ther. **246**, 902-910.
Messenger,E.A. & Warner,A.E. (1977) Br.J.Pharmacol. **61**, 607-614.
Sanders,E.H., Gardner,P.D., Berger,P.J. & Negus,N.C. (1981) Science **214**, 67-69.
Zisapel,N. & Laudon,M. (1987) Eur.J.Pharmacol. **136**, 259-260.
Sugden,D. (1991) Br.J.Pharmacol. **104**, 922-927.

196P SPECIFICITY OF OESTROGEN RECEPTORS IN RAT THYMUS CYTOSOL

E. de F. Bridges & B.D. Greenstein, Division of Pharmacology, United Medical and Dental Schools, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH.

Oestradiol exerts its effects through specific receptor proteins in target organs. The thymus may be a target tissue since it is severely atrophied after oestradiol administration (Fitzpatrick and Greenstein, 1987). Oestrogen receptors have not satisfactorily been characterised in rat thymus and, in the present study, the ligand specificity of oestrogen-binding proteins was examined in thymus and uterus cytosols using a wide range of steroids.

High speed cytosols were prepared from thymus and uterus of 21-23-day old female Wistar CSE rats, and assayed for oestrogen receptors as previously described (Ginsburg *et al*, 1974). Some incubations also contained one of a series of unlabelled steroids, and the degree of competitive displacement of [3H]-oestradiol yielded estimates of the molar dissociation constant (K_{Di}) of the inhibitor (Ginsburg *et al*, 1977).

The relative affinity for the steroids were (i) Thymus: Diethylstilboestol (DES) >> oestradiol-17b (E_2-17b)>> E_2-17a = oestriol (E_3) >> oestrone (E_1) >> testosterone (T) = corticosterone (C) >> progesterone (P); (ii) Uterus: DES = E_2 >> E_2-17a= E_1=E_3 >> T >> P = C. Receptors in both tissues exhibited identical affinity for E_2-17-b, E_1, T and P but the thymus receptor showed a significantly higher affinity for DES, E_2-17a, E_3, and C ($P< 0.001$; Student's t-test). For both tissues, the K_{Di} of the potent oestrogens DES and E_2-17b was of the order of 10^{-10}M, while for other steroids the K_{Di} was of the order of 5nM or greater.

From these results, it is concluded that oestradiol- binding macromolecules, highly specific for oestrogens are present in the thymus and may be specific oestrogen receptors. This strengthens the case for assuming that the atrophic effect of oestradiol on the thymus is a direct one. The different pattern of the ligand specificity between thymus and uterus oestrogen receptors suggests that the two may not be the same and the possibility of a functional difference should be explored.

Fitzpatrick, F.T.A. & Greenstein, B.D. (1987). J. Endocr. **113**, 51-55.
Ginsburg, M., Greenstein, B.D., MacLusky, N.J., Morris, I.D. & Thomas, P.J. (1974). Steroids **23**, 773-792.
Ginsburg, M., MacLusky, N.J., Morris, I.D. & Thomas, P.J. (1977). Br. J. Pharmacol. **59**, 397-402.

197P TEMPORAL INVOLVEMENT OF THE RENIN-ANGIOTENSIN SYSTEM IN HYPERTENSION PRODUCED BY LEFT RENAL ARTERY LIGATION IN CONSCIOUS RATS

A. Travers & A. Hilditch, Department of Peripheral Pharmacology, Glaxo Group Research Ltd., Ware, Herts., U.K.

Seven days after left renal artery ligation in the rat, resting blood pressure and plasma renin activity are elevated (Cangiano et al., 1979). In this study, we have investigated the temporal influence of the renin-angiotensin system in this model by determining the hypotensive activities of the ACE inhibitor, enalapril and the angiotensin receptor antagonist, DuP753 (losartan, Chiu et al., 1990) at periods up to 27 days after ligation.

Diastolic blood pressure (DBP) was measured via a carotid artery catheter in conscious rats that had had one renal artery ligated 6, 13 or 27 days previously. Only those animals with a DBP of 140mmHg or more were selected for experimentation, and measurements were made 1, 3, 5, 7 and 24 hours after administration of enalapril or DuP753 (both at 3mg kg^{-1} ip). DBP in 6, 13 and 27 day renal artery ligated hypertensive (RALH) rats was greater than in 6 day sham-ligated controls. Enalapril or DuP753 had little effect in sham-ligated animals, but in 6 and 13 day RALH rats, produced marked reductions in DBP with maximum effects 5-7h after administration. DBP was reduced to a level below that seen after enalapril or DuP753 in the sham-ligated controls. In 27 day RALH rats, enalapril and DuP753 also reduced DBP, but their hypotensive effects were significantly reduced ($P<0.05$). Results are shown in Table 1.

Table 1. Basal and peak changes in DBP±sem (mmHg) after administration of enalapril or DuP753 (3mg kg^{-1} ip) to RALH rats (n=5-8).

Treatment	6 day sham-ligation		6 day ligation		13 day ligation		27 day ligation	
	Basal	Change	Basal	Change	Basal	Change	Basal	Change
Enalapril	115±3	-7±3	161±3	-92±6	159±5	-88±8	150±2	-31±13
DuP753	102±2	-5±2	159±4	-84±9	163±4	-92±9	157±4	-47±15

In order to study the development of the hypertension, enalapril (3mg kg^{-1} ip) was administered to rats which had been ligated 2 or 4 days previously. Since DBP was still increasing, all animals were used. Resting DBP was significantly lower ($P<0.01$) than in 6 day RALH rats, but enalapril reduced DBP by 36±5 and 48±9mmHg, respectively, to below blood pressure values seen after enalapril administration to normotensive, sham-ligated controls.

In conclusion, the hypotensive effects of enalapril and DuP753 in ligated rats, suggests that activation of the renin angiotensin system contributes to the rise in DBP to a greater extent in 6-13 day than in 27 day RALH rats.

Cangiano, J.L., Rodriguez-Sargent, C. & Martinez-Maldonado, M. (1979) J. Pharmacol. Exp. Ther. **208**, 310-313.
Chiu, A.T., McCall, D.E., Price, W.A., Wong, P.C., Carini, D.J., Duncia, J.V., Johnson, A.L., Wexler, R.R., Yoo, S.E. & Timmermans, P.B.M.W.M. (1990) J. Pharmacol. Exp. Ther. **252**, 711-718.

198P EFFECTS OF LONG-TERM ADMINISTRATION OF THE ACE INHIBITOR, SPIRAPRIL, ON DEVELOPMENT OF HYPERTENSION AND MYOCARDIAL HYPERTROPHY IN SPONTANEOUSLY HYPERTENSIVE RATS

A. Monopoli,E. Ongini,C. Lagrasta[1] and G. Olivetti[1], Schering-Plough S.p.A., Research Labs Comazzo, Milan and [1]Dept. of Pathology, Univ. of Parma, Italy (Introduced by G.C. Folco)

Left ventricular hypertrophy (LVH) is a complex biological process which involves the architectural remodelling of the heart in response to an increase of work load. Accumulation of collagen within the interstitial and vascular compartments of the myocardium has been observed in experimental models of hypertension and in humans (Tanaka et al., 1987; Jalil et al., 1989). Many factors other than blood pressure are involved in the development or reversal of LVH. In particular, the renin-angiotensin system appears to play a relevant role in mediating cardiac cell growth (Schelling et al., 1991). For example, regression of LVH occurs after angiotensin converting enzyme (ACE) inhibition. Spirapril is a potent orally active ACE inhibitor, which lowers blood pressure in different models of hypertension (Baum et al.,1987). The present study was designed to determine whether spirapril is able to preserve myocardial structure from adverse effects of hypertension by reducing LV load in young spontaneously hypertensive rats (SHRs).

One-month old SHRs received spirapril at 5 mg/kg in the drinking water for 3 months. Age-matched SHRs were used as controls. Arterial pressure and heart rate were measured weekly by the tail-cuff technique. The hearts were then fixed by perfusion, cut into right and left ventricle (including the septum) and weighed. Myocardial damage, as evaluated by the extension of fibrotic tissue, was determined by quantitative morphometric analysis. Control SHRs developed hypertension throughout the 3-month period (from 120±3 to 209±2 mmHg). Treatment with spirapril reduced the rise of blood pressure (from 115±2 to 163±3 mmHg; -22% as compared with controls) and did not influence heart rate. The weight of LV decreased by 20% ($p<0.01$) and wall thickness by 23% ($p<0.01$). The amount of replacement fibrosis was significantly reduced in treated SHRs (-68%; $p<0.01$). These findings indicate that chronic treatment with spirapril in the early stages of development of hypertension is able to prevent cardiac hypertrophy and preserve myocardial structure.

Baum,T., Watkins,R.W., Sybertz,E.J., Ahn,H.S., Nelson,S., Coleman,W., Tedesco,R. Pula,K.K., Rivelli,M. and Sabin, C. (1987) Arch. Int. Pharmacodyn.,286,230-245.
Jalil,J.E., Doering,C.W., Janicki,J.S., Pick,R., Shroff,S. and Weber,K.T. (1989) Cir.Res. 64,1041-1050.
Tanaka,M., Fujiwara,H., Onodera,T., Wu,D., Matsuda,M., Hamashime,Y. and Kawai,C. (1987) Circulation, 75,1130-1139.
Schelling,P., Fischer,H. and Ganten,D. (1991) J. Hyper.9,3-15.

199P EFFECTS OF REPEATED ADMINISTRATION OF SCH 34826, A NEUTRAL ENDOPEPTIDASE INHIBITOR, ON MYOCARDIAL HYPERTROPHY IN THE SPONTANEOUSLY HYPERTENSIVE RAT

A. Monopoli, E. Cigola[1], R. Ricci[1] and E. Ongini, Schering-Plough S.p.A.,Research Labs. I-20060 Comazzo, Milan, and [1]Dept. of Pathology, University of Parma, Italy

Atrial natriuretic peptide (ANP) is a potent vasodilator and natriuretic hormone released from the atria in conditions of pressure or volume overload (Needleman et al.,1989). There is evidence from animal models which develop cardiac hypertrophy and from patients with congestive heart failure that left ventricular hypertrophy (LVH) is associated with increased ANP expression in the ventricles (Lee et al., 1988). Recently, inhibitors of neutral endopeptidase 24.11 (NEP), the major enzyme which inactivates ANP, have been synthesized and found to potentiate the renal and hypotensive effects of ANP. SCH 34826, (S)-N-[N-[2- [(2,2- dimethyl-1,3-dioxolan-4-yl) methoxy]-2-oxo-1-(phenylmethyl) ethyl]-L-phenylalanyl]-ß-alanine, is a potent and selective NEP inhibitor which lowers blood pressure in DOCA-salt rats and potentiates the responses to exogenous infusion of ANP in genetically hypertensive rats (SHRs) (Sybertz et al., 1990). The present study was designed to assess the effect of repeated administration of SCH 34826 on LVH and myocardial damage in SHRs.

Adult SHRs were treated with vehicle or SCH 34826 at 100 mg/kg orally twice daily over a 4-week period. Blood pressure and heart rate were measured weekly by the tail-cuff technique. The hearts were then fixed by perfusion and the weights of the right and left ventricles (including the septum) were recorded. The extent of replacement fibrosis in the left ventricle was determined by morphometric analysis.
The drug SCH 34826 did not affect blood pressure, heart rate, sodium or water excretion. Left ventricular weight decreased by 10% ($p<0.01$) and myocardial fibrosis by 42% ($p<0.01$). These results, showing that chronic inhibition of NEP by SCH 34826 reduces myocardial hypertrophy, support the possible role of ANP in the control of myocardial growth and remodelling.

Lee,R.T., Block,K.D., Pfeffer,J.M., Pfeffer,M.A, Neer,E.J. and Seidman, C.E. (1988) J. Clin. Invest. **81**, 431-434.
Needleman,P., Blaine,E.H., Greenwald,J.E., Michener,M.L., Saper,C.B., Stockmann,P.T. and Tolunay,H.E. (1989) Annu. Rev. Pharmacol. Toxicol. **29**, 23-54.
Sybertz,E.J., Chiu,P.J.S., Vemulapalli,S., Watkins,R.W. and Haslanger,M.F. (1990) Circulation **15**, 152-161.

200P REGIONAL HAEMODYNAMIC EFFECTS OF NORADRENALINE AND TYRAMINE IN CONSCIOUS LONG-EVANS RATS

S.M. Gardiner, P.A. Kemp and T. Bennett. Department of Physiology and Pharmacology, Queen's Medical Centre, Nottingham, NG7 2UH.

We have investigated the possibility that equipressor responses to i.v. noradrenaline (NA) and tyramine (Tyr) are accompanied by different regional haemodynamic changes in conscious, Long Evans rats. Animals were chronically instrumented with pulsed Doppler flow probes and intravascular catheters to monitor renal, mesenteric and hindquarters haemodynamics. All surgery was carried out under sodium methohexitone anaesthesia (60 mg kg^{-1} i.p. supplemented as required for flow probe implantation; 40 mg kg^{-1} i.p. for catheter implantation). Flow probes were implanted 7-14 days before intravascular catheters, and the latter were implanted at least 24 h before experiments were begun. Animals received randomized bolus doses of NA (1and 10 nmol) and Tyr (1 and 10 µmol) separated by at least 20 min. Peak increases in mean arterial blood pressure (MAP) occurred at about 15 s after injection of NA and corresponding haemodynamic variables are shown for NA and Tyr at this time in Table 1.

Table 1: Changes in cardiovascular variables following NA or Tyr in conscious, Long Evans rats (n=8). Values are mean ± s. e. mean.

	NA (1 nmol)	**Tyr** (1 µmol)	**NA** (10 nmol)	**Tyr** (10 µmol)
MAP (mmHg)	37±2	31±4	57±2	62±3
Renal flow (%)	-33±3	-18±4*	-69±7	-21±4*
Mesenteric flow (%)	-60±5	-52±3	-78±4	-71±6
Hindquarters flow (%)	-29±4	-5±8*	-47±8	-45±10
Renal conductance (%)	-50±2	-36±5*	-80±5	-50±4*
Mesenteric conductance (%)	-70±4	-63±2	-85±2	-81±4
Hindquarters conductance (%)	-47±4	-25±8*	-65±5	-65±7

* $P<0.05$ versus NA (Wilcoxon's test)

These results show that equipressor responses to NA and Tyr can be accompanied by significantly different haemodynamic changes. Moreover, the differences may vary according to the dose of the pressor agents used.

201P EVIDENCE THAT THE PRODRUG TRANDOLAPRIL, A NEW ACE INHIBITOR, ALSO DISPLAYS A DIRECT ACE INHIBITORY ACTIVITY

G. Hamon[1], S. Jouquey[1], C. Chevillard[2], M. Mouren[1] and J.P. Stepniewski[1], (1) Centre de Recherches Roussel Uclaf, 111, Route de Noisy, 93230 Romainville, France - (2) INSERM U.300, Faculté de Pharmacie, 34100 Montpellier, France.

It is generally believed that, except for captopril, the active form of angiotensin converting enzyme (ACE) inhibitors are the de-esterified forms, i.e. the diacids, the monoesters being considered as prodrugs. Indeed, for most of the molecules described in the literature, the monoester-monoacid forms show an ACE inhibitory activity which is several orders of potency below that of the diacid forms. The purpose of this study was to compare the *in vitro* and *in vivo* ACE inhibitory activity of trandolapril, a new ACE inhibitor, to that of its diacid metabolite, trandolaprilate. In some experiments, enalapril and enalaprilate were chosen as reference compounds.
ACE inhibitory activity of the compounds was studied *in vitro* on human serum from normotensive volunteers using furylacryloyl-L Phe-Gly-Gly as a substrate according to Harjanne's method (1984). Their enzyme inhibitory potency was also compared on normotensive rat tissue ACE using isolated aorta, atria and heart ventricle preparations ; a radiometric method using hippuryl-His-Leu as a substrate (Chevillard and Saavedra, 1982) was used. In addition, the possible direct inhibitory effect of trandolapril was evaluated *in vivo* in anaesthetized rats pretreated with an esterase inhibitor, triorthotolylphosphate (TOTP)(Gianutsos et al., 1986) (125 mg/kg i.p., 3 injections : 40, 24 and 16 hours before the experiment). The control group received injections of the vehicle only (olive oil 0.2 ml/100 g B.W.). The ACE inhibitory effect of intravenous increasing doses of trandolapril was measured as the antagonism of the pressor effect of 0.5 µg/kg angiotensin I (Ang.I) injections. Plasma levels of trandolapril and trandolaprilate were also measured by specific radio-immunoassays in the same animals.
The IC50's for human serum ACE inhibition by trandolaprilate, trandolapril, enalaprilate and enalapril were respectively (nM) : 0.93 ± 0.35 ; 7.06 ± 2.11 ; 2.85 ± 1.40 and 593 ± 208 (n = 10). However, under these conditions, no hydrolysis of trandolapril could be detected in the serum. The IC50's for rat tissue ACE inhibition were the following (nM) : trandolaprilate (aorta : 1.2 - atrium : 1.0 - ventricle : 0.4) ; trandolapril (aorta : 1.6 - atrium : 1.3 - ventricle : 0.8) ; enalaprilate (aorta : 3.3 - atrium : 2.5 - ventricle : 1.0) ; enalapril (aorta : 150 - atrium : 120 - ventricle : 5.0).
In vivo after treatment with TOTP, the ID50 for Ang.I response blockade by trandolapril was 51.3 ± 9.8 µg/kg (n = 8) as compared to 9.1 ± 1.3 µg/kg (n = 8) in control rats. The plasma levels of trandolaprilate were much lower in TOTP treated animals than in controls. The ID50 an ID80 for Ang.I response blockade were obtained at trandolaprilate plasma levels of 4.1 ± 0.4 and 57.6 ± 5.5 ng/ml respectively in control animals and 1.9 ± 0.2 and 20.7 ± 3.1 ng/ml in TOTP group. In addition, while trandolapril remained undetectable in control animals, high plasma levels were measured in TOTP treated rats.

In conclusion, these results strongly suggest that the hydrolysis of trandolapril into its diacid metabolite is not a mandatory step for producing an inhibition of ACE activity either *in vitro* or *in vivo*.

Harjanne, A. (1984) Clin Chem. **30**, 901-902
Chevillard, C. and Saavedra, J.M. (1982), J. Neurochem. **38**, 281-284
Gianutsos,G., Cohen, S.D., Carlson, G., Heyman, R., Salva, P., Morrow, G. and Hite, G.J. (1986), Toxicol. Applied Pharmacol. **82**, 14-18

202P INCREASED POTENCY OF CONTRACTILE AGONISTS IN ATHEROMA-LIKE VASCULAR LESIONS IN THE RABBIT IS NOT DUE TO ENDOTHELIAL DYSFUNCTION

Heather Giles & S.J. Lansdell, Biochemical Sciences, Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS, U.K.

5-HT and other contractile agonists have frequently been reported to have increased potency in 'atherosclerotic' vessels. In most studies the endothelium has been intact and functional and therefore it is not possible to tell whether the increased reactivity is due to a decreased release of vasodilator substances from the endothelium, or to a change in vascular smooth muscle function. The present study sought to address this issue by constructing concentration-effect curves to contractile agonists in rabbit carotid arteries denuded of endothelium, in which a lesion having some of the characteristics of an atherosclerotic vessel had been induced by placing a silastic collar around the vessel (Booth et al, 1989).

Rabbits were sedated and then anaesthetised and a hollow silastic collar (15mm long 2mm bore) was placed around one carotid artery; the contralateral artery either was similarly treated but the collar removed immediately (sham), or remained undisturbed (control). Seven days after surgery the rabbit was killed and changes in isometric force were recorded from 3mm rings of carotid artery denuded of endothelium. Following treatment with pargyline(0.5mM), and a sighting dose of KCl (80mM), cumulative concentration-effect curves were constructed to 5-HT, noradrenaline, histamine, angiotensin II and the thromboxane mimetic, U-46619. In order to confirm the absence of endothelium, acetylcholine (3µM) was added on completion of the curve.

Responses to KCl were significantly reduced on the collared vessels (0.9±0.1g collar, 3.5±0.2g sham, 5.2±0.2g control), as were the maxima achieved by the other agonists. Addition of agonists to the collared tissues frequently induced rhythmical oscillations in tone and, on occasions, unexpectedly large contractions at low agonist concentrations, resulting in asymetrical concentration-effect curves. There was a trend for an increased potency of all the agonists studied (Table 1), but this was most significant and reproducible for 5-HT. Concentration-effect curves in sham operated vessels were slightly left-shifted, compared with controls.

Table 1
Potency ($log[EC]_{50}$ ± s.e. mean) of contractile agonists
n=4-7
* significantly different from control
+ or sham

	Collar	Sham	Control
5-HT	7.92 ± 0.08*+	7.38 ± 0.07	7.12 ± 0.12
U-46619	8.38 ± 0.12 *+	7.98 ± 0.11	7.95 ± 0.09
Histamine	6.18 ± 0.17*	5.80 ± 0.17	5.52 ± 0.07
Noradrenaline	7.24 ± 0.21	7.04 ± 0.12	6.81 ± 0.07
Angiotensin II	8.73 ± 0.11+	8.30 ± 0.11	8.65 ± 0.03

These data demonstrate that the increased potency of contractile agonists in rabbit collared carotid arteries is not caused by endothelial dysfunction but is due to a change in smooth muscle sensitivity; since the potency of all the agonists was increased it seems possible that up-regulation of a transduction mechanism may be involved.

Booth, R.F.G., Martin, J.F., Honey, A.C., Hassall, D.G., Beesley, J.E. and Moncada S. (1989) Atherosclerosis **76**, 257-268.

203P SIMVASTATIN AFFECTS pH REGULATION OF SV40 TRANSFORMED MRC5 FIBROBLASTS BY INHIBITION OF Na^+/H^+ ANTIPORT ACTIVITY

Joan E. Davies & L. L. Ng, Dept. of Pharmacology, Clinical Sciences Building, Leicester Royal Infirmary, Leicester LE2 7LX.

The rate limiting enzyme of endogenous cholesterol synthesis 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase also controls the synthesis of polyisoprenoids which are important factors for control of cell growth. Cell proliferation may also depend on a permissive effect of the Na^+/H^+ antiport which regulates intracellular pH (pH_i) and cell shape. We have therefore examined changes in pH_i and Na^+/H^+ antiport activity of normal and SV40 virus transformed MRC5 fibroblasts treated for 24 h with a HMG CoA reductase inhibitor, simvastatin (5 mg/l). The dye BCECF (bis-carboxyethylcarboxy fluorescein) was used to determine pH_i, with Na^+/H^+ antiport activity measured after manipulation of pH_i to 6.2 using nigericin and monensin. Buffering capacity (ß) was measured from the change in pH_i after a pulse of NH_4Cl.

Simvastatin had no effect on pH_i or Na^+/H^+ antiport activity of MRC5 fibroblasts, which retained their characteristic shape. In contrast, in simvastatin treated SV40 transformed fibroblasts, pH_i was decreased from 7.22 ± 0.01 (mean ± SEM) to 6.99 ± 0.01 ($P<0.001$). This was due to a lowered Na^+/H^+ antiport activity (controls 42.3 ± 2.2 vs simvastatin 20.3 ± 1.5 mmol/l/min, $P<0.001$). Simvastatin also led to a rounding of cell shape. Concomitant incubation of cells with simvastatin and mevalonate prevented all these changes. The effects of simvastatin were not due to cholesterol depletion, since LDL-cholesterol was present in the culture media.

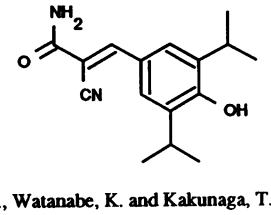
In simvastatin treated cells, mevalonate also led to a reversal of the changes in cell shape, pH_i (to 7.25 ± 0.01, $P<0.001$) and Na^+/H^+ antiport activity (to 37.6 ± 4.7 mmol/l/min, $P<0.001$) after 2 h. Further studies on the reversal of the effect of simvastatin by mevalonate showed that it was not inhibited by cycloheximide (pH_i increased to 7.17 ± 0.01, $P<0.001$ and Na^+/H^+ antiport activity to 38.0 ± 3.5 mmol/l/min, $P<0.001$). Since mevalonate is a precursor of dolichol which is essential for N-enzymatic glycosylation of proteins, tunicamycin was introduced together with mevalonate onto simvastatin treated cells. The pH_i (7.19 ± 0.02, $P<0.001$) and Na^+/H^+ antiport activity (34.2 ± 4.0 mmol/l/min, $P<0.001$) still reverted almost to control levels. The effect of pertussis toxin on mevalonate reversal was determined because the gamma subunit of some G-proteins can be isoprenylated . This toxin failed to inhibit recovery of pH_i (to 7.17 ± 0.03, $P<0.001$) and Na^+/H^+ antiport activity (to 31.9 ± 2.0 mmol/l/min, $P<0.001$). None of these inhibitors affected the recovery in cell shape after incubation of simvastatin treated cells with mevalonate. We conclude that the effects of simvastatin on pH_i, Na^+/H^+ antiport activity and cell shape are post-translational, and not dependent on N-linked enzymatic glycosylation or pertussis toxin sensitive G-proteins.

204P THE ROLE OF TYROSINE PHOSPHORYLATION IN RECEPTOR COUPLING TO PHOSPHOLIPASES IN THE HUMAN NEUTROPHIL

I.J. Uings, N.T. Thompson, R.W. Bonser, G.D. Spacey, R.W. Randall & L.G. Garland, Wellcome Research Laboratories, Beckenham, Kent.

The importance of lipid-derived second messengers in neutrophil activation is now well established. Much recent evidence has also pointed to the involvement of protein tyrosine phosphorylation in the signal transduction events leading to neutrophil activation. In this study we have investigated the role of tyrosine phosphorylation in the activation of phospholipases C and D in the human neutrophil. The tyrosine kinase inhibitors, ST271, ST638 and erbstatin (Shiraishi et al), inhibited fMet-Leu-Phe-stimulated phospholipase D (PLD) with IC_{50} values of 6.7µM±1.7, 25µM±7.6 and 63µM±24 respectively. ST271 also inhibited PAF- and LTB_4-stimulated PLD at the same concentrations (IC_{50} values 9µM ±1.5 and 9µM ±1.0) but was 100 times less effective an inhibitor of phorbol ester-stimulated PLD (IC_{50} value >100µM). These observations indicate that ST271 is acting neither as a receptor antagonist, nor as a PLD inhibitor, but is inhibiting the mechanism by which the receptors couple to the phospholipase. In contrast, the protein kinase C inhibitor, Ro-31-8220 (Twomey et al), inhibited phorbol ester-stimulated PLD (IC_{50} 0.3µM) but did not inhibit fMet-Leu-Phe-stimulated PLD at concentrations up to 30µM, arguing against the involvement of protein kinase C in the receptor-mediated activation of PLD.

ST271 had little effect on fMet-Leu-Phe-stimulated inositol 1,4,5-trisphosphate production at concentrations up to 100µM, while 100µM ST271 inhibited all increases in protein tyrosine phosphorylation stimulated by fMet-Leu-Phe. In addition the phosphotyrosine phosphatase inhibitor, pervanadate, stimulated both tyrosine phosphorylation and PLD activity. These results suggest that tyrosine phosphorylation is involved in the coupling of the fMet-Leu-Phe receptor to phospholipase D but not phospholipase C in the human neutrophil.



ST271

Shiraishi, T., Owada, M.K., Tatsuka, M., Yamashita, T., Watanabe, K. and Kakunaga, T. (1989) Cancer Res. 49, 2374-2378
Twomey, B., Muid, R.E., Nixon, J.S., Sedgwick, A.D., Wilkinson, S.E. and Dale, M.M. (1990) Biochem. Biophys. Res. Commun. 171, 1087-1092

205P LEUPEPTIN IDENTIFIES MULTIPLE PATHWAYS FOR ACTIVATION OF U937 HUMAN MONOCYTIC CELLS BY THROMBIN

S.Joseph & J. MacDermot.Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 0NN.

The mechanism by which the serine protease thrombin causes the receptor mediated activation of cells such as platelets and fibroblasts has recently been clarified, and shown to require the proteolytic cleavage of its receptor [Vu et al, 1991]. This proteolytic mechanism cannot however explain the reported biological effects of thrombin on monocytes or macrophages. In these cells, activation occurs in response to proteolytically inactive thrombin [Bar-Shavit et al,1986]. The present study set out to determine whether a non-proteolytic mechanism similarly exists in the U937 human monocytic cell line. This was assessed by comparing the dose-response effect of the serine protease and thrombin inhibitor leupeptin (acetyl-L-leucyl-L-leucyl-DL-arginal) [Brass and Shattil, 1986] on thrombin activation of U937 cells and human platelets. Platelets were used as a positive control as platelet activation by thrombin is totally dependent upon its proteolytic activity [Brass and Shattil,1986, Vu et al,1991].

U937 cells were grown in culture in RPMI-1640 medium at 37° C in an atmosphere of CO_2:air (5:95 v/v) [4]. Washed human platelets were prepared as previously described [Joseph & MacDermot,1991]. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) mobilisation was used as a marker of cell activation. In both cell types, the cells were loaded with the fluorescent Ca^{2+}-dye, Indo-1/AM (3 μM) for 45 min at 37 °C, washed and resuspended in Hepes-buffered Tyrode's solution pH 7.4 containing 1 mM $CaCl_2$ [Joseph & MacDermot,1991].

Leupeptin inhibited $[Ca^{2+}]_i$ mobilisation by thrombin in platelets which is consistent with a proteolytic mechanism. Pretreatment of platelets for 2 min with 10-500 μg/ml leupeptin inhibited by 70-96 % the rise in $[Ca^{2+}]_i$ stimulated by 2 U/ml bovine thrombin (≈17-fold increase above the resting $[Ca^{2+}]_i$ level,133±33 nM). This inhibitory effect of leupeptin could not be attributed to a non-specific action on $[Ca^{2+}]_i$ per se, as leupeptin (500 μg/ml) had no effect on the rise in $[Ca^{2+}]_i$ (≈1.8-fold increase above resting levels) stimulated by the thromboxane A_2 mimetic U46619 (1 μM). In U937 cells 10-500 μg/ml leupeptin only partially inhibited by 55-75 % the $[Ca^{2+}]_i$ response to 2 U/ml thrombin (≈3-fold increase in $[Ca^{2+}]_i$ above resting levels, 125±5 nM). Again, this inhibitory effect was not due to a non-specific action, as leupeptin had no significant effect on the PAF (1 μM) stimulated rise in $[Ca^{2+}]_i$ (≈3-fold increase above resting levels). We conclude that U937 cells may be activated by both a non-proteolytic (leupeptin insensitive) and proteolytic (leupeptin sensitive) mechanism by thrombin. The existence of a non-proteolytic mechanism in U937 cells and in other monocytes/macrophages [Bar-Shavit et al,1986] raises the possibility that these cells may possess multiple forms of the thrombin receptor [Vu et al, 1991].

Bar-Shavit,R, Kahn, AJ, Mann, KG and Wilner,GD (1986) P.N.A.S. (USA) 83, 976
Brass,LF and Shattil,SJ (1986) J.Biol.Chem. 263,5210
Joseph,S and MacDermot,J (1991) Eur.J.Pharm (Mol.Pharm Section). -in press
Vu,TH, Hung,DT, Wheaton,VI and Couglin,SR (1991) Cell 64,1057

206P MONOCLONAL ANTIBODY MEL-14 BLOCKS LYMPHOCYTE MIGRATION INTO A SITE OF CHRONIC INFLAMMATION

J. Dawson, J.C.W.Edwards*, A.D.Segwick** and P. Lees, Department of Veterinary Basic Sciences, The Royal Veterinary College, Hertfordshire, *Department of Rheumatology, University College, London and ** Department of Anti-Inflammatory Biology, Roche Products Ltd., Hertfordshire.

Lymphocyte migration is a complex process which is central to the normal functioning of the immune system. Mouse lymphocyte surface molecules specific for peripheral lymph node high endothelial venules (PLN-HEC) can be identified and blocked by the monoclonal antibody MEL-14 (Duijvestijn and Hamann, 1989). They may control lymphocyte migration pathways, including those which are likely to develop during lymphocyte infiltration into a site of chronic inflammation (Freemont, 1988). The effect of mAb MEL-14 on murine lymphocyte migration into a delayed type hypersensitivity (DTH)-like lesion produced by sensitisation and challenge to Bordetella pertussis vaccine (BPV) has been investigated.

The lymphocyte migration assay was carried out as described previously (Dawson et al., 1989). Lymphocytes were obtained from the spleen and lymph nodes of BPV sensitised mice, purified by density centrifugation, washed and incubated with RPMI 1640 (medium control), mAb MEL-14 or mAb T200 (antibody control) for 1h on ice. mAb T200 is an idiotyped matched IgG2a rat antibody. Cells were washed, then radiolabelled with ^{111}Indium oxine (5μCi per 10^7 cells), quenched, washed and then injected into recipient mice sensitised and challenged with BPV into 6 day old air pouches, 10 days previously. Migration of lymphocytes was assessed after 4 hours by counting weighed organs and tissues in a gamma counter.

Values of percentage increase in radioactivity (n=6) in inflammed tissue (compared to non-inflammed tissue) were: RPMI 1640 = 125±12 (medium control); RPMI 1640 + mAb MEL-14 = 50±5 ($p<0.001$ compared to RPMI 1640); RPMI 1640 + mAb T200 = 120±10 (not significantly different from RPMI 1640). Values for peripheral lymph nodes (PLN) expressed as a percentage of total radioactivity (n=6) were: RPMI 1640 = 23.5±2.5; RPMI 1640 + mAb MEL-14 = 7.5±2.6 ($p<0.001$ compared to RPMI 1640); RPMI 1640 + mAb T200 = 18.9±2.1 (not significantly different from RPMI 1640).

These findings suggest that lymphocyte migration into the BPV-induced site of chronic inflammation may be regulated by the same mechanism as lymphocyte migration into the PLN. They further indicate that the PLN-HEV adhesion molecule may also be expressed at the site of BPV induced chronic inflammation.

We are grateful to the Arthritis and Rheumatism Council for financial support.

Dawson, J., Sedgwick, A.D., Edwards, J.C.W. & Lees, P. (1989) Agents and Actions 27, 461-464.
Duijvestijn, A.M. & Hamman, A. (1989) Immunol. Today 10, 23-28.
Freemont, A.J. (1988) J. Pathol. 155, 225-230.

207P THE CELLULAR DISTRIBUTION OF Na/K PUMPS IN HUMAN LYMPHOBLASTS AFTER EXPOSURE TO A LOW EXTRACELLULAR CONCENTRATION OF POTASSIUM

M. Ameen[1], A.R. Peek[1], J.K. Aronson[1], & J.F. Lamb[2], [1]MRC Unit and University Dept of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE & [2]Dept. of Preclinical Medicine, St. Andrews University, Fife KY16 9TS

We have previously shown that exposing human lymphoblasts to a low extracellular concentration of potassium (0.5 mM; low K) increases the number and activity of membrane-bound Na/K pumps (Ameen et al., 1990). This could be due to an increased rate of synthesis or a reduced rate of internalization of pumps. We now report the intracellular distribution and rate of removal of Na/K pumps from the plasma membrane of lymphoblasts after exposure to low K.

Lymphoblasts were grown for 3 days in 5.5 mM K (Ameen et al., 1990). They were incubated with 40 nM [^{3}H]-ouabain either for 2 h only or for 2 h followed by chase for 2, 16, and 24 h in excess unlabelled ouabain, then disrupted by nitrogen cavitation and fractionated. Other cells were first disrupted and fractionated; the fractions were then exposed to 40 nM [^{3}H]-ouabain. Fractionation was on self-generating 27% gradients of 35 ml Percoll in a Vti-50 Beckman rotor at 35000 g for 60 min; radioactivity was analysed in 1 ml fractions. Specific maximum ouabain binding was measured at 40 nM [^{3}H]-ouabain as previously described (Oh et al., 1987). These experiments were repeated with cells that had been incubated in low K for 3 days.

Low K increased [^{3}H]-ouabain binding in plasma membrane and lysosomes after cell disruption (Fig. 1). In normal K the membrane bound 58%, the lyosomes 26%, and the soluble fraction 16% of the [^{3}H]-ouabain. In low K the total binding capacity doubled, but the membrane still bound 58%; binding rose in the lysosomes to 39% of the total and fell in the soluble fraction to 3% The half-times of removal of [^{3}H]-ouabain from the plasma membranes of cells which had been exposed to [^{3}H]-ouabain before disruption were 7.5 h (normal K) and 12.5 h (low K).

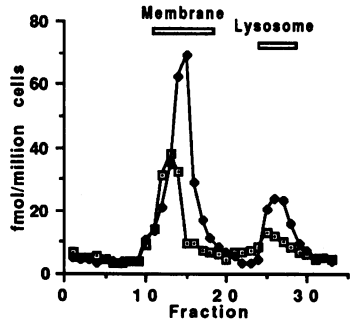


Fig. 1. The subcellular distribution of maximum [^{3}H]-ouabain binding in fractionated cells which were exposed to either 5.5 mM K (open symbols) or 0.5 mM K (closed symbols) for 3 days (n=3).

The increase in the number of Na/K pumps on exposure to low K was greater than can be explained by the insertion of already synthesized pumps from stores in the soluble fraction of the cell. Thus, this increase must be due to new synthesis and/or a reduced rate of internalization of pumps, and indeed the reduced rate of removal of pumps from the membranes of intact cells which had been exposed to low K confirms that the rate of internalization is reduced. Furthermore, the increased accumulation of pumps in the lysosomal fraction suggests that there is also a reduced rate of removal of pumps from the lysosomes after exposure to low K.

Ameen, M., Bloomfield, J., Aronson J.K. (1990) Br. Pharmacol. Soc. **100**, 470P.
Oh, V.M.S., Taylor, E.A., Ding, J.L., Boon, N.A., Aronson, J.K., Grahame-Smith, D.G. (1987) Clin. Sci. **72**, 71-79.

We are grateful to the British Heart Foundation, the Wellcome Trust, and the Medical Research Council for financial support.

208P ANTI-INFLAMMATORY ACTIONS OF BW755C INDEPENDENT OF CYCLOOXYGENASE AND 5-LIPOXYGENASE INHIBITION

N.K. Boughton-Smith, A.M. Deakin and R.L. Follenfant. Dep artment of Pharmacology, Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS.

The effects of the experimental anti-inflammatory drug BW755C have been attributed to inhibition of prostaglandin and leukotriene synthesis (Higgs et al,1980). The protective effects of BW755C against ethanol induced gastric damage in the rat are not, however, shared by specific 5-LO inhibitors (Boughton-Smith & Whittle, 1988). Actions of BW755C such as anti-oxidant (Marnett et al, 1982), may be of importance. We have investigated the mechanism of action of BW755c and the contribution of arachidonic acid metabolites and oxygen radicals to increases in vascular permeability in models of inflammation.

Paw oedema was induced in fasted rats (male,Wistar 160-200g) by carrageenan injection (1mg in 100 ul per paw) and paw thickness measured using dial calipers. In further studies, the reverse passive Arthus reaction (RPA) was induced in the skin of halothane anaesthetised rats by i.d. goat anti-rabbit IgG (50ug) and i.v. rabbit IgG (3mg), mixed with Evans blue (2.5% w/v) and ^{125}I human serum albumin (HSA, 0.5 uCi). Vascular permeability was measured as the leakage of ^{125}I HSA.

Pretreatment (30min) with indomethacin (10mg/kg p.o.) completely inhibited paw oedema ($P<0.01$, $n = 5$) for 6h, while administration 2h after carrageenan had no effect ($P> 0.05$). The specific 5-LO inhibitor, BW B70C (Salmon et al, 1989) at a dose (10 mg/kg p.o.) that maximally inhibits LTB_4 synthesis, had no effect on oedema, whether dosed before or after carrageenan. A combination of indomethacin (10mg/kg) and BW B70C (10 mg/kg) inhibited oedema when administered before ($p<0.01$, $n = 5$) but not after carrageenan. In contrast, BW755C (20 mg/kg p.o.) or SOD-PEG, (12,000U/kg i.v.) inhibited oedema when administered either before or after carrageenan ($P< 0.01$ $n=5$). Plasma leakage in the dermal RPA was inhibited by BW755c (25-100 mg/kg p.o.), the highest dose being no different from saline controls. SOD-PEG (4000-12,000 U/kg i.v.) also inhibited leakage producing 53 ± 9 % inhibition at the highest dose ($P< 0.05$, $n=4$). Indomethacin (10mg/kg p.o.) alone or in combination with BW B70c (10mg/kg p.o.) had no effect on plasma leakage.

The results indicate that the anti-inflammatory actions of BW755C in the later phase of carrageenan paw oedema or in the dermal RPA are independent of cyclooxygenase and 5-LO inhibition. Furthermore, the anti-inflammatory actions of SOD suggest that the increases in vascular permeability in these models are mediated by oxygen radicals and the anti-inflammatory actions of BW755C may, at least in part, be due to an anti-oxidant activity.

Higgs, G.A. et al (1980). Eur. J. Pharmacol., 66, 81-86.
Boughton-Smith,N.K.&Whittle,B.J.R(1988).Br.J.Pharmacol.,95, 155- 162.
Marnett, L. J. et al (1982). J Biol. Chem.,257 6957-6964.
Salmon, J. A. et al (1990). Adv Prost Thromb & Leuk Res. 21, 109-112.

209P VASODILATOR MEDIATORS INDUCED BY LIVE *E. COLI* IN THE SKIN

C. von Uexkull, S. Nourshargh, T.J. Williams, Dept. of Applied Pharmacology, National Heart and Lung Institute, London SW3 6LY

Gram negative bacteria initiate a vigorous inflammatory reaction upon invasion of host tissues, with oedema formation being a characteristic feature. We have previously reported that the magnitude of oedema formation induced by live *Escherichia coli (E.coli)* in rabbit skin is dependent on the generation of vasodilator mediators (Von Uexkull *et al.*, 1991). Pretreatment of intradermal (i.d.) sites with *E.coli* potentiated oedema responses to bradykinin (Bk). We have now extended these studies to investigate the mechanisms involved in this inflammatory reaction. In this study oedema formation in rabbit skin was measured by the local accumulation of intravenously injected ^{125}I-human serum albumin as previously described (Wedmore & Williams, 1981).

As found with live *E.coli* (10^7cells/site), pretreatment of i.d. sites with heat killed *E.coli* or *E.coli* lipopolysaccharide (LPS) (10μg/site) significantly enhanced the oedema response to i.d. BK (10^{-10}moles/site). The oedema response induced by Bk in the presence of live *E.coli* was inhibited by the Bk antagonist des-Arg[Hyp^3,Thi^5,8,D-Phe^7]Bk (Stewart & Vavrek, 1986) (10^{-10}moles/site). In addition, live *E.coli* significantly enhanced the oedema response induced by two other permeability increasing mediators, histamine (10^{-9}moles/site) and PAF (10^{-9}moles/site). Indomethacin (10^{-8}moles/site) when coinjected with live *E.coli* significantly reduced oedema formation over the 4 hour in vivo test period by 50$\pm$6.9% (n=8 experiments, $p<0.01$), however it had no significant effect on the oedema potentiating actions of *E.coli*. Further, the nitric oxide synthesis inhibitor L-N^G-nitro argine methyl ester (L-NAME) (10^{-7}moles/site) and the calcitonin gene related peptide antagonist cyclic CGRP fragment 8-37 (Chiba *et al.*, 1989) ($3x10^{-10}$moles/site) had no effect on the potentiation of Bk responses induced by live *E.coli*.

The results demonstrate that live *E.coli*, through the actions of LPS, induce vasodilatation either by a direct action or by generation of vasodilator mediators. The oedema-potentiating effect of *E.coli* does not appear to be mediated by cyclooxygenase products, nitric oxide or CGRP. The mechanisms involved in this oedema-enhancing effect of live *E.coli* remain to be elucidated.

We thank Ciba Geigy Basel, The Wellcome Trust and The National Asthma Campaign for support.

Chiba, T., Yamaguchi, A., Yamatani, T., Nakamura, A., Morishita, T., Inui, T., Fukase, M., Noda, T., & Fujita, T. (1989). *Am. J. Physiol.* **256**: E331-E335.
Stewart, J.M. & Vavrek, R.J. (1986). *Peptides of the Biological Fluids* **34**: 473-476.
Von Uexkull, C., Nourshargh, S., & Williams, T.J. (1991). *Br. J. Pharmacol.* **102**: 96P (Abstr).
Wedmore, C.V. & Williams, T.J. (1981). *Nature* **289**: 646-650.

210P THE H_3 ANTAGONIST THIOPERAMIDE INHIBITS THE PERMEABILITY EFFECT OF HISTAMINE IN RAT MICROVESSELS VIA AN H_1 RECEPTOR EFFECT

A. J. Pile & L. H. Smaje, Department of Physiology and Biophysics, St Mary's Hospital Medical School, London, W2 1PG

The permeability-increasing effects of histamine in the microcirculation have been shown to be dependent on H_1 receptors rather than H_2 receptors in most mammalian tissues (Owen et al., 1980). The recent identification of a novel H_3 receptor in mammalian brain (Arrang et al., 1983) , and the subsequent development of specific agonists and antagonists at this receptor (Arrang et al., 1987) have complicated this situation, but to date there have been no functional studies on the involvement of the H_3 receptors in permeability.

The model used involves the subcutaneous implantation of small discs of polyvinyl alcohol sponge into rats anaesthetized with 0.4 mg/kg intramuscular fentanyl fluanisone. Blood vessels grow into the sponge within a few days, and permeability changes are assessed in anaesthetized rats 28 days after implantation from the size of the extravascular albumin space measured by a dual radioisotope method. Intraperitoneal treatment with saline, 6 mg/kg mepyramine (an H_1 antagonist), 125 mg/kg cimetidine (an H_2 antagonist) and 0.5 mg/kg thioperamide (an H_3 antagonist) was followed, after 30 minutes incubation period, by 100 µl injections directly into the sponges of saline, 100 nmoles histamine, 1 µmole 2-thiazolylethylamine (2-TE, an H_1 agonist), 1µmole dimaprit (an H_2 agonist) or 1µmole R-α-methylhistamine (R-α-MH, an H_3 agonist). The results from these experiments are presented below (mean ± sem (n)).

Extravascular Albumin Space (µl per g sponge)

	Histamine	2-TE	Dimaprit	R-α-MH
Saline	35.4 ± 8.4 (7)	20.8 ± 4.6 (7)	64.2 ± 24 (4)	2.7 ± 0.4 (3)
Mepyramine	1.4 ± 0.7 (8)	1.2 ± 0.7 (6)	8.9 ± 2.3 (5)	1.5 ± 1.1 (5)
Cimetidine	7.9 ± 1.2 (8)	16.9 ± 2.4 (12)	44.2 ± 6.9 (8)	0.6 ± 0.5 (4)
Thioperamide	15.4 ± 4.3 (8)	10.8 ± 1.9 (5)	29.9, 41.9	0.7, 1.2

The lack of effect of the H_3 receptor agonist suggests that H_3 receptors play no part in the permeability response to histamine. The inhibitory effects of thioperamide on the permeability response to histamine and to the H_1 receptor agonist suggest that in this tissue the H_3 receptor antagonist thioperamide is acting via H_1 receptors.

A. J. Pile is an SERC-CASE student

Arrang, J.M., Garbarg, M. & Schwartz, J.C. (1983) Nature (Lond.) **302**, 832-837
Arrang, J.M., Garbarg, M., Lancelot, J.C., Lecompte, J.M., Pollard, H., Robba,H., Schunack, W. & Schwartz, J.C. (1987) Nature (Lond.) **327**, 117-123
Owen, D.A.A., Poy, E. & Woodward, D.F. (1980) Br. J. Pharmacol. **69**, 615-623

211P VALIDATION OF THE RAT SPONGE MODEL FOR QUANTITATIVE STUDIES ON ANGIOGENESIS

D.E. Hu, R.L. Smither, T.-P.D. Fan and G.A. Gresham*, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ and *Department of Histopathology, Addenbrooke's Hospital, Cambridge CB2 2QQ, England.

In 1987, we described a method for quantitative studies of angiogenesis in sponge implants in rats (Andrade *et al.*, 1987). The model is based on repeated measurements of relative blood flow changes through the sponges using a simple ^{133}Xe wash-out technique. Since histological observations showed that the sponges were gradually infiltrated by host blood vessels, we considered the development of blood flow to represent neovascularisation. We have now undertaken two additional approaches to validate this technique.

First, we established a good correlation between ^{133}Xe clearance, haemoglobin (Hb) and total protein concentrations in the sponges ($r=0.982$ and 0.985 respectively). For these experiments, sponges were removed from rats after ^{133}Xe measurements, homogenised in phosphate buffered saline and the Hb level quantitated colorimetrically using a Sigma Haemoglobin Kit. Sponge implants receiving daily doses of 10 pmol BK and 0.3 pmol IL-1α showed significantly higher clearance values than the control group, e.g. the 6 min ^{133}Xe clearance value of the control group on Day 8 was 30.8±1.3% (n=8) while BK/IL-1α increased it to 41.8±2.0% (n=12, $P<0.01$). Consistently higher levels of Hb were detected in the test sponges. For example, Hb concentrations (g/dL) on Day 8 were 0.185±0.04 vs 0.135±0.014 ($P<0.05$, n=6).

In a separate study, daily doses of the hetrazepine platelet-activating factor antagonist, WEB 2086 (10μg per sponge) (Casals-Stenzel *et al.*, 1987) caused a decrease in ^{133}Xe clearance relative to controls. For example, the 6 min ^{133}Xe clearance value of the control group on Day 8 was 40.6±5.3% and WEB 2086 decreased it to 22.3±6.0% ($P<0.05$, n=7-9). Serial 10μm sections of these sponges were prepared at eight levels and stained with biotinylated *Bandeiraea simplicifolia* lectin I, isolectin B_4. Using this endothelial cell marker it was possible to visualise blood vessels selectively, in particular the developing blood capillaries, and to quantitate their density using a computer-assisted image analysis system. Tissue infiltration into the sponges was shown to be significantly reduced in those treated with WEB 2086 (63.3±2.2% compared to 73.6±3.2% for controls, $P<0.05$, n=3-4). Preliminary analysis of the infiltrated areas under higher magnification suggests the vascular density in the sponges was also reduced by WEB 2086.

Thus, the good correlation between these three parameters suggests that measurements of relative blood flow changes in sponge implants by the ^{133}Xe clearance method do provide an accurate means to assess new blood vessel formation, and illustrates the general applicability of this model in angiogenesis research.

Andrade, S.P., Fan, T.-P.D. and Lewis, G.P. (1987) Br. J. Exp. Path. **68**, 755-766.
Casals-Stenzel, J., Muacevic, G. and Weber, K.-M.(1987) J. Pharmac. Exp. Ther.**241**, 974-981.

We thank the Wellcome Trust for support and Dr C. Meade for gift of WEB 2086. R.L.S. was an MRC Scholar.

212P EFFECTS OF GLUCOCORTICOIDS AND ANGIOSTATIC STEROIDS ON SPONGE-INDUCED ANGIOGENESIS IN RATS

Y. Hori, D.E. Hu, T.-P.D. Fan and G.A. Gresham*, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ and *Department of Histopathology, Addenbrooke's Hospital, Cambridge CB2 2QQ, England

In 1985, Crum et al. reported that, in the presence of heparin, angiostatic steroids inhibited angiogenesis in the chicken chorioallantoic membrane (CAM). The anti-angiogenic activity of these steroids appears to be independent of their glucocorticoid activity. However, due to its embryonic nature, the CAM may not be suitable for studying angiogenesis in chronic inflammation, a process which usually involves granulation tissue formation. Here we studied the effects of hydrocortisone (HC), dexamethasone (DX), tetrahydro-S (TS) and medroxyprogesterone (MP) on angiogenesis induced by sponge implants.

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (150-200g) and, using a ^{133}Xe clearance technique (Andrade et al., 1987), neovascularization was assessed as a function of blood flow through the implants every two days over a period of 14-20 days. The inhibitory effects of these steroids were confirmed histologically.

Daily systemic administration of HC inhibited sponge-induced angiogenesis in a dose-dependent fashion; the 6 min ^{133}Xe clearance values obtained on Day 14 for vehicle control, 2, 10 and 50 mg kg^{-1} s.c. were 42.77±5.11, 42.69±4.74, 32.8±5.77, and 21.77±3.52 %, respectively (the latter value being significant, $p<0.05$, n=10) but this inhibition disappeared by Day 20 and was accompanied by a significant decrease in the weight of either the spleen or the thymus. Daily local injection of HC (0.5, 5, 50 μg/sponge) also inhibited the angiogenesis in a dose-dependent manner up to Day 20 without affecting the weight of either organ. DX (0.5, 5, 50 μg/sponge) was at least 10 times more potent than HC, but spleen and thymus weight were significantly decreased. The cellular infiltration and neovascularisation in the test sponge sections, stained with haematoxylin & eosin or an endothelial cell marker *Bandeirea simplicifolia* lectin I, was profoundly inhibited by DX and HC. At the doses of TS and MP studied (0.5, 5, 50 μg/sponge), both of which lack glucocorticoid activity, only 5 μg of TS inhibited angiogenesis; there was no decrease in spleen and thymus weight.

Thus, unlike in the CAM assay, steroids with glucocorticoid activity do not require heparin to inhibit sponge-induced angiogenesis. The relationship between the anti-angiogenic effect of these steroids and the inhibition of angiogenic cytokine production (e.g. interleukin 1) is currently under investigation.

This work was supported by Shionogi Reserach Laboratories, Japan and the Wellcome Trust.

Andrade, S.P., Fan, T.-P.D., Lewis, G.P. (1987) Br. J. Exp. Path. 68, 755-766
Crum, R., Szaba, S., Folkman, J. (1985) Science 230, 1375-1378

213P AUGMENTATION OF A_{2b} ADENOSINE RECEPTOR-STIMULATED CYCLIC AMP FORMATION BY DL-1-AMINO-CYCLOPENTANE-TRANS-1,3-DICARBOXYLATE, A RIGID GLUTAMATE ANALOGUE

S.P.H. Alexander and A.R. Curtis, Department of Physiology & Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UNITED KINGDOM.

A_{2b} adenosine receptor-stimulated adenosine 3',5'-cyclic monophosphate (cAMP) generation in certain cells and tissues may be enhanced by activation of a second, discrete receptor type. Thus, in guinea pig cerebral cortex an augmented cAMP response is observed in the presence of noradrenaline, histamine and 5-hydroxytryptamine (Hill & Kendall, 1989), agents which also stimulate phosphoinositide turnover. We have recently observed that the selective "metabotropic" excitatory amino acid receptor agonist DL-1-amino-cyclopentane-*trans*-1,3-dicarboxylate (tACPD) stimulates phosphoinositide turnover in guinea pig cerebral cortical slices (Alexander et al., 1990), and we now report the enhancement of A_{2b} adenosine receptor-stimulated cAMP accumulation in this tissue by tACPD.

Guinea pig cerebral cortical slices were prepared as previously described (Alexander *et al.*, 1990), and incubated in Krebs-Henseleit medium with adenosine deaminase (1 U/mL), in the absence or presence of 5'-N-ethylcarboxamidoadenosine (NECA, 30 μM) for 10 minutes. Potential augmentatory agonists were added and the incubation continued for 10 minutes, before termination with ice-cold perchloric acid. The cAMP content of the neutralised supernatant layer was assessed by the method of Takeda *et al.* (1988), and protein in the NaOH-digested pellet by the method of Bradford (1978).

Basal levels of cAMP in guinea pig cerebral cortical slices (2.7 ± 0.9 pmol/mg protein, n=3) were not significantly altered in the presence of tACPD (300 μM, 3.0 ± 0.1), L-quisqualate (100 μM, 1.9 ± 0.2), DL-α-amino-3-hydroxy-5-methyl-4-isoxalone propionate (AMPA, 10 μM, 2.3 ± 0.6) or N-methyl-D-aspartate (NMDA, 100 μM, 2.3 ± 0.4). NECA-stimulated cAMP levels (77 ± 12 pmol/mg) were not altered in the presence of AMPA (110 ± 10 % NECA response) or NMDA (99 ± 13 %). In the presence of tACPD or quisqualate, however, NECA-stimulated cAMP accumulation was further enhanced to 280 ± 34 % ($P<0.01$) and 204 ± 50 % ($P<0.05$) of the NECA response, respectively. In a second series of experiments investigating the concentration-dependence of this enhancement, tACPD augmented NECA-stimulated cAMP with an EC_{50} value of 51 ± 15 μM and a maximal response of 291 ± 32 % of the NECA response (n=3).

In summary, we observe that A_{2b} adenosine receptor-induced cAMP stimulation is augmented by tACPD with an EC_{50} value comparable to that observed for tACPD-stimulated phosphoinositide turnover in this tissue (35-80 μM, Alexander et al., 1990).

The financial support of the Wellcome Trust is gratefully acknowledged.

Alexander, SPH; Hill, SJ; Kendall, DA (1990) J. Neurochem. 56, 1439-1441
Bradford, MM (1978) Anal. Biochem. 72, 248-254
Hill, SJ; Kendall, DA (1989) Cell. Signal. 1, 135-141
Takeda, T; Kuno, T; Shuntoh, H; Tanaka, C (1988) J. Biochem. 105, 327-329

214P NK1 TACHYKININ RECEPTORS ON THE U373MG CELL LINE: COUPLING TO INOSITOL PHOSPHATE FORMATION

J. MENAGER, E. HEUILLET (introduced by A. DOBLE). Rhône-Poulenc Rorer, CRVA, 13 Quai Jules Guesde, BP 14, 94403 Vitry sur Seine, France.

The U373MG cell line is a human astrocytoma expressing NK_1 tachykinin receptors. NK_1 receptors are thought to be widely coupled to phospholipase C, leading to the formation of inositol phosphates (IP). In this study, we have evaluated whether tachykinins can stimulate IP formation in this cell line. Furthermore, we have examined the ability of several recently discovered non-peptide NK_1 receptor antagonists to block the IP response (Snider et al. 1991; Garret et al. 1991): CP-96345 (±)-cis-3-(2-methoxybenzylamino)-2-benzhydrylquinuclidine; (P_I), its 2-chlorobenzylamino analogue (P_{II}), RP 67580 (7,7-diphenyl-2(1-imino-2-(2-methoxyphenylethyl)perhydroisoindole) and its inactive diastereoisomer, RP 68651. IP formation was measured by modifications of the method described by Torrens et al. (1989). The accumulation of IP was estimated using classical ion exchange chromatography. Substance P (SP) stimulated IP formation to a level 8 times above basal (10 fmol[^{3}H]-IP/10^6 cells), with an EC_{50} of 3.4 ± 0.4 nM. Maximal stimulation was reached after 2 hours incubation with 10^{-7} M SP (time used for further experiments). The NK_1-selective agonist [Pro^9]-SP also stimulated IP formation with an EC_{50} value of 3 nM, whilst the NK_3 agonist, senktide (10^{-6}M) was without effect. The NK_2-preferring agonist, [Lys^5,$MeLeu^9$,NLe^{10}]-NKA(4-10) was inactive at 10^{-7}M, but produced a moderate stimulation (16% of that seen with 10^{-6} M SP) of IP formation at 10^{-6} M; this effect could be antagonized by NK_1 antagonists. IP formation could also be evoked by carbachol (CCh) and noradrenaline (NA) (both 10^{-4}M). P_I, P_{II} and RP 67580 blocked the stimulatory effects of SP (3×10^{-9}M) with IC_{50} values (nM) of 16 ± 6, 538 ± 91 and 692 ± 37 respectively. RP 68651 had no effect. The stimulatory effects of CCh and NA were not blocked by these non-peptide NK_1 receptor antagonists. We have demonstrated that PLC stimulation leads to IP formation on this U373MG cell line and that, among the tachykinin receptors, this cell line expresses mainly NK_1 receptors. The activity of non-peptide SP antagonists could be evaluated with this convenient functional human model.

We are grateful to Drs S. Lavielle and G. Chassaing for the gifts of NK_1 and NK_2 agonists.

Garret,C.,Carruette,A.,Fardin,V.,Moussaoui,S.,Peyronel,J.F.,Blanchard,J.C. and Laduron, P.M. (1991) Proc. Natl. Acad. Sci., 88, 10208-102128.
Snider,R.M.,Constantine,J.W.,Lowe,J.A.,Longo,K.P.,Lebel,W.S.,Woody,H.A.,Drozda,S.E.,Desai, M.C.,Vinick,F.J.,Spener,R.W. and Hess,H.J. (1991) Science, 251, 435-437.
Torrens,Y.,Daguet De Montety,M.,El Etr,M.,Beaujouan,J.C. and Glowinski,J. (1989) J. Neurochem., 52, 1913-1918.

215P MONO(ADP-RIBOSYL)TRANSFERASE IN NG108-15 CELLS ADP-RIBOSYLATES A PUTATIVE ARGININE ACCEPTOR IN $G_s\alpha$

R.S. Boyd, L.E. Donnelly & J. MacDermot. Department of Clinical Pharmacology, Royal Postgraduate Medical School. London W12 0NN

Eukaryotic mono(ADP-ribosyl)transferase activity has been detected in a number of mammalian tissues. The prokaryotic forms of mono(ADP-ribosyl)transferases are known to ADP-ribosylate G protein α-subunits, and modify their activity. In chicken spleen cells a protein with a similar molecular weight to the α-subunit of Gs is ADP-ribosylated by an endogenous GTP-dependent mono(ADP-ribosyl)transferase (Obara et al., 1991). We have now examined NG108-15 cells to determine if an endogenous mono(ADP-ribosyl)transferase might ADP-ribosylate endogenous Gsα, and thus alter its abundance or activity.

NG108-15 cells were permeabilized by suspension in 300µl 50 % (w/v) PEG 1500 and 200 µCi [^{32}P]-NAD for 10 min at room temperature. The cells were then cultured in the absence or presence of known inhibitors of ADP-ribosyltransferase for 18 h. In some cases membranes were prepared and incubated in the presence of 200 µCi [^{32}P]NAD and 10µM GTP for 1 h at 37°C. ADP-ribosylated proteins were separated by polyacrylamide gel-electrophoresis. Labelled bands were identified by autoradiography. Gsα was immunoprecipitated from ADP-ribosylated membrane protein (Anderson and Blobel, 1983) using an anti-Gsα antibody. Chemical or snake venom phosphodiesterase I (PDE I) digestions were carried out on ADP-ribosylated membrane proteins (Prion and McMahon, 1990).

ADP-ribosylation of a number of proteins, including one of 45kDa, was inhibited in intact cells by nicotinamide (50mM), benzamide (25mM) or 5-bromo-2'-deoxy-uridine (25mM). Treatment of ADP-ribosylated membrane protein with snake venom PDE I yielded [^{32}P]AMP, which identified the substrates as mono(ADP-ribosylated) products. Treatment of ADP-ribosylated membrane protein with mercuric ions did not reduce the abundance of these products. In contrast, treatment of ADP-ribosylated membrane protein with hydroxylamine (2M) for 24 h cleaved the ADP-ribose linkage, which suggested adduct formation with arginine. This evidence is suggestive of arginine ADP-ribose protein linkage. Immunoprecipitation with anti-Gsα antibody resulted in the precipitation of a 45kDa labeled protein. Digestion of this protein with snake venom PDE I again revealed a mono(ADP-ribosylated) substrate.

Gsα is a substrate for mono(ADP-ribosyl)transferase in NG108-15 membranes. The stability of the (ADP-ribose)-protein linkage suggests an arginine-(ADP-ribose) bond. The functional consquences of this covalent modification of Gsα are currently under investigation.

This work is supported by grants from the Royal Society and the Wellcome Trust.

Anderson D.J. and Blobel G. (1983) Methods Enzymol. **96**, 111-112.
Obara S., Yamada K., Yoshimura Y. and Shimoyama M. (1991) Eur. J. Biochem. **200**, 75-80.
Piron K. J. and McMahon K.K. (1990) Biochem J. **270**, 591-597.

216P DETERMINING 5-HT BY REVERSE PHASE H.P.L.C. AND ELECTROCHEMICAL DETECTION AFTER PRECOLUMN DERIVATIZATION WITH OPA-2ME

Man M. Vohra & Beverly Williams, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

The current methodologies for determining serotonin (5HT) use an HPLC-EC detection technique because 5HT is electrochemically active and can thus be directly oxidized within the desired potential range. Since 5HT is also a primary amine and can be derivatized with o-phthalaldehyde-2-mercaptoethanol (OPA-2ME) under alkaline conditions (by a technique reported by Joseph and Davis [1983] and Allison et al. [1984]), we explored whether these derivatives of 5HT are electrochemically active and could be determined by EC detection, thereby providing an alternate method for assaying 5HT.

The HPLC-EC setup consisted of a Waters 501 pump coupled to a Bioanalytical LC-4B detector in oxidation mode and a nucleosil C-18 reverse phase column (150 x 4.6 mm; 5 µm particle size). Unless otherwise stated,the working electrode potential was kept at +0.70 V vs. a Ag-AgCl reference electrode. The mobile phase (buffer), which consisted of (mM) Na_2EDTA, 0.15; octylsodium sulfate, 0.43; sodium acetate • $3H_2O$, 100; citric acid, 20; and contained 30-40% acetonitrile, was filtered, degassed and pumped at 1.0-1.5 ml/min. We derivatized 5HT by adding to 390 µl of buffer (in order) 10-11 µl of 2 N NaOH (to bring the pH to 9.0-10.0), 15 µl of OPA (0.25% in methanol), 15 µl of 2ME (0.25% in methanol), and either 10-20 µl of standard 5HT or 20-100 µl of platelet extract. For platelet extract, we first centrifuged whole blood at 100 x g for 15 min (low speed spin), to prepare platelet rich plasma. Next, we centrifuged the latter at 1500 x g for 15 min (high speed spin), to obtain a platelet pellet. The pellet was thoroughly suspended in a mixture of 1 ml of 0.1 M $HClO_4$ and 0.1 ml of 10% Na_2EDTA, and this mixture was frozen at -85°C until assayed, at which time, the mixture was thawed and centrifuged and 20-100 µl of the supernatant was used to determine 5HT.

The reaction of 5HT with OPA-2ME at alkaline pH yielded electrochemically active (oxidizable) products (no such active products were formed when the reaction was carried out without NaOH, 2-ME, or OPA). The OPA-2ME derivatives of 5HT were relatively stable at the buffer pH (4.9) and did not require additional acid to stabilize them. Their peak potentials were +0.70 V, and their peak heights were linear for amounts of 0.3-26 pmol (50-4500 pg), with the minimum detection being at least 0.6 pmol (signal : noise ratio = 3:1) of 5HT. The capacity factors k' (defined as V_t-V_o/V_o, where V_t = retention volume of the compound and V_o = void volume) of various potentially interfering compounds were determined, and a comparison of the calculated k' showed that histamine (2.3), noradrenaline (3.3), dopamine (8.9), 5-hydroxytryptophan (ND) or 5-hydroxyindoleacetic acid (ND) did not interfere with the determination of 5HT (13.4). ND = Not detectable up to 5 ng amounts injected.

The mean (± SD) 5HT concentration of platelets from nine human subjects measured by our precolumn derivatization HPLC-EC procedure (530 ± 98 ng/10^9 platelets) was not significantly different from that measured by the direct oxidation HPLC-EC procedure (576 ± 93 ng/10^9 platelets) and was similar to values reported by Flachaire et al. (1990). There was also a highly significant correlation between the individual values we obtained by the two procedures (r = 0.93; p = 0.002).

We thank MRC (Canada) and the New Brunswick Heart Foundation for their support.

Allison, L.A., Mayer, G.S. & Shoup, R.E. (1984). Anal. Chem. 56, 1089-1096.
Flachaire, E., Beney, C. Berthier, A., Salandre, J., Quincy, C. & Renaud, B. (1990) Clin. Chem. 36, 2117-2120.
Joseph, M.H. & Davis, P. (1983). J. Chromatogr. 277, 125-136.

217P ELECTRICALLY STIMULATED RELEASE OF 5-HT FROM THE RAT PREFRONTAL CORTEX MEASURED BY FAST CYCLIC VOLTAMMETRY IN BRAIN SLICES

Walter Wieczorek & Zygmunt Kruk, Department of Pharmacology, Basic Medical Sciences, Queen Mary & Westfield College, Mile End Road, London, E1 4NS.

Fast cyclic voltammetry (FCV) can measure changes in stimulated endogenous dopamine (DA) overflow following single pulse electrical stimulation (Palij et al 1990). 5-HT overflow can be detected following pseudo single pulse stimulation (PSP; 5 pulses at 100Hz; O'Connor & Kruk 1991). The above experiments have been made in areas of the brain that are rich in DA or 5-HT respectively. The prefrontal cortex (PFC) which has sparse 5-HT and DA innervation has been implicated in numerous neurological mechanisms including cocaine reinforcement (Goeders & Smith 1983) and schizophrenia (Grace 1991). Using FCV we have attempted to measure neurotransmitter overflow in real time from the PFC during electrical stimulation.
Neurotransmitter overflow in a brain slice was monitored as described by Palij et al (1990). The carbonfibre electrode was positioned 100μm from a bipolar stimulating electrode (tip separation 200μm) in 350μm thick brain slice incorporating the PFC. Neurotransmitter efflux from the PFC was not detectable after a single pulse stimulation, but PSP stimulation applied every 5 min, gave reproducible voltammetric signals for up to 3h. Removal of calcium from the perfusing medium and replacement with magnesium, reversibly abolished neurotransmitter release. Neurotransmitter release was pulse width and frequency dependent. PSP stimulation gave a signal equivalent in magnitude to 5 nM. 5HT; 50p/100Hz, a signal equivalent to 20 nM. 5HT. The voltammograms at these concentrations do not allow identification of the compound released on the basis of electrochemical criteria. Pharmacological experiments were made using selective dopamine or 5HT uptake blockers (nomifensine 10^{-6}M and fluvoxamine 10^{-6}M respectively). Eleven sites were examined in different slices: at 2 sites neither nomifensine nor fluvoxamine increased the signal. At 5 sites, nomifensine only increased the signal by maximally 84 $\pm$ 10%. At 4 sites, fluvoxamine only increased the signal by maximally 37 $\pm$ 2%. At no site did addition of the second uptake blocker (in the presence of the first) further increase the signal; desmethylimipramine (10^{-6}M) had no effect either alone or in combination with either of the other uptake inhibitors. We suggest that 5HT, but not noradrenaline can be detected in the PFC but further work is needed to characterise the sites where release occurs and to substantiate dopamine release.

Grace,A.A.(1991) Neurosci. **41**, 1-24
Goeders,N.E. & Smith J.E. (1983) *Science* **221**, 773-775
O'Connor, J.J. & Kruk, Z.L. (1991). *J. Neurosci. Methods* **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**, 172-174

218P EFFECTS OF THE SULPHONYLUREA GLIQUIDONE ON THE EFFLUX OF RADIOLABELLED 5-HT AND DOPAMINE FROM RAT BRAIN SLICES

T. Zetterström, D. Finn[1], Q. Pei, & D.G. Grahame-Smith[1], Oxford University SmithKline Beecham Centre for Applied Neuropsychobiology and MRC unit of Clinical Pharmacology[1], Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE.

The presence of ATP-sensitive K^+ channels which are inhibited by a class of antidiabetic drugs, the sulphonylureas, has recently been demonstrated in the CNS (Mourre et al, 1989). Their involvement in neurosecretion has been suggested by Amoroso et al, 1990 whose studies have shown that the sulphonylureas enhance the efflux of GABA from slices of substantia nigra (SN), a brain region with high density of sulphonylurea binding sites. The purpose of this study was to investigate the action of gliquidone, a sulphonylurea previously shown to be very potent to enhance the release of ^{3}H-GABA in the CNS (Amoroso et al, 1990) on the overflow of ^{3}H-DA in striatum and ^{3}H-5HT from hippocampus, two rat brain regions with low and high number of binding sites for sulphonylureas respectively. The effect of gliquidone is compared with quinine, an unselective K^+ channel blocking agent, and TEA which is inactive on ATP-sensitive K^+ channels but potently blocks Ca^{2+} activated K^+ channels.

Male Sprague Dawley rats (250-300g) were killed and the brains were rapidly removed. Hippocampi or striata were dissected out and cut into 300μm slices which were incubated in HEPES-Ringer solution with ^{3}H-5HT or ^{3}H-DA containing pargyline (1μM) and citalopram (1μM, hippocampal slices) or nomifensine (1μM, striatal slices). The slices were then superfused with physiological medium and 20 min after the start of the superfusion were exposed to single 4 min pulse of medium containing the drug. For statistical analysis the unpaired Students T-test was used.

Gliquidone caused a dose dependent (10-100μM) increase in the efflux of ^{3}H-5HT from hippocampal slices (maximum effect by 111$\pm$17% above control values, mean$\pm$sem, n=8, $p<0.001$). In contrast gliquidone (10-100μM) had no effect on the efflux of ^{3}H-DA from striatal slices. Quinine induced a dose dependent (10-100μM) increase of both the efflux of ^{3}H-5HT in hippocampus and ^{3}H-DA striatal brain slices, maximum effects above control groups were (227$\pm$28%, n=8, $p<0.001$) and (311$\pm$92, n=8, $p<0.005$) respectively Interestingly TEA in concentrations up to 10 mM did not significantly enhance either the efflux of ^{3}H-5HT (12$\pm$18%, n=8) or ^{3}H-DA (7$\pm$15%, n=8) above control groups.

In summary these experiments show that the sulphonylurea gliquidone stimulates the efflux of ^{3}H-5HT from hippocampus but has no effect on ^{3}H-DA in striatum. This finding complements previous binding studies demonstrating high and low number of sulphonylurea binding sites in hippocampus and striatum respectively (Mourre et al, 1989). The mechanism of action of quinine is unclear, but the involvement of Ca^{2+} activated K^+ channels seems unlikely since TEA had no effect.

Mourre, C., Ben Ari, Y., Bernardi, H., Forset, M., Lazdunski , M. 1989 **Brain Res.** 486, 159-164
Amoroso, S., Schmied-Antomarchi H., Fosset, M., Lazdunski, M. 1990 **Science** 247, 852-854.

219P REGIONAL DISTRIBUTION OF 5-HT_{1C} AND 5-HT_2 RECEPTOR PEPTIDES ON THE RAT BRAIN MEASURED USING POLYCLONAL ANTISERA

A. Sharma, M. Landon[1], & K. C. F. Fone.
Departments of Physiology and Pharmacology and [1]Biochemistry, Queen's Medical Centre, Nottingham University, Nottingham NG7 2UH.

The present study characterises the binding of two antisera raised against the N-terminal, reportedly extracellular, portion of either the rat 5-hydroxytryptamine$_{1C}$ (5-HT_{1C}, Julius et al., 1988) or 5-HT_2 (Pritchett et al., 1988) receptor and reports levels of both peptides in the rat choroid plexus (ChP), frontal cortex (FC), hypothalamus (HYP), thalamus (THAL), hippocampus (HIP), septum (SEP), striatum (ST), midbrain (MB), brainstem (BS), cerebellum (CER) and spinal cord.

The N-terminal 10 amino acid sequence of the 5-HT_{1C} and 5-HT_2 receptor peptides were synthesised using Fmoc chemistry (model 431A Peptide Synthesiser, Applied Biosystems). Two additional amino acids were incorporated at the C-terminal of both peptides, to allow incorporation of ^{125}iodine (tyr_{11}) using the chloramine T method, and conjugation (cys_{12}) using m-maleimidobenzoic acid N hydroxysuccinimide ester, to Keyhole Limpet Haemocyanin (KLH) prior to repeated inoculation in sheep (5 X 2 ml i.m. and s.c. at 28 d intervals) of the purified KLH-peptide complex emulsified in Freund's Adjuvant. The resultant antisera were dialysed extensively against 0.154 M NaCl at 4°C (to remove endogenous 5-HT as confirmed by HPLC with electrochemical detection), before adding sodium azide (15 mM) and storing at 4°C.

Sensitive radioimmunoassays (detecting 10-4000 pg tube^{-1}) were developed using the 5-HT_{1C} (1:7000) and 5-HT_2 (1:3000) antisera both of which showed negligible cross-reactivity (up to 10µg tube^{-1}) with 5-HT, 5-hydroxyindoleacetic acid, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and the neuropeptides substance P and proctolin. Marked variation in the level of peptide-like immunoreactivity was observed in selected regions of adult Wistar (260-340g) rat brain (Table 1), following sonication (10 s in 0.05 M Tris HCl, pH 7.4). 5-HT_{1C} like-immunoreactive (LI) levels in ChP were four times those in any other region with intermediate levels in SEP, FC, ST and HYP and lower levels in all other regions including dorsal (96 ± 18 fmol mg wet weight^{-1}) and ventral thoraco-lumbar spinal cord (60 ± 11). In contrast, highest level of 5-HT_2-LI were found in FC and ChP with intermediate levels in SEP, ST and HYP and low levels in dorsal (59 ± 15) and ventral (55 ± 13) spinal cord.

Table 1 5-HT_{1C} and 5-HT_2-LI levels (fmol mg wet weight^{-1}) in selected rat brain regions (mean ± s.e.mean, n = 5-11).

Region	ChP	FC	HYP	ST	HIP	SEP	THAL	MB	BS	CER
5-HT_{1C}	1868±235	368±69	348±57	300±81	220±60	457±55	156±21	136±26	70±15	98±18
5-HT_2	1042±431	1490±479	479±168	427±137	73±12	254±58	70±17	96±24	36±7	24±2

The overall pattern of distribution of both peptides measured by the current radioimmunoassays is similar to that reported for 5-HT_{1C} ligand binding sites using [^{3}H] mesulergine (Pazos & Palacios, 1985) and 5-HT_2 sites (except for the high 5-HT_2-LI in ChP) estimated using [^{3}H] ketanserin (Pazos et al.., 1985). However, absolute levels of both proteins measured in the current study are approximately ten times the ligand binding Bmax values in most brain regions,which may be due to measurement by the curent method, of receptor proteins not incorporated in the membrane.

We thank the SERC (A.S.) and the Medical School Trust fund for financial support.

Julius, D., MacDermott, A.B., Axel, R. & Jessell, T.M. (1988) Science, **241**, 558-564.
Pazos, A. & Palacios, J.M. (1985) Brain Res., **346**, 205-230.
Pazos A., Cortes, R. & Palacios, J.M.(1985) Brain Res., **346**, 205-230.
Pritchett, D.B., Bach, A.W.J., Wozny, M., Taleb, O., Toso, R.D. Shih, J.C. & Seeburg, H. (1988) EMBO J., **13**, 4135-4140.

220P EFFECTS OF 8-OH DPAT ON OPERANT FOOD INTAKE IN FOOD-DEPRIVED RATS

I.S. Ebenezer. School of Pharmacy and Biomedical Sciences, Portsmouth Polytechnic, Hants.

The $5HT_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) has previously been shown to increase operant feeding in non-deprived rats (Ebenezer, 1991). The present study was carried out to investigate the effects of 8-OH-DPAT on operant food intake in food-deprived rats. Male Wistar rats (n=7) were deprived of food for 22h a day, and trained to press a lever in an operant chamber for food pellets on a fixed ratio of 6 (FR-6) as described previously (Ebenezer, 1991). During the sessions that followed, the food-deprived rats were injected s.c. with either normal saline or 8-OH-DPAT (15.625 - 500 µg kg^{-1}) and placed separately in the operant chamber for 60 min. Operant food intake was measured in 10 min bins during this period. 8-OH-DPAT (15.625 - 500 µg kg^{-1}) produced a dose-related decrease in cummulative food intake measured over 60 min ($F_{(6,36)}$= 23.6680, P<0.001) (see Table 1). Analysis of the data in 10 min bins revealed that, while 8-OH-DPAT (15.625 - 250 µg kg^{-1}) caused significant decreases in feeding during the 1st 10 to 40 min periods, there were significant increases during the last 40 to 60 min after administration. By contrast, the 500 µg kg^{-1} dose only had inhibitory effects on operant food intake.

Table 1. Effects of 8-OH-DPAT on operant food intake

Dose of 8-OH-DPAT (µg kg^{-1})	0	15.625	31.25	62.5	125	250	500
Mean Food Intake (g) ± s.e.m.	15.7±1.2	14.7±0.8	12.6±1.2*	10.4±1.6**	7.9±1.6**	5.5±1.0**	2.5±0.6**

* P<0.05 ** P<0.01 (Dunnet's t-test)

The higher doses (i.e. 125 - 500 µg kg^{-1}) produced in a dose-related manner, a number of abnormal behaviours, such as flat body posture and headweaving, which may have disrupted operant responding. Such effects were not apparent with the lower doses. The results thus indicate that 8-OH-DPAT has essentially depressant effects on operant food intake in deprived rats. The later facilitation in operant responding may be due to the depressant effects of the drug wearing off, and the occurrence of rebound eating. Alternatively, this may represent a true stimulant effect of the drug on feeding. Longer recording periods may help resolve this question. These results therefore suggest that different mechanisms may be responsible for the effects of 8-OH-DPAT on operant food intake in deprived and non-deprived rats.

Ebenezer, I.S. (1991) Br. J. Pharmacol. 104, 359P.

221P LIGHT INTENSITY INFLUENCES THE RESPONSE TO 8-OH-DPAT IN THE ELEVATED X-MAZE MODEL OF ANXIETY

J.W. McBlane[1], M.A.E.Critchley[2] and S.L. Handley[1] . [1]Pharmaceutical Sciences Institute, Aston University, Birmingham, B4 7ET and [2] Dept. Pharmacology, Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS.

The 5-HT_{1A} receptor agonists have inconsistent effects in animal models of anxiety. For instance, in the elevated X-maze model, 8-hydroxy-2-di-*n*-propylamino tetralin (8-OH-DPAT) has been reported to be anxiogenic (Critchley and Handley, 1987), inactive (Pellow et al., 1987) and anxiolytic (Soderpalm et al., 1989). The reason for this discrepancy is not known. The aim of this study was to investigate whether light intensity is one factor able to modulate the action of 8-OH-DPAT in this test.

Groups of 6 male Wistar rats (180 - 200g) were handled for 3 days prior to the experimental day, when they were injected i.p. with either 8-OH-DPAT HBr (0.2 mg/kg) or saline 10 minutes before being placed on an elevated X-maze. Their behaviour was recorded on video for 10 minutes. Light intensity, measured in the central square of the maze was either 172 lux (lower light) or 211 lux (higher light). Results were analysed by two way analysis of variance followed by Tukey's test for a direct comparison of means.

The mean open : total entries ratio ($\pm$sem) for the lower light condition were 0.19 ± 0.03 (saline) and 0.07 ± 0.01 (8-OH-DPAT) and for the higher light condition were 0.13 ± 0.04 (saline) and 0.14 ± 0.03 (8-OH-DPAT). Analysis of variance performed on these data indicated that the interaction between light intensity and drug treatment was significant ($F(120) = 4.37$; $P < 0.05$]. Post hoc analysis confirmed that 8-OH-DPAT significantly reduced the ratio in the lower, but not the higher, light condition ($P < 0.01$ and $P > 0.05$ respectively). The mean total entries made under the lower light condition were 24.3 ± 2.2 (saline) and 29.3 ± 2.4 (8-OH-DPAT) and under the higher light condition were 24.2 ± 0.8 (saline) and 30.5 ± 3.2 (8-OH-DPAT). There was a significant main effect of the drug ($F(120)=5.67$; $P < 0.05$), but not of light intensity.

This study has indicated that alteration of light intensity is able to influence the response to a 5-HT_{1A} agonist in the elevated X-maze model of anxiety. This may partly explain reported inter-laboratory differences with this drug in this test.

The support of The Wellcome Research Laboratories is gratefully acknowledged.

Critchley, M.A.E. and Handley, S.L. (1987) Psychopharmacol 93, 502-506.
Pellow,S., Johnston, A.L. and File, S.E. (1987) J. Pharm. Pharmacol. 39, 917-928.
Soderpalm, B., Hjorth, S. and Engel, J.A. (1989) Pharmacol. Biochem. Behav. 32, 259-265.

222P EFFECTS OF 5-HT ON RAT MEDIAL VESTIBULAR NUCLEUS NEURONES *IN VITRO*

A.R. Johnston, M.B. Dutia and D.S. McQueen[1], Departments of Physiology and [1]Pharmacology, Medical School, Teviot Place, Edinburgh EH8 9AG.

Intracerebroventricular injection of 5-hydroxytryptamine (5-HT, serotonin) has been shown to increase the gain of the vestibulo-ocular reflex in the alert rat, possibly through an action on second-order vestibular neurones in the medial vestibular nucleus (MVN; Ternaux and Gambarelli 1990). Steinbusch (1981) demonstrated the presence of 5-HT immunoreactivity in the MVN, while Pazos et al (1985) detected 5-HT_2-receptor ligand binding in the MVN using quantitative autoradiography. We have studied the effects of 5-HT on the spontaneous discharge of rat MVN neurones *in vitro*, using a horizontal slice preparation of the dorsal brainstem (for details see Dutia et al 1992).

Fifty-two of 55 spontaneously-active MVN cells (94%) responded to 5-HT (0.5-300uM) added to the perfusing medium, the majority being excited (42/55 cells, 76%). Seven cells (13%) showed a biphasic response consisting of an initial inhibition followed by excitation. In 3 cells (5%) 5-HT caused an inhibition of the resting discharge. The excitatory effects of 5-HT were mimicked by the selective 5-HT_2-receptor agonist alpha-methyl-5-HT (10-100 uM), and these effects were antagonised by ketanserin (3 - 50 uM; n=7) and ritanserin (3-10 uM, n=4). In a cell that showed a biphasic response to 5-HT, ketanserin enhanced the early inhibitory component and antagonised the later excitation.

5-HT thus mediates both excitatory and inhibitory effects in the rat MVN. The excitatory effects are ketanserin- and ritanserin-sensitive, suggesting a 5-HT_2-receptor linked mechanism. The inhibitory effects remain to be characterised.

Teranux, J.-P. & Gambarelli, F. (1987) Pflugers Arch. 409, 507-511.
Steinbusch, H.W.M. (1981) Neuroscience 6, 557-618.
Pazos, A., Cortes, R. & Palacios J.M. (1985) Br. Res. 346, 231-249.
Dutia, M.B., Johnston, A.R. & McQueen, D.S. (1992) Exp. Br. Res., in the press.

223P DIFFERENTIAL EFFECTS OF MONOVALENT CATIONS ON GTP-SENSITIVE STATES OF THE 5-HT_{1A} RECEPTOR

J. M. Elliott & S. Phipps, Oxford University SmithKline Beecham Centre for Applied Neuropsychobiology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE.

High affinity binding of [^{3}H]8OH.DPAT to the $5HT_{1A}$ receptor in rat brain tissue can be differentiated into GTP-sensitive and GTP-insensitive states using the stable GTP analogue guanylylimidodiphosphate (GppNHp) (Emerit *et al*, 1991). We have previously described a similar situation with regard to the binding of [^{3}H]UK14304 to α_2-adrenoceptors in rat brain (Elliott *et al*, 1991), in which we also described a selective effect of monovalent cations on the GTP-insensitive site. We have therefore now investigated the direct effects of monovalent cations on the binding of [^{3}H]8OH.DPAT in rat brain tissue in a similar manner.

Cerebral cortex tissue from the brains of male Sprague-Dawley rats (180-200g) was homogenised and washed in 5mM Tris/EDTA (pH 7.4) then resuspended in incubation buffer (50mM Tris, 1mM EGTA, 5mM $MgCl_2$, pH 7.4). Tissue was incubated with [^{3}H]8OH.DPAT for 60min at 37°C then filtered. Specific binding to the GTP-sensitive site was defined as that displaced by 100μM GppNHp and to the GTP-insensitive site as that occuring in the presence of 100μM GppNHp and displaced by 10μM 5HT.

Addition of monovalent cation salts (LiCl, NaCl, KCl) caused a concentration-dependent decrease in the binding of [^{3}H]8OH.DPAT (1.5nM) to both the GTP-sensitive and GTP-insensitive sites, with lithium (IC_{50}=35mM) being more potent than sodium (IC_{50}=116mM) or potassium (IC_{50}=119mM). The reduction in binding to the GTP-insensitive site was the greater in each case. More detailed analysis of the effects of LiCl indicated that the decreased binding of [^{3}H]8OH.DPAT resulted from a reduction in the binding capacity of both sites together with a decrease in affinity at the GTP-insensitive site but no change in affinity at the GTP-sensitive site.

Table 1: Effect of addition of LiCl *in vitro* on the binding characteristics of [^{3}H]8OH.DPAT in rat cerebral cortex.

		GTP-sensitive		GTP-insensitive	
	n	Kd	Bmax	Kd	Bmax
CONTROL	3	1.73 ± 0.39	82 ± 6	0.97 ± 0.14	77 ± 8
LiCl (10mM)	3	1.53 ± 0.33	73 ± 9	1.22 ± 0.10	70 ± 4
LiCl (50mM)	3	1.86 ± 0.32	59 ± 4 *	2.13 ± 0.28 *	42 ± 3 *

Kd - nmol/l ; Bmax - fmol/mg protein ; * p<0.05, Dunnett's t-test (repeated measures) v CONTROL

We conclude that monovalent cations reduce [^{3}H]8OH.DPAT binding at both the GTP-sensitive and GTP-insensitive sites, probably via an allosteric interaction similar to that reported for the α_2-adrenoceptor (Horstman et al, 1990). However the selective reduction in agonist radioligand affinity at the GTP-insensitive site suggests that the effect of the monovalent cations may not be identical on these two forms of the binding.

Elliott, J.M., Payvandi, N. & Heal, D.J. (1991) Br. J. Pharmacol. **102**, 6P
Emerit, M.B., Miquel, M.C., Gozlan, H. & Hamon, M. (1991) J. Neurochem. **56**, 1705-1716
Horstman, D.A., Brandon, S., Wilson, A.L., Guyer C.A., Cragoe E.J. & Limbird, L.E. (1990) J. Biol. Chem. **265**, 21590-21595

224P EFFECT OF BUSPIRONE ON REGIONAL CEREBRAL BLOOD FLOW DETERMINED IN CONSCIOUS RATS

J. Opacka-Juffry, R.G. Ahier, P. Grasby, V. Cunningham, J.E. Cremer & G.S. Sarna, MRC Cyclotron Unit, Hammersmith Hospital, Du Cane Road, London, W12 OHS.

Buspirone, an azaspirodecanedione, is a novel anxiolytic that is clinically effective. The anxiolytic properties of buspirone may be related to its properties as a partial $5HT_{1A}$ agonist but may also involve effects on other transmitter systems (Eison et al., 1991). The action of serotoninergic drugs on cerebral blood flow (CBF) is conflicting (Parsons, 1991) and the present study was undertaken to examine the effect of buspirone on regional cerebral blood flow in conscious rats.

Male Sprague Dawley rats (280-320g) were anaesthetised using isoflurane with N_2O and O_2 and two veins and an artery in the tail canulated. After surgical preparation the animals were allowed to recover for 4-5 hours. Cerebral blood flow was determined in fourteen brain regions by using 4-iodo(N-methyl-^{14}C)antipyrine as the flow indicator (Cremer & Seville, 1983) 20 min following injection of 0.9% saline (1ml/kg) or different doses of buspirone (0.1-10 mg/kg). Of the fourteen regions analysed, only those showing significant changes are given in the table. There were no systemic cardiovascular changes following 0.1 mg/Kg buspirone. 1.0 and 10 mg/Kg induced dose related falls in blood pressure and heart rate of a magnitude, due to autoregulation, be unlikely to alter CBF. All doses of buspirone induced significant and comparable decreases in the ventral hippocampus but 1.0 mg/kg of buspirone induced decreases in additional brain areas.

Table 1. Cerebral blood flow (ml/g/min)

REGION	CONTROL	BUSPIRONE		
	Saline (6)	0.1mg/kg (4)	1.0 mg/kg (4)	10 mg/kg (6)
Hippocampus (ventral)	1.06 ± 0.06	0.88 ± 0.03*	0.77 ± 0.02*	0.85 ± 0.02*
Thalamus	1.22 ± 0.03	1.12 ± 0.06	0.95 ± 0.03*	1.07 ± 0.04
Inferior colliculi	1.69 ± 0.05	1.55 ± 0.09	1.24 ± 0.12*	1.53 ± 0.07
Cerebellum	1.10 ± 0.04	0.98 ± 0.05	0.87 ± 0.02*	0.98 ± 0.04
Anterior Cingulate Cortex	1.45 ± 0.12	1.28 ± 0.05	1.14 ± 0.09*	1.61 ± 0.08

Values are s.e. mean; number of observations in brackets; *P< 0.05 (2 way ANOVA with repeated measures and Scheffe correction; comparisons made with control values)

Buspirone induced marked regional decreases in cerebral blood flow but there was no simple relationship between the magnitude of the change and the dose administered.

Cremer, J.E. & Seville, M.P. (1983) J. Cereb. Blood Flow Metab. 3, 254-256.
Eison, A.S., Yocca, F.D. & Taylor, D.P. (1991) in *Buspirone: Mechanisms and Clinical Aspects* ed. Tunnicliff, G. et al. pp 279-326. San Diego: Academic Press.
Parsons, A.A. (1991) Trends Pharmacol. Sci. 146,310-315.

225P COMPARISON OF RITANSERIN, HALOPERIDOL, PIMOZIDE AND CLONIDINE EFFECTS ON SPONTANEOUS AND DOI HEAD-SHAKE

Dursun, S. M. & Handley, S. L., Pharmaceutical Sciences Institute, Aston University, Birmingham, B4 7ET U.K.

Although head-shakes have been most studied following 5-HT agonists (Handley & Singh, 1986) they also occur spontaneously where they appear to be part of the grooming repertoire (Wei, 1981). We have therefore compared the potency of agents which reduce 5-HT-related head-shakes on the head-shake frequency occuring spontaneously and after administration of the 5-HT2/1C agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) in mice.

Male MF1 mice (20-30g, N = 6-11) received the test agents by gavage (10ml/kg). For DOI head-shakes mice received a submaximal dose of DOI (1.0 mg/kg) i.p at 60 min and were videotaped from 65-70 min inclusive. For spontaneous head-shakes, mice were videotaped from 60-89 min inclusive. The different duration of recordings resulted in a similar number of head-shakes under the two conditions.

TABLE 1. POTENCY TO REDUCE HEAD-SHAKE FREQUENCY

	DOI HEAD-SHAKES					SPONTANEOUS HEAD-SHAKES				
			LINEAR REGRESSION					LINEAR REGRESSION		
AGENT	ID50(mg/kg)	R	SLOPE	CONFIDENCE LIMITS	P	ID50(mg/kg)	R	SLOPE	CONFIDENCE LIMITS	P
RITANSERIN	**0.62**	0.75	0.45	0.29-0.62	0.001	**0.41**	0.82	0.33	0.22-0.44	0.001
HALOPERIDOL	**0.061**	0.87	0.47	0.36-0.58	0.001	**0.54**	0.82	0.58	0.36-0.79	0.001
PIMOZIDE	**1.19**	0.87	0.46	0.35-0.74	0.001	**0.83**	0.87	0.55	0.40-0.69	0.001
CLONIDINE	**0.01**	0.96	0.66	0.56-0.75	0.001	**0.021**	0.71	0.60	0.28-0.92	0.004

Table 1 shows that ritanserin possessed similar potency in reducing the frequency of both DOI and spontaneous head-shakes. The same was true for clonidine and pimozide. This suggests that the same 5-HT2/5-HT1C, α_2 and DA mechanisms may modulate both types of head-shake. In contrast, haloperidol was 10-fold more potent at reducing the frequency of DOI head-shakes. The longer observation time is not likely to account for this difference because haloperidol is very long acting. Since haloperidol binds more strongly to α_1-adrenoceptors than does pimozide the possibility that α_1-adrenoceptors modulate DOI- but not spontaneous head-shakes deserves further investigation.

These preliminary findings suggest that spontaneous head-shakes involve a serotonergic mechanism and are modulated in a similar manner to those induced by DOI by α_2- and dopamine receptors.

We are grateful to the Tourette Syndrome Association of America for financial support.

Handley, S. L. & Singh, L. (1986) Trends Pharmacol. Sci. 7, 324-328.
Wei, E. T. (1981) Federation Proc. 40, 1491-1496.

226P EFFECTS OF RITANSERIN ON THE BEHAVIOUR OF MICE DURING SOCIAL ENCOUNTERS

B. Gao and M. G. Cutler , Department of Biological Sciences, Glasgow Polytechnic, Cowcaddens Road, Glasgow G4 0BA.

The 5-HT2/ 5-HT1C receptor antagonist , ritanserin, provides an effective treatment for several syndromes related to anxiety and depression (Klieser & Strauss 1988; Paiva et al. 1988). Its mode of behavioural action differs from that of classical anxiolytics such as benzodiazepines, and ritanserin shows little activity in many of the paradigms employed to detect anxiolytic potential in laboratory animals (Gardner 1988). The present studies examine the modifications to behavioural responsiveness in mice after subchronic oral administration of ritanserin. Behaviour has been recorded in their home cage during encounters with an unfamiliar group-housed partner and in an unfamiliar neutral cage in which they acted as intruders.

Ritanserin was given in drinking fluid for 12-15 days to one group of mice at 1.6 mg/l (0.32 mg/kg daily) and to a second group at 3.1 mg/l (0.70 mg/kg daily). Adult pair-housed CD1 male mice were employed (n=16 in each group). Control animals (n=20) received tap water. Behaviour shown by each of the mice during social interactions was recorded for a 5 min period, firstly in the home cage and then in the neutral cage, using the ethological procedures described by Dixon (1986).

Ritanserin at both dose levels, (0.32 mg/kg = LD; 0.70 mg/kg = HD) significantly increased social investigation during encounters in the home cage (Control 70.1±6.2; LD 90.3±7.4; HD 91.4±7.2; $P<0.05$). In the neutral cage, ritanserin significantly increased duration of the social element "attend" (Control 4.4±0.7; LD 12.9±2.3; HD 10.3±1.2; $P<0.01$), but not of overall social investigation. In the unfamiliar circumstances of the neutral cage, although not in the home cage, ritanserin significantly increased aggressive behaviour (Control 11.0±3.9; LD 27.9±7.8; HD 27.6±7.1; $P<0.05$). The increase of social investigation by the high dose of ritanserin was associated with reductions of overall non-social activity (In the home cage; Control 220.0±8.0; HD 192.7±7.0; $P<0.05$; in the neutral cage; Control 243.0±8.3; HD 223.0±8.0; $P<0.05$). However, the time spent in digging in the neutral cage was significantly increased by ritanserin at both dose levels (Control 9.2±2.5; LD 28.0±4.8; HD 23.8±3.6; $P<0.01$).

Overall, these results suggest that ritanserin after subchronic administration may act to increase the type of behaviour stimulated by the test situation, with social stimuli having the greatest influence in resident animals and environmental stimuli such as novel sawdust being of greater importance when the environment is unfamiliar. The observed enhancement of social investigation and aggressive behaviour suggest that ritanserin can act to release behaviour that is normally suppresssed by "fear" of the unfamiliar partner .

Results expressed as mean duration (s.) ±S.E.M.. Statistics; Mann-Whitney U and Kruskal Wallis Tests.

Dixon A. K. (1986) Arch. Suisses Neurol. Psychiatry 137, 151-163.
Gardner C.R. (1988) Gen. Pharmac. 19: 347-356.
Klieser E. & Strauss W.H. (1988) Pharmacopsychiatry 21: 391-395.
Paiva T., Arriaga F., Wauquier A., Lara E., Largo R. & Laitao J.N. (1988) Psychopharmacology 96: 395-399.

227P EFFECT OF 5-HT$_3$ RECEPTOR AGONISTS ON RAT FOOD INTAKE

Kennett, G.A., & Grewal, S.S., SmithKline Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex, CM19 5AD.

Rat 5-HT$_3$ receptors are found in sites associated with nausea and emesis in higher species (Pratt et al 1990). Emesis is not seen in rats but they may experience "nausea" as indexed by hypophagia (Kennett & Curzon 1988). As this might be 5-HT$_3$ mediated, we have tested the effects of the peripheral 5-HT$_3$ receptor agonist 1,3-phenylbiguanide (PBG) and the centrally administered 5-HT$_3$ receptor agonist 2-methyl-5-HT (2-Me-5-HT); at doses found to be anxiogenic in the rat social interaction and X-maze tests (data not shown), on rat food intake.

Male SD rats 250-300g, singly housed with free access to food and water, were deprived of food 23h before testing. They were given PBG 30 min i.p. pretest or, in 3rd ventricle cannulated rats, 2-Me-5-HT i.c.v, 4 min pretest. Food was then presented and food intake measured over 1h. PBG reduced food intake (table 1) but this effect was not blocked by pretreatment with the 5-HT$_3$ receptor antagonist BRL 46470A (0.01-1 mg/kg s.c. 1h pretest) (data for 0.1 and 1.0 mg/kg in table 1) and hence is not 5-HT$_3$ receptor dependent. 2-Me-5-HT infusion (5-100 µg I.C.V.) had no effect on food intake (data not shown). The results suggest that central or peripheral 5-HT$_3$ receptor stimulation causes neither "nausea" nor loss of appetite in the rat. The role of 5-HT$_3$ receptors in those areas of the rat, thought to be associated with nausea and vomiting in higher species, is therefore unclear.

Table 1: Effect of 5-HT$_3$ receptor agonists on food intake in 23h food deprived rats

Treatment			n	1h Food Intake g (Mean and SEM)
Saline		i.p.	8	6.5 ± 0.4
PBG	50 mg/kg	i.p.	7	5.4 ± 0.7
	100 mg/kg	i.p.	8	3.7 ± 0.9*
Saline + vehicle		i.p.	8	4.8 ± 0.5
Saline + PBG	150 mg/kg	i.p.	8	2.8 ± 0.5
BRL 46470 0.1 mg/kg s.c. + PBG	150 mg/kg	i.p.	8	2.3 ± 0.7
BRL 46470 1.0 mg/kg s.c. + PBG	150 mg/kg	i.p.	8	1.2 ± 0.7*

Significantly different from relevant Saline treated group * $P<0.05$ by Dunnett's test following significant 1 way ANOVA.

Kennett, G.A. and Curzon, G. (1988) Psychopharmacology 96, 101-103.
Pratt, G.D., Bowery, N.G., Kilpatrick, G.J., Leslie, R.A., Barnes, N.M., Naylor, R.J., Jones, B.J., Nelson, D.R., Palacios, J.M., Slater, P., and Reynolds, J.M., 1990, T.I.P.S. 11, 135-137.

228P THE 5-HT$_3$ RECEPTOR ANTAGONIST, GR68755, PREVENTS A SCOPOLAMINE-INDUCED COGNITIVE IMPAIRMENT IN THE COMMON MARMOSET

Jones DNC, Carey GJ, Costall B, Domeney AM, Naylor RJ and Tyers MB*, Postgraduate Studies in Pharmacology, University of Bradford, Bradford, West Yorkshire, BD7 1DP and *Neuropharmacology Department, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 ODP.

Scopolamine (SCOP) has previously been demonstrated to disrupt the acquisition of an object discrimination task in the common marmoset, an effect which was prevented by the administration of the 5-HT$_3$ receptor antagonist, ondansetron or the cholinomimetic, arecoline (Jones et al 1990). GR68755, a novel 5-HT$_3$ receptor antagonist, has recently been reported to enhance the performance of marmosets in an object discrimination task with reversal learning (Costall et al 1991). The present study investigated the ability of this compound to influence a scopolamine-induced impairment in the acquisition of an object discrimination task.

This study used 5-6 adult marmosets (both sexes, 320-370g) which were experienced in the performance of this protocol. The animals were tested in a Wisconsin General Test Apparatus (WGTA) and were required to determine which of two distinctive junk objects was rewarded. The criterion for the acquisition of this task was 9 correct responses out of 10 trials.

The administration of SCOP (0.02 mg/kg s.c.), 30 min before testing, caused a significant increase in the trials required to reach criterion from 6.8 ± 1.2 following SAL treatment to 18.5 ± 3.1 ($P<0.05$) n = 6. Pretreatment with GR68755 hydrochloride at 0.1 and 1 µg/kg (s.c.), administered three times in the preceding 24hr, prevented the SCOP-induced deficit. The performance of GR68755/SCOP-treated marmosets was indistinguishable from that following SAL-treatment alone. For example, the lower dose of GR68755 (0.1 µg/kg) significantly reduced trials to criterion from 18.0 ± 3.8 to 5.4 ± 3.6, $P<0.05$, n = 5). Administration of a lower dose of GR68755 hydrochloride (0.01 µg/kg s.c.) had no influence upon the SCOP-induced effects.

These data provide further evidence for a role of 5-HT in the modulation of cognitive processes. The ability of ondansetron (Jones et al 1990) and GR68755 to prevent a scopolamine-induced performance impairment further indicates a potential for the 5-HT$_3$ receptor antagonists to reduce cognitive deficits including those linked with a cholinergic dysfunction.

Jones DNC, Carey GJ, Costall B, Domeney AM, Gerrard PA, Naylor RJ and Tyers MB (1990) Br J Pharmac, 100 (Suppl) 309P
Costall B, Domeney AM, Gerrard PA, Jones DNC, Naylor RJ and Tyers MB (1991) Br J Psychopharmacology (in press)

229P β-ADRENOCEPTOR SUBTYPES AND FUNCTION IN HUMAN INTERNAL MAMMARY ARTERY AND SAPHENOUS VEIN

A. Ferro, A.J. Kaumann & M.J. Brown, Clinical Pharmacology Unit, Addenbrooke's Hospital, Cambridge

β_1 and β_2 adrenoceptors (AR) coexist in human myocardium. Recently we reported that right atrial strips from patients receiving β_1 blockers exhibit marked sensitisation to β_2 but not to β_1 stimulation in vitro (Hall et al., 1990). Similar cardiac β_2 sensitisation occurs in vivo (Hall et al., 1991). We have postulated that this effect is mediated via changes occurring at the G-protein level in the cardiomyocyte, giving rise to an increase in coupling of the β_2AR to adenylyl cyclase. In order to investigate whether β_2AR sensitisation requires the presence of both receptor subtypes, we examined βAR mediated vasodilation in human internal mammary artery (IMA) and saphenous vein (SV). These were collected from patients undergoing coronary artery bypass graft surgery. Patients were divided into those who were and were not receiving β_1-selective blockers. Blood vessels were dissected free of connective tissue and fat, the endothelium was rubbed off, and 2mm-long rings were set up in organ baths with modified oxygenated Krebs' solution. Rings were stretched to a tension of 20mN and incubated with 10μM phenoxybenzamine for 1h. Following overnight incubation in Krebs' solution, the vessel rings were incubated with one of the following combinations of βAR antagonists: 1) 300nM CGP 20712A (a β_1 antagonist); 2) 50nM ICI118551 (a β_2 antagonist); 3) both; 4) neither. Contractions were elicited with 25mM KCl, and concentration-effect curves to noradrenaline (NA) and adrenaline (Adr) constructed; catecholamine-induced relaxations were expressed as a percentage of the relaxation induced by 0.1mM sodium nitroprusside (SNP). NA and Adr produced dose-dependent relaxation in both IMA and SV. In each case, most of the effect was found to be mediated by β_2AR, using the different combinations of βAR antagonists. However, a small β_1 component was also found in both IMA and SV: 0.6mM noradrenaline in the presence of 50nM ICI 118551 gave (mean ± s.e.mean) 47.5±4.1% and 32.4±5.1% respectively of maximum relaxation to SNP. No differences were found in β_2 mediated responses between β_1-blocked and non-β-blocked patients: -log EC_{50} values (mean ± s.e.mean) for adrenaline in the presence of CGP 20712A were: IMA (non-β-blocked) 6.9±0.1, IMA (β_1-blocked) 6.8±0.1, SV (non-β-blocked) 7.0±0.2, SV (β_1-blocked) 7.1±0.1. Corresponding E_{max} values (mean ± s.e.mean) were: IMA (non-β-blocked) 61.1±7.6%, IMA (β_1-blocked) 64.2±4.7%, SV (non-β-blocked) 66.7±9.7%, SV (β_1-blocked) 55.7±6.2%. All values were not significantly different between non-β-blocked and β_1-blocked vessels ($p>0.05$, Student's t test). β_1 responses were also not different between vessels from β_1-blocked and non-β-blocked patients. Our findings demonstrate that human IMA and SV both possess a small functional β_1AR component, although vasodilation is mediated mainly through β_2AR. In such tissues with a relatively minor β_1AR population, β_1 blocker treatment does not affect β_2AR mediated responses.

We thank the surgical staff of Papworth Hospital for supply of human blood vessels.

Hall, J.A., Kaumann, A.J. & Brown, M.J. (1990) Circ. Res. 66, 1610-1623.
Hall, J.A., Petch, M.C. & Brown, M.J. (1991) Circ. Res. 69, 959-964.

230P THREE α-ADRENOCEPTOR SUBTYPES EXIST IN PORCINE AORTIC SMOOTH MUSCLE

W. Erdbrügger, H.-J. Bauch & M.C. Michel[1] (introduced by O.-E. Brodde), Inst. Arteriosclerosis Res., Domagkstr. 3, D-4400 Münster, Germany, and [1]Dept. Medicine, University of Essen, Hufelandstr. 55, D-4300 Essen, Germany

Multiple subtypes of α_1- and α_2-adrenoceptors exist (Harrison et al. 1991) but little is known about their expression in different vascular beds. Therefore, we have determined the α-adrenoceptor subtypes present in porcine aortic smooth muscle in radioligand binding studies using [^{3}H]prazosin and [^{3}H]yohimbine and appropriate subtype-selective competitors. Radio-ligand binding was performed in endothelium-denuded preparations according to methods described in detail elsewhere (Michel et al. 1992).

Catecholamines competed for [^{3}H]prazosin binding (K_d 31±6 pM, B_{max} 165±11 fmol/mg protein) with a rank order of potency: adrenaline ≈ noradrenaline >> isoprenaline. The highly α_{1A}-selective antagonists 5-methyl-urapidil (K_i 24 nM), (+)-niguldipine (K_i 11 nM) and (-)-niguldipine (K_i 17 nM) competed for [^{3}H]prazosin binding with steep and monophasic curves indicating interaction with a single class of low-affinity binding sites. Pre-treatment with chloroethylclonidine reduced [^{3}H]prazosin binding by 94%. Thus, only α_{1B}-adrenoceptors appear to exist in porcine aortic smooth muscle.

Catecholamines competed for [^{3}H]yohimbine binding (K_d 0.8±0.1 nM, B_{max} 548±21 fmol/mg protein) with a rank order of potency: adrenaline > noradrenaline >> isoprenaline. The α_{2A}-selective oxymetazoline and the α_{2B}-selective prazosin competed for [^{3}H]yohimbine binding with shallow curves (Hill slopes ≈ 0.7) which could be resolved into high- and low-affinity components (oxymetazoline: K_H 1.7 nM, K_L 83 nM, 69% R_H; prazosin: K_H 28 nM, K_L 1143 nM, 27% R_H). Thus, α_{2A}- and α_{2B}-adrenoceptors appear to exist in porcine aortic smooth muscle in an approximate 70:30 ratio.

In conclusion, three α-adrenoceptor subtypes appear to exist in porcine aortic smooth muscle (≈50% α_{2A}-, ≈25% α_{2B}- and ≈25% α_{1B}-adrenoceptors). The relative contribution of these subtypes to α-adrenoceptor-mediated vasoconstriction remains to be assessed.

Harrison, J.K., Pearson, W.R. & Lynch, K.R. (1991) Trends Pharmacol. Sci. 12, 62-67.
Michel, M.C., Jäger, S., Casto, R., Rettig, R., Graf, C., Printz, M., Insel, P.A., Philipp, T. & Brodde, O.-E. (1992). Hypertension in press

231P DIRECT *IN VITRO* COMPARISON OF THE RAT ATYPICAL β-ADRENOCEPTORS MEDIATING WHITE ADIPOCYTE LIPOLYSIS AND COLONIC RELAXATION

D.M. Kirkham & J. Kelly (introduced by M. V. Sennitt),
SmithKline Beecham Pharmaceuticals, Yew Tree Bottom Road, Epsom, KT18 5XQ.

Recently the potencies of some novel β agonists for gastrointestinal relaxation were shown to correlate with stimulation of lipolysis in white adipocytes (Manara & Bianchetti, 1990). The present study has characterised and compared the atypical β-adrenoceptors responses of adipocytes and the distal colon by using the agonist potencies of known selective β-adrenoceptor agonists and the ability of cyanopindolol to antagonise the actions of isoprenaline (McLaughlin & MacDonald, 1990).

Rat white adipocytes were prepared from epididymal fat pads and lipolysis was determined by assaying glycerol release. Segments of rat distal colon were placed in an organ bath with Krebs' medium at 37°C containing phentolamine (1μM), cocaine (30μM), normetanephrine (30μM), ascorbic acid (30μM), EDTA (30μM) and contracted by the addition of a submaximal concentration of KCl (40mM). All studies were carried out in the presence of 0.1μM propranolol to block β_1 and β_2-adrenoceptors.

Isoprenaline, denopamine, salbutamol and BRL37344 induced concentration-dependent glycerol release from adipocytes and relaxations of the distal colon (Table 1). Cyanopindolol (0.1, 1.0 and 3.0μM) produced concentration-dependent rightward shifts of the concentration-response curves to isoprenaline in both adipocytes and distal colon with a schild plot not significantly different from unity (pA_2 = 7.5, slope = 1.1 ± 0.04, n=10 ; distal colon pA_2 = 7.0, slope = 1.2 ± 0.03, n=34).

Table 1

Agonist	Glycerol Release From Adipocytes EC_{50}(μM)±s.e.mean	IA	Relaxation of Distal Colon IC_{50}(μM)±s.e.mean	IA
Isoprenaline	0.12 ± 0.03	1.0	0.05 ± 0.01	1.0
Denopamine	7.50 ± 0.89	0.7	16.2 ± 3.3	1.0
Salbutamol	28.6 ± 2.0	0.8	75.9 ± 20.0	0.9
BRL 37344	0.03 ± 0.01	0.9	0.07 ± 0.03	0.7

These results show that on the basis the order of agonist potency and cyanopindolol antagonism of isoprenaline, the atypical β-adrenoceptor mediating relaxation in the distal colon is functionally similar to the white adipocyte atypical β-adrenoceptor.

Manara, L, & Bianchetti, A. (1990) Trends Pharmacol. Sci. **11**, 229-230
McLaughlin, D.P. & MacDonald, A. (1990) Br. J. Pharmacol. **101**, 569-574

232P THE NOVEL BROWN ADIPOCYTE STIMULANT, ICI D7114, REVEALS POSSIBLE DIFFERENCES IN ATYPICAL β-ADRENOCEPTOR RESERVE

JW Growcott, B Holloway, C Wilson, S Mainwaring and M Green, Bioscience Department 2, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG.

We have previously demonstrated that in histamine pre-contracted guinea-pig ileum, isoprenaline, BRL37344 and the novel atypical beta-adrenoceptor agonist, ICI D7114, cause a maximal relaxation that is mediated via atypical beta adrenoceptors (Growcott et al., 1991). BRL37344 is, however, less efficacious than isoprenaline in the rat small intestine (van der Vliet, 1990) and without effect on human colon (McLaughlin et al., 1991). These differences could be due to different levels of atypical beta-adrenoceptor reserve. We decided to investigate the pharmacology of ICI D7114 in the rat small intestine.

Segments of rat small intestine were suspended in oxygenated Krebs solution at 37°C containing CGP20712A, ICI 118551 and prazosin (all 0.1μM). In some preparations (±)propranolol (1μM) was added to the Krebs. Cumulative relaxation-responses to isoprenaline, BRL37344 and ICI D7114 were obtained in carbachol (0.5μM) pre-contracted tissues.

Isoprenaline produced a graded relaxation (EC_{50} 0.34±0.06μM) and this effect was antagonised by propranolol (at 1μM concentration ratio = 9.8±1.7). BRL37344 was more potent (EC_{50} 0.13±0.06μM) but the maximal relaxation was less (61±13% of isoprenaline). Noradrenaline was about one tenth of the potency of isoprenaline (EC_{50} 2.33±0.17μM) and salbutamol (10μM) caused a weak relaxation. ICI D7114 produced no relaxation even at 10μM. Indeed, antagonism of the responses to isoprenaline were observed (at 1μM concentration ratio = 9.7±3.5)

The relatively weak antagonist activity of propranolol and the similar agonist potencies for isoprenaline and BRL37344 suggest that atypical beta-adrenoceptors are involved in these relaxant responses. The lower efficacy of BRL37344 and the lack of any agonist effect with ICI D7114 are in contrast to the previous findings in guinea-pig ileum (Growcott et al., 1991). These data raise the possibility that guinea-pig and rat ileum possess differing numbers of spare receptors. Since ICI D7114 is an antagonist in the rat ileum then this tissue may have few, if any spare receptors, which would indicate that ICI D7114 is a partial agonist with low efficacy.

Growcott JW, Wilson C, Holloway B and Mainwaring S (1991) Br J Pharmac. 104, 155P.
McLaughlin DP, Fulton JD, MacDonald E, Scott PJW and MacDonald A (1991) Br J Pharmac. 104, 152P.
van der Vliet A, Rademaker B and Bast A (1990) J Pharmacol Exp Ther 255, 218.

233P CHARACTERIZATION OF THE α_{1B}-ADRENOCEPTOR GENE PRODUCT EXPRESSED IN A STABLY TRANSFECTED HeLa CELL-LINE

C. Pimoule, D. Graham and S.Z. Langer, Department of Biology, Synthélabo Recherche (L.E.R.S.), 31, av. Paul Vaillant Couturier, F-92220 Bagneux, France

Lefkowitz and coworkers have isolated cDNAs encoding distinct α_1-adrenergic receptor (α_1-AR) subtypes, notably α_{1A}, α_{1B} and α_{1C} (Lomasney *et al.*, 1991). In addition, this team has cloned DNA encoding the hamster α_{1B}-AR into a pBC12BI vector and transfected human epithelial carcinoma cells (HeLa) to establish a cell-line which stably expresses this AR subtype. In the present study, we have performed a detailed pharmacological characterization of the AR gene product expressed in this transfected cell-line using ^{3}H-prazosin as a probe.

The hamster α_{1B}-AR cDNA transfected HeLa cells were grown in monolayer culture and harvested between passage numbers 9-17. ^{3}H-Prazosin binding to cell membrane preparations was performed at 25°C and 10 µM phentolamine was used to define non-specific binding. Equilibrium saturation analysis of ^{3}H-prazosin binding to cell membrane preparations indicated the binding of this radioligand to a single population of non-interacting sites with a K_d of 0.052±0.008 nM and a B_{max} of 3.945±0.031 pmol/mg protein (n=3). The potency of a number of drugs to compete with ^{3}H-prazosin for these specific cell membrane binding sites was determined (Table 1). The pK_i values of drugs to inhibit ^{3}H-prazosin binding to these HeLa cells and to the α_{1B}-AR of rat liver membranes (Han *et al.*, 1987) showed a highly significant correlation (r=0.982). In contrast, a similar analysis of the pharmacological profile of ^{3}H-prazosin binding to the HeLa cell membranes and to the α_{1A}-AR of rat salivary gland membranes (Michel *et al.*, 1989) exhibited a rather poor correlation (r=0.599).

Table 1. Inhibition by selected drugs of ^{3}H-prazosin binding to membrane preparations of the transfected HeLa cells

Drug	pK_i	nH
Prazosin	10.16 ± 0.12	1.12 ± 0.09
Spiperone	9.02 ± 0.06	1.1 ± 0.04
Alfuzosin	8.84 ± 0.06	1.09 ± 0.05
WB 4101	8.31 ± 0.11	1.06 ± 0.04
Phentolamine	7.77 ± 0.07	1.06 ± 0.02
Oxymetazoline	6.63 ± 0.08	0.99 ± 0.02
Clonidine	6.30 ± 0.08	0.97 ± 0.04
Phenylephrine	5.26 ± 0.26	0.97 ± 0.02

Values represent means ± s.e.m. of at least 3 experiments

These results therefore provide evidence that the α_{1B}-adrenoceptor gene product as expressed in this stably-transfected HeLa cell-line retains the pharmacological profile of previously characterized α_{1B}-AR proteins.

Han, C., Abel, P.W. & Minneman, K.P. (1987) *Nature* **329**, 333-335.

Lomasney , J.W., Cotecchia, S., Lorenz, W., Leung, W.Y., Schwinn, D.A., Yang-Feng, T.L., Brownstein, M., Lefkowitz, R.J. & Caron, M. (1991) *J. Biol. Chem.* **266**, **10**, 6365-6369.

Michel, A.D., Loury, D.N. & Whiting, R.L. (1989) *Br. J. Pharmacol.* **98**, 883-889.

234P CARDIOVASCULAR EFFECTS OF THE THERMOGENIC β-ADRENOCEPTOR AGONIST, ICI D7114, IN CONSCIOUS TELEMETRED DOGS

C Wilson, SJ Pettinger, J Hayward, S Birtles & BR Holloway, Bioscience II, ICI Pharmaceuticals, Alderley Park, Macclesfield SK10 4TG.

ICI D7114 ((S)-4-[2-hydroxy-3-phenoxypropylaminoethoxy]-N-(2-methoxyethyl) phenoxyacetamide) is a novel β-adrenoceptor agonist which increases energy expenditure in a number of species by stimulating atypical β-adrenoceptors located in brown adipose tissue and demonstrates high selectivity for thermogenesis over responses mediated by β_1- or β_2-adrenoceptors (Holloway et al., 1991). The current studies compare the cardiovascular effects of ICI D7114 with those of two other agonists which are selective for the atypical β-adrenoceptor, BRL 35135 (Arch et al., 1984) and Ro 40-2148 (Meier et al., 1988).

Systolic and diastolic blood pressure (BP) and heart rate (HR) were recorded from free ranging Beagle dogs with previously implanted aortic Konigsberg pressure transducers and monitored remotely by means of Volland FM radio-telemetry. After a 60 min period, dogs were dosed orally with either ICI D7114, BRL 35135 or Ro 40-2148. BP and HR were monitored for up to 24 h and average values over each 20 min period displayed.

Table 1.		Heart rate (min-1)		Systolic BP (mmHg)		Diastolic BP (mmHg)	
Dose mg kg-1	n	Pre-dose	Peak	Pre-dose	Peak	Pre-dose	Peak
ICI D7114 10	6	66±4	132±11***	120±3	93±4***	75±3	60±3***
1.0	6	72±3	126±7**	118±4	101±6***	76±3	64±4***
0.1	6	69±2	111±14*	121±8	124±9	78±5	82±5
0.03	4	76±7	110±7*	123±17	134±18	81±9	83±10
0.01	4	68±6	80±8	131±13	139±11	81±5	87±6
BRL35135 0.1	3	71±6	189±10*	131±7	105±8	84+5	64±2
0.03	3	72±4	156±11**	140±7	117±9*	91±7	74±5*
Ro40-2148 1.0	3	69±2	174±18*	145±10	127±10*	93±6	78±6

Table 1 shows the average values over the 60 min pre-dose period and highest (HR) or lowest (BP) 20 min period post-dose (peak)

Data are mean±s.e.mean: *p<0.05; **p<0.01; ***p<0.001; t-test, paired data.

All peak effects were seen within 3 h and HR changes were in parallel with BP changes. ICI D7114 produced dose-related falls in BP with associated tachycardia. These effects were smaller than those seen with the same doses of BRL 35135 or Ro 40-2148. ICI D7114's lack of β_1- or β_2-adrenoceptor agonist activity (Holloway et al.1991) suggests that atypical β-adrenoceptors may mediate these cardiovascular effects.

Arch,JRS, Ainsworth,AT, Cawthorne,MA, Piercy,V, Sennitt,MV, Thody,VE, Wilson,C & Wilson,S (1984) Nature, 309, 163-165.

Holloway,BR, Howe,R, Rao,BS, Stribling,D, Mayers,RM, Briscoe,M & Jackson,J (1991) Br. J. Pharmacol., 104, 97-104.

Meier,MK, Blum-Kaelin,M, Gerold,M, Isler,D & Mueller, M (1988) In: Obesity in Europe 88, 329-338.

235P ATYPICAL β-ADRENOCEPTOR-MEDIATED RELAXATION AND CYCLIC AMP FORMATION IN TUNICA MUSCULARIS MUCOSAE (TMM) OF THE RAT OESOPHAGUS

A.P.D.W. Ford, D.T. Taneja & D.E. Clarke, *Institute of Pharmacology, Syntex Research, Palo Alto, CA 94304, USA.*

Agonist and antagonist probes were used to characterise an atypical β-adrenoceptor which mediates relaxation of longitudinal smooth muscle in the TMM of rat oesophagus. Agonist inhibition of carbachol (10μM)-induced tone was measured under equilibrium conditions with uptake (neuronal and extraneuronal), α- and classical β-adrenoceptors blocked. The following order and relativity of agonist potency was obtained: BRL 37344 (see below; 37) > (-) ISO (7.2) > SR 58611A (see below; 5.3) > (-) adrenaline (2.0) > (-) noradrenaline (1) > (+) ISO (0.2) > (±) fenoterol (0.02). A range of β-adrenoceptor antagonists was also studied to obtain an antagonist 'fingerprint' of the receptor. pA_2 values obtained versus (-) ISO were several units lower than expected for classical β-adrenoceptors: cyanopindolol (7.3), alprenolol (6.6), tertatolol (6.1), pindolol (5.9), propranolol (5.9), S(-) timolol (5.9), and nadolol (5.1), with S(+) timolol inactive. Similar affinity estimates were determined for alprenolol using the selective agonists BRL 37344 (see Hollenga *et al.*, 1991) and SR 58611A (N-[(2S)-7-ethoxycarbonylmethoxy-1,2,3,4-tetrahydronaphth-2-yl]-(2R)-2-(3-chloro-phenyl)-2-hydroxyethanamine hydrochloride), thereby demonstrating agonist independence of the pA_2 values.

Using a [^{3}H]-adenine incorporation assay (see Ford *et al.*, 1991), ISO was observed to stimulate concentration-dependently the accumulation of cyclic AMP, in the presence of 3-isobutyl-1-methylxanthine (1mM) and in the absence of β-adrenoceptor blockade, with a potency (-log EC_{50}) of 6.6 ± 0.14 (n=6). BRL 37344 demonstrated partial agonism relative to ISO, although the concentration-effect curve was bell-shaped. The concentration-inhibition curve for cyanopindolol, against the response to a single concentration of ISO (1μM), was biphasic. Iterative fitting of these data to a two-site model gave two IC_{50} values, yielding -log K_i estimations of 11.1 ± 0.4 and 7.7 ± 0.3. In percentage terms, the relative contributions of the two cyanopindolol sites were 38 : 62 (high affinity : low affinity). The atypical β-adrenoceptor component of the response was isolated pharmacologically. Under these conditions, the concentration-effect curve to ISO was shifted by cyanopindolol (1μM) in a parallel rightward fashion, permitting the estimation of a pA_2 for cyanopindolol of 7.5 ± 0.3 at the atypical β-adrenoceptor.

The present study shows that ISO-induced relaxation of the TMM of rat oesophagus is mediated predominantly by a population of β-adrenoceptors displaying a similar agonist and antagonist profile to atypical β-adrenoceptors described previously (Bond and Clarke, 1988; Hollenga *et al.*, 1991). Cyanopindolol appears to possess the greatest affinity for the receptor, making it the most useful pharmacological antagonist currently available. The receptor-effector mechanism, stimulation of adenylyl cyclase, is consistent with that reported for atypical β-adrenoceptors in rat adipocytes (Hollenga *et al.*, 1991), as well as for classical β-adrenoceptors. In this regard, the present study is the first demonstration of such coupling in smooth muscle. The TMM of rat oesophagus offers a simple and reliable assay system for studying the pharmacology of the atypical receptor.

Bond, R.A. & Clarke, D.E. (1988) *Br. J. Pharmacol.*, **95**, 723-734.
Ford, A.P.D.W., Eglen, R.M., Clarke, D.E. & Baxter, G.S. (1991) *Br. J. Pharmacol.*, (Proceedings of Glasgow BPS, July 1991).
Hollenga, Ch., Brouwer, F. & Zaagsma, J. (1991) *Br. J. Pharmacol.*, **102**, 577-580.

236P STUDIES ON THE INHIBITORY EFFECTS OF GUANABENZ ON THE FIELD-STIMULATED RAT ISOLATED ANOCOCCYGEUS MUSCLE

R.D. Smith & A.H. Weston, Smooth Muscle Research Group, Department of Physiological Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT.

Soares-Da-Silva and Villanueva (1990) showed that the α_2-adrenoceptor agonist guanabenz produced a concentration dependent relaxation of 5-HT and noradrenaline preconstricted rat aortic rings. The relaxation was unaffected by yohimbine, propranolol or by the removal of the endothelium, but was antagonized by tetraethylammonium (TEA). They concluded that the effects of guanabenz were not produced by α_2-adrenoceptor stimulation. Using ligand binding assays in rat liver, Zonnenschein et al. (1990) have shown that guanabenz has affinity for the nonadrenergic idazoxan binding site (N.A.I.B.S.). In the present investigation we have examined the effects of RX 82-1002 (a selective α_2-adrenoceptor antagonist) on the guanabenz induced inhibition of electrical field stimulated rat anococcygeus muscle and compared its effects with those of UK 14304 and of α-methylnoradrenaline (two selective α_2-adrenoceptor agonists). The effect of RX 80-1023, on the inhibition produced by guanabenz, was also investigated. In the central nervous system, this agent shows selectivity for N.A.I.B-sites as opposed to α_2-sites (Mallard et al., 1991).

Experiments were carried out using the rat isolated anococcygeus muscle set up under isometric conditions in 20ml organ baths containing bicarbonate buffered physiological salt solution (PSS) at 37°C. Mechanical changes were quantified using a MacLab recording system (W.P. Instruments). Electrical field stimulation was achieved via two platinum ring electrodes using stimulus trains of duration 10s and frequency 1 per min. Stimulus pulse width and amplitude were 0.1msec and 40V respectively and the pulse frequency was varied between 0.5 and 32Hz.

In normal PSS, electrical field stimulation (0.5-32Hz) produced frequency-dependent tonic contractions. Addition of guanabenz (30μM), UK 14304 (1μM) or α-methylnoradrenaline (1μM) produced a large sustained increase in tone with subsequent relaxations to electrical field stimulation. Addition of RX 82-1002 (1μM) in the continuing presence of either UK 14304 or α-methylnoradrenaline abolished the sustained increase in tone, and on field stimulation typical tonic contractions were once again produced. Exposure to RX 82-1002 (1μM) in the continuing presence of guanabenz (30μM) relaxed the guanabenz induced tone but subsequent field stimulation (0.5-32Hz) failed to generate contractions. Under these conditions, exposure to RX 80-1023 (10nM-100μM) was unable to restore the tonic contractions to electrical field stimulation in the presence of guanabenz.

These results suggest that the increase in smooth muscle tone produced by guanabenz is associated with stimulation of α_2-adrenoceptors located on the smooth muscle cells. However, the antagonistic effects of guanabenz on electrical field stimulation do not seem to be associated with an action at either α_2–adrenoceptors or at a N.A.I.B.S blockable by RX 80-1023. Further experiments are required to clarify these effects of guanabenz.

Mallard, N.J., Tyacke, R., Hudson, A.L. and Nutt, D.J. (1991). Br. J. Pharmacol. 102, 221P
Soares-Da-Silva, P. and Villanueva, M.M. (1990). J. Pharm. Pharmacol. 42, 209-211
Zonnenschein, R., Diamont, S. and Atlas, D. (1990). Eur. J. Pharmacol.. 190, 203-215

237P EVIDENCE FOR M_3 MUSCARINIC CHOLINOCEPTORS ON ENTEROCYTES WHICH INDUCE ELECTROGENIC SECRETION IN RAT SMALL INTESTINE

S.A. Przyborski, R.J. Levin & A. Young (introduced by A. Angel) Dept. Biomedical Science, University of Sheffield, S10 2TN, Yorkshire

Electrogenic secretion in guinea-pig ileum appears to be mediated by M3 muscarinic receptors located on enterocytes (Kachur *et al.* 1991). We have characterised the muscarinic receptor subtype mediating rat jejunal and ileal electrogenic secretion by comparing the potencies of selective M1 (pirenzepine (PIRZ)), M2 (methoctramine (METH)) and M3 (4-diphenylacetoxy-N-methylpiperidine (4DAMP) and Hexahydro-sila-difenidol (HHSiD)) antagonists as inhibitors of increases in short-circuit current (Isc, $\mu A/cm^2$) induced by carbachol.

Mid-jejunal and proximal ileal segments were removed from rats anaesthetised with pentobarbitone (Sagatal, 60mg/kg, body wt, IP). The segments were stripped of their outer muscle layers, mounted in Ussing chambers as sheets and incubated at 37°C in bicarbonate saline gassed with 95% O_2 and 5% CO_2. An automatic voltage clamp allowed continuous monitoring of Isc which was used as the index for electrogenic secretion. Cumulative concentration-response curves for carbachol over the range 10^{-7} to 10^{-3}M were obtained in the presence of serosal tetrodotoxin (1.25×10^{-6}M) to block any neural actions of carbachol. Antagonists were added to the serosal chamber, 10 mins prior to experimentation. The EC50 was obtained for each curve by non-linear regression (Table 1).

TABLE 1. EC50 values (mean (μM), ±SEM, n=8-15) obtained from rat jejunum and ileum incubated *in vitro*.

	Control	PIRZ 10-7M	METH 10^{-7}M	10^{-8}M	4DAMP 10^{-7}M	10^{-6}M	10^{-7}M	HHSiD 10^{-6}M	10^{-5}M
Jejunum	5 ± 1	7 ± 1	5 ± 1	11 ± 1	36 ± 3	424 ± 50	11 ± 2	44 ± 8	304 ± 43
Ileum	6 ± 1	4 ± 1	4 ± 1	11 ± 1	39 ± 3	451 ± 41	15 ± 2	67 ± 9	610 ± 60

Concentration-response curves for carbachol in the presence of pirenzepine and methoctramine were not significantly different compared to the control. However, 4DAMP and HHSiD significantly shifted the carbachol curve to the right with slopes parallel with the control and with the same maximal value. Schild plots were used to determine pA_2 values in jejunum/ileum for 4DAMP (8.0/7.9 respectively) and for HHSiD (7.1/7.2 respectively). The selectivity profiles of these antagonists indicate that the muscarinic receptors in the jejunal and ileal mucosa more closely resemble those to which 4DAMP and HHSiD have high affinity. In conclusion, as the neural blocker tetrodotoxin was present, carbachol appears to activate electrogenic secretion via non-neural muscarinic receptors presumably located on the rat enterocyte. The order of potency of the antagonists used suggests an M3 receptor but the absolute pA_2 values obtained appear lower than the range quoted in the literature.

SAP is supported by a SERC-CASE award with Reckitt & Colman plc, Hull.

Kachur, J.F., Sturm, B.L., Gaginella, T.S. & Noronha-Blob, L. (1991) Mol. Pharm. **38**, 836-840.

238P P_{2X}-PURINOCEPTOR AND MUSCARINIC CHOLINOCEPTOR MEDIATED CONTRACTIONS OF ISOLATED URINARY BLADDER SMOOTH MUSCLE FROM ADULT AND NEONATAL RABBITS

P. Sneddon & A. McLees, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow Gl 1XW.

Muscarinic cholinoceptors and purinoceptors on smooth muscle cells of the urinary bladder are thought to mediate the contractions of the detrusor muscle associated with the micturition process. The purinoceptors mediating contraction of rabbit bladder smooth muscle have been identified as the P_{2X}-subclass, since they can be blocked by arylazidoaminopropionyl-ATP, ANAPP3, (Thoebald & Hoffman, 1986) and desensitized by α,β,methylene-ATP (Kasakov & Burnstock, 1982). We have compared the sensitivity to cholinoceptor and P_{2X}-purinoceptor agonists in smooth muscle strips obtained from urinary bladder of adult and neonatal rabbits in order to examine the proposal that ATP is a more effective agonist in neonatal bladder than in the adult (Zderic *et al.*, 1990).

Longitudinal strips of detrusor muscle 2 mm wide and 1-2 cm long were maintained at 35°C in a physiological salt solution and bubbled with 95% O_2, 5% CO_2. Concentration-response curves were obtained to acetylcholine and ATP (10^{-7} - 10^{-3}M) or to carbachol and α,β,methylene-ATP (10^{-7} - 10^{-5}M). In each tissue the contractile response to 100 mM KCl and nerve stimulation (0.2 ms pulses at 1-8 Hz for 20 s) were also examined. The magnitude of the contractile responses to agonists were expressed as a % of the response to KCl in each tissue. In both the adult and neonatal bladder tissues, carbachol was at least ten times more potent than its hydrolysable analogue acetylcholine. Similarly, the non-hydrolysable P_{2X}-purinoceptor agonist α,β,methylene-ATP was at least ten times more potent than ATP in both the adult and neonatal tissues. At all concentrations of acetylcholine and carbachol used there was no significant difference between the responses of the adult compared to the neonatal tissues. At all concentrations of ATP the contractile responses of the neonatal tissue was greater than that from the adult, and this difference was statistically significant at concentrations of 10^{-5}M, 10^{-4}M, and 10^{-3}M ATP (e.g. at 10^{-5}M ATP the mean response in the neonate was 39.0±10.3% (n=6) and in the adult 6.2±2.6% (n=6)). Similarly, at all concentrations of α,β,methylene-ATP used, the contractile response of the neonatal tissue was greater than that of the adult, and this difference was statistically significant at concentrations of 10^{-7} and 10^{-6}M (e.g. at 10^{-6}M the mean response in the neonate was 71.1±11.8% (n=7) and in the adult 33.5±9.1% (n=6)). The magnitude of contractile responses to nerve stimulation of the neonatal tissues were also significantly greater than in the adult tissues (e.g. at 4 Hz the mean response of neonatal tissues was 119.1±20.1% (n=11) and of adult tissues 62.6±6.4% (n=11)).

We conclude that in the smooth muscle of the rabbit urinary bladder P_{2X}-purinoceptor agonists are relatively more effective in producing contraction in neonatal tissue than in the adult, which may be a significant factor in the physiological function of the neonatal bladder. The results with carbachol and α,β, methylene-ATP also suggest that both the adult and neonatal tissues have efficient mechanisms for the inactivation of acetylcholine and ATP. The reason for the enhancement of neurogenic contractions in the neonate requires further investigation.

Kasakov, L. & Burnstock, G. (1982). *Eur. J. Pharmacol.*, **86**, 291-294.
Theobald, R. J. & Hoffman, V. (1986). *Life Sci.*, **38**, 1591-1595.
Zderic, S. A., Duckett, J. W., Wein, A. T. & Snyder, H. M. (1990) *Pharmacol.*, **41**, 119-123.

239P CATHINONE, AMPHETAMINE AND NERVE-MEDIATED INHIBITION OF SPONTANEOUS CONTRACTIONS OF RABBIT SMALL INTESTINE

A. Bhupalan, I.M.L. Murray-Lyon & H. Leathard. Departments of Gastroenterology and Pharmacology, Charing Cross and Westminster Medical School, London, W6 8RF

In certain areas of the Arab Peninsula, leaves of the khat bush (Catha Edulis) are chewed widely as a stimulant. This habit appears to be associated with a high incidence of constipation, but the mechanism of this action is unknown. Approximately 12 years ago the alkaloid cathinone was isolated from khat leaves and since then animal and isolated tissue experiments have revealed that this substance is a potent amphetamine-like compound. There are well established cardiovascular, respiratory and neurological effects of cathinone, but the gastrointestinal effects are less well documented (Kalix, 1984). A recent demonstration that chewing khat significantly delays oro-caecal transit (unpublished) prompted the present study in which (-) cathinone hydrochloride was compared with dexamphetamine sulphate on rabbit sympathetically-innervated, small intestine.

Pieces of ileum with attached mesentery were dissected from freshly killed rabbits weighing 800-900 g. The segments were suspended longitudinally (counterbalanced by 1 g) in isolated organ baths at 37°C in Krebs solution gassed with 5% carbon dioxide and 95% oxygen (pH 7.4). A mesenteric branch containing perivascular nerves was passed over stimulating electrodes through which 15 s trains of 10 V, 0.5 ms square-wave pulses were delivered. Spontaneous rhythmical contractions and evoked inhibitory responses were recorded isotonically. Nerve-mediated relaxations elicited at 5, 10, 20 and 40 Hz were recorded initially, and subsequently in the presence of 0.1, 1 or 10 μM cathinone or amphetamine or equivalent volumes of their solvent (0.9% NaCl) (n=6, 6 and 9 preparations respectively).

Nerve-mediated relaxation (5-20 Hz) were enhanced by amphetamine in a concentration-dependent manner ($P<0.05$, n=6, paired 't' tests), but the apparent tendency for enhancement by cathinone of nerve-mediated relaxations was not significantly different from changes that occurred over a similar time course in control studies.

These results do not support the possibility that the delayed oro-caecal transit caused by chewing khat involves an effect of cathinone on sympathetic nerve-mediated inhibition of small intestinal muscle.

We thank the Charing Cross Research Committee for financial support and World Health Organisation for cathinone.

Kalix, P. (1984) Gen Pharmac 15, 3, pp 179-187.

240P SODIUM DECAVANADATE INHIBITS [^{3}H]InsP$_3$ BINDING IN GUINEA-PIG ILEUM SMOOTH MUSCLE AND BOVINE ADRENAL CORTEX

M.J. Allen, A. Wallace[1] & J.R. Carpenter. Smooth Muscle Research Group, Dept. Physiological Sciences, University of Manchester, M13 9PT. [1]Dept. Biochemistry, Fisons Plc., Loughborough, LE11 0RH.

Fohr *et al.* (1989) have reported that sodium decavanadate ($Na_6V_{10}O_{28}$) inhibits myo-inositol 1,4,5-trisphosphate ($InsP_3$)-induced calcium release in permeabilised secretory cells. In addition we have previously reported that a number of vanadium salts, including sodium decavanadate, were able to inhibit $InsP_3$-induced contractions of saponin-permeabilised smooth muscle (Allen & Carpenter, 1991). Here we report that sodium decavanadate, but not other vanadium salts, inhibit the binding of [^{3}H]$InsP_3$ in guinea-pig ileum smooth muscle (GPI) and bovine adrenal cortex membranes (BAC).

The source of the membranes was a crude microsomal pellet from BAC or GPI (stored at -70°C until required). The conditions for the binding assay were as follows: 25mM Tris (pH 7.0), 1mM EDTA, 1mM EGTA, 1mg/ml bovine serum albumin, 4°C. After 15 min incubation of the membrane in the presence of [^{3}H]$InsP_3$ (2.0nM) and test compound the samples were centrifuged (14,000g, 2 min) and the supernatent aspirated. Radioactivity of the pellet was counted by scintillation.

In GPI $InsP_3$ inhibited [^{3}H]$InsP_3$ binding with an IC_{50} of 120nM (n=3). The binding curve could be best fitted using a computer-generated fit to a two binding site model with affinity constants of 2.0nM and 1.1μM. In BAC $InsP_3$ inhibited [^{3}H]$InsP_3$ binding with an IC_{50} of 4.4nM (n=3). The binding curve could be best fitted using a computer-generated fit to a one binding site model with an affinity constant of 5.1nM. In GPI sodium decavanadate inhibited [^{3}H]$InsP_3$ binding with an IC_{50} of 1.5μM (n=3). Neither sodium metavanadate ($NaVO_3$), sodium orthovanadate (Na_3VO_4), vanadyl sulphate ($VOSO_4$) nor vanadium chloride (VCl_3) inhibited [^{3}H]$InsP_3$ binding (n=3). In BAC sodium decavanadate inhibited [^{3}H]$InsP_3$ binding with an IC_{50} of 1.1μM (n=3). Sodium metavanadate or sodium orthovanadate had no effect up to 1mM. 1mM vanadyl sulphate and vanadium chloride produced 36% and 51% inhibition of [^{3}H]$InsP_3$ binding (n=3).

In conclusion sodium decavanadate, but not other vanadium salts, is a potent inhibitor of [^{3}H]$InsP_3$ binding in guinea-pig ileum smooth muscle and bovine adrenal cortex. However, our previous observations that vanadium salts were able to inhibit $InsP_3$-induced contractions in smooth muscle are unlikely to be due to direct antagonism at the $InsP_3$ binding site.

M.J.Allen is in receipt of a Fisons studentship.

Allen, M.J. & Carpenter, J.R. (1991). *Br. J. Pharmacol.* **102**, 31P.
Fohr, K., Scott, J., Anhert-Hilger, G. & Gratz, M. (1989). *Biochem. J. 262, 83-89.*

241P THE EFFECTS OF PGE_2, SULPROSTONE AND AH13205 ON HAMSTER UTERUS *IN VITRO*

H.L. Yeardley, R.A. Coleman[1], K. Marshall and J. Senior. Department of Postgraduate Studies in Pharmacology, School of Pharmacy, University of Bradford, Bradford BD7 1DP, and[1] Department of Peripheral Pharmacology, Glaxo Group Research Ltd., Ware, Herts SG12 0DP.

Prostaglandin (PG)E_2 at nanomolar concentrations inhibits spontaneous and agonist evoked contractile activity in hamster isolated uterus, whereas higher concentrations induce excitatory responses (Coleman, 1983). The aim of the present study was to investigate the prostanoid EP-receptor subtype (Coleman et al., 1987) involved in the inhibition of uterine activity, using the selective synthetic agonists, sulprostone (EP_1/EP_3) and AH13205 (EP_2) (Nials et al., 1991). Data from functional studies on hamster uterus have been compared with effects on intracellular levels of cyclic AMP in the same tissue.

Hamster uterine strips were prepared for superfusion (2ml min^{-1}) with oxygenated Krebs solution (95% $C0_2$/5% 0_2, 37° C), containing indomethacin (2.8μM) (Sangha and Senior, 1989), and stimulated electrically (30s trains, 5HZ., 0.3 ms., 20V. every 2 min) to elicit phasic contractile activity. PGE_2, sulprostone and AH13205 were infused in concentrations of 0.1-1000nM over a period of at least 10 min. or until a stable response was obtained. The effect of 2 min. incubations with the agonists (1nM-10μM) on cyclic AMP levels in hamster uterine strips was investigated by RIA as described by Sumner et al (1989).

PGE_2 (1 and 10nM, n=11), but not AH13205 or sulprostone (up to 1000nM), inhibited the amplitude of electrically-induced contractions by 47±6% and 37±5% respectively. At higher concentrations, PGE_2 (100-1000nM) and sulprostone (10-1000nM), but not AH13205 (up to 1000nM), induced excitatory activity seen as an increase in basal tone and an enhancement of electrically-induced activity. In addition, PGE_2 (10nM-10μM, n=6), but not AH13205 or sulprostone (up to 10μM, n=6) caused concentration-related increases in cyclic AMP levels in hamster uterus giving a maximum of 30±8 pmol cyclic AMP mg $protein^{-1}$.

The high potency of PGE_2 in both inhibiting uterine contractions and elevating intracellular cyclic AMP supports the involvement of EP-receptors in prostanoid induced inhibitory effects in hamster uterus. However, the lack of activity of sulprostone and AH13205 casts doubt on the involvement of EP_1-, EP_2- or EP_3-subtypes in this response.

Coleman, R.A. (1983), Ph.D. Thesis, 107-121, CNAA.
Coleman, R.A., Kennedy, I., Sheldrick, R.L.G. and Tolowinska, I.Y. (1987). Br. J. Pharmacol., 91, 407P.
Nials, A.T., Coleman, R.A., Hartley, D. and Sheldrick, R.L.G. (1991), Br. J. Pharmacol., 102, 24P.
Sangha, R.K. and Senior, J. (1989), Br. J. Pharmacol., 98, 762P.
Sumner, M.J., Feniuk, W. and Humphrey, P.P.A. (1989), Br. J. Pharmacol., 97, 292-300.

242P AN INVESTIGATION OF THE SELECTIVITIES OF BRL 55834 AND BRL 38227 AS RELAXANTS OF GUINEA-PIG TRACHEAL SPIRALS RELATIVE TO PORTAL VEIN

S.G. Taylor, D.R. Buckle, D.J. Shaw, J.S. Ward & J.R.S. Arch, SmithKline Beecham Pharmaceuticals, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ

Compared to the prototype potassium channel activator (KCA) BRL 38227, BRL 55834 a novel KCA (Taylor *et al.*, 1992) is a four- to sixfold more potent bronchodilator and four- to sevenfold more selective relative to its vasodilator activity in the guinea-pig and rat (Bowring *et al.*, 1992). We have attempted to rationalize this improved *in vivo* airways selectivity of BRL 55834 by comparing the potencies of BRL 38227 and BRL 55834 as relaxants of guinea-pig tracheal spirals and portal vein *in vitro*.

Cumulative concentration-response curves were constructed in parallel for BRL 55834 and BRL 38227, at least 10 min being allowed between each addition. Geometric mean IC50 values were calculated relative to 10^{-3}M isoprenaline (tracheal spirals) or 10^{-4}M papaverine (portal vein), except for histamine-induced tone, where they are relative to the compounds' own maximum effects (n=4-9).

IC50 values (μM) and intrinsic activities (in parentheses) for BRL 55834 were 0.019 (0.94), 0.041 (0.69), 0.020 (0.88), 0.021 (0.88), 0.018 (0.92) and >20 (0.26) against spontaneous, histamine (5μM)-, LTD4 (3nM)-, PGE2 (10nM)-, 5-HT (0.8μM)- and carbachol (0.3μM)- induced tone in tracheal spirals, and 0.021 (0.95) against 30mM KCl-induced tone in portal vein, the intrinsic activities being similar to those of BRL 38227. BRL 55834 was 12-, 27-, 16-, 15- and 8- fold more potent than BRL 38227 against spontaneous, histamine-, LTD4-, PGE2- and 5HT-induced tone in tracheal spirals, but only 2.8- fold more potent in portal vein. Thus, depending on the method of inducing tone in tracheal spirals, it appears 4.1 (2.3-7.2)-, 9.6 (5.7-16.1)-, 5.6 (3.0-10.6)-,5.4 (2.4-10.2)- or 2.8 (1.5-5.3)-fold more selective than BRL 38227 for trachea (geometric means with 95% CI). Furthermore, BRL 55834 was longer-acting than BRL 38227 against histamine. The relaxant activity of BRL 38227 (10^{-5}M) against histamine peaked at 4.7±0.7 min but was no longer significant by 28min, whereas that of BRL 55834 (10^{-6}M) peaked at 11.1±0.6 min and remained significant ($P<0.05$) to 50 min (n=6).

The improved selectivity of BRL 55834 compared to BRL 38227 in protecting guinea-pigs against histamine-induced bronchoconstriction can therefore be rationalized in terms of the relative potencies of these compounds *in vitro* .

Bowring, N.E., Buckle, D.R., Taylor, J.F. & Arch, J.R.S. (1992) This meeting.
Taylor, S.G., Buckle, D.R., Foster, K.A., Shaw, D.J., Ward, J.S. & Arch, J.R.S. (1992) This meeting.

243P CHARACTERIZATION OF MUSCARINIC-PARACRINE INTERACTIONS IN THE REGULATION OF NORADRENALINE RELEASE FROM RAT ISOLATED TRACHEA

K. Racké, J. Bähring, G. Brunn, C. Hey, A. Reimann & I. Wessler, Department of Pharmacology University of Mainz, Obere Zahlbacher Str. 67, D-6500 Mainz, F.R.G.

The release of NA from rat trachea is inhibited by activation of muscarine receptors (Racké et al., 1991a), but facilitated in the presence of indomethacin (Racké et al., 1991b; Brunn et al., 1991). Moreover, indomethacin markedly affected the antagonism by the M_2 selective muscarine receptor antagonist methoctramine of the inhibitory effects of oxotremorine on NA release (slope of the Schild plot 0.4 in the absence, but 0.96 in the presence of indomethacin) (Brunn et al., 1991). The aim of the present study was to characterize further muscarinic and prostanoid mechanisms involved in the regulation of NA release.

Rat isolated tracheae with intact epithelium were incubated in 1.7 ml Krebs-HEPES solution and the release of endogenous NA was determined. Two periods of electrical field stimulation (S1, S2; 540 pulses at 3 Hz) were carried out and test drugs were added 10 min before S2. In some experiments the outflow of tritium was measured from isolated tracheae preincubated for 2 hrs with medium containing 10 µCi ^{3}H-arachidonic acid.

Spontaneous outflow of tritium from tracheae labelled with ^{3}H-arachidonic acid amounted to 0.126±0.005 %/min of tissue radioactivity (n=35, determined 40 min after end of labelling). Oxotremorine (1 µM) caused a small (by 15±2 %), but highly significant increase in the outflow of tritium. This effect of oxotremorine was abolished by 100 nM p-fluorohexahydrosiladiphenidol (pFHHSiD), but only partially antagonized by 1 µM methoctramine.

On the other hand the inhibitory effect of oxotremorine on the electrically evoked NA release in the presence of 3 µM indomethacin was only marginally affected by 1 µM pFHHSiD (apparent pA_2 value 5.93), whereas methoctramine effectively antagonized (pA_2 value 7.56) the oxotremorine mediated inhibition. In the presence of indomethacin different prostanoids concentration-dependently inhibited the evoked NA release maximally by 60-70 %. The following rank order of potencies was observed (IC_{35}): nocloprost (12 nM), sulprostone (33 nM), PGE_2 (350 nM), iloprost (3.5 µM). The EP_1 receptor antagonist AH 6809 in a concentration of 3 µM had no effect on NA release and did not affect the inhibitory action of 1 µM sulprostone.

In conclusion, noradrenergic nerve terminals in the rat trachea are endowed with inhibitory muscarine M_2 and prostaglandin EP_3 receptors. Activation of muscarine receptors, which appear to belong to the M_3 subtype, can cause liberation of arachidonic acid, an effect which may result in the release of inhibitory prostaglandins.

Supported by the Deutsche Forschungsgemeinschaft (Ra 400/3-1)

Racké, K., Bähring, A., Brunn, G., Elsner, M. & Wessler, I. (1991a) Br. J. Pharmacol. **103**, 1213-1217.
Racké, K., Brunn, G. & Elsner, M. (1991b) Naunyn-Schmiedeberg's Arch. Pharmacol. **343**, R93.
Brunn, G., Langer, C., Wessler, I. & Racké, K. (1991) Naunyn-Schmiedeberg's Arch. Pharmacol. **344**, R74.

244P INHALED LYSO-PAF CAUSES PLASMA EXUDATION IN GUINEA-PIG AIRWAYS

T. Sakamoto, W. Elwood, P. J. Barnes & K. F. Chung, Department of Thoracic Medicine, National Heart and Lung Institute, Royal Brompton Hospital, Dovehouse Street, London, SW3 6LY

Platelet activating factor (PAF) is one of the most potent inducers of bronchoconstriction and airway microvascular leakage in several species. By contrast, lyso-PAF, the precursor and metabolite of PAF, is considered inactive, although it may be converted to PAF by airway cells. We have investigated the effects of inhaled lyso-PAF on bronchoconstriction and airway microvascular leakage in anaesthetised, mechanically ventilated guinea pigs.Lung resistance (R_L), an index of bronchoconstriction, was measured for 6 minutes after inhalation of lyso-PAF aerosol (0.3, 1 and 3 mM; 30 breaths). Simultaneously airway microvascular leakage determined by extravasated Evans blue dye content was measured at the airway levels of trachea (TR), main bronchi (MB) and proximal and distal intrapulmonary airways (PIPA and DIPA). Evans blue dye extravasation was measured immediately and 2, 4, 6 and 15 minutes after lyso-PAF inhalation (3 mM, 30 breaths). We also studied the effects of a specific PAF-receptor antagonist (WEB 2086, 0.2 and 2 mg/kg i.v.) on the responses to inhaled lyso-PAF (3 mM, 30 breaths). Inhaled lyso-PAF caused an increase in R_L and extravasation of Evans blue dye at all airway levels in a dose dependent fashion (Table 1). The degree of leakage in TR, MB and PIPA was comparable to that induced by inhaled PAF (3 mM, 30 breaths). The Evans blue dye extravasation at all airway levels was detected 4 minutes after the lyso-PAF inhalation and the responses lasted over 10 minutes, whereas inhaled PAF immediately caused the extravasation after its inhalation (Tokuyama et al., 1991). WEB 2086 (2 mg/kg) completely inhibited both peak RL and extravasation of Evans blue dye at all airway levels.

Table 1. Effects of inhaled lyso-PAF on bronchoconstriction and airway microvascular leakage in guinea pig airways

	Increase in R_L (%)	Evans blue dye (ng/mg wet tissue)			
		TR	MB	PIPA	DIPA
PAF (3 mM, n = 6)	865.5 ± 114.9**	85.5 ± 1.4**	107.1 ± 8.8**	76.0 ± 6.9**	69.6 ± 7.2**
Lyso-PAF(3 mM, n = 6)	270.6 ± 55.8**	96.0 ± 4.1**	76.9 ± 8.1**	64.6 ± 6.6**	25.4 ± 1.3**
(1 mM, n = 6)	251.4 ± 73.7*	57.4 ± 8.7	58 9 ± 7.9**	45.2 ± 3.9	18.4 ± 2.9
(0.3 mM, n = 6)	256.3 ± 132.8	40.3 ± 4.2	33.9 ± 6.2	41.6 ± 4.2	13.5 ±1.1
Vehicle (n = 5)	65.8 ± 10.2	35.2 ± 5.8	16.9 ± 5.3	32.9 ± 5.1	15.8 ± 1.0

Data are shown as mean ± S.E.M. * $P < 0.05$ ** $P < 0.01$ compared with vehicle control. Our results show that inhaled lyso-PAF in the guinea pig airway causes similar increases in R_L and airway microvascular leakage as PAF. The effects of lyso-PAF may result from its metabolic transformation to PAF by PAF acetyltransferase in the airway.

Tokuyama K., Lötvall J. O., Barnes P. J., & Chung K.F.(1991) Am. Rev. Respir. Dis.143, 1345-1349.

245P A COMPARISON OF THE BRONCHOCONSTRICTOR ACTIONS OF ENDOTHELIN-1 AND HUMAN PROENDOTHELIN (1-38) IN THE ANAESTHETISED GUINEA-PIG

J.O. Curwen, P.W. Marshall & A.E. Tomlinson. ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

Some authors have shown that at least some of the bronchoconstriction caused by endothelin-1 was due to the action of thromboxane A_2 (Namba et al., 1991; Battistini et al., 1990). Other authors however, have shown endothelin-1 induced contractions of respiratory smooth muscle to have no TXA_2 component (McKay et al., 1991). In the experiments described below, the effects of both endothelin-1 and human proendothelin (1-38) on the guinea pig respiratory system were studied. The ability of indomethacin and ICI 192605, a selective TXA_2 antagonist (Jessup et al., 1988), to alter these effects was also tested.

Guinea pigs of either sex (400-500g) were anaesthetised with sodium pentobarbitone (60 mg/kg ip). They were respired with a small rodent ventilator (4ml at 55 strokes per minute) using room air. Pulmonary inflation pressure (PIP) was measured via a side-arm pressure transducer. The jugular vein was cannulated for drug administration and the carotid artery for blood pressure and heart rate measurement. Both endothelin-1 and proendothelin were given only in a single administration per animal to avoid problems caused by desensitisation (especially seen with proendothelin).

Endothelin-1 produced a dose dependent bronchoconstriction over the dose-range 0.01 to 1.0 nmoles/kg whereas proendothelin produced an "all or none" type response over the dose range 0.3 to 10.0nmoles/kg. 0.2nmoles/kg endothelin-1 and 10nmoles/kg proendothelin produced approximately equal degrees of bronchospasm (mean values of 84.6% and 77.3%, both N=9, respectively). 10.0mg/kg indomethacin or 0.1mg/kg ICI 192605 caused total inhibition of the endothelin-1 response but only partial inhibition of the proendothelin response (mean values of 39.8% and 33.8%, $P<0.05$ both N=5, respectively).

These results suggest that, although endogenously and exogenously-derived endothelin-1 have a similar bronchconstrictor effect in the anaesthetised guinea-pig, the underlying mechanisms of the bronchoconstriction are different.

Battistini, B., Filep, J. & Sirois, P. (1990) Eur. J. Pharmac. 178, 141-142
Jessup, C., Jessup, R. & Wayne, M. (1988) Br. J. Pharmac. 95, 675P
McKay, K., Black, J. & Armour, C. (1991) Br. J. Pharmac. 102, 422-428
Nambu, F., Yube, N., Omawari, N., Sawada, M., Owegawa, T., Kawasaki, A., & Ikeda, S. (1991) Adv. Prost. Throm. Leuk, Res. Vol 21, 453-456

246P PAF-INDUCED BRONCHOCONSTRICTION AND NEUTROPHIL ACCUMULATION IN THE RABBIT LUNG *IN VIVO*

JG Hanss, KM O'Shaughnessy, CT Dollery, GW Taylor, RW Fuller, Department of Clinical Pharmacology, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN.

Inhalation of platelet activating factor (PAF) causes bronchoconstriction in a number of animal species including man (Barnes *et al.*). It is thought to act indirectly on bronchial smooth muscle by the release of spasmogenic eicosanoids (Taylor *et al.*; Spencer *et al.*). Although the precise cellular origin of these eicosanoids is uncertain, the neutrophil which rapidly accumulates in the lung following inhalation of PAF aerosols is a likely candidate.

We have investigated the effect of aerosolised PAF in 9 spontaneously breathing normal rabbits anaesthetised with a combination of i.m. diazepam and Hypnorm (fentanyl/fluanisone). Tracheal air flow was measured with a pneumotachometer attached to a cuffed 3mm endotracheal tube and transpulmonary pressure (TPP) measured using an oesophageal balloon. Lung resistance and dynamic compliance were determined from the digitised flow and TPP signals using an on-line microprocessor system (Mumed pulmonary monitoring system). Neutrophils were obtained from citrated donor rabbit whole blood using plasma-Percoll gradients and labelled with [^{111}In]-tropolonate (150-200 μCi). PAF (lyophilised C16 reconstituted in 0.9% saline, Novabiochem) was administered in two doses (0.5mg/ml aerosolised for 5 min) separated by 2 min. Following PAF administration the cells (30-50 x 10^6) were injected into the ear vein of recipient rabbits. The total lung radioactivity was monitored under a gamma camera at 15 min intervals over 90 min with simultaneous measurements of lung function.

In 4 animals, PAF caused a greater rise in lung resistance 67(52-298)% [median (range)] group 1, than the remaining 4 animals where little change in lung resistance was observed 10(5-14)%, group 2. The two groups were significantly different ($P<0.05$, Mann-U-Witney). The retention of [^{111}In]-tropolonate labelled neutrophils, expressed as the % remaining in an apical (suprahilar) region of interest 30 mins after injection, was 59(50-80)% in the first group compared to 43(30-50)% in the second group of animals (which showed little response to PAF) ($P<0.05$). The retention in the second group was not significantly different from control rabbits given a saline aerosol challenge alone, 43(37-50)% ($P>0.05$). To determine whether the response in the rabbits was reproducible, single animals were taken from both group 1 and group 2 and challenged for a second time with PAF 3-4 weeks after the initial challenge: PAF still caused a rise in resistance (>50%) and a concomitant retention of [^{111}In]-tropolonate in the lung of the group 1 animal, but had little effect in the group 2 animal.

It appears that PAF-induced bronchoconstriction is intimately associated with neutrophil accumulation in the rabbit, and further studies are in progress to delineate the mechanism.

Barnes PJ, Chung KF & Page CP. (1988) Pharmacol Rev. 40, 49.
Taylor IK, Ward PS, Taylor GW, Dollery CT & Fuller RW. (1991) J. Appl. Physiol. 71, 1396.
Spencer DA, Evans JM, Green SE, Piper PJ & Costello JF. (1991) Thorax 46, 441.

247P EVIDENCE THAT THE NOVEL, AIRWAYS SELECTIVE BENZOPYRANOL, BRL 55834, IS A POTASSIUM CHANNEL ACTIVATOR

S.G. Taylor, D.R. Buckle, K.A. Foster, D.J. Shaw, J.S. Ward & J.R.S. Arch, SmithKline Beecham Pharmaceuticals, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ

BRL 55834 is a novel benzopyran that has been shown to possess potent and selective airways smooth muscle relaxant activity both *in vitro* (Taylor *et al.*, 1992) and *in vivo* (Bowring *et al.*, 1992). We now present evidence that, like BRL 38227, BRL 55834 elicits its relaxant effects in guinea-pig trachea through potassium channel activation. The possibility that BRL 55834 and BRL 38227 inhibit the mobilization of different calcium sources has also been investigated.

Low (10mM) K^+-induced contractions of tracheal spirals were enhanced by Bay K8644, to give a well-maintained tone. BRL 55834 relaxed such contractions [IC_{50}=0.051 (0.019-0.140) μM (geometric mean, 95% CI, n=4)], but had little effect against high (30mM) K+-induced contractions. BRL 55834 was a potent relaxant of 5μM histamine-induced tone [IC_{50}=0.041 (0.015-0.109) μM] and preincubation with BRL 31660 (10μM) (Taylor *et al.*, 1989) or glibenclamide (1μM) caused marked rightward shifts of the concentration-response curve [BRL 31660:IC_{50}=5.1 (2.4-10.4)μM; glibenclamide: IC_{50}=1.28 (0.25-6.50)μM, n=4]. In trachealis preloaded with $^{42/43}K+$, 0.1μM BRL 55834 stimulated potassium efflux by 31±4%, n=6.

Preincubation of tracheal spirals with either BRL 38227 or BRL 55834 had no significant effect on the histamine concentration-response curve, suggesting that neither compound influences the initial release of calcium from intracellular stores. The calcium entry blocker dazodipine (1μM) elicited a 25±7% (n=9) relaxation of spontaneous tone. In the presence of dazodipine, BRL 55834 and BRL 38227 elicited further relaxations and their IC_{50} values were unchanged [BRL 55834: control 0.027 (0.015-0.047), dazodipine 0.025 (0.017-0.036) n=6; BRL 38227: control 0.64 (0.21-1.85), dazodipine, 0.72 (0.22-1.84)μM, n=4].

These data support the notion that BRL 55834 effects smooth muscle relaxation through the opening of potassium channels. The airway selectivity of BRL 55834 compared to BRL 38227 does not appear to be due to a relatively greater influence on intracellular calcium movements.

Bowring, N.E., Buckle, D.R., Taylor, J.F. & Arch, J.R.S. (1992) This meeting.
Taylor, S.G., Buckle, D.R., Shaw, D.J., Ward, J.S. & Arch, J.R.S. (1992) This meeting.
Taylor, S.G., Foster, K.A., Shaw, D.J. & Taylor, J.F. (1989) Br. J. Pharmac. 98, 881P.

248P PROPERTIES OF RAT STELLATE GANGLION CELLS: COMPARISON OF PUTATIVE CARDIAC-PROJECTING AND NON CARDIAC NEURONES

N.Mo and D.I.Wallis, Dept. of Physiology, UWCC, Cardiff, CF1 1SS.

Intracellular recording from isolated stellate ganglia of young adult rats (3-4 months) allowed comparison of the electrical and pharmacological properties of individual sympathetic neurones. An antidromic spike evoked from cardiac nerve(s) or from a branch identified as supplying the brachial plexus was used to identify cells as putative cardiac or non-cardiac neurones, respectively. Following ether anaesthesia, ganglia and their cardiac nerves (Yasunaga & Nosaka, 1979), other nerve branches and sympathetic trunk were rapidly excised and transferred to the recording chamber. Ganglia were superfused with oxygenated, modified Krebs solution (mM): NaCl 117; KCl 4.7; $CaCl_2$ 2.5; $MgCl_2$ 1.2; $NaHCO_3$ 25; NaH_2PO_4 1.2; glucose 11.5, 34±0.5°C). Recordings were obtained from neurones of the left or right stellate ganglia by means of glass microelectrodes (35-60MΩ) filled with 2M K acetate. Cardiac nerve, brachial plexus branch and/or sympathetic trunk were drawn into suction electrodes for stimulation. 117 cells were studied in 52 ganglia.
Stellate neurones could be classified as "phasic" (53%), "tonic" (41%) or "LAH" (long after hyperpolarization, 6%) subtypes by their discharge characteristics to depolarizing current (McLachlan et al., 1989). Phasic neurones had a significantly lower input resistance (84.1±5.1MΩ, n = 62) than tonic neurones (140±12.6 MΩ, n=48), but membrane potentials did not differ significantly. The membrane properties of cardiac neurones (CN) appeared similar to those of non-cardiac neurones (NCN) (Table 1).

	R M P (mV)		Input R (MΩ)		Time constant (ms)	
	CN	NCN	CN	NCN	CN	NCN
Phasic	-57.1 ± 1.6 (n=16)	-58.4 ± 1.4 (n=22)	88.9 ± 12.7	97.2 ± 13.4	7.9 ± 0.9	6.6 ± 1.0
Tonic	-60.2 ± 1.8 (n=12)	-62.6 ± 1.4 (n=16)	142.1 ± 21.9	151 ± 20.8	9.3 ± 1.1	11.8 ± 1.8
LAH	-53, -56	-50, -64	102, 135.6	44.1, 209.5	5, 11	12.6, 13.9

TABLE 1 Membrane properties of putative cardiac and non-cardiac stellate ganglion cells.
Values are means ± s e mean. RMP = resting membrane potential, Input R = input resistance.

The pharmacological profile of cardiac and non-cardiac neurons is being examined by applying putative endogenous neurotransmitters, neuromodulators or muscarine. Preliminary results indicate that muscarine (10μM superfused for 30-60s) evoked a depolarization (5-12mV) with an increase in input resistance in 21 of 24 cells. Angiotensin II was applied either by pressure ejection or superfusion (1-10μM) to 29 cells of which 11 responsed in one of the following 3 ways: a slow depolarization, a hyperpolarization or a biphasic response. Depolarizations were evoked by Substance P (1μM) in 3 of 6 cells, by bradykinin (10μM) in 4 of 6 cells and by 5-HT (10μM) in 5 of 17 cells. Noradrenaline (10μM), 5-HT (10-100μM) and met-enkephalin (10μM), when applied to ganglia for 3-5min, reversibly depressed fast EPSPs in 8 of 9 cells, 3 of 4 cells and 3 of 4 cells, respectively.

Yasunaga, K. & Nosaka, S. (1979) Jap.J.Physiol. 29: 691-705.
McLachlan, E.M. & Meckler, R.L. (1989) J. Physiol. 415: 109-129

Supported by the British Heart Foundation.

249P PATCH-CLAMP RECORDING IN LATERAL HORN CELLS OF RAT SPINAL CORD IN A SLICE PREPARATION: MEDIATION OF SUBSTANCE P EFFECTS BY NK_1 RECEPTORS

J. Krupp & P. Feltz, Universite Louis Pasteur, URA CNRS 1446 - Laboratoire de Neurophysiologie et Neurobiologie des Systemes Endocrines, 21 rue Rene Descartes, 67084 Strasbourg Cedex, France

The undecapeptide substance P (SP) increases excitability of the sympathetic preganglionic neurones (SPNs) in the lateral horn of the spinal cord (Dun & Mo, 1988). We have studied the effects of the SP analogue and Neurokinin-1 (NK-1) receptor-agonist [Sar^9, $Met(O2)^{11}$]-SP on identified neurones recorded in the lateral horn. This study was carried out by using the nystatin perforated patch recording method (Dick & Horn, 1988) and conventional whole-cell recording in an *in vitro* spinal cord slice preparation (Edwards *et al.*, 1989).

Thin slices (200-300 µM) were prepared from neonatal rats (0-6 d) and maintained in a recording chamber continuously perfused with oxygenated Ringer solution (95% O2/5% CO2 at 35°C). Patch pipettes were filled with (mM) KCl, 140; NaCl, 9; MgCl2, 1; HEPES, 10; EGTA, 0.2; pH=7.4 with KOH. For perforated patch recording nystatin (250 µg/ml) was added. All recordings were made under immersion optics (x 400). [Sar^9, $Met(O2)^{11}$]-SP (0.5-10 µM) was applied to the recorded cell at some 500-1000 µm distance by means of a fast microperfusion through thin plastic micropipettes (i.d. 350 µm).

Stable recordings could be maintained for up to 2-3 h. Cells had resting potentials >-40 mV and input resistances in the range of 400-1400 MΩ. The neurones showed spontaneous action potential firing as well as spontaneous synaptic activity could be observed. The latter was characterised in order to test for the viability of the preparation. The synaptic activity reversed at 0 mV in equimolar Cl^--solution. When strychnine was added to the perfusing Ringer solution most of the synaptic events were reversibly suppressed. In contrast, GABA-antagonists like bicuculline or SR 95531 showed no effect. Most of the synaptic activity is likely to be due to the release of endogenous glycine as already proposed by other authors (Mo & Dun, 1987).

In perforated patch recordings two types of responses could be observed upon a short-lasting (2-5 s) application of [Sar^9, $Met(O2)^{11}$]-SP. The most common consisted of a slow and long-lasting (10-50 s) monophasic depolarisation. Biphasic responses were as well to be detected starting with a hyperpolarisation. For cells recorded in the current-clamp mode, spike discharges at high frequencies (10-15 Hz) were consistently observed on the depolarising slow wave, sometimes outlasting the changes in membrane potential for some minutes. Beside the spikes and increased excitability, small, and under the used ionic conditions, depolarising events (2-10 mV; 10-50 Hz) were observed, likely to be of synaptic origin. As Dun & Mo (1988) reported similar effects like depolarisation, spiking activity and occurence of IPSPs after application of SP in SPNs, we conclude that this excitatory effect of SP is, at least partial, mediated by NK-1 receptors.

J.K. is a Boehringer Ingelheim Fonds fellow.

Dun, N.J. & Mo, N. (1988) J. Physiol. (Lond.) **399**, 321-333.
Edwards, F.A., Konnerth, A., Sakmann, B. & Takahashi, T. (1989) Pflügers Arch. **414**, 600-612.
Horn, D. & Marty, A. (1988) J. Gen. Physiol. **92**, 145-159.
Mo, N. & Dun, N.J. (1987) Brain Res. **400**, 139-144.

250P TACHYKININS CAN DEPOLARIZE THE RAT ISOLATED SUPERIOR CERVICAL GANGLION VIA NK_1 RECEPTORS

J.M. Stables, R.M. Hagan & S.J. Ireland . Department of Neuropharmacology, Glaxo Group Research Ltd, Ware, SG12 0DP, Herts., U.K.

The tachykinins substance P (SP), neurokinin A and neurokinin B cause depolarization of the rat superior cervical ganglion (SCG) *in vitro*. Preliminary evidence, obtained by comparison of the activities of agonists, suggests that this response is mediated predominantly via NK_1 receptors (Main and Seabrook, 1991). In the present study, we have attempted to confirm the presence of NK_1 receptors by measuring the effect of the NK_1-selective antagonist GR82334 (Hagan et al., 1991a) against depolarization responses induced by the NK_1-selective agonist GR73632 (Hagan et al., 1991b). We have investigated also the effect of peptidase inhibitors on tachykinin agonist evoked depolarization of the rat SCG. These latter experiments were undertaken since this preparation has been shown to metabolize peptide agonists (Hawcock et al., 1991) and removal of agonist can influence the apparent affinity of antagonists in the rat SCG (Ireland et al., 1987).

Ganglia were excised from Lister Hooded rats (Glaxo) and desheathed. Depolarization responses were recorded extracellularly using two-compartment Perspex baths (Ireland et al., 1987). Both compartments of the bath were superfused with oxygenated Krebs-Henseleit solution at room temperature (22°C). Drugs were applied at known concentration via the superfusion stream to the cell bodies only (3min exposure repeated every 40min). Concentration-depolarization curves to tachykinin receptor agonists were constructed non-cumulatively. To avoid desensitization, no attempt was made to define the maxima of concentration-response curves.

GR73632 (0.3-10nM) and SP methylester (SPOMe 1-10nM) caused concentration-dependent depolarization of the rat SCG. GR73632 was more active than SPOMe (mean equi-potent molar ratio 4.16 ± 0.41, n = 7). The NK_1 receptor antagonist, GR82334 (1 or 3µM), produced concentration-related rightward shifts of the concentration-depolarization curve to GR73632, yielding a mean pK_B ± s.e. mean of 7.52 ± 0.12 (n=5). The peptidase inhibitors phosphoramidon (1µM), bestatin (100µM) and captopril (5µM) had no significant effect on responses to GR73632 (EC_{50} ratio 0.63 (n=6)) although responses to SPOMe were potentiated (4.5-9.0-fold (n=2)).

The apparent affinity of GR82334 as an antagonist of GR73632-induced depolarization of the rat SCG (pK_B 7.52) is comparable to that observed at NK_1 receptors in the guinea-pig ileum or guinea-pig trachea (7.64 and 7.23, respectively; Hagan et al., 1991a) confirming the presence of NK_1 receptors in the rat SCG. The lack of effect of peptidase inhibitors on responses to GR73632 suggests that the present data are unlikely to have been influenced by metabolism of the agonist. The present results do not rule out the existence of NK_2 or NK_3 receptors in the rat SCG.

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.
Hawcock, A.B., Barnes, J.C. & Michel, A.D. (1991). *Br. J. Pharmacol.*, **102**, 279P.
Ireland, S.J., Straughan, D.W. & Tyers, M.B. (1987). *Br. J. Pharmacol.*, **90**, 151-160.
Main, M.J. & Seabrook, G.R. (1991). *Br. J. Pharmacol.*, in press.

251P NK_1 RECEPTORS IN THE GUINEA-PIG MEDIATE LOCOMOTOR HYPERACTIVITY

G.S. Mason, E.A. Graham and P.J. Elliott. Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DP.

Central administration of neurokinin (NK) agonists in rodents increases locomotor activity (LMA; Elliott & Iversen, 1986) which is mediated via NK_1 receptors (Elliott et al., 1991). Recent reports have suggested that the NK_1 receptors in the rodent differ pharmacologically from those in other species, including the guinea pig (Beresford et al., 1991). In particular, the NK_1 antagonist, racemic CP-96,345 (Snider et al., 1991) shows greater activity in guinea pigs than rodents in *in vitro* preparations. The present study has investigated the effect of intracerebroventricular (icv) infusion of the NK_1 agonist, GR73632, on LMA in the guinea pig. In addition we have attempted to block the LMA response with structurally distinct NK_1 antagonists given simultaneously. The compounds used were GR73632 (Hagan et al., 1991a), GR82334 (Hagan et al., 1991b), racemic CP-96,345 and its 2-chloro analogue {3-[(2chlorophenyl)methylamino]-2-benzhydrylquinuclidine (CPQ).

Male guinea pigs (Interfauna, 200-250g) were cannulated bilaterally with stainless steel guide cannulae aimed at the lateral ventricles. One week after surgery the animals were habituated to test boxes for 30min prior to a 5μl/side infusion of either drug or vehicle. Motor activity (photocell beam breaks = counts) was subsequently recorded for 30min. After histological verification, data were analysed using an ANOVA and a post-hoc Dunnett's test to assess significance.

The NK_1 agonist, GR73632, significantly and dose-dependently (100-400pmol) increased LMA. Haloperidol (0.5mg/kg s.c.) abolished the LMA induced by the agonist (counts±sem; vehicle:198±54; GR73632:754±119; haloperidol:221±82; GR73632 & haloperidol:241±32). The NK_1 antagonists GR82334 (0.01-1nmol) and CPQ (3-30nmol) dose dependently antagonized the reponse to GR73632 (150pmol). At a dose of 1nmol, GR82334 inhibited the LMA response by 97% ($P<0.05$). In contrast, CP-96,345 (10 or 30nmol) had no significant effect.

In guinea pigs, central administration of the NK_1 selective agonist, GR73632, increases LMA. This effect was abolished by haloperidol, illustrating an involvement of dopamine in the motor stimulation. The effect was attenuated by NK_1 antagonists although in the light of the *in vitro* data, the lack of effect of the potent non-peptide NK_1 antagonist, racemic CP-96,345 in this guinea pig model is unexpected. It is however consistent with its lack of effect in other rodent CNS models (Birch et al., 1991).

Birch, P.J., Rogers, H. & Harrison, S.M. (1991) Br. J. Pharmacol. In Press
Beresford, I.J.M., Hagan, R.M. & Ireland, S.J. (1991) Br. J. Pharmacol. In Press.
Elliott, P.J. & Iversen, S.D. (1986) Brain Res. **381**, 68-76.
Elliott, P.J., Turpin, M.P., Mason, G.S. & Hagan, R.M. (1991) Neuropeptides **19**, 119-126.
Hagan, R.M., Ireland, S.J., Jordan, C.C., Beresford., I.J.M., Deal, M.J. & Ward, P. (1991a) Neuropeptides, 19,127-135.
Hagan, R.M., Ireland, S.J., McBride, C., Jordan, C.C. & Ward, P. (1991b) Br. J. Pharmacol. **102**, 168P.
Snider, R.M., Constantine, J.W., Lowe, J.A., Longo, K.P., Lebel, W.S., Woody, H.A., Drozda, S.E., Desai, M.J., Vinick, F.J., Spencer, R.W. & Hess, H.J. (1991) Science **251**, 435-437.

252P HIGH RELATIVE AFFINITY OF RX77368 FOR THE RAT RETROSPLENIAL CORTEX

A.M. McDermott, C.D. Watson[1] & D.J. Nutt, Reckitt & Colman Psychopharmacology Unit, School of Medical Sciences, Bristol, U.K. [1]Department of Physiology and Pharmacology, Queen's Medical Centre, Nottingham, U.K.

RX77368 (dimethyl proline-TRH) is a biologically stable analogue of thyrotropin releasing hormone (TRH) with enhanced in vivo potency (Metcalf, 1983) but low affinity in vitro (McDermott et al., 1990). Previously, we have shown marked differences in (a) the affinity of [^{3}H]-RX77368 for rat brain stem and spinal cord binding sites and (b) in the relative binding density of [^{3}H]-RX77368 as compared with that of the parent compound or the high affinity analogue methyl-TRH (MeTRH), (McDermott et al., 1991a,b). The present study investigates binding in pre-rhinal cortex, ventral and dorsal dentate gyri, amygdala and superior colliculus, plus the retrosplenial cortex (RSPL), an area in which we have shown enhanced binding of [^{3}H]-RX77368 (McDermott et al., 1991a).

10 μm coronal sections of rat brain were preincubated (45 mins, 21^{o}C) in buffer (50mM Tris-HCl containing 0.2% BSA and 20 μM bacitracin, pH 7.4). Sections were then incubated (3 h, 4^{o}C) with various concentrations of competing ligand (TRH, MeTRH, RX77368 or RX74355 (methyl proline-TRH)) in the presence of either 15 nM [^{3}H]-TRH (119.2 Ci/mmol) or 5nM [^{3}H]-MeTRH (87 Ci/mmol) (See McDermott et al., 1991a,b). Autoradiograms were analysed quantitatively using a Quantimet 970 Image Analysis System.

The rank order of potency for cold ligands to compete for binding was very similar for each radioligand. Versus [^{3}H]-TRH:MeTRH=RX74355>TRH>RX77368. Versus [^{3}H]-MeTRH: MeTRH>RX74355>TRH>RX77368. The table shows the range of K_i values (nM, mean of 2-3 experiments performed in duplicate) for the various regions analysed with the values for RX77368 in RSPL highlighted.

Table 1

Cold ligand	MeTRH	RX74355	TRH	RX77368
vs. [^{3}H]-TRH	1.7-2.9	1.6-2.1	5.5-21.2	77-150
vs. [^{3}H]-MeTRH	1.2-2.9	2.9-5.7	11.0-20.1	86-148

Interestingly RX77368 showed a greater relative affinity for the RSPL (Ki; TRH 7.4nm; MeTRH 40.3nm) as compared with the other brain regions analysed. These results suggest that RX77368 may distinguish subtypes of TRH receptor. That a subtype should exist in the RSPL is of particular interest because this region is believed to be involved in learning and memory, processes for which there is evidence to support an involvement of TRH.

We thank ONO for the support of these studies. C.D. Watson is a SERC CASE student.

McDermott,A.M.,Wilkin,G.P. & Dickinson,S.L. (1990) Br.J.Pharmacol., **101**, 615-620.
McDermott,A.M., Hudson,A.L., Wilkin,G.P. & Dickinson,S.L. (1991a) Br.J.Pharmacol., **102**, 270P.
McDermott,A.M., Hudson,A.L., Wilkin,G.P. & Dickinson,S.L. (1991b) Fundament. Clin. Pharmacol., **5**, 441.
Metcalf,G. (1983) In Thyrotropin Releasing Hormone. eds Griffiths,E.C. & Bennett,G.W. 315-326.

253P BEHAVIOURAL AND BIOCHEMICAL EFFECTS OF THE TRH ANALOGUE RX77368 AND SCOPOLAMINE ON RADIAL ARM MAZE PERFORMANCE IN THE RAT

C.D. Watson, M.J. Hewitt, K.C.F. Fone & G.W. Bennett. Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

The tripeptide, thyrotrophin-releasing hormone (TRH) and its analogues enhance central cholinergic function and may have therapeutic relevance in Alzheimer's Disease (Mellow *et al.*, 1989). The TRH analogue RX77368 (pGlu-His-3,3'-dimethylProNH$_2$), has previously been shown to reverse an atropine-induced performance deficit in a water maze spatial memory task (Watson *et al.*, 1990). The current study evaluated whether RX77368 could also attenuate the scopolamine-induced deficit in an 8-arm radial maze, using a similar protocol.

Male Hooded Lister rats (230-280 g) were cannulated intracerebroventricularly (i.c.v.) under Halothane anaesthesia and following 7 days recovery were placed on a 23 hr food-deprivation schedule for 5 days, which was maintained throughout the study. Testing on an 8-arm radial maze (once daily for 7 mins or until 8 arm entries were made) occurred on days 12-16, 19-23 and 26-30 post-surgery. Four groups of rats (n=8 each) were pretreated (-40 min) prior to maze testing with either saline (SAL; groups A and B; 2 µl i.c.v.) or RX77368 (groups C and D; 2 µg i.c.v.) followed 10 mins later with either SAL (A and C; 1 ml/kg i.p.) or scopolamine (SCP; B and D; 0.3 mg/kg i.p.). After the last trial, rats were decapitated and the hypothalamus, septum and medial-parietal cortex were removed to determine TRH levels by radioimmunoassay (Lighton *et al.*, 1984).

Performance of all groups significantly improved over time, as measured by unrepeated arm entries (UAE, $P<0.001$; Friedman's ANOVA), total number of arms entered (TAE, $P<0.01$) and total maze time ($P<0.01$). UAE were significantly reduced in the SAL/SCP group (Kruskal-Wallis followed by MannWhitney-U; $P<0.05$, days 13-29) compared with saline controls, indicating that SCP induced a learning deficit. However, the SAL/SCP group showed significantly fewer repeated arm entries (RAE) during the first 8 days of training ($P<0.05$) and significantly more RAE on subsequent days ($P<0.05$) compared with saline controls, suggesting that SCP may have effects on other behaviours as well as learning. Comparison of UAE, RAE and TAE for the RX77368/SCP and SAL/SCP groups showed that RX77368 attenuated the SCP-induced deficit on days 13-21 but this did not reach significance on later days. Furthermore RX77368/SAL treatment significantly increased the UAE ($P<0.05$, days 12, 15) compared with saline controls, suggesting that the TRH analogue either alone or in combination with SCP improved performance of the task. Hypothalamic TRH levels (169.2 ± 18.2 pg/mg protein, n=8) were significantly elevated in the SAL/SCP (580.5 ± 106.6, $P<0.01$), RX77368/SAL (378.5 ± 75.4, $P<0.05$) and RX77368/SCP (354 ± 94, 0.05) groups, whilst levels of TRH in the medial-parietal cortex and hippocampus appeared to be reduced compared with saline controls, although these did not reach significance.

Taken together the results suggest that the SCP-induced performance deficit on the radial maze may result from a reduction in locomotor activity rather than solely a learning impairment and that RX77368 may improve radial maze performance by increasing arousal and exploratory behaviour.

C.D.W. is a SERC CASE student in conjunction with Reckitt & Colman who kindly supplied RX77368.

Lighton, C., Marsden, C.A. & Bennett, G.W. (1984) *Neuropharmacol.* **23**, 55-60
Mellow, A.H., Sunderland, T.S., Cohen, R.M., Lawlor, B.A., Hill, L.J., Newhouse, P.A., Cohen, M.R. & Murphy, D.L. (1989) *Psychopharmacol.* **98**, 403-407
Watson, C.D., Browning, B.S., Fone, K.C.F. & Bennett, G.W. (1990) *Br. J. Pharmacol.* **100**, 312P.

254P EFFECT OF GALANIN ON HYPOTHERMIA IN MICE INDUCED BY RS86 AND 8-OH DPAT: INVOLVEMENT OF ATP-SENSITIVE POTASSIUM CHANNELS

S. Patel and P.H. Hutson, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, CM20 2QR.

Galanin, a 29 amino acid peptide, coexists with acetylcholine (ACh) and serotonin (5-HT) in the central nervous system (CNS), blocks muscarinic agonist-induced phosphatidyl inositol turnover (Palazzi *et al,* 1991) and decreases 5-HT$_{1A}$ receptor affinity (Fuxe *et al,* 1988). Centrally acting muscarinic (Freedman *et al,* 1989) and 5-HT$_{1A}$ (Goodwin *et al,* 1985) receptor agonists induce hypothermia. We have now investigated the effect of galanin, on the hypothermic responses to the muscarinic receptor agonist RS86 (2-Ethyl 8-methyl-2,8-diazaspiro[4,5]decan-1,3-dion hydrobromide) and the 5-HT$_{1A}$ receptor agonist 8-OH DPAT.

Male BKTO mice (20-30g) were housed individually at ambient temperature for at least 60 mins prior to the experiment. They were restrained, and rectal temperatures determined at 10 minute intervals using a Sensotek BAT-12 temperature probe. Mice were pretreated with the peripheral muscarinic antagonist N-methylscopolamine (NMS, 1mg/kg ip) and then injected, under metofane anaesthesia, with either galanin (0.03, 0.1, 1, 3, 10nmol/5ul icv) or vehicle (artificial CSF, 5ul icv) followed 20 mins later by either vehicle (saline, 4ml/kg ip) or RS86 (10mg/kg ip). NMS pretreatment was not used in experiments with 8-OH DPAT (0.5mg/kg ip) which was given 4 mins following icv administration of galanin. Glibenclamide (10nmol/5ul icm) or ethanol vehicle (5ul icm) were administered 15 mins prior to galanin.

RS86 (10mg/kg) significantly decreased rectal temperature with a maximum change of -3.9 ± 0.26°C ($p < 0.05$, n = 16 mean ± sem) 40-60 mins post administration. Galanin (0.1, 1, 3, 10nmol) dose-dependently blocked the hypothermia induced by RS86 (-3.3 ± 0.26, -2.3 ± 0.77, -0.96 ± -0.56, -0.60 ± 0.58°C respectively, n = ≥ 6/group) without affecting rectal temperature *per se* (MED = 3nmol). Similarly, 8-OH DPAT (0.5mg/kg) significantly decreased rectal temperature (-3.1 ± 0.30°C, $p < 0.05$, n = 6) 15-30 min post injection when compared to vehicle controls. Galanin (0.03, 0.1, 1, 3, 10nmol) significantly attenuated 8-OH DPAT induced hypothermia at 1 and 3nmol (-1.1 ± 0.3, -1.6 ± 0.3°C respectively, $p < 0.05$, n = ≥ 6/group). Administration of glibenclamide (10nmol) at a dose which did not affect temperature, prior to galanin (3nmol), prevented its ability to block the hypothermia induced by both RS86 and 8-OH DPAT.

These results demonstrate that hypothermia induced in mice by the muscarinic receptor agonist RS86 and the 5-HT$_{1A}$ receptor agonist 8-OH DPAT is dose-dependently blocked by galanin, an action which may be mediated via ATP-sensitive potassium channels.

Palazzi, E., Felinska, S., Zambelli, M., Fisone, G., Bartfai, T. and Consolo, S., (1991), J. Neurochem. 56(3), 739-747.
Fuxe, K., von Euler, G., Aganti, L.F. and Ogren, S.O., (1988), Neurosci. Letts. 85, 163-167.
Freedman, S.B., Harley, E.A. and Patel S., (1989), Eur. J. Pharmacol., 174, 253-260.
Goodwin, G.M., De Souza, R.J. and Green, A.R. (1985), Nature, 317, 531-533.

255P PRESERVATION OF [^{125}I]-GALANIN BINDING SITES DESPITE LOSS OF CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE HIPPOCAMPUS IN ALZHEIMER'S DISEASE

M. Ikeda, D. Dewar, and J. McCulloch, Wellcome Neuroscience Group, University of Glasgow, Glasgow G61 1QH, United Kingdom.

Presynaptic modulation of cholinergic release by galanin in the hippocampus of rodents has prompted the suggestion of galanin antagonism as an approach to enhancing cholinergic function in Alzheimer's disease (AD) (Crawley and Wenk, 1989). We have examined galanin binding sites in conjunction with measurements of choline acetyltransferase activity (ChAT) in the hippocampal region of patients with AD and matched controls in order to examine the possible presynaptic localisation of galanin receptors on cholinergic neurones in the hippocampus in humans.

Serial 20μm cryostat sections of the hippocampal region from 9 control subjects (age = 77 ± 4 years; postmortem delay = 8 ± 2 hours; ± s.e. mean) and 11 patients with AD (age = 78 ± 1; postmortem delay = 10 ± 3 hours) were incubated with 0.5nM [^{125}I]-galanin and autoradiography was performed as previously described (Köhler et al. 1989). Autoradiograms were analysed by image analysis and binding values were calculated by reference to [^{125}I]-microscales. ChAT activity was also determined in the same tissue samples used for autoradiographic studies.

	PHGS	PHGD	SUB	CA1	CA3	CA4	PPY	DG
CON	0.7 ± 0.1	1.7 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	0.6 ± 0.1	5.6 ± 0.7	0.8 ± 0.2
AD	0.6 ± 0.1	0.9 ± 0.2*	0.6 ± 0.1	0.8 ± 0.1	0.9 ± 1.2	0.8 ± 0.2	5.5 ± 1.5	0.6 ± 0.2

[^{125}I]-Galanin binding values are mean ± s.e. mean pmol/g tissue. *P<0.01, using an unpaired, two-tailed Student's *t*-test. Abbreviations: PHGS = parahippocampal gyrus, superficial layers; PHGD = parahippocampal gyrus, deep layers; SUB = subiculum; PPY = presubicular parvopyramidal layer; DG = dentate gyrus.

[^{125}I]-Galanin binding sites were highly concentrated in the presubicular parvopyramidal layer compared to other regions of the hippocampus. In AD there was a small reduction of [^{125}I]-galanin binding only in the deep layers of the parahippocampal gyrus compared to controls (Table).

ChAT activity was markedly reduced in AD (controls 6.8 ± 0.8; AD 2.7 ± 0.9 nmol/hr/mg protein, P<0.01). There was no significant correlation between ChAT activity and the binding levels in any of the hippocampal regions.

The striking distribution of galanin binding sites, being highly concentrated in PPY, is peculiar to galanin. The preservation of the majority of hippocampal galanin binding sites in conjunction with a severe loss of ChAT activity in AD does not support the presynaptic localisation of galanin receptors on cholinergic neurones in the human hippocampus.

Crawley, J.N., Wenk, G.L. (1987) TINS, 12, 278-282.
Köhler, C., Hallman, H., Melander, T., Hökfelt, T. and Norheim, E. (1989) J. Chem. Neuroanat. 2, 269-284.

256P CHOLECYSTOKININ-INDUCED DEPOLARIZATION OF NEONATE RAT SPINAL CORD *IN VITRO*

S.K. Long & W.C.M. Cramer. Solvay Duphar B.V., Postbox 900, 1380 DA Weesp, The Netherlands.

The depolarizing action of three cholecystokinin fragments, the sulphated octapeptide (CCK8S), pentapeptide (CCK5) and tetrapeptide (CCK4) have been investigated on an in vitro neonatal spinal cord preparation.
Hemicords were prepared from neonate rats and perfused with artificial cerebrospinal fluid containing no added magnesium ions. Conventional D.C. recordings were made from a ventral root using Ag/AgCl wick electrodes (Long et al., 1990). Peptide fragments were added as 2 minute doses of known concentration (2 min contact time). Other compounds were added to the fluid reservoir. Results are presented as mean ± s.e.mean (n).
CCK8S depolarized the preparation with a threshold concentration of between 10 and 30 nM. Low doses of CCK8S (<100 nM) were repeatable with time at a dosing interval of 20 min, although more frequent application of CCK8S or higher concentrations induced desensitization. Consequently following doses of CCK8S >300 nM an interval of at least 60 min was used between doses, for lower concentrations 30 min was considered adequate. CCK8S (30 nM) produced a mean depolarization of 0.32 mV ± 0.04 s.e.mean (35). Depolarizations induced by N-methyl-D-aspartate (NMDA) did not alter responses to CCK8S. CCK5 was equipotent with CCK8S in 3 preparations. In contrast CCK4 was weaker, with a mean equipotent molar ratio (EPMR) of 17.5 ± 0.5 (3). Under control conditions NMDA was also weaker than CCK8S, EPMR 10.5 ± 1.8 (15). The NMDA receptor antagonist 2-amino-5-phosphonopentanoate (10 μM) was more effective at depressing CCK8S- than NMDA-induced depolarizations. (Respective dose ratios 6.2 ± 1.5 (4) and 3.4 ± 0.9 (4)). The addition of 0.75 mM magnesium ions to the medium produced a rightward shift (>1 log unit) in the CCK8S dose response curve in 4 preparations. In the presence of the synaptic blocker tetrodotoxin (0.1 μM) CCK8S-induced responses were also depressed with measured dose ratios >22 in 5 preparations. The corresponding mean dose ratio for NMDA on the same preparations was 3.53 ± 0.34 (5).
These results suggest that a large proportion of the CCK8S-induced response, recorded in the absence of TTX, is mediated through the release of an endogenous excitatory amino acid acting at NMDA receptors on both inter- and motoneurones. From the agonist profile it is likely that that the CCK receptor involved is a CCK-B receptor although this awaits clarification with antagonist studies.

Long, S.K., Smith, D.A.S., Siarey, R.J. & Evans, R.H. (1990) Brit. J. Pharmacol. 100, 850-854.

257P HYPOTENSIVE EFFECTS OF THE ANGIOTENSIN II RECEPTOR ANTAGONIST DuP 753 IN RENAL HYPERTENSIVE RATS

J.C.A.van Meel, W.Wienen & M.Entzeroth. A.Pharma Research, Dr.Karl Thomae GmbH, 7950 Biberach/Riss, Germany.

DuP 753 (2-n-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl] imidazole) is a nonpeptide angiotensin II receptor antagonist (Wong et al,1990). The aim of this study was to investigate the blood pressure lowering effects of DuP 753 in a conscious rat model of renovascular hypertension (2-kidney,1-clip). The compound was given orally via the drinking water during 5 days as well as by single dosages once daily or b.i.d. to conscious freely moving rats which have been implanted with a chronic-use pressure transmitter to monitor blood pressure and heart rate continuously (PhysioTel Telemetry, Data Science Int.).

Male rats (Chbb:THOM;140-150 g) were anaesthetized with pentobarbitone-Na (50 mg/kg,ip). A solid silver clip (0.20 mm diameter) was applied to the left renal artery as close as possible to the aorta. The contralateral kidney was not disturbed. The catheter of a pressure transmitter (TA11PA-C40) was inserted in the abdominal aorta and the transmitter was fixed to abdominal musculature. The animals were allowed to recover for several weeks. After implantation of the chronic-use device, blood pressure and heart rate were transmitted by telemetry and the signals were received by a RA 1010 General Purpose Receiver. Data were acquired with Dataquest IV 1.11 on a HP Vectra ES/12 386 computer. Analysis of the data was performed with a software package developed at Thomae. Results are presented as mean values ± SEM.

DuP 753 given in a dose of 3 mg/kg b.i.d. lowered mean arterial blood pressure (mBP) after 4 days of treatment by -41±11 mm Hg (n=3). Pretreatment values of mBP were 173±21 mm Hg (n=3). The vehicle had no effect on mBP. Heart rate was not changed in the vehicle-treated group (288±22 bpm,n=4) and the DuP-treated group (289±19 bpm). Administration of DuP 753 via the drinking water (3 mg/kg/day) had a small but significant effect on mBP. Pre-treatment values of mBP were 170±5 mm Hg (n=5). A reduction of -17±4 mm Hg (n=5) was observed after 5 days of treatment. The vehicle had no effect on mBP. Heart rate did not change in either group. Administration of 10 mg/kg of DuP 753 initially lowered mBP by -83±13 mm Hg (n=4) (pretreatment values 193±11 mm Hg). The maximal reduction after 4 days of treatment with 10 mg/kg of DuP 753 once daily amounted to -55±8 mm Hg. Heart rate was insignificantly increased by 27±27 bpm. An increase in plasma angiotensin II levels is probably responsible for the attenuation of the hypotensive effects of DuP 753 as recently observed in human volunteers (Christen et al,1991)

The results demonstrate antihypertensive activity of DuP 753 when administered orally as single dosages or via the drinking water during several days to conscious chronically-instrumented renovascular hypertensive rats.

Wong,P.C.,Price,W.A.,Chiu,A.T.,Duncia,J.V.et al.(1990) J.Pharmacol.Exp.Ther.252,726-732.
Christen,Y.,Waeber,B.,Nussberger,J.,Porchett,M.,et al.(1991) Circulation 83,1333-1342.

258P CALCITONIN GENE-RELATED PEPTIDE POTENTIATES NK_1 RECEPTOR-MEDIATED PAW OEDEMA BUT NOT INTRATHECAL NK_1 AGONIST-INDUCED BEHAVIOUR IN THE RAT

H. Rogers, J.A. Hill, and P.J. Birch. Department of Neuropharmacology, Glaxo Group Research Ltd, Ware, Herts, SG12 0DP, UK.

Calcitonin gene-related peptide (CGRP) has been reported to potentiate the effects of substance P in rat skin (Gamse and Saria, 1985; Brain and Williams, 1985) and in the rat spinal cord (Wiesenfeld-Hallin et al., 1984) but not in the mouse spinal cord (Gamse and Saria, 1986). In the present study, we compare the ability of CGRP to potentiate neurokinin- or 5-hydroxytryptamine (5-HT)-induced paw oedema, and caudally-directed biting/scratching (CDBS) induced by intrathecal injection of the NK_1 agonist, GR73632 (Hagan et al., 1989) or substance P in the rat.

Male, Glaxo-bred, Random-Hooded rats were used for the paw oedema experiments (weight range 75-120g) and for intrathecal studies (weight range 50-75g). Paw volume was measured by plethysmometry before and 30min after intraplantar injection of drugs (dose volume 100µl). Paw oedema was calculated as the percentage change in paw volume post-injection and ED_{50} values (with 95% confidence limits) were calculated when appropriate. A Student's unpaired t-test was used to assess significance ($p<0.05$). In the intrathecal studies, GR73632 or substance P were administered by direct injection to the lumbar region of conscious weanling rats (dose volume 10µl). The appearance of CDBS was noted up to 5min post-injection.

The NK_1 agonist, GR73632 (0.3-3nmol/paw) caused a dose-dependent paw oedema (ED_{50} 1.2 (0.7-2.3)nmol/paw; n=5). The mean increase in paw volume produced by GR73632 (3nmol/paw) was 23±3%. Co-administration of CGRP (0.1nmol/paw) with GR73632 caused a marked leftward displacement of the GR73632 dose-effect curve. The ED_{50} for GR73632 in the presence of CGRP was 0.05 (0.02-0.09)nmol/paw (n=5) and the maximum paw volume change induced by GR73632 was 38±3%. CGRP (0.1nmol/paw) alone had little effect on paw volume (8±2% increase), which was insufficient to account for the potentiation of GR73632-induced paw oedema. The ability of CGRP to enhance GR73632 induced paw oedema was selective, since CGRP (0.1nmol/paw) was without effect on oedema produced by intraplantar 5-HT (ED_{50} values in the presence and absence of CGRP were 0.8(0.5-1.3) and 1.0(0.6-1.7)nmol/paw respectively). Neither the NK_2-agonist, GR64349 (Hagan et al., 1989; 0.01-10nmol/paw; n=5) nor the NK_3-agonist, senktide (0.1-10nmol/paw; n=5) co-injected with CGRP (0.1nmol/paw) produced significant paw oedema. In the rat, intrathecal GR73632 (1-32pmol/rat) or substance P (8-64pmol/rat) caused a dose-dependent increase in the proportion of rats exhibiting CDBS with ED_{50} values of 1.2(0.2-2.3) and 41(20-4045)pmol/rat respectively (n=6). The proportion of rats responding did not markedly change on co-administration of CGRP (0.01-1nmol/rat; n=6). CGRP (0.01-1nmol/rat) did not induce CDBS (n=6).

This study shows that CGRP selectively potentiates GR73632-induced paw oedema. However, a similar potentiation of CDBS induced by intrathecal GR73632 or substance P in the rat was not observed.

Brain, S.D. and Williams, T.J. (1985) Br. J. Pharmacol., **86**, 855-860
Gamse, R. and Saria, A. (1985) Eur. J. Pharmacol., **114**, 61-66
Gamse, R. and Saria, A. (1986) Neurosci. Letts, **70**, 143-147
Hagan, R.M, Ireland, S.J., Jordan, C.C., Bailey, F., Stephens-Smith, M., Deal, M., and Ward, P. (1989) Br. J. Pharmacol., **98**, 717P
Wiesenfeld-Hallin, Z., Hökfelt, T., Lundberg, J.M., Forssmann, W.G., Reinecke, M., Schopp, F.A., and Fischer, J.A. (1984) Neurosci. Letts, **52**, 199-204

259P EFFECT OF SR 47436 ON ANGIOTENSIN II-INDUCED PRESSOR RESPONSES IN PITHED RATS

B. Christophe, R. Libon, P. Chatelain & A.S. Manning, SANOFI-Pharma Research Centre, 1, avenue de Béjar, 1120 Brussels (Belgium)

SR 47436 (2-n-butyl-4-spirocyclopentane-1-[((2'-tetrazol-5-yl)biphenyl-4-yl)methyl]2-imidazolin-5-one) is a new specific, non-peptide angiotensin II antagonist (Nisato et al., 1991). In this study, we have investigated the effect of SR 47436 on angiotensin II-induced pressor effects in pithed rats and compared these effects to that of another non-peptide angiotensin II antagonist, DuP 753 (Wong et al., 1990)

Male Wistar rats were pithed under pentobarbital anaesthesia and ventilated with air. Left carotid arterial diastolic blood pressure was measured via a catheter attached to a pressor transducer. Drugs were injected via the posterior vein of the penis. After stabilization, SR 47436 or DuP 753 were injected i.v.. Fifteen min later, cumulative doses of angiotensin II were injected i.v. Pressor responses were assessed by changes in diastolic blood pressure expressed (mean ± S.E.M.) in mm Hg.

In the pithed rat, SR 47436 or DuP 753 dose-dependently inhibited the pressor response to angiotensin II. DuP 753 behaved as a competitive antagonist (*in vivo* pA_2 = 5.85 ± 0.12, n=16) as previously reported (Wong et al., 1990). SR 47436 reduced both the maximal amplitude of the angiotensin II dose-pressor curve and also the slope of this curve (*in vivo* pD'_2 = 4.98 ± 0.10, n=29 ; apparent *in vivo* pA_2 = 6.39 ± 0.12, n=32).

These results suggest that the antagonism existing between SR 47436 and angiotensin II is insurmountable as reported with other angiotensin II antagonists such as EXP3892 (Wong and Timmermans, 1991), EXP3174 (Wong et al., 1990) or DuP 532 (Chiu et al., 1991) and also support the recent findings of Nisato et al. (1991) that SR 47436 is a novel non-peptide angiotensin II antagonist more potent that DuP 753.

Chiu A.T., Carini D.J., Duncia J.V., Leung K.H., McCall D.E., Price W.A., Wong P.C., Smith R.D., Wexler R.R. & Timmermans P.B.M.W.M. (1991) *Biochem. Biophys. Res. Com.*, **177**, 209-217
Nisato D., Cazaubon C., Lacour C., Gougat J., Guiraudou P., Bernhart C., Perraut P., Brelière J.C. & Le Fur G. (1991) *Brit. J. Pharmacol.* (This meeting)
Wong P.C., Price W.A. Chiu A.T., Duncia J.V., Carini D.J., Wexler R.R., Johnson A.L. & Timmermans P.B.M.W.M. (1990) *J. Pharmacol. exp. Ther.*, **255**, 211-217
Wong P.C., Price W.A., Chiu A.T., Duncia J.V., Carini D.J., Wexler R.R., Johnson A.L. & Timmermans P.B.M.W.M. (1990) *J. Pharmacol. exp. Ther.* **252**, 719-725
Wong P.C. & Timmermans P.B.M.W.M. (1991) *J. Pharmacol. exp. Ther.*, **258**, 49-57

260P CHARACTERISATION OF THE CONTRACTILE RESPONSES TO ANGIOTENSIN II ANALOGUES IN THE GUINEA-PIG ILEUM

A.B. Hawcock and J.C. Barnes; Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DP.

Early studies showing that angiotensin II (AII) contracts guinea-pig ileum (GPI) indirectly through the release of acetylcholine have not been substantiated and are confounded by the occurrence of tachyphylaxis in this tissue (see Paiva et al, 1976). In the present study, we have investigated the nature of the responses to AII and its non-desensitising analogue, [Lys^2]AII (Miasiro et al, 1983), using tetrodotoxin (TTX), atropine and the tackykinin NK_1 antagonist, CP-96,345 (Snider et al, 1991), in the presence of raised [K^+]. Increased [K^+] has been shown to prevent tachyphylaxis (Barnes et al, 1991). Furthermore, the availability of the AT_1 and AT_2 selective non-peptide antagonists, losartan (Dup 753) and PD123177 respectively (Bumpus et al, 1991) has provided the opportunity to characterise further the AII contractions in this tissue.

Longitudinal muscle strips of ileum from male guinea-pigs (200-250g) were prepared and mounted in 5ml organ baths under 0.5g tension. Experiments were carried out in modified Krebs buffer, containing 10mM KCl, aerated with 5% CO_2 in oxygen. Responses were recorded isotonically and repeated cumulative concentration-response curves (CRC) to AII and [Lys^2] AII were constructed every 60min until superimposable. Antagonists were applied for 45min, prior to reconstructing the curves. Agonist potency was expressed as pD_2 and where appropriate antagonist potency as pK_B (with 95% confidence limits) obtained from Schild analysis. Cumulative CRC to [Lys^2]AII and AII were obtained, giving pD_2 estimates of 7.42±0.06 and 9.15±0.14 respectively. The maximum response (Emax) to AII relative to [Lys^2]AII was 99±5% TTX (0.1μM) reduced the Emax [Lys^2]AII by 63±5% and to AII by 88±3%. Atropine (0.1μM) reduced the Emax to [Lys^2]AII by 33±4% and to AII by 60±7%. Atropine, combined with CP-96,345 (30nM) reduced the Emax to [Lys^2]AII by 72±6% and to AII by 84±2%. These results indicate that AII acts predominantly indirectly via the release of both acetylcholine and a tachykinin and that [Lys^2]AII possesses a more substantial direct compound. Losartan antagonised responses to both AII (acting mainly pre-synaptically) and [Lys^2]AII in the presence of TTX (acting on the smooth muscle). pK_B vs AII = 8.56 (8.45-8.68), slope 1.03±0.1; pK_B vs [Lys^2]AII+TTX = 9.13 (8.94-9.32), slope 0.99±0.21. Neither slope was significantly different from unity. PD123177 (3μM) had very little effect on the CRC to either agonist.

These studies show that in the absence of tachyphylaxis, angiotensin agonists contract GPI by the activation of presynaptic AT_1 receptors as well as AT_1 receptors located on the smooth muscle. The small but statistically significant ($P<0.05$) difference in potency of losartan at these two sites may be indicative of a receptor difference.

Barnes, J.C., Brown, J.D., Hawcock, A.B. & Michel, A.D. (1991) Br. J Pharmacol. **104**, 43P.
Bumpus, F.M., Catt, K.J., Chui, A.T., DeGasparo, M., Goodfriend, T., Husain, A., Peach, M.J., Taylor, D.G. & Timmermans, P.B.M.W.M. (1991) Hypertension **17**, 720-721.
Miasiro, N., Oshiro, M.E.M., Paiva, T.B. & Paiva, A.C.M. (1983) Eur. J. Pharmacol. **87**, 3970406
Paiva, T.B., Mendes, G.B., Aboulafia, J. & Paiva, A.C.M. (1976) Pflugers Arch. **365**, 129-133.
Snider, R.M., Constantine, J.W., Lowe, J.A., Longo, K.P., Lepel, 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.

261P CARDIOVASCULAR EFFECTS OF THE ANGIOTENSIN II RECEPTOR ANTAGONIST ICI D8731 IN THE DOG AND MARMOSET

C.P Allott, A.A. Oldham, J.A. Steel, V. Worrall, R.J. Pearce[1], D.A. Roberts[1], S.T. Russell[1], Bioscience II and Chemistry II[1] Departments, ICI Pharmaceuticals, Alderley Park, Macclesfield, SK10 4TG.

ICI D8731 is a potent and specific angiotensin II receptor antagonist in vitro in guinea-pig and rabbit tissues and in vivo in conscious rats (Oldham et al, 1991). This study investigates the effects of ICI D8731 as an antagonist of angiotensin II-induced pressor responses in conscious dogs and its effects as a hypotensive agent in sodium-deplete anaesthetised marmosets.

Male Alderley Park Beagle dogs were prepared with indwelling carotid artery and jugular vein cannulae. Dogs were trained to lie quietly whilst blood pressure was monitored. Pressor responses to bolus intravenous injections of angiotensin II (30 ng kg^{-1}) were measured before and after cumulative intravenous administration of ICI D8731 in the dose range 0.1-3.0 mg kg^{-1}. ICI D8731 produced dose-related inhibition of the angiotensin II pressor responses with an ED50 of 1.1 ± 1.0 mg kg^{-1}. There were only small changes in baseline blood pressure.

Marmosets were pretreated with a low sodium diet and furosemide (25 mg kg^{-1} day^{-1} for 3 days). Following the final dose of diuretic, the animals were anaesthetised with thiobutabarbital 110 mg kg^{-1} i.p. Carotid arterial blood pressure was monitored whilst ICI D8731 was administered cumulatively in the dose range 0.1-3.0 mg kg^{-1} i.v. ICI D8731 produced dose-related reductions in mean arterial pressure with an ED50 of 0.81 ± 0.19 mg kg^{-1}.

In conclusion, the above studies indicate that the potency of ICI D8731 as an angiotensin II antagonist in dog and marmoset is similar to that reported in the rat (Oldham et al, 1991).

Oldham, A.A., Allott C.P., Major J.S., Smith C.F.C. et al., This Meeting.

262P CAPTOPRIL-INDUCED DIPSOGENICITY IN THE RAT: EFFECTS OF THE NON-PEPTIDE ANGIOTENSIN II ANTAGONISTS LOSARTAN (DuP 753) AND PD123177

R.P. Dennes, J.J. Irlam-Hughes and J.C. Barnes, Neuropharmacology Department, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 0DP

It has previously been shown that the angiotensin converting enzyme (ACE) inhibitor captopril, administered peripherally, has a dipsogenic action in the rat (Evered & Robinson, 1984), an effect which is paradoxical to the known dipsogenic actions of angiotensin II in its own right. The present study was designed to further investigate the nature of the ACE inhibitor-induced dipsogenic response, employing captopril, enalapril and SQ29852. We also report the effects of the non-peptide angiotensin II receptor antagonists losartan (DuP 753) and PD123177, selective for the AT_1 and AT_2 subtypes respectively (Bumpus *et al*, 1991).

Male Lister Hooded rats (250-300g) were used in all experiments. Dipsogenic responses were measured by recording the volume of water consumed by individual animals over a 20 minute test period. The ACE inhibitors captopril (0.01 - 30$mgkg^{-1}$ sc); enalapril (0.003 - 1.0$mgkg^{-1}$ sc), and SQ29852 (0.0005 - 50$mgkg^{-1}$ sc), were administered 1h prior to testing, during which time animals were denied access to water. In antagonist studies, losartan (0.1 - 30$mgkg^{-1}$ sc) or PD123177 (0.1 - 30$mgkg^{-1}$), were administered 1 hr prior to captopril, (3$mgkg^{-1}$). In a further study to examine the role of central ACE activity, captopril was administered icv, in animals with stereotaxically implanted guide cannulae which allow direct injections to the lateral ventricles. In this experiment, captopril was administered icv (100nmols in 2μl) 30min after captopril (3$mgkg^{-1}$ sc). Data is expressed as mean volume ± s.e.mean (ml) water consumed during the 20 minute test period, n = 6-16 per treatment group.

All ACE inhibitors produced a dose-dependent increase in water intake. Peak dipsogenic effects were achieved with 10$mgkg^{-1}$ captopril (2.6 ± 0.5), 0.03$mgkg^{-1}$ enalapril (3.8 ± 0.3) and 0.5$mgkg^{-1}$ SQ29852 (2.2 ± 0.5), compared to vehicle responses (0.6 ± 0.2). Higher doses of all three ACE inhibitors were much less effective. The dipsogenic response to captopril was dose-dependently blocked by losartan, (captopril alone 3.7±0.4; captopril with losartan 3, 10 & 30$mgkg^{-1}$, 2.3±0.3, 1.7±0.3 & 1.6±0.4 respectively, $p<0.05$), these doses being in line with to those required to block the drinking response to icv angiotensin II, (Dennes *et al* 1991). PD123177 was without effect in modifying the captopril-induced response. Both losartan and PD123177 failed to modify water intake when administered alone. When captopril was administered icv, it blocked the dipsogenic response induced by peripheral captopril, (captopril sc 3.4±0.1; captopril sc+icv 1.7±0.4, $p<0.05$)

In conclusion, we show the ACE inhibitors captopril, enalapril and SQ29852 induce a dipsogenic response in the rat. While our results suggest central ACE activity and an action at AT_1 receptors are involved in mediating the captopril-induced response, further work is necessary to fully elucidate the mechanisms by which ACE inhibitors exert their effects.

Bumpus, F.M., Catt, K.J., Chiu, A.T., DeGasparo, M., Goodfriend, T., Husain, A., Peach, M.J., Taylor. D.G. & Timmermans, P.B.M.W.M. (1991) Hypertension 17 720-721
Dennes, R.P., Barnes, J.C., Michel, A.D. & Tyers, M.B. (1991) This meeting
Evered, M.D. & Robinson, M.M. (1984) J.Physiol 348, 573-588

263P EXPRESSION OF CGRP IN CULTURED ENDOTHELIAL CELLS WITH PLASMID DNA

B. Sun, C. Plumpton, J. Sinclair[1] and M.J. Brown, Clinical Pharmacology Unit, and [1] Department of Medicine, University of Cambridge, Cambridge CB2 2QQ

Calcitonin gene-related peptide (CGRP) is a potent vasodilator peptide. It has also been shown to stimulate the proliferation of endothelial cells (EC; Hægerstrand et al., 1990), and postulated to inhibit the proliferation of vascular smooth muscle cells by increasing intracellular level of cAMP (Kubota et al., 1985). The deficiency of CGRP-containing nerve endings may play a role in some diseases such as Raynaud's Phenomenon. The introduction of an extra source of CGRP may be of benefit in such disease or for the prevention of coronary artery restenosis after angioplasty or by-pass. EC have been proven to be the best recipient for gene transfer for the delivery of recombinant products to the cardiovascular system (Nabel et al., 1990). Expression of human alpha-CGRP (α-hCGRP) was carried out by introducing CGRP cDNA into cultured human EC (hEC).

Transfection vector (pRR-CGRP1)was constructed by insertingα-hCGRP cDNA into pUC18 under the promotion of the major IE genomic region of HCMV. hEC were isolated from umbilical vein and cultured as described previously (Jaffe et al., 1973). First subcultured hEC were used for the transfection. Co-transfection of pRR-CGRP1 and SV2neo was carried out with calcium precipitation or lipofectin. 48 h later, supernatants from transfected hEC were collected. After 12 days selection with G418, the supernatants from stably transfected hEC were collected and stored at -70°C. CGRP in the supernatants were measured using a two-site ELISA with monoclonal antibodies against the N and C termini of syntheticα-hCGRP. The supernatants from hEC before transfection, or after transfection with a similar vector but without the CGRP cDNA insert served as negative control while the supernatant from Ben cells (a CGRP-producing lung carcinoma cell line) served as positive control. The CGRP content of transfected and control cells were also studied immuno-histochemically.

CGRP was detected in the supernatants of hEC 48 h after transfection with both calcium precipitation (2.4 pM) and lipofectin (5.0 pM). After selection with G418, two clones of stably transfected hEC were obtained, and pooled together for subculture. The CGRP concentration in the supernatants ranged from 3.0 pM to 6.5 pM after the cells grew to near-confluence during 12 passages. Stimulation of the cloned hEC by $CaCl_2$ (20 mM) for 4 h caused the CGRP in the supernatant to increase from non-detectable to 2.4 pM; there was no response to substance P (10 uM), nonadrenaline (1 uM), KCl (6 mM), endothelin-1 (0.1 uM), 5-HT (1 uM) or acetylcholine (10 uM). CGRP was not detected in the stably transfected hEC. The transfected hEC still expressed large amounts of von Willebrand factor.

These results showed successful expression ofα-hCGRP in hEC in vitro, and that the expressed CGRP could be secreted.

Hægerstrand, A., Dalsgaard, C.J., Jonzon, B., Larsson, O. & Nilsson, J. (1990) Proc. Natl. Acad. Sci. USA 87, 3299-3303.
Jaffe, E.A., Nachman, R.L., Becker, C.G. & Minick, C.R. (1973) J. Clin. Inves. 52, 2745-2756.
Kubota, M., Moseley, J.M., Butera, L., Dusting, G.J., MacDonald, P.S. & Martin, T.J. (1985) Biochem. Biophysi. Res. Commun. 132, 88-94.
Nabel, E.G., Plautz, G. & Nabel, G.J. (1990) Science 249, 1285-1288.

264P USE OF SELECTIVE INHIBITORS OF [^{125}I]-ENDOTHELIN-1 BINDING SHOWS THAT RAT AORTIC HOMOGENATES CONTAIN BOTH ET_A AND ET_B RECEPTORS

C. Robin Hiley[1], C. Richard Jones[2] & John T. Pelton, Marion Merrell Dow Research Institute, BP 447/R9, 16 rue d'Ankara, 67009 Strasbourg Cédex, France and [1]Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ

The two receptors for the endothelins (ETs) are defined by their selectivity for ET-1 and ET-3; ET-1 is more potent than ET-3 at the ET_A receptor but they are equipotent at the ET_B receptor (Vane, 1990). It has been proposed, on the basis of their actions in tissues with these selectivities for ET-1 and ET-3, that [Ala^{1,3,11,15}]ET-1 is a selective ET_B receptor agonist (Saeki *et al.*, 1991) and that BQ-123, cyclo{D-Trp-D-Asp-Pro-D-Val-Leu} (Ishikawa *et al.*, 1991), is an ET_A antagonist with a K_d at that receptor of 22 nM and of 23 μM at the ET_B receptor. These ligands have been used to inhibit [^{125}I]ET-1 binding to rat aorta in order to see which ET receptors it contains.

Aortae from Male Sprague-Dawley rats (200-300 g; Charles River France) were pulverised in liquid N_2 in a SPEX Industries (Edison, NJ, USA) 6700 Freezer Mill and then homogenized in 7.5 vol. ice-cold buffer (50 mM HEPES; 1 mM 1,10 phenanthroline; pH 7.4) with 2 x 20 s bursts in a Polytron at setting 7. Homogenates were centrifuged at 1000 g for 10 min at 4°C and the supernatants used for binding experiments. 1 mg protein (as determined by the Lowry method) was incubated for 2 h at 25°C in 0.5 ml of 50 mM HEPES buffer (pH 7.4) containing 1 mM 1,10 phenanthroline, 0.1% bovine serum albumen and 140 μg/ml bacitracin (all from Sigma). Bound and free ligand (20-30 pM [^{125}I]ET-1 from Amersham) were separated by filtration through Whatman GF/B filters. Non-specific binding was defined by 1 μM ET-1. ET-1 and ET-3 were from The Peptide Institute, Osaka, Japan; [Ala^{1,3,11,15}]ET-1 was from Neosystem Laboratoire, Strasbourg, and BQ-123 was synthesized by solid state synthetic techniques.

ET-1 inhibited [^{125}I]ET-1 binding with a K_i of 0.73±0.10 nM and a Hill slope (n_H) = 1.0±0.1 (n = 6) suggesting binding was to a single site. In contrast the n_H for ET-3 was 0.67±0.05 (n = 7) and the data were best fitted ($P < 0.01$) by a 2-site model with K_i values of 0.24±0.09 nM and 70.6±2.6 nM. In the presence of 100 nM [Ala^{1,3,11,15}]ET-1, used to inhibit ET_B receptor binding, the inhibition curve for ET-3 had an n_H = 0.9±0.1 (n = 4) and the data did not fit to a 2-site model better than to a single site; the K_i value was 150±15 nM. Both [Ala^{1,3,11,15}]ET-1 and BQ-123 had shallow inhibition curves with n_H values of 0.7±0.1 (n = 7) and 0.5±0.1 (n = 7), and IC_{50} values of 300±27 and 1.57±0.36 nM, respectively. These data were best fitted to 2-site models and the K_i values for [Ala^{1,3,11,15}]ET-1 were 10.5±7.8 and 496±131 nM which shows a high degree of selectivity between the two sites.

The low n_H values for inhibition of [^{125}I]ET-1 binding to rat aorta by ET-3, [Ala^{1,3,11,15}]ET-1 and BQ-123 are probably due to binding to both ET_A and ET_B receptors. The 50 fold difference in the K_i values for [Ala^{1,3,11,15}]ET-1 suggests that the two sites might represent binding to the two receptors but, more importantly, the binding of ET-3 approximated to a single site in the presence of a concentration [Ala^{1,3,11,15}]ET-1 sufficient to block binding of the radioligand to more than 90% of any ET_B receptors present (Ishikawa *et al.*, 1990).

[2] Present address: F.Hoffman-La Roche & Co. Ltd, CH-4002 Basel, Switzerland.

Ishikawa, K., Fukami, T., Hayama, T., Niiyama, K., Nagase, T., Mase, T., Fujita, K., Kumagai, U., Urakawa, Y., Ihara, M., Kimura, S. & Yano, M. (1991). Proc. 12th Amer. Peptide Symp., Abstract P506.
Saeki, T., Ihara, M., Fukuroda, T., Yamigawa, M. & Yano, M. (1991). Biochem. Biophys. Res. Commun., **179**, 286-292.
Vane, J. (1990). Nature, **348**, 673.

265P PREVENTION AND REVERSAL OF ANGIOTENSIN II-INDUCED TACHYPHYLAXIS IN THE RABBIT ISOLATED AORTA

A. Wragg, M.J. Robertson and K.L. Clark. Department of Peripheral Pharmacology, Glaxo Group Research Ltd. Ware, Herts., U.K.

Tachyphylaxis can occur to the contractile effects of angiotensin II (Ang II) on vascular smooth muscle in vitro. The present study has investigated whether it was possible to induce angiotensin tachyphylaxis in the rabbit aortic strip and, if so, to study the effect of the non-peptide angiotensin receptor antagonist, DuP 753 (losartan; Chiu et al., 1990), on this phenomenon.

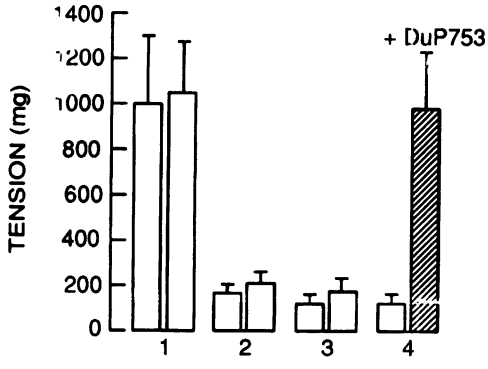


Figure 1. Effects of four successive Ang II (10μM) challenges at hourly intervals in rabbit aortic strips. Open columns: Ang II alone. Hatched column: Ang II in the presence of DuP 753 (300nM). Paired, control/test experiments shown.

De-endothelialised helical strips (1.5-2cm) of thoracic aorta from male, New Zealand White rabbits were suspended under a resting tension of 0.5g in Krebs solution at 37°C, containing indomethacin (30μM) and ascorbic acid (100μM), and gassed with 95% O_2 / 5% CO_2. Administration of a supra-maximal concentration of Ang II (10μM : a concentration which is 100 fold greater than that required to elicit a maximum contractile response) for 20 min at one hour intervals induced a pronounced tachyphylaxis (Figure 1) which was neither prevented, nor reversed, by extensive washing between Ang II challenges. Preparations tachyphylactic to Ang II responded normally to phenylephrine (3μM) and, furthermore, a correspondingly supra-maximal concentration of phenylephrine (1mM), did not show tachyphylaxis on successive challenges. DuP 753 (300nM) incubated for 45 min before each Ang II (10μM) challenge had no effect on the initial maximum contraction produced by Ang II, but prevented the onset of tachyphylaxis to subsequent Ang II (10μM) administrations. In tissues previously made tachyphylactic to Ang II (10μM), DuP 753 (300nM) caused a reversal of the tachyphylaxis when given 45 min before a final Ang II (10μM) challenge (Figure 1). The peptide angiotensin receptor antagonist, saralasin (30nM), caused a similar prevention of the tachyphylaxis. Saralasin also substantially reversed previously established tachphylaxis.

In conclusion, supramaximal concentrations of Ang II cause tachyphylaxis in rabbit aortic strips. The reduction in contractile reactivity to Ang II may be a consequence of receptor desensitisation, but the mechanism of the desensitisation is unknown. DuP 753 may prevent the loss of contractility by preventing Ang II from binding to its receptor in a manner which induces desensitisation. However, since DuP 753 can also reverse the loss of contractility, DuP 753 may act by restoring the receptor to a state which allows Ang II to fully activate the contractile pathway.

Chiu, A.T., McCall, D.E., Price, W.A., Wong, P.C., Carini, D.J., Duncia, J.V. Wexler, R.R., Yoo, S.E., Johnson, A.L., Timmermans, P.B.M.W.M. (1990) J. Pharmacol. Exp. Ther. **252** (2), 711-718.

266P DEVELOPMENT AND APPLICATION OF A MODEL OF ANGIOTENSIN-II RECEPTOR LIGAND INTERACTIONS IN ISOLATED SMOOTH MUSCLE ASSAYS

Y.J. Liu, N.P. Shankley[1], N.J.Welsh & J.W. Black, Department of Analytical Pharmacology, King's College School of Medicine & Dentistry, Rayne Institute, 123 Coldharbour Lane, London SE5 9NU. 1 - James Black Foundation, 68 Half Moon Lane, London SE24 9JE.

We have described how eight peptide analogues of angiotensin II (AII) produced a combination of rightward shift and saturable depression of AII concentration-effect (E/log[A]) curves on the rabbit aorta assay (Liu *et al.*, 1991). These results have been analysed by extracting those experimental parameters which have been previously shown to be formally-related to the underlying parameters in a model of agonism (Black & Leff, 1983). Thus, the peptide ligands were found to produce changes in the upper asymptote, midpoint location and slope of the AII E/log[A] curves. Because the ligands (B) appeared to be acting in a reversible, syntopic manner at the AII-receptor (Liu *et al.*, 1991), the model included the usual expression for competitive antagonism by multiplying the agonist (A) equilibrium dissociation constant (K_A) by the factor ($1+[B]/K_B$). It can be shown that the additional effects of the peptide ligands can be accounted for by reducing the value of the efficacy parameter, τ, as a linear function of the ligand receptor occupancy. An excellent goodness-of-fit was obtained when the data were fitted directly to the following system of equations using the BMDP derivative-free non-linear regression programme (Dixon, 1981):

$$E=(E_m \cdot \tau_B^n \cdot [A]^n)/((K_A(1+[B]/K_B)+[A])^n+\tau_B^n \cdot [A]^n) \quad \text{where,} \quad \tau_B=\tau(1-(\beta \cdot [B]/(K_B+[B]))).$$

E_m is the maximum effect in a system and β, the proportionality constant which governs the reduction of efficacy by a peptide ligand. The model-fit allowed estimation of the antagonist-dependnet pK_B and β (table 1), agonist-dependent pK_A (8.94±0.10) and τ (1.41±0.20) and, system-dependent E_m (9.23±0.96) and n (2.20±0.30). The model was satisfactorily tested by applying it to the results of a cross-tissue analysis of the interactions between AII and [Sar^1,Ala^8]AII on the rabbit aorta, guinea-pig aorta, ileum and stomach.

Table 1.

Ligand	pK_B(s.e.)	β(s.e.)	Ligand	pK_B(s.e.)	β(s.e.)
[Sar^1,Ile^8]AII	8.97(0.05)	0.68(0.06)	[Sar^1,Thr^8]AII	8.49(0.05)	0.35(0.05)
[Sar^1,Leu^8]AII	9.31(0.05)	0.69(0.05)	[Sar^1,Ala^8]AII	8.74(0.06)	0.43(0.05)
[Sar^1,$Phe(2,3,4,5,6\text{-}Br_5)^8$]AII	8.28(0.06)	0.41(0.06)	[Sar^1,Val^5,Ala^8]AII	8.69(0.05)	0.34(0.05)
[Sar^1]AII(1-7)NH_2	7.33(0.06)	0.49(0.06)	[Sar^1,$Tyr(Me)^4$]AII	6.75(0.08)	0.22(0.05)

Black, JW & Leff, P (1983). Proc. Roy. Soc. Lond. B, 200, 141-162.
Dixon, WJ (1981). BMDP Statistical Software. University of California Press.
Liu YJ, Shankley NP, Welsh NJ & Black JW (1991). This Meeting.

267P BIOLOGICAL ACTIVITY OF NEUROMEDIN U-8 FRAGMENTS ON RAT FUNDIC CIRCULAR MUSCLE *IN VITRO*

M.A. Benito-Orfila, K.A. Nandha & S.R. Bloom (introduced by J. MacDermot). Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 ONN, U.K.

We have previously reported that Neuromedin U-like peptides induce concentration-dependent contractions of strips of rat fundic circular muscle (Benito-Orfila et al., 1991a). We have recently studied the bioactivity of neuromedin U-8 (NmU-8) fragments on the rat uterus *in vitro* which suggested that the C-terminal fragment NmU(2-8) constitutes the minimum length required to activate the smooth muscle of the rat uterus (Benito-Orfila et al., 1991b).
The aim of the present study was to examine the effect of NmU-8 fragments on the circular smooth muscle of rat fundus, in order to distinguish the receptor types in both rat stomach and uterus.
Female Wistar rats (150-200 g) were used. The animals were sacrified by asphyxiation in CO_2. The fundus was removed and strips of the circular muscle were mounted in an organ bath and maintained in Krebs solution at 37°C and pH 7.4, aerated with 95% O_2/5% CO_2. The tissues were equilibrated under a resting tension of 1 g and the isometric muscular contractions were recorded as previously described (Benito-Orfila et al., 1991a).
Porcine NmU-8 (custom synthesis; IAF Bio-Chem, Quebec, Canada), NmU(4-8), NmU(3-8), NmU(2-8) and Phe^1-NmU-8 (custom synthesis; Palliard Chemical Company Ltd, Twinstead, Sudbury, Suffolk, UK) were tested in the dose range 1 nM to 1 μM. All peptides, except NmU(3-8) and NmU(4-8), showed a contractile effect in a concentration dependent manner on the rat fundus.
The fragment NmU(2-8)($EC_{50}=199\pm29$nM) was less potent than porcine NmU-8($EC_{50}=68\pm9$nM). The effect of Phe^1-NmU-8($EC_{50}=105\pm20$nM) showed the same magnitude of response as that obtained with porcine NmU-8.
The results show that as with the uterus bioassay, NmU(2-8) is the shortest bioactive fragment on the rat fundus and the fragment Phe^1-NmU-8 also has a similar response. We have thus not been able to demonstrate heterogeneity between receptors of the two tissues studied.

Benito-Orfila, M.A., Domin, J., Nandha, K.A. & Bloom, S.R. (1991a) Eur. J. Pharmacol. 193, 329-333.
Benito-Orfila, M.A., Nandha, K.A. & Bloom, S.R. (1991b) Br. J. Pharmacol. 104 (S1): 148P.

268P EFFECTS OF PP, PYY, NPY AND SELECTIVE ANALOGUES ON THE FIELD-STIMULATED RAT AND RABBIT VAS DEFERENS

H.N. Doods, Preclinical Research, Dr. Karl Thomae GmbH, P.O. Box 1755, 7950 Biberach 1, Germany.

The pancreatic polypeptide (PP)-like receptor family consist of at least three putative subtypes. One receptor population exhibits high affinity for PP and low affinity for neuropeptide Y (NPY) as well as peptide YY (PYY) and is classified as the PP-receptor. Receptors with the opposite profile are termed NPY-receptors (Jorgensen et al., 1990). In addition receptors with a high affinity for NPY and PYY can be further subdivided into Y1 and Y2 based on their different affinity or potency towards selective analogs. For instance, (Leu^{31}, Pro^{34})-NPY appears to be a selective Y1-agonist whereas the C-terminal fragments NPY(13-36) and NPY(18-36) possess a higher potency for Y2-receptors (Fuhlendorff et al., 1990). In the present study we investigated the role of prejunctional PP- and NPY-receptors in the rat and rabbit vas deferens. Prostatic portions of rat and rabbit vasa deferentia were suspended in a Krebs buffer solution at 37°C gassed with 5 % CO_2 / 95 %0_2. Contractions were elicited by electrical field stimulation (0.15 Hz, 1 ms, 3-5 V) and were recorded isometrically under an initial tension of 10 mN.

Table 1. -log EC50 (mean of 4-6 experiments) values for PP, PYY, NPY and some selective analogs to inhibit the electrically stimulated twitch response in rat and rabbit vas deferens.

compound	**-log ED50 (mol/l)**	
	rat	**rabbit**
NPY	8.02	8.77
(Leu^{31}, Pro^{34}) NPY	6.10	8.39
NPY(18-36)	6.62	5.22
NPY(13-36)	7.16	6.02
PYY	8.89	9.64
PP	8.52	9.28

Both NPY and PYY inhibited in a concentration dependent manner the twitch response due to electrical stimulation in rat and rabbit vas deferens exhibiting a slight preference for NPY receptors in rabbit vas deferens. The selective Y1-agonist (Leu^{31}, Pro^{34})-NPY showed a pronounced selectivity for the rabbit vas deferens whereas the opposite was observed for the Y2-selective C-terminal fragments. PP could inhibit the twitch response in rabbit vas deferens almost completely whereas in the rat vas deferens the maximal effect amounted to 60%. In conclusion: (Leu31,Pro34)-NPY and the C-terminal fragments NPY(13-36) or NPY(18-36) exhibit selectivity for prejuntional NPY-receptors in rabbit and rat vas deferens, respectively. Therefore it is suggested that NPY-receptors in the rabbit vas deferens are of the Y1-like subtype and those in rat vas deferens belong to the Y2-type. Moreover, it appears that PP-receptors in the rabbit vas deferens have a stronger inhibitory effect on neurotransmitter release than those in the rat vas deferens.

Jorgensen, J.C. et al. (1990). Eur. J. Pharmacol., **186**, 105--144.
Fuhlendorff, J. et al. (1990). Proc. Natl. Acad. Sci., **87**, 182-186.

269P FOCAL INCREASES IN CEREBROVASCULAR PERMEABILITY FOLLOWING MK801 ADMINISTRATION IN THE RAT

J. Sharkey , I.M. Ritchie & P.A.T. Kelly, Department of Clinical Neurosciences, University of Edinburgh, Western General Hospital, Edinburgh. EH4 2XU.

The non-competetive NMDA receptor antagonist (MK801) has been shown in a variety of animal models to be highly effective in reducing the volume of brain damage produced by focal cerebral ischemia. However, in a recent study, Nehls et al. (1990), reported that at neuroprotective doses, MK801 can also produce focal disturbances in the normal physiological mechanisms which regulate local cerebral blood flow. Whether these abnormally high flow rates are associated with pathological changes in the physiology of cerebral blood vessels is unknown. Therefore in the present study we have examined the effects of acute MK801 administration on the integrity of the blood-brain barrier.

Local cerebrovascular permeability was measured using the [^{14}C]-α-aminoisobutyric acid ([^{14}C]-AIB) quantitative autoradiographic technique (Blasberg et al., 1983) in 20 neuroanatomically discrete regions of the conscious rat brain, 10 minutes after a single intravenous infusion of either MK801 (0.5mg/kg; n=5) or saline vehicle (2ml/kg; n=5)

The administration of MK801 was associated with a marked increase in arterial blood pressure (baseline=119±4mmHg ; 153±4 mmHg 3-5minutes post drug) which accompanied (both in timecourse and intensity) the characteristic head weaving stereotypies reported elsewhere (Nehls et al., 1990). Both the behavioural and hypertensive responses persisted throughout the 35 minute experimental period.

MK801 produced increases in the local vascular permeability to [^{14}C]-AIB in only 5 of the 20 brain regions examined. Focal increases (3 fold) in permeability were observed within small punctate areas of the cerebellar cortex. In contrast, a two-fold increase in the local AIB blood-to-brain transfer constant was found throughout the lateral portion of the caudate nucleus whereas the medial portion of the nucleus remained relatively unaffected. Significant increases in permeability were also observed within primary visual, parietal and posterior cingulate cortex. With the exception of the cerebellum, these areas also exhibit marked hyperaemia in response to MK801 administration (Nehls et al., 1990). The distribution of these increases in blood-brain barrier permeability does not exactly parallel the anatomical distribution of highest NMDA receptor densities. However, the pattern was similar to that found in response to the acute hypertension induced by noradrenaline (Ellison et al., 1986), and the disturbances in blood-brain barrier permeability observed here may also reflect vascular changes resulting from the hypertensive actions of MK801.

Blasberg, R.G., Fernstermacher, J.D., Patlak, C.S. (1983) J Cereb. Blood Flow Metabol. 3, 8-32.
Ellison, M.D., Povlishock, J.T., Hayes, R.L. (1986) J Cereb. Blood Flow Metabol. 6, 471-480.
Nehls, D.G., Park, C.K., MacCormack, A.G., McCulloch, J. (1990), Brain Res. 511, 271-279.

270P THE HYPOCALCAEMIC RESPONSE TO INTRAVENOUS CALCITONIN IN JUVENILE RATS: THE IMPORTANCE OF AGE AND OTHER FACTORS

J.A. Phillips & A. Steward, Ciba-Geigy Pharmaceuticals, Horsham, West Sussex

Since measurement of actual calcitonin levels in plasma is expensive and time consuming, the hypocalcaemic response in juvenile rats is used as an alternative method for looking for raised levels of exogenous calcitonins in plasma, (Kumar et al., 1965). I have looked at two areas in which the hypocalcaemic response may be affected by variations in methodology:

1. Sensitivity to the age of the rats.
2. The effect of other variables in the treatment of the animals.

The method used involved anaesthetising the animals after overnight starvation using 0.2ml/kg i.m. Hypnorm (fentanyl/fluanisone) followed by 0.5ml/kg i.m. Sagatal (pentabarbitone). The dose of human calcitonin (500ng/Kg), which was chosen as it is on the hypocalcaemic borderline, was administered i.v. in sterile saline. Blood was collected from the cannulated carotid artery over a 2 hour period after dosing and the plasma assayed for calcium using a colorimetric method (Sigma).

1. Groups of animals (4-6) were dosed at starved weights of 40, 60, 80, 100, 120, and 140g (ages 23-49 days). Plasma calcium levels showed a variation in response from basal values of 1.8 ± 0.18 mmol/l. This varied from a maximum calcium lowering (30%) in 60-80g animals to reduced or no response in other groups. The results showed that 60-80g gives the maximum lowering over 2 hours and indicated that using animals outside the limits of 60-100g would compromise the assay.

2. The effects of the sedative Hypnorm and overnight starvation on the hypocalcaemic response were also examined. Hypnorm is routinely used for sedation when bleeding from the tail vein as it also dilates blood vessels. Our work showed that Hypnorm increases the hypocalcaemic response to hCT, presumably by the same mechanism. The 30% drop in plasma calcium in Hypnorm treated animals was completely lost when Sagatal alone was used. Although starving the animals had no effect on the response to hCT, it did affect the basal plasma calcium levels in the animals, which were over 50% higher in the fed animals.

These results indicate that the performance of the hypocalcaemic assay is very sensitive to the age of the rat used and care must be taken in comparing results from different groups since variation in the methodology can affect the results.

1. Kumar M.A. et al.(1965), J. Endrocrinol. 33, 469-475.

271P RELEASE OF [^{3}H]-5-HT FROM RABBIT ISOLATED AORTA EVOKED BY ELECTRICAL-FIELD STIMULATION

A.H. Gouliaev & O.A. Nedergaard, Department of Pharmacology, School of Medicine, Odense University, J.B. Winsløws Vej 19, DK-5000 Odense C, Denmark

5-Hydroxytryptamine (5-HT) is present in peripheral blood vessels. 5-HT is found in circulating plasma where it is derived from thrombocytes (storage) and enterochromaffine cells (synthesis). Blood vessels can accumulate 5-HT which is then located in postganglionic sympathetic neurones, endothelial cells and smooth muscle. The purpose of the present investigation was to study the release of [^{3}H]-5-HT from a peripheral blood vessel *in vitro*.

Rings of rabbit aorta were used. The method has been described in detail (Nedergaard, 1980). Rings were preloaded with [^{3}H]-5-HT (10^{-6}M) or [^{3}H]-(-)noradrenaline ([^{3}H]-NA; 10^{-6}M). In some experiments the passive [^{3}H]-outflow was examined. In other experiments, after a wash-out period the rings were subjected to electrical-field stimulation (S_1-S_7; 600 pulses; 3 Hz; 0.5 msec; 200 mA) 35 min apart. S_1 or S_3 was used as an initial control value (~ 100%). [^{3}H]-outflow and [^{3}H]-overflow were collected fractionally (2 ml; 5 min).

The passive [^{3}H]-outflow from aorta preloaded with either [^{3}H]-5-HT or [^{3}H]-NA levelled off about 70 min after the onset of wash-out and remained almost constant thereafter (70-240 min). The [^{3}H]-outflow from tissues preloaded with [^{3}H]-5-HT was almost 3-fold higher (70-240 min) than that seen after preload with [^{3}H]-NA. Cocaine ($3x10^{-5}$M) did not alter the [^{3}H]-outflow (15-240 min) from tissues preloaded with [^{3}H]-5-HT, while pargyline ($5x10^{-4}$M) decreased it by about 66% (100-240 min).

The initial stimulation-evoked [^{3}H]-overflow from aorta preloaded with [^{3}H]-5-HT was higher than the subsequent ones (S_1-S_6: 100, 35, 35, 35, 37, and 40%). Similar results were obtained with tissues preloaded with [^{3}H]-NA. Tetrodotoxin decreased the [^{3}H]-overflow from aorta preloaded with [^{3}H]-5-HT (S_2-S_6) by about 60%, while S_1 was not affected. The inhibitory effect of tetrodotoxin was fully reversed by washing the aorta with drug-free salt solution. Pargyline ($5x10^{-4}$M), cocaine ($3x10^{-5}$M) and removal of endothelium did not alter the [^{3}H]-overflow evoked by stimulation (S_1-S_6) of aorta preloaded with [^{3}H]-5-HT. In the following experiments S_3 was used as the initial control value (~ 100%). The stimulation-evoked [^{3}H]-overflow increased linearly with the amount of current used (50-200 mA). This was also the case for the relationship between number of pulses (100-900) in the stimulus and [^{3}H]-overflow. The [^{3}H]-overflow depended in part on the stimulation frequency: unchanged at 2-8 Hz; and a 15-fold increase at 16 Hz.

It is concluded that [^{3}H]-5-HT can be released by depolarisation as a "false transmitter" from rabbit isolated aorta. Most of the release is probably of neuronal origin. However, some of the [^{3}H]-overflow is derived from extraneuronal sites.

This work was supported by a grant from the Danish Medical Research Council and P. Carl Petersen's Fund.

Nedergaard, O.A. (1980) J. Cardiovasc. Pharmacol. **2**, 629-643.

272P SUMATRIPTAN DOES NOT MODIFY CAPSAICIN-INDUCED RELAXATION OF GUINEA-PIG ISOLATED BASILAR ARTERY

G.J. Waldron, C.T. O'Shaughnessy & H. Connor; Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DP.

Capsaicin releases substance P (SP) and CGRP from trigeminal sensory nerves and this results in intracranial vasodilatation (Jansen et al, 1990) and plasma protein extravasation (Buzzi and Moskowitz, 1990). The 5-HT_1-like receptor agonist, sumatriptan, blocks capsaicin-induced extravasation in dura, possibly via inhibition of SP and CGRP release (Buzzi and Moskowitz, 1990). We have therefore studied the effect of sumatriptan on capsaicin-induced relaxations of guinea-pig isolated basilar arteries (GPBA).

Male guinea-pigs (400g) were exsanguinated under pentobarbitone anaesthesia, brains removed, basilar arteries dissected free and placed in well oxygenated Krebs-Henseleit solution. Artery segments 2-3mm long were mounted between 2 L-shaped wires in 10ml tissue baths at 37° C under a resting tension of 0.2g for measurement of isometric tension changes. After a 90min equilibration period, tissues were exposed to $PGF_{2\alpha}$ (0.3-10μM). Capsaicin, SP or CGRP (each at 0.1-100nM) were applied in cumulative concentrations to vessels submaximally contracted to $PGF_{2\alpha}$. Tissues (2 per animal) were paired. Antagonists were added 15min before capsaicin, SP or CGRP. The maximum relaxable tone was assessed using papaverine (200μM). Data were expressed as mean pEC_{50} values ± s.e.mean.

Capsaicin, SP and CGRP produced concentration-dependent relaxations of GPBA. Ruthenium red (RR; 3μM), which inhibits capsaicin-evoked release of neuropeptides (Maggi et al, 1988), blocked the effect of capsaicin (Table 1). The selective neurokinin NK1 antagonist GR82334 (10μM; Hagan et al, 1991), blocked the effects of exogenous SP and produced a small inhibition of the response to capsaicin (response to capsaicin 30nM was -36 ± 7% and 1 ± 6% max relaxable tone in the absence and presence of GR82334, n=7, $p<0.05$, paired Student's t-test). Sumatriptan produced small contractions under conditions of resting tone(21 ± 8mg at 3μM, n=12) but in the presence of $PGF_{2\alpha}$ had no further contractile effect. Sumatriptan (0.3 and 3μM) had no effect on capsaicin-induced relaxations (Table 1). Lowering the calcium concentration in the Krebs from 1.3 to 0.65mM did not reveal any inhibitory effects of sumatriptan.

Table 1 Effect of various treatments on relaxations to capsaicin in GPBA (n=3-5).

Treatment	pEC_{50}	
	Control	Treated
RR (3μM)	7.7 ± 0.2	<6
Sumatriptan (0.3μM)	7.1 ± 0.1	7.3 ± 0.1
Sumatriptan (3μM)	7.5 ± 0.1	7.3 ± 0.1

These results provide no evidence for 5-HT_1-like receptor mediated inhibition of sensory neurotransmission in GPBA.

Buzzi, M.G. & Moskowitz, M.A. (1990) Br. J. Pharmacol. 99:202-206.
Hagan, R.M., Ireland, S.J., Bailey, F., McBride, C., Jordan, C.C. & Ward, P. (1991) Br. J. Pharmacol. 102: 168P.
Jansen, I. Alafaci, C., Uddman, R & Edvinsson, L. (1990) Regulat. Peptides, 31:167-178.
Maggi, C.A., Santicioli, P., Gepetti, P., Paralani, M., Astolfi, M., Pradelles, P., Patacchini, R. & Meli, A. (1988) Eur. J. Pharmacol., 154:1-10.
Moskowitz, M.A., Buzzi, M.G., Sallas, D.E. & Linnik, M.D. (1989) Rev. Neurol. (Paris) 145:181-193.

273P SUMATRIPTAN DOES NOT ATTENUATE SENSORY NERVE-MEDIATED RELAXATIONS OF GUINEA-PIG ISOLATED PULMONARY ARTERY

A. Butler, S. Worton and H.E. Connor. Department of Neuropharmacology, Glaxo Group Research, Ware, Herts U.K. SG12 0DP.

It has been proposed that the anti-migraine activity of the 5-HT_1-like receptor agonist, sumatriptan, lies in its ability to inhibit the release of neuropeptides from sensory neurones in intracranial blood vessels (Buzzi and Moskowitz ,1990). Maggi et al (1990) have recently demonstrated neurogenic, capsaicin-sensitive relaxations of guinea-pig isolated pulmonary artery (GPPA). In the present study we have examined the effects of sumatriptan on electrically-induced relaxations in GPPA to assess whether 5-HT_1-like receptors are involved in the modulation of sensory neurotransmission in this tissue.

Rings of pulmonary artery were prepared from male, Dunkin Hartley guinea-pigs and suspended between two L-shaped tissue holders. The tissues were bathed in Krebs-Henseleit medium at 37° C, gassed with 95%O_2/5%CO_2 and containing atropine and guanethidine (3μM each). A submaximal concentration of histamine (HA;usually 10μM) was used to apply a tone of 1.5-2g to the tissues. Rectangular-wave pulses of 4Hz, 20V and 0.1ms for 10s applied via two platinum electrodes placed on either side of the artery ring caused relaxations of HA-contracted GPPA. These relaxations were reproducible when a 15min cycle was used. Relaxations were markedly attenuated by TTX (0.3μM) and capsaicin (1μM) suggesting that they were sensory-nerve mediated. The nitric oxide synthase inhibitor, L-NAME (100μM), and the neurokinin-1 (NK1) receptor antagonist, CP 96,345 (0.1μM), had no effect on the relaxations. These results are summarized in table 1. Sumatriptan (0.1 and 1μM) had no effect on the stimulation-induced relaxations (table 1). At the higher concentration only, it caused relaxation of HA tone in 3 out of 5 tissues (mean relaxation of 12.7 ± 4.1% of histamine tone; n=3). In a separate study sumatriptan (0.1-3μM) had no relaxant effect on tissues contracted to $PGF_{2\alpha}$ but did cause relaxations at 1-10μM when the same tissues were contracted to histamine. In contrast to the lack of effect of sumatriptan the α_2 adrenoceptor agonist, UK14304 (0.1 μM), significantly attenuated the stimulation-induced relaxations (table 1).

Electrical stimulation-induced non-adrenergic, non-cholinergic relaxations of GPPA appear to be mediated by activation of sensory neurones. Sumatriptan had no effect on the electrically-induced relaxations. The direct relaxant effect of sumatriptan was seen only when HA was used as the pre-contracting agent. This probably represents a weak antagonism of histamine H_1 receptors since sumatriptan has been shown to have some affinity for H_1 receptors (MacLennan et al., 1991). In conclusion, there is no evidence that sumatriptan modulates the release of sensory neuropeptides in GPPA.

Table 1. The effect of various agents on the electrical stimulation-induced relaxations of GPPA. (values are mean ± s.e.; n=3-5)

		% Relaxation of histamine tone		
TREATMENT	CONC.	Before treatment	After treatment	n
T.T.X.	3×10^{-7}M	35.4 ± 6.8	3.8 ± 1.8*	5
CP 96,345	1×10^{-7}M	24.3 ± 3.3	23.7 ± 3.0	3
L-NAME	1×10^{-4}M	24.6 ± 10.6	26.2 ± 3.0	5
Sumatriptan	1×10^{-7}M	28.4 ± 4.3	29.5 ± 4.4	5
Sumatriptan	1×10^{-6}M	30.3 ± 4.4	25.6 ± 4.1	5
Capsaicin	1×10^{-6}M	41.5 ± 2.6	3.5 ± 3.5*	4
UK14304	1×10^{-7}M	43.0 ± 5.5	12.3 ± 6.4*	3

* = P < 0.05 Students 't' test.

Buzzi, M.G.and Moskowitz, M.A.(1990).Br.J.Pharmacol., 99, 202-206.
MacLennan, S.J., Turner, M.A., Prentice, D.J. and Martin, G.R. (1991). Br.J.Pharmacol., 102, 16P.
Maggi, C.A., Patacchini, R., Perretti, F., Tramontana, M., Manzini, S., Geppetti, P. and Santicioli, P.(1990). N-S Arch. Pharmacol., 342, 78-84.

274P THE EFFECT OF CISPLATIN ON PLASMA 5-HT, 5-HIAA AND 5-HTP LEVELS DURING EMESIS IN THE FERRET

J.A. Rudd, K.T. Bunce[1], C.H.K. Cheng & R.J. Naylor. Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, BD7 1DP. [1]Dept. of Gastrointestinal Pharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 ODP.

Cisplatin has been hypothesised to facilitate a release of 5-hydroxytryptamine (5-HT) to trigger the emetic reflex via 5-HT_3 receptors located at central sites or on the afferent vagus nerve (see Andrews *et al.*, 1988). A preliminary study in man reported that plasma 5-HT levels are increased in some patients receiving cisplatin based chemotherapy (Barnes *et al.*, 1990) and, in a separate study, 5-hydroxyindoleacetic acid (5-HIAA) has been reported to be elevated in the urine, the increases correlating well with emesis (Cubeddeu *et al.*, 1990). In the present study we investigate the effect of an emetogenic dose of cisplatin on the plasma levels of 5-HT, 5-HIAA and 5-hydroxytryptophan (5-HTP) in an attempt to clarify the relationship between 5-HT and cisplatin-induced emesis in the ferret.

Male albino ferrets (0.7-1.7 kg) with a chronic indwelling jugular cannula were administered either cisplatin (10 $mgkg^{-1}$) or saline (0.9%w/v) in a volume of 5 $mlkg^{-1}$. Immediately before administration (t=0) and at 30, 60, 90, 150, 240 min time intervals, blood samples were withdrawn from the cannula. The first 0.2 ml of each sample was discarded and a further 1 ml of blood was withdrawn into monovette® vials (Sarstedt) and briefly mixed before being centrifuged at 15000 g for 3 min (room temperature). A 200µl aliquot of resulting plasma was then taken and the 5-HT, 5-HIAA and 5-HTP content assayed by HPLC-ECD by a method similar to Kumar *et al.*, 1990. The cannula was refilled with 0.1 ml of heparin/saline (200 $IUml^{-1}$) after each sample was withdrawn. Each animal was also monitored for the development of retching and vomiting during the course of the experiment.

Table 1. The effect of cisplatin (10 $mgkg^{-1}$) on plasma 5-HT, 5-HIAA and 5-HTP levels in the plasma of the ferret.

	5-HT (ng200 μl^{-1} plasma)		5-HIAA(ng200 μl^{-1} plasma)		5-HTP(ng200 μl^{-1} plasma)	
Time (min)	Saline	Cisplatin	Saline	Cisplatin	Saline	Cisplatin
0	25.0±12.1	38.2±16.2	8.4±1.5	7.2±1.3	47.6±4.3	46.1±9.6
30	31.4±12.2	31.8±12.7	10.2±2.7	8.2±3.0	35.8±8.0	43.9±6.2
60	24.2±11.0	35.1±17.3	10.0±3.4	11.8±7.4	52.4±12.5	43.0±10.8
90	30.0±16.0	26.9±13.8	9.3±3.1	11.1±7.1	61.6±18.7	45.2±11.3
150	28.5±18.9	23.8±13.4	13.0±4.0	6.4±2.6	38.2±10.6	36.8±12.0
240	20.0±10.0	33.4±14.5	10.3±5.0	6.2±1.8	38.7±7.0	38.7±12.2

Results represent the means±s.e.m.s of 3-4 determinations. There were no significant differences between the levels of 5-HT, 5-HIAA and 5-HTP in cisplatin and saline treated animals (Mann-Whitney U test).
Cisplatin failed to affect the plasma levels of 5-HT, 5-HIAA and 5-HTP, but did induce retching and vomiting within 68.9±1.8 min of administration, the most intense periods of retching/vomiting occurring between 75-90 min. (total retches 108.5±33.3; vomits 9.8±2.3).
In conclusion, cisplatin-induced emesis is independent of changes in plasma 5-HT, 5-HIAA and 5-HTP levels in the ferret.

Andrews, P. L. R., Rapeport, W. G. and Sanger, G. J. (1988) T.I.P.S. 9, 334-341.
Barnes, N.M., Ge, J., Jones, W.G, Naylor, R.J. & Rudd, J.A. (1990) Br. J. Cancer 62, 862-864.
Cubeddu, L. X., Hoffmann, I. S., Fuenmayor, N. T. and Finn, A. L. (1990) New Eng. J. Med. 322, 810-816.
Kumar, A.M., Kumar, M., Deepika, K., Fernandez, J.B. and Eisdorfer, C. (1990) Life sci. 47, 1751-1759.

275P CHARACTERIZATION OF THE INTERACTION OF SDZ 205,557 AT PUTATIVE 5-HT$_4$ RECEPTORS

R.M. Eglen, R. Alvarez, E.H.F. Wong, L. Johnson, L.A. Perkins, S. Ramsey, D.N. Loury, H-M. Tang, S. Wallace, & R.L. Whiting
Institute of Pharmacology, Syntex Research, 3401 Hillview Ave., Palo Alto, CA 94304, USA

SDZ 205,557 (2-diethylamino ethyl-(2-methoxy-4-amino-5-chloro)benzoate; Buchheit & Gamse, 1991) is an antagonist at putative 5-HT$_4$ receptors in guinea-pig ileum (-log K_B = 7.3-7.5). Receptors exhibiting a similar pharmacology to ileal 5-HT$_4$ receptors are present in guinea-pig hippocampus, rat oesophagus and porcine myocardium (see Clarke et al., 1989 for review). It was therefore of interest to determine the activity of SDZ 205,557 at these sites.

5-HT (-log EC_{50} = 6.0)stimulated guinea-pig hippocampal adenylyl cyclase activity was inhibited in a concentration-dependent manner by SDZ 205,557 (-log IC_{50} = 6.5). SDZ 205,557 (3 µM) surmountably antagonized relaxant responses in rat isolated oesophagus to 5-HT, 5-methoxytryptamine, (S)zacopride, (R)zacopride, renzapride, BIMU-1 and BIMU-8 (Dumuis et al., 1991) with -log K_B values of 7.1, 7.0, 6.8, 7.1, 7.3, 7.3 and 7.2, respectively. No agonist response was seen in these preparations to SDZ 205,557 (1 nM - 3 µM). At functional 5-HT$_3$ receptors in guinea-pig ileum, SDZ 205,557 exhibited weak antagonism (-log K_B = 6.3), whereas at 5-HT$_3$ receptors in NG108-15 cells, the apparent binding (using displacement of [^{3}H]-quipazine) affinity (-log K_I) was 6.9, Hill coefficient 1.3.

In vagotomized, anesthetized micropigs, 5-HT elicited a dose-dependent tachycardia (ED_{50} = 4 µg/kg, i.v. max. response 95 beats/min), which was antagonized by 5 mg/kg, i.v. ICS 205,930. These responses were probably mediated by stimulation of putative 5-HT$_4$ receptors since they were unaffected by 5-HT$_{1\text{-like}}$, 5-HT$_2$, 5-HT$_3$ or β adrenoceptor blockade (Villalon et al., 1990) but were mimicked by renzapride (ED_{50} 20 µg/kg, i.v.; max. response 76 beats/min) and (R,S)zacopride (ED_{50} 28 µg/kg, i.v.; max. response 100 beats/min). Administration of SDZ 205,557 (0.3-6 mg/kg, i.v. administered 30-45 min prior to 5-HT) failed to antagonize the tachycardic effects of 5-HT (10-100 µg/kg, i.v.). However, blockade by SDZ 205,557 (3 and 6 mg/kg, i.v.) of 5-HT responses was seen 3 min after dosing.

SDZ 205,557, therefore, antagonized putative 5-HT$_4$ receptor mediated-responses in rat oesophagus in vitro, with similar values to those at putative 5-HT$_4$ receptors in guinea-pig ileum (Buchheit & Gamse, 1991). The compound did not discriminate between putative 5-HT$_4$ receptors and 5-HT$_3$ sites in NG108-15 cells. The brief blockade of putative 5-HT$_4$ receptor mediated responses in vivo may limit the utility of the antagonist.

Buchheit, K.H. & Gamse, R. (1991) Naunyn-Schmiedeberg's Arch. Pharmacol. **343** (Suppl), abstract 402P.
Clarke, D.E., Craig, D.A. & Fozard, J.R. (1989) Trends Pharmacol. Sci. **10**, 385-386.
Dumuis, A., Sebben, M., Monferini, E., Nicola, M., Turconi, M., Ladinsky, H. & Bockaert, J. (1991). Naunyn-Schmiedeberg's Arch. Pharmacol. **343**, 245-251.
Villalon, C.M., den Boer, M.O., Heiligers, J.P.C. & Saxena, P.R. (1990) Br. J. Pharmacol., **100**, 665-667.

276P BRL 46470A ANTAGONISES 5-HT$_3$ RECEPTOR-MEDIATED RESPONSES ON THE RAT VAGUS AND ON NG108-15 CELLS

N. R. Newberry, C.J. Watkins & T.S.Sprosen, Oxford University-SmithKline Beecham Centre for Applied Neuropsychobiology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE.

BRL 46470A is a potent and selective 5-HT$_3$ antagonist (Thomas *et al*, 1990). We have examined its antagonism of the 5-HT induced depolarising response on the rat vagus and the 5-HT-induced fast, transient inward current on murine neuroblastoma - rat glioma (NG108-15) cells. We have compared its action on these 5-HT$_3$ mediated responses with that of other 5-HT$_3$ antagonists.

Grease-gap recordings were made from Sprague-Dawley rat desheathed vagus nerves *in vitro* (Ireland and Tyers, 1987) using methods similar to those for superior cervical ganglia (Newberry *et al*, 1991). 5-HT was superfused for 1 min periods at 10 - 20 min intervals and the antagonists were preincubated for 1 h. Whole cell patch-clamp recordings (at -60 mV) from NG108-15 cells (passages 33-39) were made using electrodes filled with CsF/EGTA (*inter alia*). At 10 min intervals, 5-HT (0.05 ml of 3 - 100 µM) was rapidly applied to the cell from a plastic tube (internal diameter 0.86 mm) positioned approximately 200 µm away. Antagonists were applied in the bath perfusate for at least 5 min. Response amplitudes were normalised to the response to 10 µM 5-HT on the rat vagus and 100 µM 5-HT on NG108-15 cells. When appropriate, the pKb of an antagonist was estimated using $pK_b = \log_{10}(DR-1) - \log_{10}$(molar concentration of antagonist). The percentage effects quoted are geometric means.

BRL 46470A (0.3 - 3.0 nM) antagonised the 5-HT$_3$ receptor induced depolarisation of the rat vagus nerve in an insurmountable manner (n = 12) having an IC_{50} of 0.3 - 1.0 nM. We have also observed insurmountable antagonism of this response by BRL 43694 (granisetron, Fake *et al*, 1987, IC_{50} ca. 1.0 nM, n = 4) and ICS 205-930 (Richardson *et al*, 1985, IC_{50} ca. 0.3 nM, n = 3). This type of antagonism contrasted with the parallel shifts of the 5-HT concentration-response curve seen with GR38032F (Butler *et al*, 1988, IC_{50} ca. 10 nM, pKb = 9.3, n = 4) and (+)-tubocurarine (IC_{50} = 0.3 - 1.0 µM, pK_b = 7.2 , n = 13). BRL 46470A (1.0 nM) also antagonised the 5-HT$_3$ induced fast inward current of patch-clamped NG108-15 cells in an insurmountable manner (n = 3), the maximum response (to 100 µM 5-HT) being reduced by 89% (n = 10). By comparison, 10 nM (+)-tubocurarine reduced this response by 42% (n = 4). The response to 100 µM 5-HT recovered from antagonism by (+)-tubocurarine after 10 min of washing with drug free solution. In contrast the inhibition by BRL 46470A did not recover after 60 min of washing, it was still reduced by 80%.

The present findings indicate that BRL 46470A is a potent antagonist of the 5-HT$_3$ receptor mediated response on the rat vagus and on NG108-15 cells. Its action does not appear to be competitive.

Butler, A., Hill, J.M., Ireland, S.J., Jordan, C.C. & Tyers, M.B. (1988) Br. J. Pharmac. **94**, 397-412.
Fake, C.S., King, F.D. & Sanger, G.J. (1987) Br. J. Pharmac. **81**, 34P.
Ireland, S.J. & Tyers, M.B. (1987) Br. J. Pharmac. **90**, 229-238.
Newberry, N.R., Cheshire, S.H. & Gilbert, M.J. (1991) Br. J. Pharmac. **102**, 615-620.
Richardson, B. P., Engel, G., Donatsch, P. & Stadler, P.A. (1985) Nature **316**, 126-131.
Thomas D.R., Nelson, D.R., Blackburn, T.P. & Wood, M.D. (1990) Proc. BAP meeting 115P.

277P EFFECT OF MDL 73005EF ON 5-HT1$_A$ RECEPTOR-MEDIATED BLOOD PRESSURE RESPONSES IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS

M. van den Buuse and S. Buisson-Defferier (introduced by R.C. Miller). Marion Merrell Dow Research Institute, 16, rue d'Ankara, B.P. 447 R/9, 67009 Strasbourg, France.

MDL 73005EF is a potent and selective ligand for central 5-hydroxytryptamine-1A (5-HT_{1A}) receptors which exerts significant anxiolytic effects in animal models (Moser *et al.* 1990). MDL 73005EF acts as a 5-HT_{1A} agonist or antagonist at pre- and postsynaptic 5-HT_{1A} receptors, respectively (see Van den Hooff and Galvan, 1991). As 5-HT_{1A} receptors play an important role in central cardiovascular control, we studied the effect of MDL 73005EF on blood pressure in conscious, catheterized, spontaneously hypertensive rats (SHR). Body weight of these rats was 250-300 g and basal mean arterial pressure (MAP) was 150-160 mm Hg.

Intravenous (iv) injection of MDL 73005EF caused a dose-dependent decrease in MAP. The decrease was maximum at 5-10 min after injection: 0.1, 0.3, 1 and 3 mg/kg decreased MAP by -6±5, -12±3, -15±3 and -29±3 mm Hg, respectively (all data are means ± s.e. mean). At 15 min after injection of 0.1-1 mg/kg MDL 73005EF, MAP was not significantly different from saline-injected control-SHR. The iv injection 0.1 mg/kg of the 5-HT_{1A} receptor agonist 8-OH-DPAT at this time-point caused a reduction of MAP, which was significantly smaller than that occurring in controls. Thus, in SHR pretreated with MDL 73005EF, 0.03, 0.1, 0.3, or 1 mg/kg 8-OH-DPAT reduced MAP by -4±3, -3±5, -13±5, and -16±4 mm Hg, respectively. In saline-treated SHR these values were -10±6, -27±4, -33±3, and -28±5 mm Hg, respectively. Similarly, 30 min after the administration of 3 mg/kg MDL 73005EF, when MAP had returned to baseline, the effect of 0.1 mg/kg 8-OH-DPAT was reduced (-5±3 mm Hg vs. -29±4 mm Hg in controls). Pretreatment with 1 mg/kg MDL 73005EF also significantly reduced the hypotensive action of 0.3 mg/kg flesinoxan (-1±3 vs. -19±4 mm Hg) and that of 0.1 mg/kg 5-methyl-urapidil (-1±2 vs. -20±4 mm Hg) administered 15 min later. In contrast, pretreatment with MDL 73005EF did not significantly affect the decrease in MAP caused by administration of 0.01 mg/kg clonidine, 0.3 mg/kg hydralazine or 0.2 mg/kg nifedipine. Pretreatment with 0.3 mg/kg hydralazine, which caused a significant fall in MAP, did not affect the hypotensive response to injection of 8-OH-DPAT. However, pretreatment with 0.1 mg/kg 8-OH-DPAT caused a partial reduction of the hypotensive effect of another 8-OH-DPAT injection 30 min later (-13±3 vs. -23±3 mm Hg), suggesting the occurrence of desensitization.

These results suggest that MDL 73005EF has mixed properties at 5-HT_{1A} receptors involved in central cardiovascular control. The initial decrease in MAP after administration of especially higher doses of MDL 73005EF could be caused by its agonist properties. The inhibition of the hypotensive response to 5-HT_{1A} agonists, which was seen with both low and higher doses of MDL 73005EF, could be caused by a longlasting antagonistic effect. In addition, however, desensitization of the hypotensive effect of 5-HT_{1A} activation could play a role in these inhibitory responses.

Moser, P.C., Tricklebank, M.D., Middlemiss, D.N., Mir, A.K., Hibert M.F. & Fozard, J.R. (1990). Br. J. Pharmacol. **99**, 343-349.
Van den Hooff, P. & Galvan, M. (1991). Eur. J. Pharmacol. **196**, 291-298.

278P VARIATION IN THE RELAXATION RESPONSE TO 5-HT IN DIFFERENT REGIONS OF THE RAT SMALL INTESTINE

B. R. Tuladhar, B. Costall & R. J. Naylor, Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, BD7 1DP

5-Hydroxytryptamine (5-HT) causes a relaxation response in the terminal region of the rat ileum which is mediated via putative 5-HT_4 receptors (Tuladhar *et al.*, 1991). In the present study we investigate the variation in this response in the different regions of the small intestine.

2 cm segments were taken from different regions of the small intestine of female Hooded Lister rats (200-250 g) and mounted in 10 ml organ baths containing oxygenated (95% O_2 / 5% CO_2) Krebs-Henseleit solution (37°C) containing methysergide (1 µM) and atropine (0.1 µM) under an initial tension of 0.75 g. The tissues were allowed to equilibrate for 1 hour. Changes in tissue tension were recorded isometrically using force displacement transducers and a Grass polygraph.

Concentration response curves constructed to 5-HT (0.001-10 µM) in the terminal ileum indicated a maximal relaxation response at 1 µM. Using this concentration, in the duodenum (Duo) and proximal ileum an initial brief contraction preceded a small relaxation response (Figure 1). Whilst the contraction was not observed in tissues taken from regions approaching the ileocaecal junction, the relaxation response increased and maximised in the most distal 20 cm section of the ileum. Treatment with indomethacin (3 µM) markedly reduced the contraction response without modifying the relaxation response. Ondansetron (1 µM) failed to modify the relaxation response which was inhibited by ICS 205-930 (10 µM). The results indicate that 5-HT has a varying ability to cause relaxation in different areas of the rat ileum. This may be related to a varying density of putative 5-HT_4 receptors with highest density in the terminal ileum.

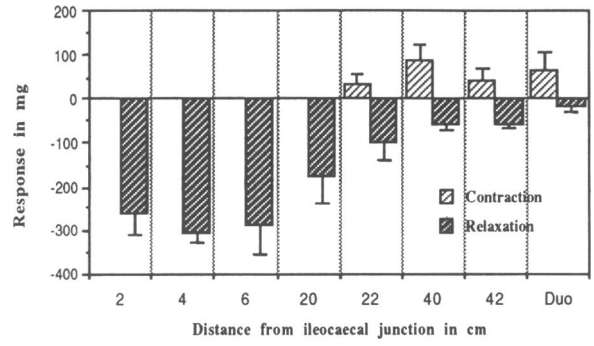


Figure 1. Relaxation responses to 5-HT in different regions of the rat small intestine. Values are the mean and vertical bars indicate the s.e.mean of 6 determinations.

Tuladhar, B. R., Costall, B. & Naylor R. J. (1991). Br. J. Pharmacol., **104**, 151P.

279P GUINEA-PIG DISTAL COLON: A SENSITIVE MODEL OF THE PUTATIVE 5-HT$_4$ RECEPTOR

Kay Wardle and Gareth Sanger, SmithKline Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex, CM20 5AD

In guinea-pig ileum , the concentration response curve to 5-HT is biphasic (Buchheit *et al.;* 1985). The second, and by much the larger phase is 5-HT$_3$ receptor mediated while the first, smaller phase is mediated by the putative 5-HT$_4$ receptor. We now report a similar biphasic response to 5-HT on guinea-pig distal colon, however here, the initial 5-HT$_4$ response is much pronounced.

Longitudinal muscle-myenteric plexus (LMMP) preparations of guinea-pig distal colon were suspended under 0.5g load in Krebs solution containing methiothepin (10^{-7}M). Concentration response curves to agonists were constructed non-cumulatively (30s exposure; 15 min dose-cycle). Antagonists were equilibrated for at least 30 min. Results are expressed as mean ± sem % of a maximum acetylcholine (ACh) evoked contraction.

Concentration response curves to 5-HT (10^{-11}-10^{-3}M) were biphasic. For the first phase (10^{-11}-10^{-7}M) - log EC_{50}=10.33±0.07 with a max response of 46 ± 1 % ACh. ; For the second phase ($3x10^{-7}$-10^{-3}M) - log EC_{50} = 6.42±0.04, with an overall max response of 85.0±2.5% ACh. Both were blocked (up to 98%) by atropine (10^{-6}M) or TTX ($3x10^{-7}$M). Granisetron (10^{-6}M) selectively antagonised the second phase and was thus added routinely in all subsequent experiments. Under these conditions, the rank order of agonist potency was similar to that seen in the rat oesophagus model of the 5-HT$_4$ receptor (Baxter *et al.;* 1991), although the sensitivity to 5-HT was almost 100-fold greater.

Agonist (all tested at 10^{-12}-10^{-4}M)	Intrinsic activity	p value	-Log EC_{50}
5-HT	1.0		10.08±0.19
5-MeOT	0.9	0.35	9.95±0.32
Renzapride	1.0	0.60	8.71±0.14
Cisapride	0.7	0.04	8.16±0.16
5-CT	0.8	0.16	6.67±0.09
2-Me-5-HT	0.9	0.43	4.92±0.17
8-OH-DPAT	0.1	0.0001	5.44±0.33

ICS 205-930 (10^{-6}, $3x10^{-6}$, 10^{-5}M) concentration-dependently and surmountably antagonised the 5-HT-evoked contraction; pA_2 6.39±0.12. While such a value is consistent with activity at the 5-HT$_4$ receptor the slope of the Schild plot (1.3±0.10) was greater ($p<0.05$) than one. This may be attributed to the fact that, in this tissue, 10^{-5}M ICS 205-930 also antagonised the response to DMPP, suggesting a degree of non-selectivity. Nevertheless, our data suggests that in contrast to the ileum where the 5-HT$_4$ response is usually very small, in the colon the 5-HT$_4$ response is much pronounced. The results indicate a regional difference in magnitude of the response evoked by activation of the putative 5-HT$_4$ receptor along the guinea-pig intestine.

Buchheit, K.H., Engel G., Mutschler E. and Richardson B. (1985) N-S's Arch Pharmacol, 329, 36-41
Baxter G.S., Craig D.A. and Clarke D.E. (1991). N-S's Arch Pharmacol, 343, 439-446

280P 5-HT$_1$-LIKE RECEPTORS IN THE PORCINE CAROTID CIRCULATION: DO THEY BELONG TO THE 5-HT$_{1D}$ RECEPTOR SUBTYPE?

M.O. Den Boer, C.M. Villalón & P.R. Saxena, Department of Pharmacology, Erasmus University, P.O. Box 1738, 3000 DR, Rotterdam, The Netherlands.

Both the reduction of porcine carotid arteriovenous anastomotic flow (AVAF) and the increase in skin blood flow (SBF) by 5-HT are mediated by 5-HT$_1$-like receptors and the decrease in AVAF also by 5-HT$_2$ receptors (Saxena & Verdouw, 1982; Saxena *et al.*, 1986). Sumatriptan, a 5-HT$_1$-like agonist with some selectivity for the 5-HT$_{1D}$-receptor subtype, reduces AVAF, without much changing SBF (Den Boer *et al.*, 1991). Therefore, we have attempted to determine whether the 5-HT$_1$-like receptor, mediating the decrease in AVAF, belongs to the 5-HT$_{1D}$ subtype. We used intracarotid injection of radioactive microspheres in pentobarbitone-anaesthetized pigs to study the distribution of carotid blood flow. 5-HT (2 μg kg^{-1} min^{-1}) was infused in one carotid artery before and after metergoline (MTG; 1 mg kg^{-1}), an antagonist of 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D}$ and 5-HT$_2$ receptors. Cumulative i.v. dose responses were obtained for sumatriptan in animals, pretreated with saline (SAL; n=8) or MTG (1 mg kg^{-1};n=7).

Table 1. Effect of intracarotid infusions of 5-HT before and after MTG.

	Control	5-HT	MTG	5-HT
AVAF	150±21	-95±1%	116±20	-59±7%*
SBF	2±0	711±233%	3±1	2340±406%*

Flow in ml min^{-1}; effects of 5-HT in % change of baseline; data as means ± s.e.mean; *, $P<0.05$ vs. first 5-HT response.

Table 2. Effect of cumulative i.v. doses of sumatriptan after SAL or MTG treatment.

	Sumatriptan (mg kg^{-1})				
	0	0.01	0.03	0.1	0.3
SAL					
AVAF	140±25	125±21	105±22	80±19	53±16
SBF	1±0	2±0	2±0	3±1	3±0
MTG					
AVAF	127±19	117±17	100±15	80±13	62±11
SBF	2±0	3±1	3±1	4±0	4±0

Flow in ml min^{-1}; data as means ± s.e.mean

As shown in Table 1, the reduction in AVAF by 5-HT was attenuated by MTG, whereas its cutaneous vasodilator effect was markedly enhanced. This pattern was similar to cyproheptadine and ketanserin in our former experiments (Saxena & Verdouw, 1982; Verdouw *et al.*, 1984), so antagonism of MTG of constrictor 5-HT$_2$-receptors seems to be involved. Sumatriptan reduced dose-dependently AVAF and slightly raised SBF (Table 2). Neither of these effects was changed by MTG-pretreatment. We conclude that the 5-HT$_1$-like receptor involved in the reduction of arteriovenous anastomotic flow and in the increase in cutaneous flow in the carotid circulation of the pig, is not equal to the 5-HT$_{1D}$-subtype that is antagonized by metergoline.

Den Boer, M.O., Villalón, C.M., Heiligers, J.P.C., Humphrey, P.P.A. & Saxena, P.R. (1991) Br. J. Pharmacol. **102**, 323-330.
Saxena, P.R., Duncker, D.J., Bom, A.H., Heiligers, J. & Verdouw, P.D. (1986) Naunyn-Schm. Arch. Pharmacol. **333**, 198-204.
Saxena, P.R. & Verdouw, P.D. (1982) J. Physiol. (Lond). **332**, 501-520
Verdouw, P.D., Jennewein, H.M., Heiligers, J., Duncker, D.J. & Saxena, P.R. (1984) Eur. J. Pharmacol. **102**, 499-509.

281P THE ISOLATED ATRIA FROM MICE AND RATS AS MODELS FOR TESTING CARDIOACTIVE DRUGS: A COMPARATIVE STUDY

L.B. Andersen, C. Dyerberg & K. Hermansen, Department of Biological Sciences, Pharmacology and Toxicology, Royal Danish School of Pharmacy, 2100 Copenhagen, Denmark.

The most commonly used in vitro preparations to investigate the effects of cardioactive drugs are isolated atria from guinea-pigs and rats. (Politi et al., 1985; Rasmussen et al., 1989). In the present study some pharmacodynamic characteristics of isolated atria from mice as a new model for testing cardioactive drugs (Wong, 1987; Hermansen et al.,1990) were compared with similar data obtained from rat atria.

The stability of the contractile rate of spontaneously beating atria from the two species was compared at organ bath temperatures of 28 and 38° C during a 4 h experimental period. Also the chronotropic response to noradrenaline, carbachol and histamine was compared.

The initial contractile rate of the mouse atria was 325±15 and 535±48 beats/min (mean ±s.e. mean) at 28 and 38° C respectively. These values declined to 74 and 82% of the initial values after 1h and to 53 and 71% after 4h incubation. On the contrary the contractile rate of atria from rats remained fairly constant both at 28 and 38° C the minimum rate being 91% of the initial values after 4h incubation.

Noradrenaline caused a concentration-dependent, propranolol-sensitive increase of the atrial rate both in mice and rats with a K_d of 0.35±0.31μM and 0.19±0.08μM ($p>0.05$) respectively.

Carbachol caused a concentration-dependent decrease of the atrial rate both in mice and rats with a K_d of 19±19 nM and 75±34 nM ($p>0.05$) respectively.

Histamine caused a concentration-dependent, cimetidine sensitive increase of the atrial rate in mice with a K_d of 3.6±0.7μM and an E_{max} of 123±15 beats/min. On the contrary rat atria were virtually insensitive to histamine as concentrations up to 10^{-2} M only caused a maximal increase of 11±2.9 beats/min. The lack of chronotropic response to histamine in rat atria was observed in two different strains of rats both at 28 and 38° C and is surprising in view of previously published results by Frkovic et al. (1988) and others.

From the findings in the present study it is concluded that the spontaneously beating isolated mouse atria may be a useful alternative to isolated rat- and guinea pig atria for testing cardioactive drugs.

Frkovic, A., Skrobonja, A. & Atanackovic, D. (1988) Acta Physiol. Pharmacol. Bulg. 14, 43-48.
Hermansen, K., Rasmussen, I.M.N. & Schou, H.S. (1990) Pharmacol. Toxicol. 66, 299-302.
Politi, P.M., Rothlin, R.P. & Pinto, J.E.B. (1985) Cancer. Treatm. Rep. 69, 859-865.
Rasmussen, I.M.N., Schou, H.S. & Hermansen, K. (1989) Pharmacol. Toxicol. 65, 69-72.
Wong, K.K. (1987) Med. Sci. Res. 15, 261-262.

282P DISTRIBUTION OF β_1- AND β_2-ADRENOCEPTORS IN RIGHT AND LEFT ATRIA AND VENTRICLES OBTAINED POST-MORTEM FROM NONFAILING AND FAILING HUMAN HEARTS

M. Steinfath, J. Lavický, W. Schmitz & H. Scholz (introduced by J. Neumann), Abteilung Allgemeine Pharmakologie, Universitäts-Krankenhaus Eppendorf, Universität Hamburg, Germany

The human heart contains a heterogeneous population of β_1- and β_2-adrenoceptors in atria as well as in ventricles, and both subtypes can mediate positive inotropic effects (Lemoine et al., 1988; Brodde, 1991). Studies comparing total number of ß-adrenoceptors and subtype distribution in all cardiac chambers of nonfailing and failing human hearts have as yet not been published. We determined total ß-adrenoceptor density and subtype distribution in right and left atria and ventricles obtained from 7 nonfailing hearts and 15 patients with end-stage heart failure due to idiopathic dilated cardiomyopathy (IDC, n=9) or ischaemic cardiomyopathy (ICM,n=6). (-)-(^{125}I)-iodocyanopindolol was used as radioligand and CGP 20712A (1-(2-(3-carbamoyl-4-hydroxy)phenoxyethylamino)-3-(4-(1-methyl-4-triflouromethyl-2-imidazolyl)phenoxy)-2-propanol methanesulfonate, β_1-selective antagonist) to calculate the β_1:β_2-ratio.

In nonfailing hearts the total number of ß-adrenoceptors was similar in atria and ventricles (mean ± SEM): right atrium 77.9 ± 6.5, left atrium 82.1 ± 7.3, right ventricle 72.4 ± 6.9, and left ventricle 70.1 ± 6.0 fmol/mg protein. The β_1:β_2-adrenoceptor ratio (%) in the nonfailing atria was slightly smaller than in ventricles indicating a higher β_2-adrenoceptor population in both atria: right atrium 71:29 %, left atrium 69:31 %, right ventricle 80:20 %, and left ventricle 79:21 %. In patients with end-stage heart failure due to IDC or ICM the total ß-adrenoceptor density was markedly reduced by about 60 % in all cardiac chambers. On the other hand, the β_1:β_2-adrenoceptor ratio was differently influenced depending on the aetiology of heart failure. In tissues obtained from patients with IDC the β_1:β_2-ratio was shifted in atria and ventricles to about 60:40 % due to a selective reduction of β_1-adrenoceptors ($p<0.05$). The β_2-adrenoceptor population was not downregulated. In patients with ICM the β_1:β_2-adrenoceptor ratio was unchanged in all cardiac chambers as compared with nonfailing hearts indicating a similar reduction of both subtypes β_1- and β_2-adrenoceptors.

It is concluded that downregualtion of ß-adrenoceptors in patients with IDC or ICM is homogeneously distributed in all chambers of the human heart. A change in β_1:β_2-adrenoceptor ratio was observed in IDC only. This may support the hypothesis that changes in β_1- and β_2-adrenoceptor subtypes might be related to the aetiology of heart failure.

Brodde, O.E. (1991) Pharmacol. Rev. 43, 203 - 242
Lemoine, H., Schönell, H. & Kaumann, A.J. (1988) Br. J. Pharmacol. 95, 55 - 66

283P STIMULATION OF β_1- AND β_2-ADRENOCEPTORS IN HUMAN VENTRICULAR MYOCARDIUM FROM FAILING HEARTS HASTENS THE ONSET OF RELAXATION

A.J. Kaumann*[1,2], L. Sanders[1], J. A. Hall[1], K.J. Murray[2] & M.J. Brown[1]
[1]Clinical Pharmacology Unit, Addenbrooke's Hospital, Cambridge CB2 2QQ & [2]SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts AL6 9AR

In isolated ventricular preparations obtained from patients with mild heart failure (-)-adrenaline and (-)-noradrenaline augment contractile force predominantly through β_1-adrenoceptors (β_1AR) but to a smaller degree also through β_2-adrenoceptors (β_2AR) (Kaumann & Lemoine, 1987). It has recently been reported in feline right ventricular muscle that for matching increases in contractile force mediated through β_1AR and β_2AR onset of relaxation is hastened by catecholamines only through β_1AR and not through β_2AR, suggesting a cyclic AMP-dependent pathway for β_1AR but not for β_2AR (Lemoine & Kaumann, 1991).

What are the relative contributions of β_1AR and β_2AR to catecholamine-evoked increases in contractile force of ventricular muscle obtained from patients with terminal heart failure? Is relaxation hastened and if so, through which β-adrenoceptor subtype? To answer these questions we studied the responses of trabeculae, usually from the right ventricle, obtained from patients undergoing heart transplants. Six to 24 tissues from each patient were set up and paced at 0.2 Hz to contract isometrically at 37°C. Following incubation for 2 h in phenoxybenzamine, 5 μM, β_1AR responses were determined from cumulative concentration-effect curves to (-)-noradrenaline in the presence of ICI 118551, 50 nM, to block β_2AR; β_2AR responses were obtained, using tissues from the same patients, from cumulative concentration-effect curves to (-)-adrenaline in the presence of CGP 20712A, 300 nM, to block β_1AR, as previously described (Kaumann & Lemoine, 1987). Time to peak force and contractile force were measured from fast-speed tracings. β_1AR stimulation by (-)-noradrenaline decreased time to peak force (pEC_{50} = 6.4 ± 0.4, n = 5, $\bar{x}$ ± sem) and increased contractile force (pEC_{50} = 6.0 ± 0.3) with a maximum effect of 73 ± 5% of that of 6.75 mM $CaCl_2$ added to terminate the experiment. β_2AR stimulation by (-)-adrenaline had very similar effects: time to peak force was decreased (pEC_{50} = 6.9 ± 0.1, n = 5) and contractile force was increased (pEC_{50} = 6.2 ± 0.3) with a maximum of 61 ± 6% of that of 6.75 mM $CaCl_2$. These results suggest that both β_1AR and β_2AR function via a cyclic AMP-dependent pathway. To test this we measured cyclic AMP-dependent protein kinase (cA-PrK) activity in tissues from an additional 4 patients, using the method of Murray et al. (1990). Stimulation of both β_1AR and β_2AR increased the activity of cA-PrK in tissues from each patient.

We conclude that in isolated ventricular myocardium from failing human hearts: (i) β_1AR and β_2AR mediate similar positive inotropic effects of physiological catecholamines; (ii) Activation of both β_1AR and β_2AR hastens the onset of relaxation; (iii) The stimulation of cA-PrK suggests that activation of both β_1AR and β_2AR leads to phosphorylation of phospholamban and troponin I thereby hastening relaxation.

We thank the Heart and Lung Transplant Research Group of Papworth Hospital for the supply of human ventricle.

Kaumann, A.J. & Lemoine, H. (1987) Naunyn-Schmiedeberg's Arch Pharmacol 335, 27-39
Lemoine, H. & Kaumann, A.J. (1991) Naunyn-Schmiedeberg's Arch Pharmacol 344, 56-69
Murray, K.J., England, P.J., Lynham, J.A., Mills, D., Schmitz-Pfeiffer, C. & Reeves, M.L. (1990) Biochem. J. 267, 703-708

284P THE PHOSPHODIESTERASE IV INHIBITOR ROLIPRAM ENHANCES MECHANICAL AND ELECTRICAL EFFECTS OF THE PHOSPHODIESTERASE III INHIBITOR R 80122

D. Wilhelm, M. Ebbert, A. Leidig, B. Wilffert & Th. Peters, Janssen Research Found., W-4040 Neuss 21, FRG

R 80122 ((E)-N-cyclohexyl-N-methyl-2-[[[phenyl (1,2,3,5-tetrahydro-2 oxoimidazo [2,1-b]-quinazolin-7-yl) methylene] amino]oxy] acetamide) is a cardiotonic drug with a selective inhibitory action on phosphodiesterase (PDE) III (Wilhelm et al., 1991). In contrast to milrinone, which discriminates less between inhibition of PDE III and PDE IV, R 80122 did not significantly reduce action potential duration. To assess whether inhibition of PDE IV modifies the mechanical and electrophysiological effects of PDE III inhibition by R 80122, we used the selective PDE IV inhibitor rolipram.
Isometric contraction measurements were performed on guinea-pig isolated left atria paced at 0.2 Hz. After an equilibration period of 60 min and pretreatment with either solvent or compound (R 80122 and/or rolipram) for 45 min cumulative concentration-response curves of isoprenaline were obtained. Action potential duration and force of contraction were assessed in guinea-pig isolated right papillary muscles paced at 1 Hz. After an equilibration period of 90 min during which control action potentials were recorded the muscles were incubated with either solvent or rolipram for 45 min before R 80122 was added for 60 min in the continuous presence of rolipram.
Contraction measurements: Pretreatment with R 80122 (10^{-7} - 10^{-6} mol/l) dose dependently shifted the concentration-response curve of isoprenaline to the left. R 80122 (10^{-7}, 3 x 10^{-7}, 10^{-6} mol/l) increased contractile force by 7 ± 5%, 26 ± 7%, and 43 ± 5%, respectively (mean ± SEM, n = 5 - 6). Rolipram (10^{-6} mol/l) had a small positive inotropic effect of 10 ± 2% and had no effect on the concentration-response curve to isoprenaline. The combination of R 80122 (3 x 10^{-7} or 10^{-6} mol/l) and rolipram (10^{-6} mol/l) shifted the concentration-response curve further to the left than R 80122 alone, but depressed the maximum.
Electrophysiological measurements: R 80122 (10^{-6} mol/l) decreased the action potential duration by 13 ± 3 ms and increased force of contraction from 1.2 ± 0.2 mN to 2.5 ± 0.3 mN (mean ± SEM, n = 7). Resting membrane potential, action potential amplitude and maximal rate of rise were not affected by R 80122. Rolipram (10^{-6} mol/l) did not modify action potential configuration and contractile force. In the presence of rolipram, R 80122 shortened the action potential duration by 32 ± 4 ms and enhanced force of contraction from 1.7 ± 0.2 mN to 4.8 ± 0.6 mN (n = 7).
The results are consistent with a role of both PDE III and PDE IV in guinea-pig left atria. The sensitivity to isoprenaline and the positive inotropic effect of the PDE III inhibitor R 80122 are enhanced by the PDE IV inhibitor rolipram. Furthermore, inhibition of PDE IV seems to be responsible for the marked shortening of the action potential duration in guinea-pig papillary muscle.

Wilhelm, D., De Chaffoy de Courcelles, D., Leidig, A., Ebbert, M. & Meuter, C. (1991) Naunyn-Schmiedeberg's Arch. Pharmacol. 343, R54, 216.

285P INFLUENCE OF ISCHAEMIA ON BINDING BEHAVIOUR OF MUSCARINIC AGONISTS AND ANTAGONISTS IN RAT VENTRICULAR PREPARATIONS

R. van den Ende, H.D. Batink & P.A. van Zwieten. Department of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands.

In this study we investigated the influence of global ischaemia on muscarinic receptor binding characteristics in the rat Langendorff heart (LH). Using the radioligand [^{3}H]NMS ([^{3}H]-N-methylscopolamine) and dexetimide to determine specific binding, the muscarinic receptor density and affinity were assessed in left ventricular homogenates of rat LH under normoxic circumstances, after 30 min of ischaemia and after 30 min of ischaemia plus reperfusion, respectively. Neither ischaemia nor post-ischaemic reperfusion caused any change in receptor density or affinity. In displacement experiments in normoxic and ischaemic left ventricular rat heart preparations low concentrations ($2x10^{-10}$-10^{-7} M) of various muscarinic agonists and antagonists increased (to values of 120 to 145 %) or did not affect [^{3}H]NMS binding (Table 1). The aforementioned increase in [^{3}H]NMS binding was abolished in the presence of 10^{-4} M guanylyl-imidodiphosphate (Gpp(NH)p) which is known to shift receptors to the low-affinity state. At higher concentrations (10^{-7} -10^{-4} M) of the substances the usual displacement was observed.

Table 1 Maximal increase in [^{3}H]NMS binding (% change from initial binding) induced by various muscarinic agonists and antagonists ($2x10^{-10}$-10^{-7} M) as observed during displacement experiments. Values are means ± s.e.mean. *$p<0.05$ as compared to initial binding.

		NORMOXIC Δ%	n	ISCHAEMIC Δ%	n
AGONISTS :	Acetylcholine	42.1 ± 7.6*	3	38.8 ± 5.1*	3
	Carbachol	43.2 ± 5.7*	3	32.7 ± 6.0*	3
	Oxotremorine	6.4 ± 0.1	6	22.0 ± 8.0*	6
ANTAGONISTS :	AF-DX 116	26.6 ± 8.9*	6	11.0 ± 2.0	3
	Atropine	4.2 ± 3.7	3	22.1 ± 2.5*	3
	Gallamine	31.0 ± 7.2*	4	20.8 ± 4.8*	4
	Pirenzepine	23.9 ± 9.9*	5	21.2 ± 6.3*	6
	(-)Scopolamine	4.5 ± 1.8	3	24.0 ± 15.5*	4

Scatchard plots confirmed the potentiation of [^{3}H]NMS binding in the presence of low concentrations of either oxotremorine or AF-DX 116, since oxotremorine ($3x10^{-9}$ M) increased B_{max} in ischaemic preparations only, while AF-DX 116 ($3x10^{-9}$ M) increased B_{max} in the normoxic preparations only. In conclusion, global ischaemia did not change the density and affinity of cardiac muscarinic receptors for [^{3}H]NMS, but low doses of muscarinic agonists and antagonists potentiated the binding of [^{3}H]NMS to ventricular homogenates. This potentiation was abolished in the presence of 10^{-4} M Gpp(NH)p.

286P REDUCED VENTRICULAR α-ADRENOCEPTOR DENSITY DURING ENHANCED RESPONSIVENESS TO PHENYLEPHRINE INDUCED BY CHRONIC NORADRENALINE INFUSION IN THE RAT

M.C. Butterfield+ and R. Chess-Williams. Departments of Biomedical Science, University of Sheffield and Pharmacology, University of Liverpool. +Present address: Department of Cardiology, Washington University Medical Center, St. Louis, U.S.A.

We have previously shown that chronic noradrenaline infusion in rats enhances papillary muscle responses to phenylephrine (Butterfield & Chess-Williams, 1990). The present study examines whether this enhanced responsiveness is associated with an increase in the density of ventricular α-adrenoceptor binding sites.
Osmotic minipumps delivering noradrenaline (1 mg/kg/hr), isoprenaline (40 μg/kg/hr) or saline were implanted subcutaneously in rats. Three days later ventricular papillary muscles were removed and set up in aerated Krebs solution at 37 °C and tissues paced at 1 Hz. Concentration-response curves to isoprenaline and phenylephrine (in the presence of propranolol 1 μM) were obtained in the presence of cocaine (10 μM) and metanephrine (10 μM). A crude membrane fraction was prepared from the remaining ventricular tissue and assayed for [^{3}H]dihydroalprenolol ([^{3}H]DHA) and [^{3}H]prazosin binding.
Papillary muscle responses to isoprenaline were depressed following catecholamine infusion, the maximum increase in developed tension to isoprenaline being reduced from 0.26 ± 0.03 g (n=6) to 0.14 ± 0.02 g (m=5; $P<0.001$) and 0.12 ± 0.02 g (n=10;$P<0.01$) by noradrenaline and isoprenaline treatment respectively. Isoprenaline EC_{50} values were not significantly altered by either treatment. Papillary muscle responses to phenylephrine were enhanced by noradrenaline and isoprenaline pretreatment, the EC_{50} values being reduced from 24.9[12.1 - 51.7]μM (n=6) to 4.0[3.0 - 5.3]μM (n=5;$P<0.001$) by noradrenaline and from 13.4(8.1 - 22.1)μM (n=6) to 3.5(1.5 - 8.0)μM (n=10, $P<0.01$) by isoprenaline pretreatment.

INFUSION	[^{3}H]PRAZOSIN Bmax	Kd	n	[^{3}H]DHA Bmax	Kd	n
Saline	79.9 ± 3.8	0.06 ± 0.01	7	102.6 ± 7.5	0.52 ± 0.10	8
Noradrenaline	58.6 ± 2.7*	0.06 ± 0.01	5	66.8 ± 5.3*	0.43 ± 0.08	6
Isoprenaline	83.2 ± 5.2	0.04 ± 0.01	5	64.4 ± 5.0*	0.66 ± 0.07	7

Table 1: Density of binding sites (Bmax in fmol/mg protein) and dissociation constants (Kd in nM) for [^{3}H]prazosin and [^{3}H]DHA binding to ventricular membranes. $P<0.05$.

Infusions of noradrenaline, but not isoprenaline, reduced the density of ventricular [^{3}H]prazosin binding sites (Table 1). Three day infusions of noradrenaline or isoprenaline reduced the density of [^{3}H]DHA binding sites. Dissociation constants for binding were not affected by either pretreatment.

The results demonstrate that the enhanced α-adrenoceptor responsiveness observed with papillary muscles following chronic noradrenaline infusion is accompanied by a *reduction* in ventricular α-adrenoceptor density.

This work was supported by the British Heart Foundation and the Smithkline Foundation.
Butterfield, M.C. & Chess-Williams, R. (1990) J. Auton. Pharmacol., 10, 12-13.

287P EVIDENCE FOR ALTERED EXPRESSION OF $G_s\alpha$ SUBUNIT AS A FACTOR IN THE ENHANCED COUPLING OF β ADRENOCEPTORS TO ADENYLYL CYCLASE IN HUMAN ATRIUM

MJ Brown, A Ferro, H Jia, S Monteith.
Clinical Pharmacology Unit, University of Cambridge Clinical School, Cambridge CB2 2QQ.

β_1-adrenoreceptor (β_1AR) and β_2-AR stimulation of human atrium achieves the same maximal inotropic response, and treatment with β-blockade induces a further 5-10 fold increase in β_2AR (but not β_1AR) sensitivity, both in vitro and in vivo. Pharmacological experiments have pointed to increased coupling of β_2AR to adenyl cyclase following β-blockade, possibly because there is a greater reserve of adenyl cyclase coupled in human atrium to β_2AR than β_1AR. We have therefore investigated whether human atrium differs from other species in the expression of the stimulatory GTP-binding protein, G_s, for whose α-subunit ($G_s\alpha$) there are 4 splice variants: 2 long ($G_{s_L}\alpha$) and 2 short ($G_{s_S}\alpha$). Predominantly $G_{s_S}\alpha$ is found in most species when measured by cholera-toxin stimulated ADP-ribosylation. In pieces of atrium removed from patients undergoing bypass surgery, the pattern of mRNA encoding $G_s\alpha$ was measured by reverse transcription and amplification of the cDNA (RT/PCR); the primers were synthesized to distinguish each of the 4 variants by spanning the 45-bp region which is absent from $G_{s_S}\alpha$, and including the variable serine codon at the 3' end of one of the primers. The PCR products were separated on agarose gels, blotted, probed with a complementary digoxigenin labelled RNA, and revealed by alkaline phosphatase conjugated anti-serum and laser densitometry of the colour product. Semi-quantification was possible for PCR product from cycles 10-20 where amplification was found to be linear. The identity of some of the PCR products was also confirmed by sequencing. The expression of the 3 $G_i\alpha$ subunits was similarly investigated, although the need for separate pairs of primers for each of these excludes relative quantification. The G-proteins themselves were detected by immunoblotting, using separate antisera kindly donated by Dr. G. Milligan which recognise either $G_s\alpha$ (all forms) or the α subunit of G_{i1} and G_{i2}.

The PCR provided evidence for all 4 $G_s\alpha$ variants in human atrium. The amount of $G_{s_L}\alpha$ and $G_{s_S}\alpha$ mRNA was approximately equal in each of 8 atria. This contrasted with the finding of predominantly $G_{s_S}\alpha$ mRNA in atria from dog and guinea-pig (n=1). The greatest contrast, however, was with the immunoblots from 13 human atria, in which the amount of $G_{s_L}\alpha$ was 8 fold greater than that of $G_{s_S}\alpha$. In these small numbers, no difference was found between atria from β-blocked and untreated patients. RT/PCR demonstrated expression of all three $G_i\alpha$ subunits; immunoblotting with the antiserum against G_i1 and G_i2 demonstrated mainly G_i2.

These findings suggest that the greater functional importance of β_2AR in human atrium (compared to other species), and their greater coupling to adenyl cyclase, may be associated with increased expression of $G_{s_L}\alpha$. The higher ratio of $G_{s_L}\alpha$ to $G_{s_S}\alpha$ on the immunoblots than predicted from their mRNA levels also suggests that $G_{s_L}\alpha$ is more stable. We postulate therefore that $G_{s_L}\alpha$ is a poorer substrate for endogenous ribosyl transferases (as it is for cholera toxin), and that cAMP-dependent activation of these may be a mechanism of receptor cross-regulation.

288P 5-HT-INDUCED RESPONSES AFTER N^G-NITRO-L-ARGININE METHYL ESTER IN PITHED RATS

E.M. van Gelderen and P.R. Saxena, Department of Pharmacology, Erasmus University,
P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

The vascular endothelium regulates smooth muscle responsiveness by releasing vasoactive factors. One of these factors, the endothelium dependent relaxing factor (EDRF) has been characterized as nitric oxide (EDNO). The release of EDNO can be inhibited by N^G-Nitro-L-arginine methyl ester (L-NAME). Relaxations induced by 5-hydroxytryptamine (5-HT) are inhibited by L-NAME in the rat kidney (Verbeuren *et al.*, 1991). Furthermore contractions to 5-HT are potentiated in isolated endothelium denuded vessels from hypertensive and normotensive rats (Dohi & Lüscher, 1991). In this study we investigated the role of EDNO in 5-HT induced responses in normotensive pithed rats.

Hypotensive responses to 5-HT (0.1 to 3.0 μg kg^{-1}) were studied before and after L-NAME (1, 3 and 10 mg kg^{-1}) in rats pretreated with ketanserin (0.1 mg kg^{-1}). L-NAME increased mean blood pressure from 59 ± 1 mmHg to 126 ± 8 mmHg, which was unaffected by ketanserin pretreatment. Peak hypotensive responses to 5-HT (1.0 and 3.0 μg kg^{-1}) were augmented by L-NAME. At similar blood pressure levels, during phenylephrine infusion (160, 240 and 400 μg kg^{-1} hr^{-1}), 5-HT responses were significantly less augmented (Table 1.). With the two highest doses of L-NAME the time to reach 50 % recovery of the 5-HT (1.0 μg kg^{-1}) hypotensive effect was significantly prolonged.

Hypertensive responses were studied with higher doses of 5-HT (3.0, 10.0 and 30.0 μg kg^{-1}). Both L-NAME, up to 10 mg kg^{-1}, and phenylephrine, up to 400 μg kg^{-1} hr^{-1}, failed to alter the magnitude of the pressor response. However, the duration of the response to 5-HT (30 μg kg^{-1}) was prolonged by L-NAME (3.0 and 10 mg kg^{-1}).

Table 1. Decrease in MAP (mmHg) by 5-HT in pithed ketanserin pretreated rats

5HT	Saline (injection; n=7)			L-NAME (mg kg^{-1}; n=9)			Phenylephrine (μg kg^{-1} hr^{-1})		
(μg kg^{-1})	1	2	3	1	3	10	160	240	400
0.1	10±1	9±2	8±2	13±3	15±3	18±3	6±0	9±1	9±2
0.3	13±4	8±2	11±4	10±1	17±2	15±3	10±2	12±2	12±2
1.0	14±3	15±2	14±2	21±3	33±3*	28±5*	12±2	14±2	15±2
3.0	14±4	18±2	19±2	33±5*	45±5*	42±9*	24±3	31±4*	34±3*

mean ± sem; *, significantly different ($p<0.05$) from saline value.

In conclusion, the basal release of EDNO is inhibited by L-NAME in pithed rats. However, there seems to be little contribution of EDNO to the hypotensive effect of 5-HT in these animals.

Dohi, Y. & Lüscher, T.F.(1991) J. Cardiovasc. Pharmacol. **18**, 278-284
Verbeuren, J., Mennecier, P. & Laubie, M.(1991) Eur. J. Pharmacol. **201**, 17-27.

289P THE ROLE OF CALCIUM IN THE INDEPENDENT REGULATION OF THE RELEASE OF EDRF AND PROSTACYCLIN FROM BOVINE AORTIC ENDOTHELIAL CELLS

H. Parsaee, S. Joseph, J. MacDermot & J.R. McEwan, Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W12 ONN

Bradykinin (BK) receptor activation triggers the release of both prostacyclin (PGI_2) and endothelium derived relaxing factor (EDRF) from endothelial cells. This is accompanied by a rise in free ionized intracellular calcium $[Ca^{2+}]_i$. The calcium ionophore, ionomycin, mimics the effects of BK with an associated rise in $[Ca^{2+}]_i$. We have shown previously that direct or receptor mediated activation of adenylate cyclase by forskolin or isoprenaline selectively reduces the BK-stimulated release of PGI_2, leaving EDRF release unaltered, and that adenylate cyclase activation also reduces the BK-stimulated rise in $[Ca^{2+}]_i$ (Parsaee et al, 1991). We have now examined the relationship between the $[Ca^{2+}]_i$ and the differential release of PGI_2 and EDRF.

Bovine aortic endothelial cells, AG7680A, from the Institute of Aging Cell Repository, USA, were cultured to confluence on glass coverslips. $[Ca^{2+}]_i$ was measured using the fluorescent indicator Fura-2AM, and a curve was generated relating concentration of ionomycin to the $[Ca^{2+}]_i$. In further experiments endothelial cells were cultured on microcarrier beads and placed in a column and selected concentration of ionomycin added to the perfusing buffer. The superfusate from the cell-covered beads was dripped on to rings of de-endothelialized rat aorta for measurement of EDRF release. Small amounts of superfusate were diverted from the column for the simultaneous measurement of PGI_2 release by RIA of 6-oxo-$PGF_{1\alpha}$.

The basal level of endothelial $[Ca^{2+}]_i$ was 141 ± 12 nM, n = 12. Ionomycin at concentrations between 10 and 500 nM caused increases in endothelial $[Ca^{2+}]_i$ between 300 nM and 2600 nM. Both EDRF and PGI_2 were released by ionomycin but the $[Ca^{2+}]_i$ threshold for release of PGI_2 was approximately 350 nM, while that for release of EDRF was less than 200 nM. Direct activation of adenylate cyclase by 5 μM forskolin reduced the $[Ca^{2+}]_i$ stimulated by 500 nM BK from 517 ± 56 nM, n = 5 to 177 ± 23 nM, n = 3, while pretreatment with isoprenaline (1 μM) reduced the BK-stimulated $[Ca^{2+}]_i$ to 237 ± 15 nM, n = 4.

In conclusion, we propose that the BK-stimulated $[Ca^{2+}]_i$ is most probably reduced by protein kinase A activity to a level above the threshold for nitric oxide synthase, but below that for phospholipase A_2 activation.

Parsaee,H., McEwan,J., Hardwick,S., Warren,J. & MacDermot,J.(1991) Br.J.Pharmacol. 102, 325P.

290P A ROLE FOR EDRF IN COLLATERAL PERFUSION FOLLOWING ARTERIAL OCCLUSION

M. D. Randall* & T. M. Griffith, Department of Diagnostic Radiology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN. Introduced by C.R. Hiley.

An *in vitro* model has been developed in which filling of a segment of the rabbit central ear artery, isolated between ligatures, is assessed by X-ray microangiography. The influence of EDRF activity on collateral perfusion was examined by using N^G-nitro-L-arginine methyl ester (L-NAME), a potent inhibitor of nitric oxide synthesis.

Arterial diameters were determined by X-ray microangiography and perfusion pressure was measured by means of a pressure transducer proximal to the ligation. The central artery was ligated at its midpoint and apex. 2 minutes after arterial occlusion 21.6±4.2% of the segment was filled with no significant change in the perfusion pressure of the intact ear. Filling of the segment increased to 46.6±5.3% 90 minutes after ligation. Segmental filling was also accompanied by an increase in diameter of the first order branch vessels proximal (pG1) to the ligation feeding the collateral vessels. Addition of 100μM L-NAME to some preparations 60 minutes after ligation reduced filling to 17.8±3.8%, reduced the diameter of pG1 and increased perfusion pressure significantly. In another group of preparations 100μM L-NAME added prior to ligation did not significantly affect perfusion pressure. Following arterial ligation in these preparations perfusion pressure rose substantially above basal and filling of the isolated segment was 15.6±5.9%, similar to that observed in the absence of L-NAME and there was no change in pG1. Filling did not however alter significantly with time being 14.8±7.4% at 90 minutes after ligation.

The results of this study indicate sufficient pre-existing collateral supply to partially fill an arterial segment isolated between occlusions, an effect which increased to a plateau around 60 minutes after ligation. Collateral filling and maintenance of perfusion appeared to be dependent on EDRF activity as they were reversed by L-NAME. Similarly after pretreatment with L-NAME collateral perfusion remained constant at a level comparable to initial filling in the absence of L-NAME. We conclude that EDRF activity has a central role in promoting and maintaining collateral arterial perfusion to an occluded artery.

This work was funded by a grant from the British Heart Foundation.

291P THE EFFECT OF POTASSIUM CHANNEL BLOCKING AGENTS ON ENDOTHELIUM-DEPENDENT RELAXATION IN THE RABBIT AORTA

R.A. Evans, D.Lang and M.J. Lewis, Department of Pharmacology & Therapeutics, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN.

The role of potassium channels in the regulation of endothelium-derived relaxing factor (EDRF) release is poorly understood. In the present study we have examined the effects of various potassium channel blocking agents on endothelium-dependent and -independent relaxations in isolated rabbit aortic preparations.

Endothelium-intact or -denuded 2-3mm wide aortic ring preparations were isometrically mounted (resting tension 2g) in 7ml tissue baths containing Holman's solution of the following composition (mM):- Na Cl 120; K Cl 5; Ca Cl_2 2.5; Na H_2PO_4 1.3; Na HCO_3 25; glucose 11; sucrose 10 and indomethacin 0.01 at 37°C gassed with 95% O_2 and 5% CO_2. Following equilibration, the rings were preconstricted to 80-90% maximum tension development with phenylephrine (10^{-6}M). Cumulative relaxation concentration-responses to acetylcholine (ACh, 10^{-7} - 3 X 10^{-5}M), or the calcium ionophore A23187 (10^{-8} - 3 X 10^{-6}M) in endothelium-intact preparations and sodium nitroprusside (SNP, 10^{-7} - 10^{-4}M) in endothelium-denuded preparations, were obtained in the absence and in the presence of the potassium channel blockers glibenclamide (10^{-6}M), 4-aminopyridine (4-AP, 5 X 10^{-5}M) or tetrapentylammonium bromide (TPA, 10^{-6}M).

The relaxation-response curve for ACh (ED_{50} = 8.1 X 10^{-8}M; peak relaxation = 63.08 ± 0.96%; n=20) was significantly ($p<0.01$) shifted to the right and the asymptote reduced in the presence of each of the blocking agents; the ED_{50} and peak relaxation in the presence of glibenclamide, 4-AP and TPA were 9.8 X 10^{-7}M; 3.5 X 10^{-7}M; 1.2 X 10^{-7}M and 40.4 ± 2.3%; 44.4 ± 1.7%; 47.9 ± 2.6% resp., (n>7). The relaxation-response curve for A23187 (ED_{50} = 9.4 X 10^{-8}M; peak relaxation = 62.3 ± 1.7%; n=17) was unaffected by TPA (ED_{50} = 1.0 X 10^{-7}M; peak relaxation = 65.5 ± 3.2% n=6) but significantly ($p<0.01$) shifted to the left by glibenclamide (ED_{50} = 7.7 X 10^{-8}M; peak relaxation = 76.8 ± 3.3%; n=7) and to the right by 4-AP (ED_{50} = 1.6 X 10^{-7}M; peak relaxation = 51.7 ± 2.4%; n=6).

In the absence of endothelium the relaxation response curve to SNP (ED_{50} = 4.6 X 10^{-7}M; peak relaxation = 93.4 ± 1.0%; n=37) was unaffected by the presence of either 4-AP or TPA but shifted slightly but significantly ($p<0.01$) to the right in the presence of glibenclamide (ED_{50} = 5.5 X 10^{-7}M; peak relaxation = 84.4 ± 3.3%; n=14). Neither ACh nor A23187 produced relaxation in endothelium-denuded preparations.

These results show that the potassium channel blockers can either inhibit or enhance endothelium-dependent relaxation of the rabbit aorta, the effect depending on the blocking agent and EDRF releasing agent used. Furthermore, glibenclamide exerts a small direct inhibitory effect on the vascular smooth muscle in response to SNP-induced relaxation.

292P RECOMBINANT HUMAN TNF INDUCES A NON-CONSTITUTIVE FORM OF NITRIC OXIDE (NO)-SYNTHASE IN THE ENDOTHELIUM AND SMOOTH MUSCLE

R. Foulkes and S. Shaw., Celltech Ltd, 216 Bath Road, Slough, Berkshire.

Previous studies have shown that Lipopolysaccharide induced hypotension in rats can be reversed by inhibition of NO- synthase (Kilbourn et al., 1990). In isolated aortic rings from rats the effects of LPS on NO- synthase are mediated by the induction of a non-constitutive form of the enzyme within the smooth muscle, but not the endothelium (Flemming et al., 1991). Since tumour necrosis factor α (TNF) is a primary mediator in LPS induced shock in man and animals (Beutler et al., 1988) the present study investigated the effects of recombinant human TNF (rhTNF) on the L-arginine-NO pathway in isolated aortic rings removed from rats either 1 or 24h following saline or rhTNF administration.

Male Sprague-Dawley rats (300-400g) were given either saline (1.0ml/kg) or rhTNF (1μg/kg) via the tail vein and killed by a blow to the head and cervical dislocation either 1 or 24h later. Aortas were quickly removed and rings of tissue mounted between 2 hooks in a 10ml organ bath filled with Krebs solution containing 10^{-5}M indomethacin maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Some rings were left with their endothelium intact (EI) and some had their endothelium removed by gentle rubbing of the lumen with the barrel of a syringe needle. All tissues were left to equilibrate for 90 min under a resting tension of 1g, constricted with 10^{-6}M phenylephrine (Phe) and the presence of functional endothelium tested with 10^{-6}M ACh. Tissues were washed, re-equilibrated and a cumulative concentration response curve (CRC) to Phe (10^{-10} - 10^{-4}M) performed. Rings were then washed repeatedly and 60 min later reconstricted with a concentration of Phe giving a 70% maximal constriction and a cumulative CRC to L-arginine (L-arg) carried out. Following a further 60 min washing and re-equilibration period tissues were preincubated with 10^{-3}M N -nitro-L-arginine methylester (L-NAME) and 10 min later reconstricted with the same concentration of Phe that gave a 70% constriction (above).

	TISSUES REMOVED AT 60MIN			TISSUES REMOVED AT 24h.		
	Phe EC_{50} (nM)	% relaxation to L-arg.	% Δ in Phe constriction by L-NAME (10^{-3}M)	Phe EC_{50} (nM)	% relaxation to L-arg.	% Δ in Phe constriction by L-NAME (10^{-3}M)
EI SALINE	167±35	53±10	+78±22	108±36	41±2	+66±14
EI TNF	513±216	53±10	+81±18	241±75	81±3##	+138±29##
DENUDED SALINE	20.5±3.1#	0	0	11.5±3#	0	0
DENUDED TNF	29.5±3.8#	0	0	11.2±3#	0	+31±15##

#Sig different ($P<0.05$) from respective EI group, ##sig different ($P<0.05$) from respective saline only group, ANOVA. Values are mean ± s.e.mean, n=6 per group.

Hence in rings from rats given rhTNF 24h previously, there was an enhanced relaxation to L-ARG IN EI tissues only, but a greater potentation of the Phe-induced constriction in the presence of L-NAME in both EI and denuded tissues. These data suggest that rhTNF administration can induce a form of NO synthase which is present within both the endothelium and smooth muscle itself.

1) Beutler, B. & Cerami, A. (1988) Ann.Rev.Biochem. 57, 505-518.
2) Fleming, I., Jolou-Schaeffer, G., Gray, G.A., Parratt, J.R. & Stoclet, J.C. (1991) Br.J.Pharmacol. 103, 1047-1052
3) Kilbourn, R.G., Jubran, A., Gross, S.S., (1990) Biochem.Biophys.Res.Comm., 172 (3), 1132-1138.

293P DIFFERENTIAL EFFECTS OF VARIOUS DIHYDROPYRIDINE CALCIUM ANTAGONISTS ON THE CONTRACTION PATTERN OF RAT CORONARY ARTERY STIMULATED WITH HIGH POTASSIUM

M. Pfaffendorf, M.J. Mathy & P.A. van Zwieten. Department of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands.

In coronary arteries, potassium-induced contractions are known to be biphasic. Although both the initial transient phase and the subsequent stable tonic phase of contraction are dependent on extracellular calcium, the second phase was found to be more sensitive to dihydropyridine calcium antagonists (Godfraind *et al.*, 1989).
We investigated the effect of three dihydropyridine calcium antagonists, lacidipine, nifedipine and nisoldipine, in order to explore whether the differences in the reactivity of the two phases depend on the dihydropyridine used, or whether they are drug independent. Circular preparations of septal coronary arteries with an average diameter of 273 ± 4.3 µm (n=77) were taken from 9 week old wistar rats and mounted in an isometric wire myograph. A normalization procedure (Nyborg & Mikkelsen, 1987) was used in order to standardize the degree of passive wall tension. After an equilibration period of 60 min the calcium antagonists were added. Subsequently every 30 min the physiological salt solution was replaced for 5 min by an iso-osmotic 120 mM potassium containing solution. The peak of the initial contraction (1st phase) and the tension after 3 minutes (2nd phase) were recorded. Lacidipine, a new lipophilic dihydropyridine calcium-antagonist, requires more than 4 h of exposure to reach the equilibrium effect. Accordingly the standard protocol for all experiments had to last for 6 h. In control experiments we could demonstrate that the magnitude of the first as well as the second phase remained constant during this 6 h period. Only one concentration of the calcium antagonists was used in each experiment. The results of at least 4 experiments per concentration were averaged and concentration-response curves for the two phases were constructed. The calculated EC_{50}-values (nM) are shown in table 1.

Table 1	lacidipine	nifedipine	nisoldipine
1st phase (phasic)	4.5	40	13.2
2nd phase (tonic)	0.15	4.7	1.14

The ratio of the EC_{50}-values for the first and the second phase was approximately 29 for lacidipine, 9 for nifedipine, and 12 for nisoldipine, respectively. In conclusion, the ratio between the effect on the two phases of the potassium-induced contraction in rat coronary artery appears to depend strongly on the calcium-antagonist used. Lacidipine, a potent new dihydropyridine compound, was found to be more selective in suppressing the second phase compared to nifedipine or nisoldipine.

Godfraind, T. *et al.* (1989) Am.J.Cardiol. **64**, 58I-64I
Nyborg, N.C.B. & Mikkelsen, E.O. (1987) J.Cardiovasc.Pharmacol. **9**, 519-524

294P THE EFFECT OF NEDOCROMIL SODIUM ON CITRIC ACID AND CAPSAICIN COUGH CHALLENGE IN THE GUINEA-PIG

E.A. Laude and A.H. Morice, Department of Medicine and Pharmacology, Royal Hallamshire Hospital, University of Sheffield, S10 2JF.

Nedocromil sodium is a pyranoquinoline dicarboxylate used in the treatment of asthma. Its mode of action is unknown, but an effect on sensory nerves has been proposed. We have studied the effect of 2% nebulised nedocromil sodium on the cough response of guinea pigs (400-550g) placed in a perspex chamber and exposed to citric acid (100mM and 300mM) and capsaicin (10uM and 30uM). Ultrasonically generated aerosols (Devilbis 99) were administered for 2 minutes at a flow rate of 0.75ml/min. Cough frequency during a 10 minute period was measured by a pneumotachograph connected to a manometer and recorded on a Riba recorder Par 1000. A minimum of 24 hours was allowed between each cough challenge at each dose to eliminate any short term tachyphylaxis. Control responses were determined by 0.9% saline pretreatment 24 hours before and repeated 24 hours after 2% nedocromil sodium inhalation. The drug was administered 10 minutes before challenge with tussive agent.

Pretreatment with nedocromil sodium had no significant effect on cough threshold. At a low dose (10mM) of tussive agent, citric acid produced a mean total cough (± S.E.) of 2.14 (± 1.46) on control days compared with 2.29 (± 2.43) (n=7) with nedocromil sodium pretreatment. In contrast with 300mM citric acid cough frequency was significantly ($p<0.01$) reduced by nedocromil sodium from 20.46 (± 3.35) to 13.54 (± 2.75) (n=13). Due to the individual variability in cough sensitivity to capsaicin the dose causing greater than 5 coughs was used in analysis of capsaicin challenges. Nedocromil sodium pretreatment significantly ($p<0.002$) reduced the number of coughs from 9.31 (± 1.04) to 4.08 (± 0.84) (n=13).

In this study nedocromil sodium caused a reduction in cough to both citric acid and capsaicin probably by an inhibitory action on the sensory pathways of the cough reflex.

295P PHARMACOLOGICAL RESPONSIVENESS OF HUMAN UMBILICAL VESSELS FROM NORMAL AND PRE-ECLAMPTIC PREGNANCIES

E. Breslin, T.G. Teoh[1], M. Darling[1] & J.R. Docherty. Departments of Physiology and Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2 & [1]Rotunda Hospital Dublin 1.

Pre-eclampsia is associated with increased maternal peripheral resistance and decreased utero-placental blood flow. In this study we examine the responses of umbilical vessels to vasoactive agents such as 5-hydroxytryptamine (5-HT), a potent vasoconstrictor in human umbilical vessels (McGrath *et al.*,1985), to endothelin 1 (ET-1), which has been implicated in umbilical artery vasospasm (Hartikainen-Sorri *et al.*, 1991), and to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), as pre-eclampsia is believed to be associated with prostaglandin imbalance (Remuzzi *et al.*, 1980). Other authors have reported a decreased responsiveness to $PGF_{2\alpha}$ in umbilical vessels from pre-eclamptic patients (Di Tommaso *et al.*, 1988).

Vessels were obtained from normotensive pregnant women (n=9) and patients with pre-eclampsia (n=8) and were dissected free from Wharton's jelly within 2-12 hours of delivery, mounted in an organ bath at 37^0C and bubbled with $95\%0_2$ and $5\%CO_2$. After equilibration, responses to the various agonists were recorded isometrically, and cumulative dose response curves were constructed.

There were no significant differences in the responses of the normal and pre-eclamptic vessels to 5-HT, $PGF_{2\alpha}$ or ET-1, in terms of EC_{50} values (contraction producing 50% of maximum response, relative to KCl).

Table 1. EC_{50} values obtained to vasoactive agents in human umbilical vessels.

		5-HT	$PGF_{2\alpha}$	ET-1
normotensive	artery	7.1±0.16	5.5±0.13	7.9±0.20
	vein	6.6±0.22	5.3±0.18	7.8±0.08
pre-eclamptic	artery	6.8±0.14	5.4±0.16	7.6±0.20
	vein	6.8±0.13	5.2±0.18	7.8±0.13

Values shown are mean ± s.e. mean (-log M).

In conclusion, we are unable to find differences between normal and pre-eclamptic umbilical vessels in responses to the above mentioned vasoactive agents.

Di Tommaso, M., Branconi, F, Mello, G., Borri, P. (1988) Clin Exp Hypertens B-Hyperten **7**: 37-41.
Hartikainen-Sorri, A., Vuolteenaho, O., Leppaluoto J. & Ruskoaha, H. (1991) Lancet **337**: 619.
McGrath, J.C., MacLennan, S.J. & Stuart-Smith, K. (1985) Br J. Pharmac **84**:199-202.
Remuzzi, G., Marchesi, D., Zoja, C., Muratore, D., Mecca, G., Misiani, R., Rossi, E., Barbato, M., Capetta, P., Donati, M.B., and deGaetano, G. (1980) Prostaglandins **20**: 105-110.

296P INVESTIGATIONS OF CONTRACTIONS MEDIATED BY NORADRENALINE IN RAT AORTA

Richard W. Aboud & James R. Docherty, Department of Physiology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2.

At least 3 genes code for $alpha_1$-adrenoceptor subtypes (Lomasney et al., 1991), and from ligand binding studies 2 major subtypes have been identified ($alpha_{1A}$, $alpha_{1B}$:Han et al., 1990). There are differing reports that rat aorta contains predominantly $alpha_{1B}$-adrenoceptors (Han et al., 1990) or both $alpha_{1A}$ and $alpha_{1B}$ (Piascik et al., 1991), or that an $alpha_{1A}$-site is expressed (Lomasney et al., 1991). In the present study, we have examined the $alpha_1$-adrenoceptors of rat aorta, comparing them with those mediating contractions of rat spleen, ($alpha_{1B}$: Han et al., 1987).

Ring preparations of rat descending aorta (endothelium denuded) and longitudinal strips of rat spleen were mounted in organ baths for recording of isometric tension. Cumulative concentration-response curves were obtained to noradrenaline (NA: aorta) or phenylephrine (spleen), before and after 1 h exposure to competitive antagonists, or before and after washout of irreversible antagonists.

The alpha1A selective competitive antagonists WB 4101, benoxathian and 5-methyl-urapidil showed relatively high potency in rat aorta, with pA_2 values (and 95% confidence limits) of 9.06 (7.99-10.63), 8.57 (7.72-9.96) & 8.12 (7.20-10.99), respectively. Benoxathian was an order of magnitude less potent in rat spleen with a pA_2 of 7.58 (7.24-8.00), but WB 4101 had similar potency in both tissues, with a pA_2 in spleen of 8.85 (8.21-10.13). The irreversible antagonist phenoxybenzamine (0.01-0.3 μM, 10 min) was able to reduce or abolish contractions of rat aorta to NA in a non-competitive manner. The irreversible antagonist chloroethylclonidine (10 μM, 30 min) produced apparently parallel shifts in the potency of NA.

The results obtained with competitive antagonists suggest that an $alpha_{1A}$-adrenoceptor is present in rat aorta, but the actions of chloroethylclonidine, which selectively inactivates $alpha_{1B}$-adrenoceptors (Han et al., 1990), are difficult to explain in terms of a single population of receptors.

Supported by the Health Research Board (Ireland) and by RCSI.

Han, C., Li, J. & Minneman, K.P. (1990) Eur. J. Pharmac. 190: 97-104
Lomasney, J.W., Cotecchia, S., Lorenz, W., Schwinn, D.B., Caron, M.G. & Lefkowitz, R.J. (1991) in *Adrenoceptors: Structure, Mechanisms, Function* ed. Szabadi, E. & Bradshaw, C.M. pp67-78. Basel: Birkhauser.
Piascik, M.T., Sparks, M.S., Pruitt, T.A. & Soltis, E.E. (1991) Eur. J. Pharmac. 199: 279-289

297P RELEASE OF PROSTACYCLIN BUT NOT EDRF FOLLOWS ACTIVATION OF THROMBOXANE RECEPTORS ON BOVINE ENDOTHELIUM

G. Clesham, H. Parsaee, S. Joseph, J. MacDermot & J.R. McEwan, Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W12 ONN

Activation of thromboxane receptors has been shown previously to trigger release of prostacyclin (PGI_2) from endothelial cells (Hunt et al., 1989). We have now examined the capacity of U46619 (a stable thromboxane A_2 mimetic) to mediate the release of endothelium derived relaxing factor (EDRF) from bovine aortic endothelial cells. Bovine aortic endothelial cells, AG4762, were obtained from the Institute of Aging Cell Repository (USA) and cultured on microcarrier beads which were then placed in a column and perfused with Krebs/Henseleit buffer. The cells were challenged with U46619, bradykinin (BK) or the calcium ionophore, ionomycin, and the release of PGI_2 measured by RIA of its stable hydrolysis product 6-oxo-$PGF_{1\alpha}$. EDRF release was measured by superfusion of the column eluate on to a ring of de-endothelialised rat aorta. Direct effects of U46619 on the aorta were prevented by simultaneous addition of the specific thromboxane antagonist SQ29548 to the ring. In further experiments, cells were cultured on glass coverslips, loaded with Fura 2-AM and the intracellular Ca^{2+} ($[Ca^{2+}]_i$) measured after challenge with U46619, BK or ionomycin.

U46619 (1 nM-1 μM) triggered a concentration-dependent release of PGI_2, but did not release EDRF at concentrations up to 10 μM. In contrast, BK released both PGI_2 and EDRF. Ionomycin did not release PGI_2 but did release EDRF. U46619 (1 μM) produced a small increase in $[Ca^{2+}]_i$ (57 ± 8 nM above basal levels, mean ± SE, n = 3), which was less than that produced by 100 nM BK (120 ± 24 nM) or 100 nM ionomycin (243 ± 46 nM). Staurosporine (an inhibitor of protein kinase C) substantially inhibited both the U46619 and BK-dependent release of prostacyclin.

In conclusion, while activation of BK receptors on AG4762 endothelial cells triggers release of both prostacyclin and EDRF, activation of thromboxane A_2 receptors on these cells mediates selectively the release of PGI_2 but not EDRF. Further, the effects of ionomycin and staurosporine suggest that agonist-stimulated PGI_2 release from AG4762 bovine endothelial cells is mediated predominantly by protein kinase C, rather than by rises in $[Ca^{2+}]_i$.

Hunt, J.A, Hallam, T.J. & MacDermot, J. (1989) Br. J. Pharmacol. 96, 170P.

298P SIMULTANEOUS REGIONAL VASODILATOR AND VASOCONSTRICTOR EFFECT OF ENDOTHELIN ET_B RECEPTOR STIMULATION IN ANAESTHETIZED RATS

M. Bigaud (introduced by B.A. Callingham). Marion Merrell Dow Research Institute, BP 447/R9, 16 rue d'Ankara, 67009 Strasbourg Cédex, France.

Two receptor binding sites for endothelin-1 (ET-1), termed ET_A and ET_B have been described (Arai *et al.*, 1990; Sakurai *et al.*, 1990), and recently the linear ET-1 analogue, [$Ala^{1,3,11,15}$]ET-1 (4AlaET-1), has been described as an ET_B selective agonist (Saeki *et al.*, 1991). The ability of this analogue to induce regional vasodilatation and vasoconstriction was examined as an attempt to discriminate between ET_A and ET_B mediated effects in male anaesthetized (pentobarbitone 60 mg/kg i.p.) Sprague-Dawley rats, of 300-400 g, instrumented with ultrasonic Doppler flow probes on the carotid (Ca), coeliac (Ce), mesenteric (M), renal (R) and iliac (I) arteries.

Bolus injections of 4AlaET-1 (0.1 to 3 nmol/kg) provoked an immediate, dose-dependent, vasodilatation of the Ca, Ce and I vascular beds, i.e. increases in regional vascular conductance, expressed as regional blood flow divided by systemic arterial blood pressure. Striking regional differences were observed regarding the magnitude and the duration of the local vasodilatations (Table 1). The vasodilator response was most prominent in the Ca, Ce and I beds, and was maintained for about 5 min. This was followed by a mild vasoconstrictor response. For the M and R beds, vasodilatation was weak and transient, and was followed within 30 seconds by a marked and prolonged vasoconstriction.

Table 1

	Immediate vasodilator effect[a]					Secondary vasoconstrictor effect[a]				
4AlaET-1 (nmol/kg)	Ca	Ce	M	R	I	Ca	Ce	M	R	I
0.1	40.5 ± 9.8	30.3 ± 20.3	4.9 ± 8.1	2.1 ± 1.5	34.4 ± 6.9	-1.6 ± 2.7	-2.1 ± 4.7	-14.6 ± 4.9	-8.7 ± 4.7	-2.8 ± 3.8
0.3	105.8 ± 30.2	60.8 ± 18.8	26.4 ± 11.5	22.4 ± 7.8	52.4 ± 28.3	-7.3 ± 2.2	-11.2 ± 5.6	-28.3 ± 3.0	-15.1 ± 2.5	-13.7 ± 4.7
1	101.5 ± 26.7	70.1 ± 17.1	8.4 ± 5.5	1.1 ± 4.1	63.5 ± 14.9	-18.4 ± 7.3	-24.1 ± 5.1	-38.5 ± 5.1	-60.5 ± 11.1	-9.6 ± 5.0
3	89.2 ± 13.8	69.9 ± 8.9	5.6 ± 7.6	1.6 ± 1.3	83.5 ± 26.8	-18.6 ± 3.2	-24.8 ± 6.8	-45.5 ± 8.4	-67.8 ± 18.1	-7.9 ± 5.4

[a] Results expressed as percent changes from baseline conductance ± s.e. mean.

These results demonstrate that stimulation of ET_B receptors by 4AlaET-1, in anaesthetized rats, induces both vasodilatation (mainly of Ca, Ce and I beds), and vasoconstriction (mainly of R and M beds). Thus, the idea that ET_A receptors mediate vasoconstriction, and ET_B receptors vasodilatation (Ihara *et al.*, 1991) is questionable, or perhaps, 4AlaET-1 is not a sufficiently selective ET_B agonist.

Arai, H., Hori, S., Aramori, I., Ohkubo, H. & Nakanishi, S. (1990). Nature **348**, 730-732.
Ihara, M., Fukuroda, T., Saeki, T., Nishikibe, M., Kojiri, K., Suda, H. & Yano, M. (1991). Biochem. Biophys. Res. Comm. **178**, 132-137.
Saeki, T., Ihara, M., Fukuroda, T., Yamagiwa, M. & Yano, M. (1991). Biochem. Biophys. Res. Comm. **179**, 286-292.
Sakurai, T., Yanagisawa, M., Takuwa, Y., Miyazaki, H., Kimura, S., Goto, K. & Masaki, T. (1990). Nature **348**, 732-735.

299P INVESTIGATION OF THE MECHANISM UNDERLYING DRUG-INDUCED UNCOVERING OF ENHANCED CONTRACTIONS TO UK-14,304 IN RAT TAIL ARTERY RINGS *IN VITRO*

D.M. Aidulis , A. Fuglsang & D. Pollock, Department of Pharmacology, University of Glasgow, G12 8QQ, Scotland.

The existence of post-junctional α_2-adrenoceptors in vascular smooth muscle can readily be demonstrated in vivo but is less obvious in isolated vasculature (MacLean & McGrath, 1990).Various procedures such as agonist-induced pre-contraction have been used to demonstrate post-junctional α_2-adrenoceptor-mediated responses in isolated blood vessels but the mechanism(s) underlying the uncovering of these responses remain(s) unknown. One possible explanation is that enhanced α_2-adrenoceptor-mediated responses occur when there is an opportunity (e.g. in the presence of a pre-contracting agonist) to inhibit an inhibitory, possibly cyclic nucleotide-mediated mechanism, that normally restrains the response. This study sought to determine whether enhanced α_2-adrenoceptor-mediated responses in the rat tail artery could be explained by inhibition of the synthesis or the effects of cyclic nucleotides.

Segments (3-4 mm) of proximal tail artery from male Wistar rats (150-200g) were suspended between pairs of stainless steel hooks, inserted through the lumen, in Krebs buffer (37 °C), gassed with 95%O_2 /5% CO_2 . The resting tension on each ring was set at 1g. After two hours equilibration, responses to drugs were recorded isometrically. Contractile responses to a standard submaximal concentration of the specific α_2-adrenoceptor agonist , UK-14,304 (50nM) were examined in rat tail artery rings pre-contracted with a submaximal concentration of vasopressin (VP, 0.4 miu ml^{-1}) then relaxed with forskolin (FOR, 36 nM), sodium nitroprusside (SNP,0.2 μM), dibutyryl cyclic adenosine monophosphate (dibutyryl cAMP, 0.2 mM), or isobutylmethylxanthine (IBMX, 10 μM). These vasprelaxants inhibited the VP-induced tone and in each case the subsequent superimposed response to UK-14,304 was usually similar to that produced by the pre-contracting agonist, VP, and was enhanced with respect to the control response obtained in the absence of VP and the vasorelaxant (FOR, 799 ± 167%, $P<0.001$; SNP, 111± 15%, $P<0.001$: dibutyryl cAMP 673 ± 140%, $P<0.001$; IBMX, 870 ± 272%, $P<0.05$).

These results confirm that in the isolated tail artery of the rat, enhanced contractile responses to activation of α_2-adrenoceptors can be uncovered following pre-contraction with VP and then relaxation with drugs that either increase the synthesis of cAMP (FOR) or of cGMP (SNP) or prevent the breakdown of these nucleotides (IBMX). It seems likely that the mechanism underlying the uncovering of enhanced responses to UK,14,304 involves inhibition of the effects of cyclic nucleotides since enhanced responses also occurred in the presence of dibutyryl cAMP but the possibility that the mechanism might also involve inhibition of the synthesis of cAMP cannot be excluded.

MacLean, M.M. & McGrath, J.C. (1990) Br. J. Pharmacol. 101, 205-211.

300P THE ENDOTHELIN ISOPEPTIDES DO NOT RELEASE EDRF OR PROSTACYCLIN (PGI_2) FROM HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECS)

D.G. White, M.J. Sumner and I.S. Watts. Department of Peripheral Pharmacology, Glaxo Group Research Ltd., Ware, Herts SG12 0DP.

Endothelins (ETs) produce both vasoconstriction and vasodilatation. Experiments in bovine cultured endothelial cells suggest that the vasodilator response may occur via the release of EDRF and PGI_2 (Emori et al., 1991, Filep et al., 1991). We examined therefore whether (ET-1, -2, or -3) could release EDRF and PGI_2 from HUVECS. Endothelial cell cGMP content was measured as a marker of EDRF formation and 6-keto $PGF_{1\alpha}$ as a marker of PGI_2 release. Histamine was studied for comparison.

HUVECS were isolated by collagenase digestion and grown to confluence. The medium was replaced with Krebs solution containing the phosphodiesterase inhibitor IBMX (100μM). Compounds under test were added to the cells at the concentrations and times indicated, after which the Krebs solution was removed for assay of 6-keto $PGF_{1\alpha}$ and the cells were extracted for determination of cGMP and protein content. Basal levels of cGMP and 6-keto $PGF_{1\alpha}$ were 6.2 ± 1.0 pmol/mg protein (n=19) and 95 ± 13.5 ng/mg protein (n=19) respectively. Histamine (1μM, 3 min) produced a ~13 fold and ~5 fold increase in cGMP and 6-keto $PGF_{1\alpha}$ levels respectively. Pretreatment (30 min) with the EDRF inhibitors haemoglobin (Hb) and L-N^G-monomethylarginine (L-NMMA) attenuated the histamine-induced rise in cGMP (Table 1). In contrast, the ETs (0.1nM-1μM) produced no significant change in either the cGMP content or 6-keto $PGF_{1\alpha}$ release from the HUVECS (Table 1).

Table 1 Modulation of cGMP and PGI_2 production in HUVECS

TREATMENT	RATIO TEST/CONTROL (Control = 1.0)		
	pmol cGMP/mg protein	ng 6-keto $PGF_{1\alpha}$/mg protein	
Histamine (1μM, 3 min)	12.6 ± 2.0*	4.7 ± 0.6*	*$p<0.05$ (paired
Histamine + Hb (10μM, 30 min)	1.4 ± 0.3*	-	t-test) compared
Histamine + L-NMMA (10μM, 30 min)	6.3 ± 1.9*	-	to control; mean
Endothelin -1 (1μM, 3 min)	1.4 ± 0.5	1.4 ± 0.5	± s.e.mean, n=4-19
Endothelin -2 (1μM, 3 min)	1.0 ± 0.1	1.0 ± 0.3	
Endothelin -3 (1μM, 3 min)	1.6 ± 0.4	1.3 ± 0.2	

In conclusion, histamine but not ETs release EDRF and PGI_2 from HUVECS, suggesting ET receptors are either absent from HUVECS or not coupled to EDRF or PGI_2 production. These data differ from recent findings in bovine endothelial cells (Emori et al., 1991).

Emori T., Hirata Y., Kanno K., Ohta K., Eguchi S., Imai T., Shichiri M. & Marumo F. (1991) Biochem. Biophys. Res. Comm., 174, 228-235.
Filep J.G., Battistini B., Côte Y.P., Beaudoin A.R. & Sirois P. (1991) Biochem. Biophys. Res. Comm., 177, 171-176.

301P A POSSIBLE ROLE FOR POLYAMINES IN THE GROWTH OF ENDOTHELIAL CELLS

Heather M Wallace, Stephanie A Millican and Thidar Aung Clinical Pharmacology Unit, Department of Medicine and Therapeutics and School of Biomedical Sciences, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen AB9 2ZD

Vascular endothelial cells line the heart and blood vessels and in normal adult mammals exist in a quiescent, non-growing state. Endothelial growth is, however, a pre-requisite for vascular repair and wound healing with abnormal growth being associated with tumour angiogenesis and atherosclerosis. Endothelial cells respond to mitogenic signals by alterations in ion flux including Ca^{2+} and protein kinase C activity. The cellular polyamines, putrescine, spermidine and spermine, are positive regulators of cell growth and metabolism and an increase in polyamine biosynthesis is an early event in the response of cells to a growth stimulus. The aim of this study was to determine if polyamine biosynthesis was involved in the mitogenic response of human endothelial cells to the peptide growth factor, epidermal growth factor (EGF).

Human endothelial cells were cultured from umbilical cord veins as described previously (Jaffe *et al*, 1973). Cells were grown for 16h prior to the addition of EGF and harvested after a 48h exposure. Protein and polyamine content were determined as described elsewhere (Wallace *et al*, 1988). DNA synthesis was measured as the incorporation of [^{3}H]-thymidine into acid-insoluble material. The maximum response to EGF was observed using 10%(v/v) serum and a concentration of 50ng/ml. Cell growth, as measured by protein content and DNA synthesis, was increased by treatment with EGF, as was both spermidine and spermine content (Table 1).

Table 1: Effect of EGF on growth of human endothelial cells in culture

Treatment	Protein Content (mg/plate)	DNA Synthesis (dpm x 10^{-5}/mg P)	Total Polyamine Content (nmol/mg protein)
None	0.066 ± 0.004	1.37 ± 0.26	7.86 ± 1.30
EGF (50ng/ml)	0.094 ± 0.003	4.78 ± 0.50	11.26 ± 1.10

Pretreatment of the cells with difluoromethylornithine, an inhibitor of polyamine biosynthesis prevented the EGF-induced increase in protein content, DNA synthesis and polyamine content suggesting an essential role for polyamines in induced endothelial cell growth.

Jaffe EA, Nachman RL, Becker RL and Minick CUR (1973) J. Clin. Invest. **52,** 2745-2756
Wallace HM, Nuttall ME and Robinson FC (1988) Biochem. J. **253,** 223-227

302P HYDROGEN PEROXIDE-MEDIATED DAMAGE IN HUMAN VASCULAR ENDOTHELIAL CELLS

Stephanie A Millican, J.Webster & Heather M Wallace, Clinical Pharmacology Unit, Department of Medicine & Therapeutics & School of Biomedical Sciences, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen AB9 2ZD

Vascular endothelial cells are targets for attack from reactive oxygen species which is thought to play a contributory role in pathological conditions such as ischaemia/reperfusion injury, inflammation and arteriosclerosis. The cellular concentrations of oxidants such as Hydrogen peroxide (H_2O_2) are known to increase several fold in such conditions. Although a reactive species itself, H_2O_2 can also act as a precursor for the more reactive hydroxyl radical ($^{\bullet}OH$). The aim of this study was to assess the contribution of both H_2O_2 and Fe dependent $^{\bullet}OH$ generation in the promotion of lytic injury in a cell culture model of vascular damage.

Human vascular endothelial cells were isolated from umbilical cord veins as previously described (Jaffe et al, 1973). Confluent monolayers of cells were exposed to bolus administration of H_2O_2 (50-500μM) for 5 hours. Lytic cell damage was determined by the release of lactate dehydrogenase, with the ratio of released LDH:total LDH being expressed as % cell viability. Dimethylthiourea (DMTU), a low molecular weight free radical scavenger and the iron chelator O-phenanthroline were added at the time of exposure to H_2O_2.

Hydrogen peroxide was found to cause a dose-dependent increase in lytic injury. DMTU caused a dramatic reduction in the H_2O_2 concentration of the extracellular media and prevented the lytic damage induced by H_2O_2. O-phenanthroline also caused a significant reduction in the lytic injury (Table 1).

Table1: Effect of DMTU and O-Phenanthroline on lytic injury

Treatment	Viability(%)	
250μM H_2O_2	35 ± 5	
DMTU(10mM)	100 ± 8 *	
O-phenanthroline(40μM)	88 ± 6 *	(* $p<0.01$)

The results suggest, that in this system, lytic cell damage is due to both H_2O_2 and a iron dependent reaction which is most probably intracellular hydroxyl radical generation. DMTU which scavenges both H_2O_2 and $^{\bullet}OH$, in this system probably breaksdown H_2O_2 before the conversion into $^{\bullet}OH$ and therefore in the abscence of such scavengers the most likely candidate for the major part of the damage produced is intracellular $^{\bullet}OH$.

Jaffe EA, Nachman RL, Becker RL and Minick CUR (1973) J. Clin. Invest. **52,** 2745-2756

303P POTENCIES OF ADENOSINE RECEPTOR-BLOCKING DRUGS ON DOG ISOLATED CORONARY ARTERY AND GUINEA-PIG ISOLATED AORTA

M.D.R. Croning, M.F. Gurden and I. Kennedy. Peripheral Pharmacology Department, Glaxo Group Research, Ware, Herts, SG12 0DP.

We have shown (Gurden & Kennedy, this meeting) that the adenosine receptors mediating relaxation of the dog coronary artery and the guinea-pig isolated aorta can be distinguished on the basis of agonist potencies, the former appear to be of the A_{2a} subtype, whilst the latter may be of the A_{2b} subtype. In the present study, we have compared the potencies of two adenosine receptor-blocking drugs on these preparations. The compounds tested were 1,3-diethyl-8-phenylxanthine (DPX) which was reported to have 22-fold higher affinity for A_{2b} receptors than for A_{2a} receptors (Bruns *et al.*, 1986) and 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-C] quinazoline-5-amine (CGS15943) which is a potent antagonist at the A_2 receptor (Ghai *et al.*, 1987), but which has not been investigated at A_2 receptor subtypes.

Ring preparations were suspended in modified Krebs solution at 37°C, gassed with 5% CO_2 in O_2 and contracted with phenylephrine ($3x10^{-6}$M, aorta) or U-46619 ($1x10^{-8}$M, coronary artery). A cumulative concentration-effect curve for 5'-N-ethylcarboxamidoadenosine (NECA) was obtained on each preparation in the absence or presence of an antagonist. An antagonist contact time of 60 min was used throughout and three concentrations of each antagonist were tested. The results were subjected to Schild analysis and antagonist potencies expressed as pA_2 values, which, together with the slopes of the regressions, are shown in Table 1.

Table 1:

PREPARATION	DPX		CGS15943	
	pA_2	slope	pA_2	slope
Guinea-pig aorta	7.60 (7.19-8.01)	0.75 (0.66-0.85)	7.55 (7.33-7.77)	1.19 (1.05-1.33)
Dog coronary artery	7.23 (6.51-7.94)	0.61 (0.44-0.78)	9.24 (8.54-9.95)	0.76 (0.47-1.05)

Figures in parentheses are 95% confidence limits; all values are means of at least 4 observations.

Both compounds caused rightward displacements of the concentration-effect curve to NECA with no apparent change in the maximum responses. DPX had similar potencies on both preparations although the slopes of the Schild regressions were significantly less than unity ($P<0.05$). For these reasons, this compound cannot be used to differentiate between the receptors in these preparations. In the case of CGS15943, the results obtained on the coronary artery were somewhat variable, and the slope of the Schild regression for the aorta was slightly but significantly ($P<0.05$) greater than unity. Nevertheless, CGS15943 was approximately 50 times more potent on the coronary artery than on the aorta. These results demonstrate that CGS15943 is a potent antagonist at the A_{2a} receptor and may be useful in differentiating between A_2 adenosine receptor sub-types.

Bruns, R.F., Lu, G.U. & Pugsley, T.A. (1986). Mol. Pharmacol., *29*, 331-346.
Ghai, G., Francis, J.E., Williams, M., Dotson, R.A., Hopkins, M.F., Cote, D.T., Goodman, F.R. & Zimmerman. (1987). J. Pharmacol. exp. Ther., *242*, 784-790.

304P SR 47063, A NEW K^+ CHANNEL OPENER: EFFECT ON ^{86}Rb EFFLUX FROM RAT AORTA

B. Christophe, P. Chatelain & A.S. Manning, SANOFI-Pharma Research Centre, 1, avenue de Béjar, 1120 Brussels, Belgium.

A common characteristic of all K^+ channel openers is the ability to increase $^{86}Rb^+$ efflux (Cook, 1988; Giudicelli & Richer,1989). In this study, we have investigated the ability of a new K^+ channel opener (Gautier & Bertrand, 1991; Guiraudou et al.,1991), SR 47063 (4-(2-cyanimino-1,2-dihydropyrid-1-yl)-6-nitro-2,2-dimethyl-2H-1-benzopyran) to modulate $^{86}Rb^+$ efflux from rat aorta in comparison to that of cromakalim and bimakalim.

The method was adapted from that of Weir & Weston (1986). Strips of thoracic aorta from male Wistar rats were mounted in an organ bath containing a modified Krebs medium and isometric tension was measured. $^{86}Rb^+$ was used as a marker of K^+ fluxes. After a 30 min equilibration period, tissues were loaded with $^{86}Rb^+$ (1 μCi/ml for 180 min). $^{86}Rb^+$ was then measured throughout a 16 min period. The tissue were then superfused for 8 min with medium containing 10^{-5} M SR 47063, cromakalim or bimakalim. Water or ethanol were used as controls. After this period, the tissues were superfused with buffer alone for 16 min. 1 ml aliquots of physiological medium were collected every 2 min and used for assessement of radioactivity. The aorta were dissolved at 75 °C with 100 μl $HClO_4$ - H_2O_2 and the remaining radioactivity was measured. Efflux data was expressed (mean ± S.E.M.) in terms of $^{86}Rb^+$ efflux rate coefficient (fractional loss of $^{86}Rb^+$ from the tissue standardised for a 1 min period). After 16 min, the basal $^{86}Rb^+$ efflux rate coefficient was 8.70 ± 0.54 min^{-1} (n=18). SR 47063 (10^{-5} M) induced a significant increase in $^{86}Rb^+$ efflux rate coefficient (maximal effect measured 6 min after addition of SR 47063: 54.07 ± 4.70 min^{-1}, n=4) This increase was not significantly different from that produced under the same experimental conditions by cromakalim or bimakalim (48.32 ± 3.47 min^{-1}, n=4 and 49.62 ± 1.54 min^{-1}, n=4 respectively). In all three cases, the $^{86}Rb^+$ efflux rate coefficient returned to basal values 16 min after termination of drug administration.

In conclusion, SR 47063 produces, in isolated rat aorta, a significant increase in the $^{86}Rb^+$ efflux rate coefficient similar to that induced by cromakalim and bimakalim. This result confirms that SR 47063 is a K^+ channel opener.

Cook N.S. (1988) *Trends Pharmacol. Sci.* **9** , 21-28
Gautier P. & Bertrand J.P. (1991) *Fund. Clin. Pharmacol.*, (in press)
Giudicelli J.F. & Richer C. (1989) *Thérapie* **44** , 419-422
Guiraudou P., Rizzoli G., Giraud R., Nisato D. & Gautier P. (1991) *Fund Clin. Pharmacol.*, (in press)
Weir S.W. & Weston A.H. (1986) *Br J. Pharmacol.* **88** , 121-128

305P EFFECTS OF AGEING ON CONTRACTIONS TO NORADRENALINE AND 5-HT IN HUMAN SAPHENOUS VEIN

J. R. Docherty, Department of Physiology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland.

Ageing is associated with a variety of changes in the cardiovascular system (see Docherty, 1990). However, investigations of vasoconstriction mediated by noradrenaline (NA) or 5-hydroxytryptamine (5-HT) have failed to find age-related differences in responsiveness of human blood vessels (Scott & Reid, 1982; Stevens et al., 1982). The objects of this study were to investigate the effects of ageing on responses of human saphenous vein to NA and 5-HT.

Human saphenous veins were obtained from female patients aged 32-67 years, undergoing surgical removal of varicose veins, and rings (3-5mm) from apparently healthy segments were attached to myograph transducers under 1g tension for isometric tension recording. A cumulative concentration-response curve was carried out to NA or 5-HT (0.5 log unit increments) until a maximum response was reached. Agonist potency was expressed as an EC_{50} (concentration producing 50% of maximum contraction) or as a pD2 (-log EC_{50}).

NA produced isometric contractions with an EC_{50} of 0.46 μM (95% confidence limits of 0.28-0.74 μM, n=21 veins) and a maximum contraction of 1.49±0.24g. 5-HT produced isometric contractions with an EC_{50} of 0.17 μM (0.08-0.35 μM, n=10) and a maximum contraction of 1.00±0.14g. There was a significant negative correlation between agonist potency and age for NA ($r=0.52$, $n=21$, $P<0.05$) and 5-HT ($r=0.89$, $n=10$, $P<0.01$), so that both agonists were less potent with increasing age. There were no age-related changes in maximum response to either agonist.

In conclusion, this study demonstrates that the potencies of NA and 5-HT are reduced with increasing age in saphenous veins obtained from females undergoing varicose vein surgery.

Supported by the Irish Heart Foundation, the Health Research Board (Ireland) and the Royal College of Surgeons in Ireland.

Docherty, J.R. (1990) Pharmacol. Rev. **42**, 103-125.
Scott, P.J.W. & Reid, J.L. (1982) Br. J. Clin. Pharmacol. **13**, 237-239.
Stevens, M.J., Lipe, S., & Moulds, R.F.W. (1982) Br. J. Clin. Pharmacol. **14**, 750-751.

306P U46619, A STABLE THROMBOXANE MIMETIC, CAN CONSTRICT THE CANINE SPLENIC ARTERIAL VASCULATURE WITHOUT SIGNIFICANT EFFECT ON CAPSULAR SMOOTH MUSCLE

P.G.Withrington[1], E.Antunes, Dora M.Grassi-Kassisse & G. de Nucci, Departments of Pharmacology, UNICAMP, Campinas, Sao Paulo, Brazil and [1]Queen Mary Westfield College, Mile End Rd, London E.1

Thromboxane (TX) is released from the spleen in response to the administration of endothelin 1 (ET-1; Rae et al, 1989). ET-1 causes marked and persistent splenic arterial vasconstriction accompanied by capsular contraction (Withrington et al, 1992). The present experiments were designed to assess whether TX had any intrinsic actions on splenic smooth muscle.

Dogs (mean weight 14.2±1.3 Kg) were anaesthetized with a mixture of Dormonid (Midazolam, 0.3-1.0mg kg^{-1} i.v.) and Fentanyl (50-100μg kg^{-1} i.v.) and the spleen (mean weight 114±14 g) removed after heparinisation. The splenic artery and vein were cannulated and the spleen perfused at constant flow (mean 42.4±2.7 ml min^{-1}) with Krebs' solution equilibrated with 95% O_2/5% CO_2 at 37°C. The splenic arterial perfusion pressure (SAPP) was measured and changes in splenic arterial vascular resistance (SAVR) calculated. The spleen was continuously weighed on a digital balance to provide an indirect estimate of changes in spleen volume (SV).

The stable thromboxane mimetic, U46619 (Coleman et al, 1981), was injected as a bolus over the dose range 0.1 to 500 ng in 7 perfusions and the splenic capsular and vascular responses compared with those of adrenaline (AD). Injections of U46619 above 10 ng caused graded increases in SAVR and splenic arterial vasoconstriction. Vasodilatation was never observed. The mean molar dose of U46619 to increase the basal SAVR by 50% was 0.31±0.08 nmol, significantly less ($p<0.05$) than the molar ED50 for AD (30.3±12.8 nmol). Whilst bolus injections of AD were accompanied by substantial reductions in spleen weight (mean maximum 32.9±6.3 g) there were very small changes in spleen weight to injections of U46619 (mean max increase 3.6±1.4 g; mean max decrease 3.0±0.9 g).

The results indicate that TX, as assessed by the actions and potency of U46619, has marked splenic vasoconstrictor properties and could exert an important role in modulation of splenic circulation. In contrast, U46619 has no action on capsular smooth muscle.

Coleman, R.A., Humphrey, P.P.A., Kennedy, I., Levy, G.P. & Lumley, P. (1981). Br.J.Pharmacol. 73, 773-778.
Rae, G.A, Trybulec, M., de Nucci, G. & Vane, J.R (1989). J.Cardiovasc.Pharmacol. 13 (suppl. 5), 589-592.
Withrington, P.G., Ansari, N., Croxton, R., de Nucci, G. & Vane, J.R (1992). Br. J. Pharmacol (in press).

307P VASORELAXANT ACTIONS OF THIAZIDES AND RELATED DRUGS

J.A Calder, M. Schachter & P.S. Sever, Department of Clinical Pharmacology, St. Mary's Hospital, Imperial College of Science, Technology and Medicine, London, W2 1NY

It is well established that thiazide diuretics produce diuresis by inhibiting the reabsorption of NaCl in the distal tubule; however, the mechanism by which thiazides lower peripheral resistance is unresolved.Indapamide (IND) is an atypical thiazide-like diuretic and it appears to reduce blood pressure by a combined diuretic and direct vascular action (Campbell & Brackman, 1990). The novel furopyridine compound cicletanine (CIC) is also an antihypertensive agent and at high concentrations acts like a diuretic (Bippi & Guinot, 1988). The mechanisms by which cicletanine produces its hypotensive effects are not known although several hypotheses have been put forward including an action on potassium channels or interaction with the vascular eicosanoid system. The aim of this study was to compare the acute relaxation produced by the agents IND, CIC and the thiazide diuretic hydrochlorothiazide (HTZ) on isolated vessels from human, rat and guinea pig tissue.

Resistance vessels (n=16 human subcutaneous arteries mean diameter 366±34μm: n=15 rat mesenteric vessels mean diameter 265±11μm: n=17 guinea pig mesenteric vessels mean diameter 430±25μm) were dissected free and mounted on a Mulvany microvascular myograph (Mulvany & Halpern, 1977) for the measurement of isometric tension. The arteries were normalised to a standard resting tension in aerated physiological saline solution (PSS) maintained at 37°C. After an hour the vessels were tested for viability using depolarising potassium solution (KPSS=K^+ 118mM) and noradrenaline (NA); those vessels failing to produce a tension equivalent to 90mmHg were discarded. The functional integrity of the endothelium was determined by application of 10μM acetylcholine to a vessel preconstricted with NA; relaxation of the preconstricted vessel was assumed to indicate the presence of functional endothelium. Concentration response (i.e. relaxation) curves were constructed for all three drugs in human, rat and guinea pig vessels following preconstriction with either NA or KPSS. At the end of the experiment the vessels were again challenged with KPSS in a final test for viability: vessels which failed to produce a tension equivalent to at least 80% of the original tension produced by KPSS were discarded and the experiment repeated using different vessels.

Responses to IND,CIC and HTZ were found not to be dependent upon the presence of functional endothelium. Table 1 shows the maximal relaxant effects of the agonists on isolated vessels.

Table 1	HUMAN		GUINEA PIG		RAT	
	NA	KPSS	NA	KPSS	NA	KPSS
IND	0	0	60%	57%*	0	0
CIC	77%	57%*	95%	30%*	70%	20%*
HTZ	58%	12%*	74%	8%*	0	0

All values are mean percentage relaxations of a maximal (30μM) concentration of agonist. * Relaxation of NA and KPSS constricted vessels are significantly different ($P<0.001$).

Following noradrenaline preconstriction CIC was the most efficient vasodilating agent in all three species and IND the least effective. The acute relaxant action of IND, CIC and HTZ appears to be dependent upon species and constricting agent. The actions of these drugs suggest inter-species differences and multiple mechanisms of action. Functional studies of these mechanisms are in progress.

Bippi, H. & Guinot, P.H. (1988) Drugs Exp. Clin. Res. **14**., 215-220
Campbell, D.B. & Brackman, F. (1990) Am. J. Cardiol. **65**., 11H-27H
Mulvany, M.J. & Halpern, W. (1977) Circ. Res. **41**., 19-26

308P HYPOXIA-INDUCED VASOCONSTRICTION IN THE ISOLATED RABBIT PULMONARY ARTERY: TOTAL INOSITOL PHOSPHATE ACCUMULATION AND PROTEIN KINASE C ACTIVATION

Margaret R. MacLean & J. Nally, Institute of Physiology, University of Glasgow, Glasgow G12 8QQ

We have previously demonstrated hypoxia-induced pulmonary vasoconstriction (HPV) in the isolated rabbit pulmonary artery. This is dependent on pre-constriction with an agonist such as noradrenaline (NA) and is transient, being followed by a prolonged vasodilation. It is dependent on release of Ca^{2+} from intra-cellular stores (MacLean & McGrath, 1991). Here we investigate the second messenger systems involved in this response by measuring accumulation of total inositol phosphates and assessing protein kinase C (PKC) activation during HPV.

Isolated rings (3-5mm) of the first left branch of the pulmonary artery were dissected from NZW rabbits (2-3 kg), newly killed by stunning and cervical dislocation. Rings were suspended in organ baths containing Krebs saline at 37°C under 2g force and gassed with 5% O_2, 6% CO_2 balance N_2 (bath O_2 tension=45.3±0.5mmHg, CO_2=35.6±0.4mmHg, pH=7.39±0.04, n=5). Contraction to hypoxia in vessels pre-constricted with 10^{-7}M NA ($HPV^{[+NA]}$) was studied by gassing with 0% O_2, 6% CO_2 balance N_2 (bath O_2 tension=8±0.6mmHg, CO_2=35.8±0.5mmHg, pH=7.37±0.01, n=5) for 5 min. The effects of the PKC inhibitor, staurosporine (10^{-8}M, 3 x 10^{-8}M and 10^{-7}M) and the PKC activator, phorbol dibutyrate (PDBu, 3 x 10^{-8}M - 3 x 10^{-6}M) on $HPV^{[+NA]}$ was studied. Other rings were incubated for 3 h with 6μCi ml^{-1}[^{3}H]-myo-inositol and total inositol phosphates were assayed in each ring by accumulation in 10mM LiCl essentially as described by Downes *et al.*, 1986. This was after incubation for 45 min with either NA (3 x 10^{-8}M) or endothelin-1 (Et-1, 10^{-8}M) in normoxia or incubated for 45 min in hypoxia. Significant differences were assessed using a Student's paired *t*-test.

Staurosporine inhibited $HPV^{[+NA]}$ in a concentration-dependent fashion, i.e. a control $HPV^{[+NA]}$ of 550 ± 132 mg wt tension (n=5) was reduced to 400 ± 108 mg wt by 3 x 10^{-8}M ($P<0.05$) and to 120 ± 97 mg wt by 10^{-7}M ($P<0.05$). PDBu alone induced a dose-dependent vasoconstriction of the pulmonary vessels, i.e. 10^{-7}M induced an increase of 550 ± 114 mg wt tension (n=5) whilst 10^{-6}M increased tone by 1700 ± 478 mg wt. Pre-constriction with PDBu instead of NA did not allow the induction of HPV but when PDBu was administered after tone had already been raised with NA, the $HPV^{[+NA]}$ was potentiated (relative to $HPV^{[+NA]}$ before administration of PDBu) and the secondary vasodilatory response inhibited such that the HPV was no longer transient but well maintained. Hypoxia increased total inositol phosphate accumulation by 58.8% (± 11.5%, n=5). NA and Et-1 also increased total inositol phosphate accumulation by 83.6% (± 20.7%) and 79.8% (± 14%) respectively (n=5, $P<0.05$, calculated using the absolute values).

The results suggest that HPV involves the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) and subsequent accumulation of inositol phosphates as well as activation of PKC. PIP_2 is cleaved into two second messengers, *sn*-1,2-diacylglycerol (DAG) and inositol 1,4,5 trisphosphate (IP_3). IP_3 releases Ca^{2+} from internal stores whilst DAG activates PKC. Activation of PKC alone will not support HPV and therefore the two second messenger processes must act synergistically to elicit the full physiological response to hypoxia.

The authors acknowledge The Wellcome Trust and National Asthma Campaign for funding and thank Professor J.C. McGrath for his support.

Downes, C. P., Hawkins, P.T. & Irvine, R. F. (1986). *Biochem. J.*, **238**, 501-506.
MacLean, M. R. & McGrath, J. C. (1991). *Br. J. Pharmacol.*, **104**, 119P.

309P ABSENCE OF EFFECT OF ADENOSINE ON THE DISCHARGE OF ARTICULAR MECHANORECEPTORS IN NORMAL AND ARTHRITIC RATS

A.U.R. Asghar, D.S. McQueen & A.E. Macdonald. Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ

It has been shown that adenosine and adenosine A2-selective agonists produce hyperalgesia when injected intra-dermally into the rat hind paw (Taiwo and Levine, 1990). The present study was undertaken to determine whether agonists and antagonists selective for adenosine receptors would influence neural discharge recorded from high threshold mechanoreceptor afferent fibres innervating the ankle joint in normal rats and those with unilateral adjuvant-induced arthritis.

Adjuvant arthritis was induced in male Wistar rats by injecting 0.1mg (1mg/ml) Freund's complete adjuvant subdermally around the left ankle joint under halothane anaesthesia. Normal and arthritic (15-30 days postinjection) rats (250-500g) were anaesthetized with urethane (25% w/v solution 0.6ml $100g^{-1}$, I.P.) and "spontaneous" (on-going) discharge of C- and A-delta afferent fibres was recorded from the left ankle joint together with responses to a standard mechanical indent applied to the joint capsule every 2min. Details of the induction of arthritis, surgical procedures and electrophysiological recordings are as described by Birrell et al. (1991). We attempted to construct log dose-response curves for the effect on discharge ("spontaneous" and mechanically-evoked activity) of close intra-arterial injections of adenosine, the non-selective agonist NECA (5'-(N-ethyl)-carboxamidoadenosine), the A_1 selective agonist CPA (N^6-cyclopentyladenosine) and the agonist metrifudil ($A_2>A_1$) in both normal (n=2-6) and arthritic (n=2-7) rats. These dose-response curves were also repeated in the presence of the A_1/A_2 antagonists, theophylline and 8-phenyltheophylline (8-PT).

In both normal and arthritic rats, adenosine (0.37-37nmoles), NECA (0.32-97 nmoles), CPA (0.30-30nmoles) and metrifudil (0.27-80nmoles) did not cause any change in the responsiveness to the mechanically-evoked discharge nor did they affect the level of "spontaneous" discharge; they did cause changes in systemic blood pressure. There was also no change in the discharge of articular sensory receptors with these agonists in the presence of the antagonists, theophylline (550nmoles) or 8-PT (390nmoles), and neither theophylline nor 8-PT affected the discharge of articular sensory receptors in normal or in arthritic rats. All of the units examined in the experiments were excited by capsaicin (0.32-32 nmoles) thus confirming the chemosensitivity of the preparations used.

These results show that neither adenosine nor A_1 and A_2 adenosine receptors influence the discharge of mechanoreceptors recorded from normal or arthritic ankle joints in the rat.

Taiwo, Y.O. and Levine, J.D. (1990). Neuroscience., 38, 757-762.
Birrell, G.J., McQueen, D.S., Iggo, A. & Grubb, B.D. (1991). Br.J.Pharmacol., 101, 715-721.

310P YOHIMBINE POTENTIATES AND ADENOSINE INHIBITS THE NERVE-MEDIATED RESPONSE IN A BLOOD VESSEL EXHIBITING CO-TRANSMISSION

J.M. Bulloch, Autonomic Physiology Unit, Institute of Physiology, University of Glasgow, Glasgow G12 8QQ.

The role of presynaptic α_2-adrenoceptor-mediated autoinhibition of transmitter release has been studied previously in a system where noradrenaline (NA) and ATP act as co-transmitters, namely, the ileocolic artery of the rabbit (Bulloch & Starke, 1990). The present study has extended this to an examination of the neuromodulating role of both of the co-transmitters in this tissue.

Ring segments of rabbit ileocolic artery were placed in physiological salt solution maintained at 37°C and gassed with 95%O_2:5%CO_2. Isometric contractile responses to electrical field stimulation (4-32 Hz, 25-40 V, 0.1 msec pulse width, for 1 s and 20 s) were recorded under a resting tension of 0.5 g wt.

Nerve mediated responses of the rabbit ileocolic artery can be blocked by a combination of α,β–methylene ATP (mATP) and prazosin: stimulations of 1s duration produced monophasic responses and stimulations of 20s duration produced biphasic responses (Bulloch & Starke, 1990). Antagonist drugs (prazosin 0.3μM and mATP 15 μM) were used to separate the purinergic and adrenergic contributions involved in the sympathetic vasopressor responses produced by electrical field stimulation in the ileocolic artery of the rabbit. Blocking drugs were applied either alone or in various combinations and sequences. The effect of yohimbine (0.3μM) was studied in conditions of α_1-adrenoceptor blockade (n=5), P_{2x}-purinoceptor desensitisation (n=6), or in the absence of antagonists (n=5).

On its own and under conditions of P_{2x}-purinoceptor desensitisation, yohimbine (0.3μM) potentiated nerve mediated responses at 16Hz for 1 s as well as both components of the biphasic response at 5Hz 20s and the first component at 10Hz 20s. In the presence of prazosin (0.3μM) the subsequent addition of yohimbine (0.3μM) potentiated nerve mediated responses at 32Hz for 1 s as well as both components of the biphasic response at 5Hz 20s and 10Hz 20s. On its own yohimbine (0.3μM) potentiated nerve mediated responses at 8, 16 and 32Hz for 1 s as well as both components of the biphasic response at 5Hz 20s and 10Hz 20s. Adenosine (100μM and 1000μM) produced concentration-dependent inhibitions of the nerve-mediated responses at 8,16 and 32Hz for 1s and both components of the response at 5Hz and 10Hz for 20s: the subsequent addition of 8-phenyltheophylline (10μM) potentiated the residual responses. However, on its own 8-phenyltheophylline (10μM) inhibited the nerve-mediated responses at most parameters studied.

From these studies it is concluded that both of the components of the response attributed to the co-transmitters ATP and NA are subject to α_2-adrenoceptor-mediated autoinhibition and that adenosine also has a neuromodulatory role in this tissue.

J.M.B. holds a British Heart Foundation Intermediate Fellowship and acknowledges the support of Prof. J.C. McGrath.

Bulloch, J.M. & Starke, K. (1990). Br. J. Pharmacol. **99**, 279-284.

311P ADENOSINE-STIMULATED ACCUMULATION OF TOTAL [^{3}H]-INOSITOL PHOSPHATES IN HAMSTER VAS DEFERENS DDT$_1$MF-2 CELLS

T.E. White & S.J. Hill. Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

Adenosine can inhibit and stimulate adenylate cyclase in a number of tissues via activation of A_1- and A_2-receptors respectively (Stiles, 1986). Recently, evidence has been presented which suggests that adenosine receptors can modulate (both positively and negatively) histamine H_1-receptor-mediated inositol phospholipid hydrolysis (Alexander *et al.*, 1989). In this communication we provide evidence that adenosine A_1-receptor stimulation alone can directly stimulate the accumulation of total ^{3}H-inositol phosphates in hamster vas deferens derived smooth muscle cell line DDT$_1$MF-2 cells.

DDT$_1$MF-2 cells were grown in Dulbecco's modified Eagles medium (DMEM) containing 10% foetal calf serum (FCS) and 2mM 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 2mM 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 10µl medium and the incubation continued for 5-45 min. 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).

2-Chloroadenosine (2CA, 10µM) produced a marked and significant accumulation of total ^{3}H-inositol phosphates in DDT$_1$MF-2 cells (7.2 ± 0.8 fold, n=15, $p< 0.05$). Similar responses were obtained with N^6-cyclopentyladenosine (CPA), adenosine (AD) and 5'-N-ethylcarboxamidoadenosine (NECA). The rank order of agonist potency (EC_{50}, nM) was: 26 ± 4 (CPA, n=6); 290 ± 80 (NECA, n=4); 480 ± 140 (2CA, n=6); 38,000 ± 9,000 (AD, n=6). The response to 10µM 2CA was antagonised by the selective A_1-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (K_d 1.7 ± 0.6 nM, n=4) and by PD 115,199 (Bruns *et al.*, 1987; K_d 55 ± 9 nM, n=4).

These results suggest that adenosine can stimulate the hydrolysis of inositol phospholipid hydrolysis in DDT$_1$MF-2 cells via an action on adenosine A_1-receptors. The DDT$_1$MF-2 cell line should therefore be an important model system in which to investigate adenosine receptor-effector coupling.

We thank the Wellcome Trust for financial support.

Alexander, S.P.H., Kendall, D.A. & Hill, S.J. (1989) *Br. J. Pharmacol.* **98**, 1241-1247.
Bruns, R.F., Fergus, J.H., Badger, E.W., Bristol, J.A., Sanay, L.A. & Hays, S.J. (1987) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **335**, 64-69.
Dickenson, J.M. & Hill, S.J. (1991) *Biochem. Pharmacol.* in press
Hawley, J., Rubin, P.C. & Hill, S.J. (1991) *Biochem. Soc. Trans.* in press
Stiles, G.L. (1986) *Trends Pharmacol. Sci.* **7**, 486-490

312P PRESYNAPTIC A_2 ADENOSINE RECEPTORS OF THE MOTOR NERVE ENDINGS OF THE RAT ARE COUPLED TO ADENYLATE CYCLASE/CYCLIC AMP TRANSDUCING SYSTEM

P. Correia-de-Sá, M.A. Timóteo & J.A. Ribeiro[1], Laboratory of Pharmacology, ICBAS, Oporto University and [1]Laboratory of Pharmacology, Gulbenkian Institute of Science, Oeiras, PORTUGAL.

A_2 adenosine receptor agonists increase acetylcholine evoked release from rat phrenic nerve endings by activating xanthine-sensitive adenosine receptors (Correia-de-Sá *et al.*, 1991). In the present work we investigated whether substances that activate adenylate cyclase, forskolin (FSK), that mimic cAMP, dibutyryl cyclic AMP (db-cAMP), or that inhibit cAMP metabolism, the non-xanthine phosphodiesterase inhibitor rolipram (Rol), could affect the excitatory action of CGS 21680C (A_2-selective adenosine agonist).

The experiments were performed at 37°C on rat phrenic nerve-hemidiaphragm preparations bathed with Tyrode solution. After incubation with [^{3}H]-choline (2.5 µCi/ml, 1 Hz, 40 min), hemicholinium-3 (10 µM) was added to the bathing solution and [^{3}H]-acetylcholine ([^{3}H]-ACh) release was evoked by nerve stimulation at 5 Hz during 3 min. Two stimulation periods (S_1 and S_2) separated by a resting period of 24 min were used. Test drugs were added 15 min before S_2 and were present up to the end of the experiments. Their effects on transmitter release were expressed by the ratios S_2/S_1. When testing the ability of FSK, Rol or db-cAMP to modify the effect of CGS 21680C, those drugs were added 15 min before each stimulation period.

In the absence of test drugs or in the presence of FSK (3 µM), Rol (300 µM) or db-cAMP (3 mM) the S_2/S_1 ratios were of similar magnitude. FSK (3 µM), Rol (300 µM) and db-cAMP (3 mM) maximally increased evoked [^{3}H]-ACh release by 29.3±7.5%(n=4), 26.7±5.0%(n=3) and 99.5±13.8%(n=3) and attenuated the excitatory effect of CGS 21680C (3 nM) (n=4) by 22.5%, 67.1% and 48.2%, respectively. The FSK analogue, 1,9-dideoxyFSK (3 µM), which does not activate adenylate cyclase, did not modify evoked [^{3}H]-ACh release.

The results suggest that an adenylate cyclase / cAMP transducing system operates the excitatory effect of A_2 adenosine agonist, CGS 21680C, on transmitter release.

Aknowlegements: P.Correia-de-Sá is in receipt of a JNICT Ph.D studentship (BD/721/90-ID). The work performed in ICBAS was supported by a project Grant (PCMT/C/SAU/792/90) from JNICT. CGS 21680C and rolipram were gifts from Dr. M. Williams of Ciba-Geigy (USA) and from Schering AG, Berlin (Germany), respectively.

P. Correia-de-Sá, A.M. Sebastião & J.A.Ribeiro (1991). *Br. J. Pharmacol.*, **103**, 1614-1620.

313P A COMPUTER SIMULATION TO TEACH THE ESSENTIALS OF RESPIRATORY PHARMACOLOGY

D.G. Dewhurst, J. Mazzoni[2], J. Morley[2], C. Page[3] & R.T. Ullyott.
Faculty of Health, Leeds Polytechnic, Calverley Street, Leeds LS1 3HE, U.K. and [3]Biomedical Sciences Division, King's College London, Manresa Road, London, SW3 6LX, U.K. and [2]Sandoz Pharma Ltd., CH-4002 Basle, Switzerland.

Computer simulations of animal experiments are increasingly being used in teaching undergraduate students. Here we present details of a program based on pulmonary function data obtained from guinea pig designed to teach fundamental respiratory pharmacology.

The program is written in Turbo Pascal IV (Borland) for IBM-compatible microcomputers to run under a range of graphics cards (Hercules,CGA,EGA,VGA). It utilises an easy-to-use, windows-like menu display from which a number of options may be selected. INTRODUCTION and METHODS sections present background theoretical information as text supported by high-resolution colour graphics and describe the essential pharmacology of the airways, the preparation and the recording system used. The main section of the program, EXPERIMENTS, offers a choice of investigative experiments which demonstrate:

i) dose response relationship for histamine;
ii) the action of a range of drugs (bronchodilators and bronchoconstrictors);
iii) the neural control of the airways.

The results (airway resistance, compliance and systemic arterial blood pressure) are presented, using high-resolution graphics, on a chart-recorder like display on the monitor from which students may take measurements directly.

We would like to acknowledge the financial support of The Lord Dowding Fund (NAVS, U.K.).

314P A COMPUTER SIMULATION TO TEACH THE PRINCIPLES OF NUTRIENT TRANSPORT IN THE SMALL INTESTINE

D.G. Dewhurst, J.E. Hardcastle[1], P.T. Hardcastle[1] & A.D. Williams.
Faculty of Health, Leeds Polytechnic, Calverley Street, Leeds LS1 3HE, U.K. and [1]Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield S10 2TN, U.K.

Computer software is increasingly being incorporated into undergraduate teaching programs where it is used either to support traditional teaching methods or, in some instances, as an alternative to experiments normally performed on animals or animal tissue. Here we present details of a program designed to teach the essentials of the intestinal absorption of hexoses (galactose) and amino acids (glycine and methionine) by simulating experiments which may be performed on one of the classical *in vitro* techniques used to study intestinal absorption, the isolated everted intestinal sac of the rat.

The program is written in C++ (Borland) for IBM compatible microcomputers and will run under a range of graphics cards (Hercules,CGA,EGA,VGA). It utilises an easy-to-use, windows-like menu display from which a number of options may be selected. INTRODUCTION and METHODS sections present background theoretical information as a combination of text and high-resolution colour graphics and describe the essential physiology of carrier-mediated diffusion, the preparation and the apparatus used. The main section, EXPERIMENTS, offers a choice of investigative experiments which allow students to collect data to:

measure the transport of each of the nutrients using a simulated radiolabelled tracer method;
demonstrate the sodium dependence of the transport process;
investigate the mutual interaction of the hexose and amino acid transport systems.

Results are presented in graphical form and students are expected to gather data and use it to calculate transport parameters.

This work was supported by The Lord Dowding Fund (NAVS).

ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work

1P **Soares-da-Silva P** Role of actin cytoskeleton in the regulation of the renal synthesis of dopamine

2P **Dalley JW & Webster RA** Comparative effects of neuroleptics and apomorphine on dopamine release and metabolism in the rat caudate nucleus and medial prefrontal cortex

3P **Rose S, Hindmarsh JG, Steiger MJ, Quinn NP, Jenner P & Marsden CD** Plasma HVA levels following debrisoquine administration do not reflect brain dopamine loss in Parkinson's disease

4P **Stamford JA, Hafizi S & Palij P** Actions of the nomifensine metabolite 8-amino-2-methyl-4-(3-methoxy-4-hydroxyphenyl)-1,2,3,4-tetrahydro-isoquinoline on striatal dopamine efflux and uptake

5P **Thomas K, Jenner P & Marsden CD** Striatal D_2 receptors are uncoupled from adenylyl cyclase following 6-hydroxydopamine lesion of the nigrostriatal pathway

6P **Hicks GA & Henderson G** The effects of dopamine on rat substantia nigra zona compacta neurones studied using the whole cell recording technique

7P **Daly SA & Waddington JL** Distinct antagonist profiles of two D_1 agonist-induced behaviours: differential involvement of D_1 receptor subtypes?

8P **Richards JG, Bertocci B, Da Prada M & Malherbe P** Cellular expression of catechol-O-methyltransferase (COMT) in rat CNS and peripheral organs revealed by *in situ* hybridization histochemistry

9P **Mitchell SN & Gray JA** Systemic administration of nicotine increases hippocampal noradrenaline release *in vivo* largely by an action at the locus caeruleus

10P **Ruck A, Millns PJ, Hill SJ & Kendall DA** Iprindole down-regulates β_1–adrenoceptors in cultured rat C_6 glioma cells

11P **Bonanno G & Raiteri M** $GABA_B$ receptor heterogeneity in rat cerebral cortex

12P **Prince RJ & Simmonds MA** Antagonism of the steroid recognition site of the $GABA_A$ receptor by epipregnanolone

13P **Anderson SMP, De Souza RJ & Cross AJ** Interactions of chlormethiazole with the GABA receptor in IMR-32 human neuroblastoma cells

14P **Whittington MA, Lambert JDC & Little HJ** Ethanol withdrawal hyperexcitability in isolated hippocampal slices is not due to decreases in GABA-mediated inhibition

15P **Eaton SA & Salt TE** Actions of four endogenous excitatory amino acids on NMDA receptors of rat ventrobasal thalamic neurones *in vivo*

16P **Scott G & Mason R** The effect of excitatory amino acid antagonists on retinogeniculate neurotransmission in the rat

17P **Schoemaker H** Further characterization of polyamine-sensitive [^{3}H]-ifenprodil binding to the NMDA receptor

18P **Carter C, Minisclou C & Rivy JP** Glycine receptor status determines the effects of ifenprodil and spermidine on [^{3}H]-TCP binding to the NMDA receptor

19P **Gibson IC, Spanswick D, Pickering AE & Logan SD** Some pharmacological properties of non-NMDA receptors in rat sympathetic preganglionic neurones *in vitro*

20P **Cross AJ, Misra A, Snape MF, Murray TK & Green AR** Chlormethiazole inhibits the effects of N-methyl-DL-aspartate *in vivo* but does not inhibit NMDA receptor binding

21P **Smith SE & Meldrum BS** Cerebroprotective effects of GYKI52466 (1-(4-aminophenyl-4-methyl-7,8-methylen-dioxy-5H-2,3-benzodiazepine) after focal ischaemia in the rat

22P **Alexander SPH** Excitatory amino acid-stimulated accumulation of inositol 1,4,5-trisphosphate mass in guinea-pig cerebral cortex is rapid and transient

23P **Arias-Montaño JA & Young JM** Different patterns of Ca^{2+}-dependence of histamine-induced inositol phosphate formation in HeLa cells and rat brain

24P **Challiss RAJ, Patel N & Nahorski SR** Inhibition of noradrenaline-stimulated inositol polyphosphate accumulation by lithium in depolarised rat cerebral cortex slices

25P **Krane A & Keen M** Coupling of prostacyclin (IP) receptors to Gs during IP receptor desensitization

26P **Malkhandi J & Keen M** The influence of GTP concentration on the dose/response curves for prostacyclin receptor agonists

27P **MacDermot J, Donnelly LE & Boyd RS** ADP-ribosyltransferase activity of NG108-15 cells modifies the structure, activity and abundance of $G_s\alpha$

28P **Roberts MHT, Lopez-Garcia JA & Long SK** Modulatory and depolarising actions of 5-HT in rat neocortex *in vitro*

29P **Hoyer D, Schoeffter P, Palacios JM, Kalkman HO, Bruinvels AT, Fozard JR, Siegl H, Seiler MP & Stoll A** SDZ 216-525: a selective, potent and silent 5-HT_{1A} receptor antagonist

30P **Anderson IK, Martin GR & Ramage AG** Evidence to suggest that activation of central 5-HT_2/5-HT_{1C} receptors causes the release of vasopressin in anaesthetized rats

31P **Kirkman E, Marshall HW & Little RA** Modulation by anaesthetic agents of the reflex cardio-inhibitory responses to activation of the baroreflex and the cardiopulmonary C-fibre afferents in the rat

32P **Davis S, Heal DJ, Luscombe GP & Stanford SC** Short- and long-latency effects of a brief swim test on central 5-HT_2 receptor function and binding

33P **Wong EHF, Wu I, Eglen RM & Whiting RL** Labelling of species variants of 5-HT_3 receptors by a novel 5-HT_3 receptor ligand, [^{3}H]RS-42358-197

34P **Tadipatri S & Saxena PR** Is 2-methyl-5-HT a selective 5-HT_3 receptor agonist?

35P **Higgins GA & Sellers EM** Behavioural effects of the 5-HT_3 receptor agonists 1-phenyl-biguanide and m-chlorophenylbiguanide in rats

36P **Cheng CHK, Barnes NM, Costall B, Ge J & Naylor RJ** Differential activities of (R)- and (S)-zacopride to modify extracellular levels of 5-HT in the rat frontal cortex

37P **Rueff A & Dray A** Pharmacological characterization of 5-HT-induced effects on peripheral nociceptors of the neonatal rat *in vitro*

38P **Ali Z, Foster G, Barasi S, Wu G & Butcher S** Reduction in the antinociceptive effects of intrathecal noradrenaline following 5,7 dihydroxytryptamine lesions of the dorsolateral funiculus

39P **Wheeler-Aceto H & Cowan A** Studies with CI-977 and ICI 204448 support a role for peripheral receptors in κ-mediated antinociception and diuresis

40P **Jones DNC & Holtzmann SG** Evidence for the involvement of central δ opioid receptors in the interaction between opioid antagonists and amphetamine

41P **Kennedy C** Chronic exposure to morphine does not induce dependence at the level of the calcium current in human SH-SY5Y cells

42P **Clapham J & Kilpatrick GJ** Histamine H_3 receptor-mediated modulation of [^{3}H]-acetylcholine release from slices of rat entorhinal cortex

43P **Aspley S, Fone KCF, Bennett GW & Marsden CA** Effect of galanin on hippocampal acetylcholine release and performance of rats in the Morris water maze

44P **Perretti M & Flower RJ** Lipocortin-1 inhibits the neutrophil infiltration elicited by interleukin-1 in the mouse air-pouch

45P **Armstrong RA** PGE_2 and EP_3 agonists induce chemotaxis of human neutrophils *in vitro*

46P **Stevens EJ, Willars GB & Tomlinson DR** Basal and angiotensin II-stimulated prostacyclin release by perfused rat lung in experimental diabetes: effects of insulin and aldose reductase inhibition

47P **Forster LA, Katsura M & Änggård EE** The oxidative modification of low-density lipoprotein by polymorphonuclear leukocytes

48P **Peers SH, Duncan GS, Bolton C & Flower RJ** Importance of endogenous corticosteroids in the control of the cellular immune response in the Brown Norway rat

49P **Rossi AG, Donigi-Gale D, Shoupe TS, Edwards R, Norman KE & Williams TJ** The role of complement, PAF and leukotriene B_4 in the reversed passive Arthus reaction in rabbit skin

50P **Berry CN & Choppin A** Inhibition of arachidonic acid-induced rabbit platelet aggregation *in vitro* by ligands which selectively bind peripheral-type benzodiazepine sites

51P **Jeremy JY, Sifakis G, Gill J & Kontoghiorghes G** Copper chelators inhibit cyclooxygenase and lipoxygenase activity by washed human platelets, *in vitro*

52P **Murphy CT & Westwick J** Modulation of the activation of platelets by genistein, an inhibitor of tyrosine kinase(s)

53P **Bourne AD, Watson ML, Smith D & Westwick J** Dissociation of agonist-induced intracellular free calcium elevation from hydrogen peroxide production in guinea-pig eosinophils

54P **Joseph S & MacDermot J** Thrombin stimulates actin polymerization in U937 human monocytic cells

55P **Ennis M & Trimble ER** Histamine release from mast cells derived from obese and lean Zucker rats

56P **van Giezen JJJ & Jansen JWCM** Dexamethasone inhibits fibrinolysis *in vivo* by modulation of t-PA and PAI-1 levels

57P **Taylor AD, Loxley HD, Cowell A-M, Flower RJ & Buckingham JC** Evidence that lipocortin 1 mediates the inhibitory effect of dexamethasone on the secretion of prolactin by the rat anterior pituitary gland

58P **Munavvar AS & Johns EJ** Evaluation of the α_1-adrenoceptor subtypes in the renal vasculature of the stroke-prone spontaneously hypertensive rat

59P **Smith K & Docherty JR** Evidence for an alpha$_{2A}$-ligand binding site in rat sub-mandibular gland

60P **Rump LC & Bohmann C** Evidence that sympathetic nerve endings of rat kidney possess α_2-autoreceptors of the α_{2A}-subtype

61P **Schwietert HR, Wilhelm D, Wilffert B & van Zwieten PA** The contractile effect of some α-adrenoceptor antagonists in the rat portal vein and the putative involvement of ATP-sensitive K^+ channels

62P **Ibbotson T & Weston AH** Interaction of potassium channel openers with [^{3}H]-idazoxan binding at the non-adrenergic idazoxan binding site in the rat liver

63P **Murphy TV, Foucart S & Majewski H** Prejunctional α_2-adrenoceptors in mouse atria function through G-proteins which are sensitive to N-ethylmaleimide, but not pertussis toxin

64P **Michel MC, Broede A, Michel-Reher M, Zerkowski H-R & Brodde O-E** Chronic β_1-adrenoceptor antagonist treatment does not affect activity of G_s-protein or adenylate cyclase in human right atrium

65P **Tobin AB, Lambert DG & Nahorski SR** Desensitisation of recombinant muscarinic M_3 receptor responses following short term exposure to agonist

66P **Marsh KA & Hill SJ** Bradykinin-induced phosphoinositide hydrolysis and calcium mobilisation in cultured bovine tracheal smooth muscle cells

67P **Dickenson JM & Hill SJ** Histamine H_1-receptor-mediated Ca^{2+} influx in the hamster vas deferens cell line DDT_1MF-2

68P **Tomlinson DR & Ettlinger CB** Protein kinase C and modulation of the sodium pump of peripheral nerve

69P **Wessler I, Pohan D, Maclagan J & Racké K** Cromakalim inhibits [^{3}H]acetylcholine release from parasympathetic nerves of the isolated rat trachea via an epithelium-dependent mechanism

70P **Tomkinson A, Dupuy A, Karlsson J-A & Raeburn D** Comparison of the *in vitro* airway smooth muscle relaxant properties of rolipram, isoprenaline and salbutamol

71P **Hall IP, Townsend P, Daykin K & Widdop S** Control of tissue cyclic AMP content in primary cultures of human airway smooth muscle cells

72P **Hall IP & Widdop S** Desensitisation of isoprenaline-induced [^{3}H]-cyclic AMP formation in primary cultures of human airway smooth muscle cells

73P **Bowring NE, Buckle DR, Taylor JF & Arch JRS** BRL 55834: a novel potassium channel activator (KCA) that is more selective for the airways in the guinea-pig and rat compared with BRL 38227

74P **Khan RM & Zar MA** Effect of stimulation-frequency on inhibition by neuropeptide Y of electrically-evoked twitches in rat isolated vas deferens

75P **Trezise DJ & Weston AH** Comparison of the relaxant and hyperpolarising actions of calcitonin-gene related peptide (CGRP) and BRL38227 in rabbit isolated basilar artery: studies using glibenclamide

76P **Cambridge H & Brain SD** The effect of calcitonin gene related peptide on blood flow and plasma extravasation in the rat knee joint

77P **Advenier C, Emonds-Alt X, Vilain P, Goulaouic P, Proietto V, Van Broeck D, Naline E, Neliat G, Le Fur G & Brelière JC** SR 48968, a potent and selective non-peptide antagonist of the neurokinin $_A$ (NK_2) receptor

78P **Watson N, Maclagan J & Barnes PJ** Demonstration of a facilitatory role of endogenous tachykinins in cholinergic neurotransmission in guinea-pig trachea

79P **Pradier L, Laville M, Le Guern S, Hubert JP, Doble A & Blanchard JC** NK_1 tachykinin receptors on the U373MG cell line: antagonist effects of RP 67580

80P **Fardin V, Foucault F, Bock MD & Garret C** Binding profile of RP 67580, a new non-peptide Substance P antagonist: comparison with CP-96,345

81P **Rogers H, Harrison SM & Birch PJ** Behavioural effects of direct intrathecal administration of neurokinins in the weanling rat

82P **Briand V, Riva L & Galzin AM** Angiotensin II induces DNA synthesis in cultured vascular smooth muscle cells

83P **Oldham AA, Allott CP, Major JS, Pearce RJ, Roberts DA & Russell ST** ICI D8731: a novel, potent and orally-effective angiotensin II antagonist

84P **Nisato D, Cazaubon C, Lacour C, Gougat J, Guiraudou P, Bernhart C, Perreaut P, Brelière JC & Le Fur G** Pharmacological properties of SR 47436, a non-peptidic angiotensin II receptor antagonist

85P **Zhang JS & Van-Zwieten PA** Characterization of two novel nonpeptide angiotensin II antagonists

86P **Widdop RE, Gardiner SM, Kemp PA & Bennett T** PD 123177-evoked inhibtion of the haemodynamic effects of angiotensin II in conscious rats after exposure to EXP 3174

87P **Liu YJ, Shankley NP, Welsh NJ & Black JW** Pharmacological analysis of angiotensin-II receptor ligand interactions in the isolated rabbit aorta assay

88P **Dennes RP, Barnes JC, Michel AD & Tyers MB** The effect of the AT_1 receptor antagonist, losartan (DuP 753) on cognitive performance in the radial maze and in a delayed non-matching to position task in the rat

89P **Banks RJA, O'Neill MF & Dourish CT** Effect of the angiotensin receptor antagonist DuP 753 on hyperlocomotion induced by D1 and D2 receptor agonists in the rat

90P **Hobbs AJ, Tucker JF & Gibson A** A comparison of NANC- and nitrosothiol-induced relaxations of the mouse anococcygeus muscle

91P **Bogle RG, Baydoun AR, Pearson JD & Mann GE** Induction of L-arginine transport in activated macrophages and its role in nitric oxide production

92P **Shaw AM, Pollock D, McGrath JC & Thomson JE** L-N^{ω}-nitro arginine methyl ester and haemoglobin expose or enhance pressor response to 5-HT in the rat isolated perfused lung

93P **Gardiner SM, Kemp PA, Bennett T, Palmer RMJ & Moncada S** Renal vasodilator responses to acetylcholine in Brattleboro rats before, during and after chronic oral ingestion of N^G-monomethyl-L-arginine (L-NMMA)

94P **McCarthy AL, Raju KS, Woolfson RG & Poston L** Endothelial function and sensitivity to vasoconstrictor agents in isolated small human feto-placental arteries

95P **Lopez-Belmonte J, Whittle BJR & Moncada S** Effects of close-arterial infusion of nitro-vasodilators on rat gastric mucosal integrity

96P **Brady AJB, Warren JB, Poole-Wilson PA & Harding SE** Differential metabolism of nitrovasodilators by guinea-pig cardiac myocytes: nitroprusside reduces myocyte contractility

97P **Salvemini D, Mollace V, Pistelli A, Änggård E & Vane J** Metabolism of glyceryl trinitrate to nitric oxide in endothelial and smooth muscle cells and the effect of *E. coli* lipopolysaccharide

98P **Gupta P** A 5-HT_1-like receptor mediates an endothelium-dependent relaxation in guinea-pig isolated jugular vein

99P **Bax WA, van Heuven-Nolsen D, Bos E, Simoons ML & Saxena PR** 5-HT_2 receptors and a receptor resembling the 5-HT_{1D} receptor subtype mediate contractions in human saphenous vein

100P **Pfaffendorf M & van Zwieten PA** Characteristics of muscarinic receptors in the rat isolated portal vein

101P **Hendriks MGC, Pfaffendorf M & van Zwieten PA** Characterization of muscarinic receptors in isolated perfused mesenteric vascular bed of SHR and normotensive control rats

102P **Doggrell SA & Bishop BE** The effects of (±), (+) and (-)-pinacidil on the membrane potential of the rat aorta

103P **Wermelskirchen D, Wilffert B, Nebel U, Wirth A & Peters T** Veratridine-induced [$^{45}Ca^{2+}$] uptake as an indication of electrically silent Na^+ channels in isolated rat aorta

104P **Cirino G, Cicala C, Sorrentino R, Miranda L & Pinto A** Indomethacin impairs *ex vivo* aorta contractions to phenylephrine and KCl of lipopolysaccharide treated rats

105P **Eglen RM, Harris GC, Cox H, Stefanich E, Sullivan A & Whiting RL** Characterization of the interaction of imperialine at muscarinic receptors *in vitro*

106P **Forster C, Naik G & Armstrong PW** Sequential measurement of plasma dihydroxyphenylalanine and dihydroxyphenylglycol from dogs with pacing-induced heart failure

107P **Hoey A, Lehmkuhl A, Wettwer E & Ravens U** Differences between DPI 201-106 and BDF 9148 in positive inotropic responses of cardiac preparations from guinea-pig, rat and man

108P **Brodde O-E, Bals S, Broede A, Kunde K, Schäfer E & Zerkowski H-R** Receptor systems mediating positive inotropic effect in isolated human right atrium

109P **Mertens MJF, Batink HD, Pfaffendorf M & van Zwieten PA** Impaired response of SHR hearts to α_1-adrenoceptor stimulation caused by changes in post-receptor events

110P **Aceto JF, Condrescu M, Nelson H, Nelson N, Nicoll D, Phillipson KD & Reeves JP** Na^+/Ca^{++} exchanger from bovine heart: cloning and expression in *Xenopus* oocytes and mammalian cells

111P **Pijl AJ, Pfaffendorf M & van Zwieten PA** Nifedipine, lacidipine and nisoldipine in the rat isolated working heart: a comparative study

112P **Leboeuf J, Baissat J, John G & Massingham R** Inhibition of veratrine-induced contracture by dopropidil, a new antianginal drug

113P **Castle NA** Two distinct potassium currents are activated by depolarization in adult rat ventricular myocytes

114P **Waugh CJ, Dockrell MEC, Haynes WG, Olverman HJ, Williams BC & Webb DJ** Inhibition of ^{125}I-endothelin-1 binding to rat cardiac membranes by BRL 38227

115P **Gurden MF & Kennedy I** Characterization of the adenosine receptors in dog coronary artery, guinea-pig aorta and guinea-pig fundus

116P **Martin PL & Cusack NJ** Halogen substituted N^6-endonorbornyl-9-methyladenines are potent and selective A_1-adenosine receptor antagonists

117P **Wainwright CL & Parratt JR** The anti-arrhythmic effects of the A_1-adenosine agonist R-PIA are mediated by bradycardia

118P **Scott RH, Stapleton SR & Currie KPM** Ca^{2+}-dependent actions of palmitoyl-DL-carnitine on cultured rat DRG neurones

119P **Iredale PA, Kendall DA, Martin KF & Hill SJ** Comparison of calcium mobilisation responses to different agonists in undifferentiated N1E-115 cells: modulatory effects of phorbol esters

120P **Rodger C & Pleuvry BJ** Anticonvulsant effects of calcium channel antagonists in combination with established anti-epileptic drugs

121P **Ikeda M, Dewar D & McCulloch J** [^3H]-PN200-110 binding sites are reduced in discrete regions of the hippocampus in Alzheimer's disease

122P **Saura J, Shih JC, Da Prada M, Chan-Palay V, Ulrich J, Huber G, Löffler J & Richards JG** Differential cellular expression of MAO-A and MAO-B in control versus Alzheimer brains

123P **Peirce MJ, Warner JA & Peachell PT** Effect of okadaic acid on histamine release from activated human lung mast cells and basophils

124P **Fairbairn SM, Lees P, Page CP & Cunningham FM** Neutrophil recruitment to the airways of normal horses in response to intravenous PAF

125P **Russell VP, Thompson AM, Sturton RG & Fitzgerald MF** Extent of antigen-induced eosinophilia and enhanced production of reactive oxygen species in aerosol and parenterally sensitized guinea-pigs

126P **Coyle AJ, Ackerman SJ & Irvin CG** Cationic proteins induce airway hyperresponsiveness dependent on charge interactions

127P **Weg VB, Watson ML, Faccioli LH & Williams TJ** [^{111}In]-eosinophil accumulation during passive cutaneous anaphylaxis in the guinea-pig

128P **Smith D, Watson ML, Bourne AD, Thompson RC & Westwick J** An interleukin-1 receptor antagonist inhibits antigen-induced eosinophil accumulation and bronchial hyperreactivity in guinea-pigs

129P **Shahid M, Philpott AJ, de Boer J, Zaagsma J & Nicholson CD** Cyclic nucleotide phosphodiesterase (PDE) isoenzyme activities in human peripheral bronchi

POSTER COMMUNICATIONS

130P **Rees H, Terenzi MG & Roberts MHT** The involvement of ACh on antinociception evoked by electrical stimulation of the anterior pretectal nucleus

131P **Lynn B & Cotsell B** Action of topical capsaicin on blood flow and C-fibre nociceptors in the skin of anaesthetized rats

132P **Fleetwood-Walker S, Hope PJ, Molony V, Parker RMC, Munro F & Mitchell R** Distinct effects of somatostatin-14 and somatostatin-28 on nociceptive responses of spinal dorsal horn neurones

133P **Puttick RM** Excitatory action of prostaglandin E_2 on rat neonatal cultured dorsal root ganglion cells

134P **Birch PJ, Beresford IJM, Rogers H, Hagan RM, Bailey F, Hayes AG, Harrison SM & Ireland SJ** Profile of activity of the peptide NK_1 receptor antagonist, GR82334, in acute nociceptive tests

135P **Beresford IJM & Birch PJ** Antinociceptive activity of the bradykinin antagonist HOE-140 in rat and mouse

136P **Patel IA, Rueff A, Urban L & Dray A** Concanavalin-A prevents cyclic-GMP-induced desensitization of bradykinin on peripheral nociceptors in the neonatal rat *in vitro*

137P **Thompson SWN, Gerber G, Sivilotti L & Woolf CJ** NMDA and non-NMDA receptor-mediated slow ventral root potentials recorded from the neonatal rat hemisected spinal cord *in vitro*

138P **Chapman V & Dickenson AH** Antinociceptive effects in the spinal cord of morphine and NMDA antagonists in combination

139P **Dickenson AH, Kalso EA, Sullivan AF & McQuare HJ** Cross-tolerance between α-adrenergic and μ opioid but not δ opioid antinociception in the rat spinal cord

140P **Herrero JF & Headley PM** Functional evidence for κ receptor heterogeneity between spinal and supraspinal sites

141P **Bardaji E & Traynor JR** [^{3}H]-DPDPE binding to mouse brain membranes: further evidence for δ opioid receptor binding site heterogeneity

142P **Patel D, Jennewein HK, Schmidhammer H & Traynor JR** *In vitro* evaluation of the opioid antagonist 3-hydroxycyprodime

143P **Gray AM, Kirkham LJ & Sewell RDE** Potentiation of amitryptyline-induced antinociception by acetorphan

144P **Jackson HC & Nutt DJ** Opioid receptor involvement in the anticonvulsant action of phenytoin

145P **Paterson SJ & Cunningham JR** Opioid binding sites in bovine retina

146P **Patten C & Bull DR** Apamin differentiates between quinpirole- and BHT920-induced inhibition of dopamine overflow *in vitro*

147P **Lalies MD & Holman RB** Effects of acute and chronic buprenorphine treatment on dopamine release in the nucleus accumbens of conscious rats

148P **Cammack C & Sheehan MJ** Dopamine D_2 receptor characterisation in the corpus striatum and nucleus accumbens using acetylcholine release

149P **Philip R, Sharkey J & Kelly PAT** Acute cocaine produces focal uncoupling of blood flow from metabolic demand in sub-regions of the rat striatum

150P **McCreary AC & Marsden CA** Antagonism of cocaine-induced hyperactivity and stereotypy by the D_1 antagonist SCH 23390

151P **Dalley JW & Webster RA** Neuroleptic effects on apomorphine-induced inhibition of striatal neuronal firing and passage across the rat blood brain barrier

152P **Lange KW, Löschmann P-A, Wachtel H, Jenner P & Marsden CD** Locomotor activity in behaviourally disabled and recovered MPTP-treated marmosets after administration of the dopamine partial agonist terguride

153P **Smith LA, Jenner P & Marsden CD** Reversal of MPTP-induced motor deficits in common marmosets following oral or transdermal administration of piribedil

154P **Cheetham SC, Kettle CJ, Martin KF & Heal DJ** Effects of various treatments which modify central dopaminergic function on striatal D_1 receptor binding

155P **Clapham J & Kilpatrick GJ** Evidence for histamine H_3 receptor regulation of drinking in the rat

156P **Arbuckle JB, Docherty RJ & James IF** Inhibition of voltage-activated calcium current in NG108-15 cells by selective α_2 adrenoceptor agonists

157P **Sastre M & García-Sevilla JA** α_2-Adrenoceptors in the human brain postmortem: ratio of high affinity agonist sites to antagonist sites and effect of age

158P **Hudson AL, Mallard NJ, Wilson J, Tyacke R & Nutt DJ** Affinity and selectivity of 6 and/or 7 aromatic substituted analogues of idazoxan for rabbit brain non-adrenoceptor idazoxan binding sites

159P **Gao B & Cutler MG** An ethopharmacological study of the effects in mice of the β-adrenoceptor antagonist, propranolol

160P **Watson WP, Misra A, Little HJ & Green AR** Felodipine antagonises cerebral [^{3}H]-PN 200-110 binding, but not the ethanol withdrawal syndrome

161P **Wright PW, Rogers DC, Roberts JC, Rothaul AL & Hunter AJ** Photothrombic lesions of the frontal cortex impair the acquisition of a spatial memory task in rats

162P **Graham D, Darles G & Langer SZ** The cytoprotective properties of ifenprodil in neuronal cell culture injury models

163P **Wood IC, Caulfield MP, Buckley NJ & Brown DA** Pharmacological profile of the muscarinic receptor in a mouse neuroblastoma (N18TG2) which expresses the m_4 muscarinic receptor gene

164P **Boddeke HWGM & Shapiro G** SDZ-ENS 163, a selective M_1 muscarinic receptor agonist, facilitates LTP in rat hippocampal slices

165P **Reynolds DJM, Lowenstein PR, Harvey JM & Leslie RA** Cholinergic vagal afferent fibres and presynaptic muscarinic receptor binding sites are present in the dorsal vagal complex of the ferret

166P **Smith CPS, Chape A, Hunter AJ & Bennett GW** Effects of chronic administration of atropine and scopolamine on elevated plus-maze behaviour in the rat

167P **Shoaib M & Stolerman IP** Locomotor activity after nicotine infusions into the fourth ventricle of rats

168P **Johnston HB, Thomas SM & Atterwill CK** Are changes in neural metabolism following exposure to aluminium chloride in cell culture associated with free radical production?

169P **Durcan MJ & Morgan PF** Naloxone attenuates caffeine-induced hypothermia

170P **Qume M, Davies J & Fowler LJ** Chronic treatment with the GABA-transaminase inhibitors EOS and GVG enhance basal GABA release from cross-chopped hippocampal slices

171P **Goudie AJ, Harrison AA, Leathley MJ & Taylor MAI** Somatic and affective benzodiazepine withdrawal signs after short term chlordiazepoxide treatment in rats

172P **Brett RR & Pratt JA** Autoradiographic analysis of [^{3}H]-flunitrazepam binding after chronic low dose diazepam treatment in rats

173P **Kennett GA, Grewal SS, Upton N & Blackburn TP** Reversal of diazepam withdrawal anxiety by BRL 46470A, a novel 5-HT_3 receptor antagonist

174P **Eigenhuis JJ, Leuwin RS, Piek T & van Wilgenburg H** Diazepam causes a voltage-dependent block of action potentials in isolated frog muscles

175P **Cascalheira JF & Sebastião AM** Role of ecto-adenylate kinase on AMP formation from ADP at the innervated sartorius muscle of the frog

176P **Oliveira JC, Sebastião AM & Ribeiro JA** The frog brain possesses one binding site for [^{3}H]-DPCPX different from the A_1 or the A_2 adenosine receptors

177P **Beckett SRG, Curwen JO, Marshall PW & Marsden CA** Anaesthetic sensitivity affects the cardiovascular defence response in different breeding strains of rat

178P **Sian J, Dexter DT, Jenner P, Marsden CD & Cohen G** Comparison of HPLC and enzymatic recycling assays for the measurement of oxidised glutathione levels in brain

179P **Zempléni É, Jones CA, Pearson S & Reynolds GP** Glutamate modulates endogenous dopamine release from superfused minislices of both olfactory tubercle and striatum of rat

180P **Davis B, Jones CA & Reynolds GP** Glutamate-stimulated release of [^{3}H]-dopamine from minislices of rat frontal cortex involves more than one receptor subtype

181P **Collins GGS** Agonists of metabotropic glutamate receptors enhance NMDA receptor-mediated components of potentials in olfactory cortex

182P **Oblin A, Bidet S & Schoemaker H** Polyamines modulate the binding of the NMDA antagonist [^{3}H]CGP 39653 via the glycine site

183P **Marshall FH, Barnes JC & Michel AD** Characterization of angiotensin II stimulation of phosphoinositide metabolism in rat hepatocytes

184P **Büscher R, Siepmann F, Philipp T, Michel MC & Brodde O-E** α_{1A}- and α_{1B}-adrenoceptors stimulate inositol phosphate generation in rat kidney

185P **Hughes B, Gozzard N & Higgs G** Differential effects of phosphodiesterase isoenzyme inhibitors (PDEIs) in reversing induced tone in guinea-pig trachea

186P **Souness JE & Scott LC** Stimulation of eosinophil cyclic AMP phosphodiesterase (PDE) by vanadate/glutathione

187P **Tonner PH, Poppers D & Miller KW** Anaesthetic potency of propofol is reduced by hydrostatic pressure

188P **Lowe KC & Armstrong FH** Effects of perfluorochemicals on liver microsomal enzymes in rats

189P **Ismail S, Back DJ & Edwards G** The effect of malaria infection on the glucuronidation of AZT and paracetamol *in vitro*

190P **Lambert MB, Omara AAM & Deasy PB** Feasibility of non-steroidal anti-inflammatory drugs for transdermal delivery: effect of vehicles *in vitro*

191P **Zhang B-L, Liu K-L, Sacquet J, Benzoni D, Wiernsperger N & Sassard J** Effects of naftidrofuryl on the renal function and the synthesis of prostanoids in isolated perfused kidneys of spontaneously hypertensive rats

192P **Martin SW & Broadley KJ** Comparison of the vascular responses to dopexamine and fenoldopam in noradrenaline- and U46619-constricted rat kidney

193P **Quilley CP, Lin Y-SR & McGiff JC** Chloride anion concentration as a determinant of renal vascular responsiveness to vasoconstrictor agents

194P **Carroll MA, Balzy M, Margiotta P & McGiff JC** Vasodilator responses to 5,6-epoxy-eicosatrienoic acid (5,6-eet): metabolism by cyclooxygenase and release of prostaglandins

195P **Sugden D** Luzindole (2-benzyl N-acetyltryptamine) is a competitive melatonin receptor antagonist in *Xenopus laevis* melanophores

196P **Bridges E de F & Greenstein BD** Specificity of oestrogen receptors in rat thymus cytosol

197P **Travers A & Hilditch A** Temporal involvement of the renin-angiotensin system in hypertension produced by left renal artery ligation in conscious rats

198P **Monopoli A, Ongini E, Lagrasta C & Olivetti G** Effects of long-term administration of the ACE inhibitor, spirapril, on development of hypertension and myocardial hypertrophy in spontaneously hypertensive rats

199P **Monopoli A, Cigola E, Ricci R & Ongini E** Effects of repeated administration of SCH 34826, a neutral endopeptidase inhibitor, on myocardial hypertrophy in the spontaneously hypertensive rat

200P **Gardiner SM, Kemp PA & Bennett T** Regional haemodynamic effects of noradrenaline and tyramine in conscious Long-Evans rats

201P **Hamon G, Jouquey S, Chevillard C, Mouren M & Stepniewski JP** Evidence that the prodrug trandolapril, a new ACE inhibitor, also displays a direct ACE inhibitory activity

202P **Giles H & Lansdell SJ** Increased potency of contractile agonists in atheroma-like vascular lesions in the rabbit is not due to endothelial dysfunction

203P **Davies JE & Ng LL** Simvastatin affects pH regulation of SV40 transformed MRC5 fibroblasts by inhibition of Na^+/H^+ antiport activity

204P **Uings IJ, Thompson NT, Bonser RW, Spacey GD, Randall RW & Garland LG** The role of tyrosine phosphorylation in receptor coupling to phospholipases in the human neutrophil

205P **Joseph S & MacDermot J** Leupeptin identifies multiple pathways for activation of U937 human monocytic cells by thrombin

206P **Dawson J, Edwards JCW, Sedgwick AD & Lees P** Monoclonal antibody MEL-14 blocks lymphocyte migration into a site of chronic inflammation

207P **Ameen M, Peek AR, Aronson JK & Lamb JF** The cellular distribution of Na/K pumps in human lymphoblasts after exposure to a low extracellular concentration of potassium

208P **Boughton-Smith NK, Deakin AM & Follenfant RL** Anti-inflammatory actions of BW755C independent of cyclooxygenase and 5-lipoxygenase inhibition

209P **von Uexkull C, Nourshargh S & Williams TJ** Vasodilator mediators induced by live *E. coli* in the skin

210P **Pile AJ & Smaje LH** The H_3 antagonist thioperamide inhibits the permeability effect of histamine in rat microvessels via an H_1 receptor effect

211P **Hu DE, Smither RL, Fan T-PD & Gresham GA** Validation of the rat sponge model for quantitative studies on angiogenesis

212P **Hori Y, Hu DE, Fan T-PD & Gresham GA** Effects of glucocorticoids and angiostatic ster-oids on sponge-induced angiogenesis in rats

213P **Alexander SPH & Curtis AR** Augmentation of A_{2b} adenosine receptor-stimulated cyclic AMP formation by DL-1-amino-cyclopentane-*trans*-1,3-dicarboxylate, a rigid glutamate analogue

214P **Menager J & Heuillet E** NK_1 tachykinin receptors on the U373MG cell line: coupling to inositol phosphate formation

215P **Boyd RS, Donnelly LE & MacDermot J** Mono(ADP-ribosyl)transferase in NG108-15 cells ADP-ribosylates a putative arginine acceptor in $G_s\alpha$

216P **Vohra MM & Willams B** Determining 5-HT by reverse phase h.p.l.c. and electrochemical detection after precolumn derivatization with OPA-2ME

217P **Wieczorek W & Kruk Z** Electrically stimulated release of 5-HT from the rat prefrontal cortex measured by fast cyclic voltammetry in brain slices

218P **Zetterström T, Finn D, Pei Q & Grahame-Smith DG** Effects of the sulphonylurea gliquidone on the efflux of radiolabelled 5-HT and dopamine from rat brain slices

219P **Sharma A, Landon M & Fone KCF** Regional distribution of 5-HT_{1C} and 5-HT_2 receptor peptides on the rat brain measured using polyclonal antisera

220P **Ebenezer IS** Effects of 8-OH-DPAT on operant food intake in food-deprived rats

221P **McBlane JW, Critchley MAE & Handley SL** Light intensity influences the response to 8-OH-DPAT in the elevated x-maze model of anxiety

222P **Johnston AR, Dutia MB & McQueen DS** Effects of 5-HT on rat medial vestibular nucleus neurones *in vitro*

223P **Elliott JM & Phipps S** Differential effects of monovalent cations on GTP-sensitive states of the 5-HT_{1A} receptor

224P **Opacka-Juffry J, Ahier RG, Grasby P, Cunningham V, Cremer JE & Sarna GS** Effect of buspirone on regional cerebral blood flow determined in conscious rats

225P **Dursun SM & Handley SL** Comparison of ritanserin, haloperidol, pimozide and clonidine effects on spontaneous and DOI head-shake

226P **Gao B & Cutler MG** Effects of ritanserin on the behaviour of mice during social encounters

227P **Kennett GA & Grewal SS** Effect of 5-HT_3 receptor agonists on rat food intake

228P **Jones DNC, Carey GJ, Costall B, Domeney AM, Naylor RJ & Tyers MB** The 5-HT_3 receptor antagonist, GR68755, prevents a scopolamine-induced cognitive impairment in the common marmoset

229P **Ferro A, Kaumann AJ & Brown MJ** β-adrenoceptor subtypes and function in human internal mammary artery and saphenous vein

230P **Erdbrügger W, Bauch H-J & Michel MC** Three α-adrenoceptor subtypes exist in porcine aortic smooth muscle

231P **Kirkham DM & Kelly J** Direct *in vitro* comparison of the rat atypical β-adrenoceptors mediating white adipocyte lipolysis and colonic relaxation

232P **Growcott JW, Holloway B, Wilson C, Mainwaring S & Green M** The novel brown adipocyte stimulant, ICI D7114, reveals possible differences in atypical β-adrenoceptor reserve

233P **Pimoule C, Graham D & Langer SZ** Characterization of the α_{1B}-adrenoceptor gene product expressed in a stably transfected HeLa cell-line

234P **Wilson C, Pettinger SJ, Hayward J, Birtles S & Holloway BR** Cardiovascular effects of the thermogenic β-adrenoceptor agonist, ICI D7114, in conscious telemetred dogs

235P **Ford APDW, Taneja DT & Clarke DE** Atypical β-adrenoceptor-mediated relaxation and cyclic AMP formation in tunica muscularis mucosae (TMM) of the rat oesophagus

236P **Smith RD & Weston AH** Studies on the inhibitory effects of guanabenz on the field-stimulated rat isolated anococcygeus muscle

237P **Przyborski SA, Levin RJ & Young A** Evidence for M_3 muscarinic cholinoceptors on enterocytes which induce electrogenic secretion in rat small intestine

238P **Sneddon P & McLees A** P_{2X}-purinoceptor and muscarinic cholinoceptor mediated contractions of isolated urinary bladder smooth muscle from adult and neonatal rabbits

239P **Bhupalan A, Murray-Lyon IML & Leathard H** Cathinone, amphetamine and nerve-mediated inhibition of spontaneous contractions of rabbit small intestine

240P **Allen MJ, Wallace A & Carpenter JR** Sodium decavanadate inhibits [^{3}H]$InsP_3$ binding in guinea-pig ileum smooth muscle and bovine adrenal cortex

241P **Yeardley HL, Coleman RA, Marshall K & Senior J** The effects of PGE_2, sulprostone and AH13205 on hamster uterus *in vitro*

242P **Taylor SG, Buckle DR, Shaw DJ, Ward JS & Arch JRS** An investigation of the selectivities of BRL 55834 and BRL 38227 as relaxants of guinea-pig tracheal spirals relative to portal vein

243P **Racké K, Bäring J, Brunn G, Hey C, Reimann A & Wessler I** Characterization of muscarinic-paracrine interactions in the regulation of noradrenaline release from rat isolated trachea

244P **Sakamoto T, Elwood W, Barnes PJ & Chung KF** Inhaled lyso-PAF causes plasma exudation in guinea-pig airways

245P **Curwen JO, Marshall PW & Tomlinson AE** A comparison of the bronchoconstrictor actions of endothelin-1 and human proendothelin (1-38) in the anaesthetised guinea-pig

246P **Hans JG, O'Shaughnessy KM, Dollery CT, Taylor GW & Fuller RW** PAF-induced bronchoconstriction and neutrophil accumulation in the rabbit lung *in vivo*

247P **Taylor SG, Buckle DR, Foster KA, Shaw DJ, Ward JS & Arch JRS** Evidence that the novel, airways selective benzopyranol, BRL 55834, is a potassium channel activator

248P **Mo N & Wallis DI** Properties of rat stellate ganglion cells: comparison of putative cardiac-projecting and non cardiac neurones

249P **Krupp J & Feltz P** Patch-clamp recording in lateral horn cells of rat spinal cord in a slice preparation: mediation of substance P effects by NK_1 receptors

250P **Stables JM, Hagan RM & Ireland SJ** Tachykinins can depolarize the rat isolated superior cervical ganglion via NK_1 receptors

251P **Mason GS, Graham EA & Elliott PJ** NK_1 receptors in the guinea-pig mediate locomotor hyperactivity

252P **McDermott AM, Watson CD & Nutt DJ** High relative affinity of RX77368 for the rat retrosplenial cortex

253P **Watson CD, Hewitt MJ, Fone KCF & Bennett GW** Behavioural and biochemical effects of the TRH analogue RX77368 and scopolamine on radial arm maze performance in the rat

254P **Patel S & Hutson PH** Effect of galanin on hypothermia in mice induced by RS86 and 8-OH DPAT: involvement of ATP-sensitive potassium channels

255P **Ikeda M, Dewar D & McCulloch J** Preservation of [^{125}I]-galanin binding sites despite loss of choline acetyltransferase activity in the hippocampus in Alzheimer's disease

256P **Long SK & Cramer WCM** Cholecystokinin-induced depolarization of neonate rat spinal cord *in vitro*

257P **van Meel JCA, Wienen W & Entzeroth M** Hypotensive effects of the angiotensin II receptor antagonist DuP 753 in renal hypertensive rats

258P **Rogers H, Hill JA & Birch PJ** Calcitonin gene related peptide (CGRP) potentiates NK_1 receptor-mediated paw oedema, but not intrathecal NK_1 agonist-induced behaviour in the rat

259P **Christophe B, Libon R, Chatelain P & Manning AS** Effect of SR 47436 on angiotensin II-induced pressor responses in pithed rats

260P **Hawcock AB & Barnes JC** Characterisation of the contractile responses to angiotensin II analogues in the guinea-pig ileum

261P **Allott CP, Oldham AA, Steel JA, Worrall V, Pearce RJ, Roberts DA & Russell ST** Cardiovascular effects of the angiotensin II receptor antagonist ICI D8731 in the dog and marmoset

262P **Dennes RP, Irlam-Hughes JJ & Barnes JC** Captopril-induced dipsogenicity in the rat: effects of the non-peptide angiotensin II antagonists losartan (DuP 753) and PD123177

263P **Sun B, Plumpton C, Sinclair J & Brown MJ** Expression of CGRP in cultured endothelial cells with plasmid DNA

264P **Hiley CR, Jones CR & Pelton JT** Use of selective inhibitors of [^{125}I]-endothelin-1 binding shows that rat aortic homogenates contain both ET_A and ET_B receptors

265P **Wragg A, Robertson MJ & Clark KL** Prevention and reversal of angiotensin II-induced tachyphylaxis in the rabbit isolated aorta

266P **Liu YJ, Shankley NP, Welsh NJ & Black JW** Development and application of a model of angiotensin-II receptor ligand interactions in isolated smooth muscle assays

267P **Benito-Orfila MA, Nandha KA & Bloom SR** Biological activity of neuromedin U-8 fragments on rat fundic circular muscle *in vitro*

268P **Doods HN** Effects of PP, PYY, NPY and selective analogues on the field-stimulated rat and rabbit vas deferens

269P **Sharkey J, Ritchie IM & Kelly PAT** Focal increases in cerebrovascular permeability following MK801 administration in the rat

270P **Phillips JA & Steward A** The hypocalcaemic response to intravenous calcitonin in juvenile rats: the importance of age and other factors

271P **Gouliaev AH & Nedergaard OA** Release of [^{3}H]-5-HT from rabbit isolated aorta evoked by electrical-field stimulation

272P **Waldron GJ, O'Shaughnessy CT & Connor H** Sumatriptan does not modify capsaicin-induced relexation of guinea-pig isolated basilar artery

273P **Butler A, Worton S & Connor HE** Sumatriptan does not attenuate sensory nerve-mediated relaxations of guinea-pig isolated pulmonary artery

274P **Rudd JA, Bunce KT, Cheng CHK & Naylor RJ** The effect of cisplatin on plasma 5-HT, 5-HIAA and 5-HTP levels during emesis in the ferret

275P **Eglen RM, Alvarez R, Wong EHF, Johnson L, Perkins LA, Ramsey S, Loury DN, Tang H-M, Wallace S & Whiting RL** Characterization of the interaction of SDZ 205,557 at putative $5\text{-}HT_4$ receptors

276P **Newberry NR, Watkins CJ & Sprosen TS** BRL 46470A antagonises $5\text{-}HT_3$ receptor-mediated responses on the rat vagus and on NG108-15 cells

277P **van den Buuse M & Buisson-Defferier S** Effect of MDL 73005EF on $5\text{-}HT_{1A}$ receptor-mediated blood pressure responses in conscious spontaneously hypertensive rats

278P **Tuladhar BR, Costall B & Naylor RJ** Variation in the relaxation response to 5-HT in different regions of the rat small intestine

279P **Wardle K & Sanger G** Guinea-pig distal colon: a sensitive model of the putative $5\text{-}HT_4$ receptor

280P **Den Boer MO, Villalón CM & Saxena PR** $5\text{-}HT_1$-like receptors in the porcine carotid circulation: do they belong to the $5\text{-}HT_{1D}$ receptor subtype?

281P **Andersen LB, Dyerberg C & Hermansen K** The isolated atria from mice and rats as models for testing cardioactive drugs: a comparative study

282P **Steinfath M, Lavicky J, Schmitz W & Scholz H** Distribution of β_1- and β_2-adrenoceptors in right and left atria and ventricles obtained post-mortem from nonfailing and failing human hearts

283P **Kaumann AJ, Sanders L, Hall JA, Murray KJ & Brown MJ** Stimulation of β_1- and β_2-adrenoceptors in human ventricular myocardium from failing hearts hastens the onset of relaxation

284P **Wilhelm D, Ebbert M, Leidig A, Wilffert B & Peters Th** The phosphodiesterase IV inhibitor rolipram enhances mechanical and electrical effects of the phosphodiesterase III inhibitor R 80122

285P **van den Ende R, Batink HD & van Zwieten PA** Influence of ischaemia on binding behaviour of muscarinic agonists and antagonists in rat ventricular preparations

286P **Butterfield MC & Chess-Williams R** Reduced ventricular α-adrenoceptor density during enhanced responsiveness to phenylephrine induced by chronic noradrenaline infusion in the rat

287P **Brown MJ, Ferro A, Jia H & Monteith S** Evidence for altered expression of $G_s\alpha$ subunit as a factor in the enhanced coupling of β-adrenoceptors to adenyl cyclase in human atrium

288P **van Gelderen EM & Saxena PR** 5-HT-induced responses after N^G-nitro-L-arginine methyl ester in pithed rats

289P **Parsaee H, Joseph S, MacDermot J & McEwan JR** The role of calcium in the independent regulation of the release of EDRF and prostacyclin from bovine aortic endothelial cells

290P **Randall MD & Griffith TM** A role for EDRF in collateral perfusion following arterial occlusion

291P **Evans RA, Lang D & Lewis MJ** The effect of potassium channel blocking agents on endothelium-dependent relaxation in the rabbit aorta

292P **Foulkes R & Shaw S** Recombinant human TNF induces a non-constitutive form of nitric oxide-synthase in the endothelium and smooth muscle

293P **Pfaffendorf M, Mathy MJ & van Zwieten PA** Differential effects of various dihydropyridine calcium antagonists on the contraction pattern of rat coronary artery stimulated with high potassium

294P **Laude EA & Morice AH** The effect of nedocromil sodium on citric acid and capsaicin cough challenge in the guinea-pig

295P **Breslin E, Teoh TG, Darling M & Docherty JR** Pharmacological responsiveness of human umbilical vessels from normal and pre-eclamptic pregnancies

296P **Aboud RW & Docherty JR** Investigations of contractions mediated by noradrenaline in rat aorta

297P **Clesham G, Parsaee H, Joseph S, MacDermot J & McEwan JR** Release of prostacyclin but not EDRF follows activation of thromboxane receptors on bovine endothelium

298P **Bigaud M** Simultaneous regional vasodilator and vasoconstrictor effect of endothelin ET_B receptor stimulation in anaesthetized rats

299P **Aidulus DM, Fuglsang A & Pollock D** Investigation of the mechanism underlying drug-induced uncovering of enhanced contractions to UK-14,304 in rat tail artery rings *in vitro*

300P **White DG, Sumner MJ & Watts IS** The endothelin isopeptides do not release EDRF or prostacyclin (PGI_2) from human umbilical vein endothelial cells (HUVECS)

301P **Wallace HM, Millican SA & Aung T** A possible role for polyamines in the growth of endothelial cells

302P **Millican SA, Webster J & Wallace HM** Hydrogen peroxide-mediated damage in human vascular endothelial cells

303P **Croning MDR, Gurden MF & Kennedy I** Potencies of adenosine receptor-blocking drugs on dog isolated coronary artery and guinea-pig isolated aorta

304P **Christophe B, Chatelain P & Manning AS** SR47063, a new K^+ channel opener: effect on ^{86}Rb efflux from rat aorta

305P **Docherty JR** Effects of ageing on contractions to noradrenaline and 5-HT in human saphenous vein

306P **Withrington PG, Antunes E, Grassi-Kassisse D & de Nucci G** U46619, a stable thromboxane mimetic, can constrict canine splenic arterial vasculature without significant effect on capsular smooth muscle

307P **Calder JA, Schachter M & Sever PS** Vasorelaxant actions of thiazides and related drugs

308P **MacLean MR & Nally J** Hypoxia-induced vaso-constriction in the isolated rabbit pulmonary artery: total inositol phosphate accumulation and protein kinase C activation

309P **Asghar AUR, McQueen DS & Macdonald AE** Absence of effect of adenosine on the discharge of articular mechanoreceptors in normal and arthritic rats

310P **Bulloch JM** Yohimbine potentiates and adenosine inhibits the nerve-mediated response in a blood vessel exhibiting co-transmission

311P **White TE & Hill SJ** Adenosine-stimulated accumulation of total [^{3}H]-inositol phosphates in hamster vas deferens DDT_1MF-2 cells

312P **Correia-de-Sá P, Timóteo MA & Ribeiro JA** Presynaptic A_2 adenosine receptors of the motor nerve endings of the rat are coupled to adenylate cyclase/cyclic AMP transducing system

DEMONSTRATIONS

313P **Dewhurst DG, Mazzoni J, Morley J, Page C & Ullyott RT** A computer simulation to teach the essentials of respiratory pharmacology

314P **Dewhurst DG, Hardcastle JE, Hardcastle PT & Williams AD** A computer simulation to teach the principles of nutrient transport in the small intestine

British Journal of Pharmacology

Proceedings Supplement

Proceedings of the British Pharmacological Society Meeting

University College, London/Sandoz Institute for Medical Research

17–19 December, 1991

Author Index

Aboud RW & Docherty JR Investigations of contractions mediated by noradrenaline in rat aorta 296P

Aceto JF, Condrescu M, Nelson H, Nelson N, Nicoll D, Phillipson KD & Reeves JP Na^+/Ca^{++} exchanger from bovine heart: cloning and expression in *Xenopus* oocytes and mammalian cells 110P

Ackerman SJ *see* Coyle AJ 126P

Advenier C, Emonds-Alt X, Vilain P, Goulaouic P, Proietto V, Van Broeck D, Naline E, Neliat G, Le Fur G & Brelière JC SR 48968, a potent and selective non-peptide antagonist of the neurokinin$_A$ (NK_2) receptor 77P

Ahier RG *see* Opacka-Juffry J 224P

Aidulus DM, Fuglsang A & Pollock D Investigation of the mechanism underlying drug-induced uncovering of enhanced contractions to UK-14,304 in rat tail artery rings *in vitro* 299P

Alexander SPH & Curtis AR Augmentation of A_{2b} adenosine receptor-stimulated cyclic AMP formation by DL-1-amino-cyclopentane-*trans*-1,3-dicarboxylate, a rigid glutamate analogue 213P

Alexander SPH Excitatory amino acid-stimulated accumulation of inositol 1,4,5-trisphosphate mass in guinea-pig cerebral cortex is rapid and transient 22P

Ali Z, Foster G, Barasi S, Wu G & Butcher S Reduction in the anti-nociceptive effects of intrathecal noradrenaline following 5,7-dihydroxytryptamine lesions of the dorsolateral funiculus 38P

Allen MJ, Wallace A & Carpenter JR Sodium decavanadate inhibits [^{3}H]InsP_3 binding in guinea-pig ileum smooth muscle and bovine adrenal cortex 240P

Allott CP, Oldham AA, Steel JA, Worrall V, Pearce RJ, Roberts DA & Russell ST Cardiovascular effects of the angiotensin II receptor antagonist ICI D8731 in the dog and marmoset 261P

Allott CP *see* Oldham AA 83P

Alvarez R *see* Eglen RM 275P

Ameen M, Peek AR, Aronson JK & Lamb JF The cellular distribution of Na/K pumps in human lymphoblasts after exposure to a low extracellular concentration of potassium 207P

Andersen LB, Dyerberg C & Hermansen K The isolated atria from mice and rats as models for testing cardioactive drugs: a comparative study 281P

Anderson IK, Martin GR & Ramage AG Evidence to suggest that activation of central 5-HT_2/5-HT_{1C} receptors causes the release of vasopressin in anaesthetized rats 30P

Anderson SMP, De Souza RJ & Cross AJ Interactions of chlormethiazole with the GABA receptor in IMR-32 human neuroblastoma cells 13P

Änggård E *see* Salvemini D 97P

Änggård EE *see* Forster LA 47P

Antunes E *see* Withrington PG 306P

Arbuckle JB, Docherty RJ & James IF Inhibition of voltage-activated calcium current in NG108-15 cells by selective α_2 adrenoceptor agonists 156P

Arch JRS *see* Bowring NE 73P

Arch JRS *see* Taylor SG 242P

Arch JRS *see* Taylor SG 247P

Arias-Montaño JA & Young JM Different patterns of Ca^{2+}-dependence of histamine-induced inositol phosphate formation in HeLa cells and rat brain 23P

Armstrong FH *see* Lowe KC 188P

Armstrong PW *see* Forster C 106P

Armstrong RA PGE_2 and EP_3 agonists induce chemotaxis of human neutrophils *in vitro* 45P

Aronson JK *see* Ameen M 207P

Asghar AUR, McQueen DS & Macdonald AE Absence of effect of adenosine on the discharge of articular mechanoreceptors in normal and arthritic rats 309P

Aspley S, Fone KCF, Bennett GW & Marsden CA Effect of galanin on hippocampal acetylcholine release and performance of rats in the Morris water maze 43P

Atterwill CK *see* Johnston HB 168P

Aung T *see* Wallace HM 301P

Back DJ *see* Ismail S 189P

Bailey F *see* Birch PJ 134P

Baissat J *see* Leboeuf J 112P

Bals S *see* Brodde O-E 108P

Balzy M *see* Carroll MA 194P

Banks RJA, O'Neill MF & Dourish CT Effect of the angiotensin receptor antagonist DuP 753 on hyperlocomotion induced by D_1 and D_2 receptor agonists in the rat 89P

Barasi S *see* Ali Z 38P

Bardaji E & Traynor JR [^{3}H]-DPDPE binding to mouse brain membranes: further evidence for δ opioid receptor binding site heterogeneity 141P

Bäring J *see* Racké K 243P

Barnes JC *see* Dennes RP 88P

Barnes JC *see* Dennes RP 262P

Barnes JC *see* Hawcock AB 260P

Barnes JC *see* Marshall FH 183P

Barnes NM *see* Cheng CHK 36P

Barnes PJ *see* Sakamoto T 244P

Barnes PJ *see* Watson N 78P

Batink HD *see* Mertens MJF 109P

Batink HD *see* van den Ende R 285P

Bauch H-J *see* Erdbrügger W 230P

Bax WA, van Heuven-Nolsen D, Bos E, Simoons ML & Saxena PR 5-HT_2 receptors and a receptor resembling the 5-HT_{1D} receptor subtype mediate contractions in human saphenous vein 99P

Baydoun AR *see* Bogle RG 91P

Beckett SRG, Curwen JO, Marshall PW & Marsden CA Anaesthetic sensitivity affects the cardiovascular defence response in different breeding strains of rat 177P

Benito-Orfila MA, Nandha KA & Bloom SR Biological activity of neuromedin U-8 fragments on rat fundic circular muscle *in vitro* 267P

Bennett GW *see* Aspley S 43P

Bennett GW *see* Smith CPS 166P

Bennett GW *see* Watson CD 253P

Bennett T *see* Gardiner SM 93P

Bennett T *see* Gardiner SM 200P

Bennett T *see* Widdop RE 86P

Benzoni D *see* Zhang B-L 191P

Beresford IJM & Birch PJ Antinociceptive activity of the bradykinin antagonist HOE-140 in rat and mouse 135P

Beresford IJM *see* Birch PJ 134P

Bernhart C *see* Nisato 84P

Berry CN & Choppin A Inhibition of arachidonic acid-induced rabbit platelet aggregation *in vitro* by ligands which selectively bind peripheral-type benzodiazepine sites 50P

Bertocci B *see* Richards JG 8P

Bhupalan A, Murray-Lyon IML & Leathard H Cathinone, amphetamine and nerve-mediated inhibition of spontaneous contractions of rabbit small intestine 239P

Bidet S *see* Oblin A 182P

Bigaud M Simultaneous regional vasodilator and vasoconstrictor effect of endothelin ET_B receptor stimulation in anaesthetized rats 298P

Birch PJ, Beresford IJM, Rogers H, Hagan RM, Bailey F, Hayes AG, Harrison SM & Ireland SJ Profile of activity of the peptide NK1 receptor antagonist, GR82334, in acute nociceptive tests 134P

Birch PJ *see* Beresford IJM 135P

Birch PJ *see* Rogers H 81P

Birch PJ *see* Rogers H 258P

Birtles S *see* Wilson C 234P

Bishop BE *see* Doggrell SA 102P
Black JW *see* Liu YJ 87P
Black JW *see* Liu YJ 266P
Blackburn TP *see* Kennett GA 173P
Blanchard JC *see* Pradier L 79P
Bloom SR *see* Benito-Orfila MA 267P
Bock MD *see* Fardin V 8P
Boddeke HWGM & Shapiro G SDZ-ENS 163, a selective M_1 muscarinic receptor agonist, facilitates LTP in rat hippocampal slices 164P
Bogle RG, Baydoun AR, Pearson JD & Mann GE Induction of L-arginine transport in activated macrophages and its role in nitric oxide production 91P
Bohmann C *see* Rump LC 60P
Bolton C *see* Peers SH 48P
Bonanno G & Raiteri M $GABA_B$ receptor heterogeneity in rat cerebral cortex 11P
Bonser RW *see* Uings IJ 204P
Bos E *see* Bax WA 99P
Boughton-Smith NK, Deakin AM & Follenfant RL Anti-inflammatory actions of BW755C independent of cyclooxygenase and 5-lipoxygenase inhibition 208P
Bourne AD, Watson ML, Smith D & Westwick J Dissociation of agonist-induced intracellular free calcium elevation from hydrogen peroxide production in guinea-pig eosinophils 53P
Bourne AD *see* Smith D 128P
Bowring NE, Buckle DR, Taylor JF & Arch JRS BRL 55834: a novel potassium channel activator (KCA) that is more selective for the airways in the guinea-pig and rat compared with BRL 38227 73P
Boyd RS, Donnelly LE & MacDermot J Mono(ADP-ribosyl)transferase in NG108-15 cells ADP-ribosylates a putative arginine acceptor in $G_s\alpha$ 215P
Boyd RS *see* MacDermot J 27P
Brady AJB, Warren JB, Poole-Wilson PA & Harding SE Differential metabolism of nitrovasodilators by guinea-pig cardiac myocytes: nitroprusside reduces myocyte contractility 96P
Brain SD *see* Cambridge H 76P
Brelière JC *see* Advenier C 77P
Brelière JC *see* Nisato D 84P
Breslin E, Teoh TG, Darling M & Docherty JR Pharmacological responsiveness of human umbilical vessels from normal and pre-eclamptic pregnancies 295P
Brett RR & Pratt JA Autoradiographic analysis of [^{3}H]-flunitrazepam binding after chronic low dose diazepam treatment in rats 172P
Briand V, Riva L & Galzin AM Angiotensin II induces DNA synthesis in cultured vascular smooth muscle cells 82P
Bridges E de F & Greenstein BD Specificity of oestrogen receptors in rat thymus cytosol 196P
Broadley KJ *see* Martin SW 192P
Brodde O-E, Bals S, Broede A, Kunde K, Schäfer E & Zerkowski H-R Receptor systems mediating positive inotropic effect in isolated human right atrium 108P
Brodde O-E *see* Büscher R 184P
Brodde O-E *see* Michel MC 64P
Broede A *see* Brodde O-E 108P
Broede A *see* Michel MC 64P
Brown DA *see* Wood IC 163P
Brown MJ, Ferro A, Jia H & Monteith S Evidence for altered expression of $G_s\alpha$ subunit as a factor in the enhanced coupling of ß-adrenoceptors to adenyl cyclase in human atrium 287P
Brown MJ *see* Ferro A 229P
Brown MJ *see* Kaumann AJ 283P
Brown MJ *see* Sun B 263P
Bruinvels AT *see* Hoyer D 29P
Brunn G *see* Racké K 243P
Buckingham JC *see* Taylor AD 57P
Buckle DR *see* Bowring NE 73P
Buckle DR *see* Taylor SG 242P
Buckle DR *see* Taylor SG 247P
Buckley NJ *see* Wood IC 163P
Buisson-Defferier S *see* van den Buuse M 277P
Bull DR *see* Patten C 146P
Bulloch JM Yohimbine potentiates and adenosine inhibits the nerve-mediated response in a blood vessel exhibiting co-transmission 310P
Bunce KT *see* Rudd JA 274P
Büscher R, Siepmann F, Philipp T, Michel MC & Brodde O-E α_{1A}- and α_{1B}-adrenoceptors stimulate inositol phosphate generation in rat kidney 184P
Butcher S *see* Ali Z 38P
Butler A, Worton S & Connor HE Sumatriptan does not attenuate sensory nerve-mediated relaxations of guinea-pig isolated pulmonary artery 273P
Butterfield MC & Chess-Williams R Reduced ventricular α-adrenoceptor density during enhanced responsiveness to phenylephrine induced by chronic noradrenaline infusion in the rat 286P

Calder JA, Schachter M & Sever PS Vasorelaxant actions of thiazides and related drugs 307P
Cambridge H & Brain SD The effect of calcitonin gene related peptide on blood flow and plasma extravasation in the rat knee joint 76P
Cammack C & Sheehan MJ Dopamine D_2 receptor characterisation in the corpus striatum and nucleus accumbens using acetylcholine release 148P
Carey GJ *see* Jones DNC 228P
Carpenter JR *see* Allen MJ 240P
Carroll MA, Balzy M, Margiotta P & McGiff JC Vasodilator responses to 5,6-epoxyeicosatrienoic acid (5,6-eet): metabolism by cyclooxygenase and release of prostaglandins 194P
Carter C, Minisclou C & Rivy JP Glycine receptor status determines the effects of ifenprodil and spermidine on [^{3}H]-TCP binding to the NMDA receptor 18P
Cascalheira JF & Sebastião AM Role of ecto-adenylate kinase on AMP formation from ADP at the innervated sartorius muscle of the frog 175P
Castle NA Two distinct potassium currents are activated by depolarization in adult rat ventricular myocytes 113P
Caulfield MP *see* Wood IC 163P
Cazaubon C *see* Nisato D 84P
Challiss RAJ, Patel N & Nahorski SR Inhibition of noradrenaline-stimulated inositol polyphosphate accumulation by lithium in depolarised rat cerebral cortex slices 24P
Chan-Palay V *see* Saura J 122P
Chape A *see* Smith CPS 166P
Chapman V & Dickenson AH Antinociceptive effects in the spinal cord of morphine and NMDA antagonists in combination 138P
Chatelain P *see* Christophe B 259P
Chatelain P *see* Christophe B 304P
Cheetham SC, Kettle CJ, Martin KF & Heal DJ Effects of various treatments which modify central dopaminergic function on striatal D_1 receptor binding 154P
Cheng CHK, Barnes NM, Costall B, Ge J & Naylor RJ Differential activities of (R)- and (S)-zacopride to modify extracellular levels of 5-HT in the rat frontal cortex 36P
Cheng CHK *see* Rudd JA 274P
Chess-Williams R *see* Butterfield MC 286P
Chevillard C *see* Hamon G 201P
Choppin A *see* Berry CN 50P
Christophe B, Chatelain P & Manning AS SR 47063, a new K^+ channel opener: effect on ^{86}Rb efflux from rat aorta 304P
Christophe B, Libon R, Chatelain P & Manning AS Effect of SR 47436 on angiotensin II-induced pressor responses in pithed rats 259P
Chung KF *see* Sakamoto T 244P
Cicala C *see* Cirino G 104P
Cigola E *see* Monopoli A 199P
Cirino G, Cicala C, Sorrentino R, Miranda L & Pinto A Indomethacin impairs *ex vivo* aorta contractions to phenylephrine and KCl of lipopolysaccharide treated rats 104P
Clapham J & Kilpatrick GJ Evidence for histamine H_3 receptor regulation of drinking in the rat 155P

Clapham J & Kilpatrick GJ Histamine H_3 receptor-mediated modulation of [^{3}H]-acetylcholine release from slices of rat entorhinal cortex 42P
Clark KL *see* Wragg A 265P
Clarke DE *see* Ford APDW 235P
Clesham G, Parsaee H, Joseph S, MacDermot J & McEwan JR Release of prostacyclin but not EDRF follows activation of thromboxane receptors on bovine endothelium 297P
Cohen G *see* Sian J 178P
Coleman RA *see* Yeardley HL 241P
Collins GGS Agonists of metabotropic glutamate receptors enhance NMDA receptor-mediated components of potentials in olfactory cortex 181P
Condrescu M *see* Aceto JF 110P
Connor H *see* Waldron GJ 272P
Connor HE *see* Butler A 273P
Correia-de-Sá P, Timóteo MA & Ribeiro JA Presynaptic A_2 adenosine receptors of the motor nerve endings of the rat are coupled to adenylate cyclase/cyclic AMP transducing system 312P
Costall B *see* Cheng CHK 36P
Costall B *see* Jones DNC 228P
Costall B *see* Tuladhar BR 278P
Cotsell B *see* Lynn B 131P
Cowan A *see* Wheeler-Aceta H 39P
Cowell A-M *see* Taylor AD 57P
Cox H *see* Eglen RM 105P
Coyle AJ, Ackerman SJ & Irvin CG Cationic proteins induce airway hyperresponsiveness dependent on charge interactions 126P
Cramer WCM *see* Long SK 256P
Cremer JE *see* Opacka-Juffry J 224P
Critchley MAE *see* McBlane 221P
Croning MDR, Gurden MF & Kennedy I Potencies of adenosine receptor-blocking drugs on dog isolated coronary artery and guinea-pig isolated aorta 303P
Cross AJ, Misra A, Snape MF, Murray TK & Green AR Chlormethiazole inhibits the effects of *N*-methyl-DL-aspartate *in vivo* but does not inhibit NMDA receptor binding 20P
Cross AJ *see* Anderson SMP 13P
Cunningham FM *see* Fairbairn SM 124P
Cunningham JR *see* Paterson SJ 145P
Cunningham V *see* Opacka-Juffry J 224P
Currie KPM *see* Scott RH 118P
Curtis AR *see* Alexander SPH 213P
Curwen JO, Marshall PW & Tomlinson AE A comparison of the broncho-constrictor actions of endothelin-1 and human proendothelin (1-38) in the anaesthetised guinea-pig 245P
Curwen JO *see* Beckett SRG 177P
Cusack NJ *see* Martin PL 116P
Cutler MG *see* Gao B 159P
Cutler MG *see* Gao B 226P

Da Prada M *see* Richards JG 8P
Da Prada M *see* Saura J 122P
Dalley JW & Webster RA Comparative effects of neuroleptics and apomorphine on dopamine release and metabolism in the rat caudate nucleus and medial prefrontal cortex 2P
Dalley JW & Webster RA Neuroleptic effects on apomorphine-induced inhibition of striatal neuronal firing and passage across the rat blood brain barrier 151P
Daly SA & Waddington JL Distinct antagonist profiles of two D_1 agonist-induced behaviours: differential involvement of D_1 receptor subtypes? 7P
Darles G *see* Graham D 162P
Darling M *see* Breslin E 295P
Davies J *see* Qume M 170P
Davies JE & Ng LL Simvastatin affects pH regulation of SV40 transformed MRC5 fibroblasts by inhibition of Na^+/H^+ antiport activity 203P
Davis B, Jones CA & Reynolds GP Glutamate-stimulated release of [^{3}H]-dopamine from minislices of rat frontal cortex involves more than one receptor subtype 180P
Davis S, Heal DJ, Luscombe GP & Stanford SC Short- and long-latency effects of a brief swim test on central 5-HT_2 receptor function and binding 32P
Dawson J, Edwards JCW, Sedgwick AD & Lees P Monoclonal antibody MEL-14 blocks lymphocyte migration into a site of chronic inflammation 206P
Daykin K *see* Hall IP 71P
de Boer J *see* Shahid M 129P
de Nucci G *see* Withrington PG 306P
De Souza RJ *see* Anderson SMP 13P
Deakin AM *see* Boughton-Smith NK 208P
Deasy PB *see* Lambert MN 190P
Den Boer MO, Villalón CM & Saxena PR 5-HT_1-like receptors in the porcine carotid circulation: do they belong to the 5-HT_{1D} receptor subtype? 280P
Dennes RP, Barnes JC, Michel AD & Tyers MB The effect of the AT_1 receptor antagonist, losartan (DuP 753) on cognitive performance in the radial maze and in a delayed non-matching to position task in the rat 88P
Dennes RP, Irlam-Hughes JJ & Barnes JC Captopril-induced dipsogenicity in the rat: effects of the non-peptide angiotensin II antagonists losartan (DuP 753) and PD123177 262P
Dewar D *see* Ikeda M 255P
Dewar D *see* Kieda M 121P
Dewhurst DG, Hardcastle JE, Hardcastle PT & Williams AD A computer simulation to teach the principles of nutrient transport in the small intestine 314P
Dewhurst DG, Mazzoni J, Morley J, Page C & Ullyott RT A computer simulation to teach the essentials of respiratory pharmacology 313P
Dexter DT *see* Sian J 178P
Dickenson AH, Kalso EA, Sullivan AF & McQuare HJ Cross-tolerance between α-adrenergic and μ opioid but not δ opioid antinociception in the rat spinal cord 139P
Dickenson AH *see* Chapman V 138P
Dickenson JM & Hill SJ Histamine H_1-receptor-mediated Ca^{2+} influx in the hamster vas deferens cell line DDT_1MF-2 67P
Doble A *see* Pradier L 79P
Docherty JR Effects of ageing on contractions to noradrenaline and 5-HT in human saphenous vein 305P
Docherty JR *see* Aboud RW 296P
Docherty JR *see* Breslin E 295P
Docherty JR *see* Smith K 59P
Docherty RJ *see* Arbuckle JB 156P
Dockrell MEC *see* Waugh CJ 114P
Doggrell SA & Bishop BE The effects of (±), (+) and (-)-pinacidil on the membrane potential of the rat aorta 102P
Dollery CT *see* Hans JG 246P
Domeney AM *see* Jones DNC 228P
Donigi-Gale D *see* Rossi AG 49P
Donnelly LE *see* Boyd RS 215P
Donnelly LE *see* MacDermot J 27P
Doods HN Effects of PP, PYY, NPY and selective analogues on the field-stimulated rat and rabbit vas deferens 268P
Dourish CT *see* Banks RJA 89P
Dray A *see* Patel IA 136P
Dray A *see* Rueff A 37P
Duncan GS *see* Peers SH 48P
Dupuy A *see* Tomkinson A 70P
Durcan MJ & Morgan PF Naloxone attenuates caffeine-induced hypothermia 169P
Dursun SM & Handley SL Comparison of ritanserin, haloperidol, pimozide and clonidine effects on spontaneous and DOI head-shake 225P
Dutia MB *see* Johnston AR 222P
Dyerberg C *see* Andersen LB 281P

Eaton SA & Salt TE Actions of four endogenous excitatory amino acids on NMDA receptors of rat ventrobasal thalamic neurones *in vivo* 15P
Ebbert M *see* Wilhelm D 284P
Ebenezer IS Effects of 8-OH-DPAT on operant food intake in food-deprived rats 220P
Edwards G *see* Ismail S 189P
Edwards JCW *see* Dawson J 206P
Edwards R *see* Rossi AG 49P
Eglen RM, Alvarez R, Wong EHF, Johnson L, Perkins LA, Ramsey S, Loury DN, Tang H-M, Wallace S & Whiting RL Characterization of the interaction of SDZ 205,557 at putative 5-HT_4 receptors 275P
Eglen RM, Harris GC, Cox H, Stefanich E, Sullivan A & Whiting

RL Characterization of the interaction of imperialine at muscarinic receptors *in vitro* 105P
Eglen RM *see* Wong EHF 33P
Eigenhuis JJ, Leuwin RS, Piek T & van Wilgenburg H Diazepam causes a voltage-dependent block of action potentials in isolated frog muscles 174P
Elliott JM & Phipps S Differential effects of monovalent cations on GTP-sensitive states of the 5-HT_{1A} receptor 223P
Elliott PJ *see* Mason GS 251P
Elwood W *see* Sakamoto T 244P
Emonds-Alt X *see* Advenier C 77P
Ennis M & Trimble ER Histamine release from mast cells derived from obese and lean Zucker rats 55P
Entzeroth M *see* van Meel JCA 257P
Erdbrügger W, Bauch H-J & Michel MC Three α-adrenoceptor subtypes exist in porcine aortic smooth muscle 230P
Ettlinger CB *see* Tomlinson DR 68P
Evans RA, Lang D & Lewis MJ The effect of potassium channel blocking agents on endothelium-dependent relaxation in the rabbit aorta 291P

Faccioli LH *see* Weg VB 127P
Fairbairn SM, Lees P, Page CP & Cunningham FM Neutrophil recruitment to the airways of normal horses in response to intravenous PAF 124P
Fan T-PD *see* Hori Y 212P
Fan T-PD *see* Hu DE 211P
Fardin V, Foucault F, Bock MD & Garret C Binding profile of RP 67580, a new non-peptide Substance P antagonist: comparison with CP-96,345 80P
Feltz P *see* Krupp J 249P
Ferro A, Kaumann AJ & Brown MJ ß-adrenoceptor subtypes and function in human internal mammary artery and saphenous vein 229P
Ferro A *see* Brown MJ 287P
Finn D *see* Zetterström T 218P
Fitzgerald MF *see* Russell VP 125P
Fleetwood-Walker S, Hope PJ, Molony V, Parker RMC, Munro F & Mitchell R Distinct effects of somatostatin-14 and somatostatin-28 on nociceptive responses of spinal dorsal horn neurones 132P
Flower RJ *see* Peers SH 48P
Flower RJ *see* Perretti M 44P
Flower RJ *see* Taylor AD 57P
Follenfant RL *see* Boughton-Smith NK 208P
Fone KCF *see* Aspley S 43P
Fone KCF *see* Sharma A 219P
Fone KCF *see* Watson CD 253P
Ford APDW, Taneja DT & Clarke DE Atypical ß-adrenoceptor-mediated relaxation and cyclic AMP formation in tunica muscularis mucosae (TMM) of the rat oesophagus 235P
Forster C, Naik G & Armstrong PW Sequential measurement of plasma dihydroxyphenylalanine and dihydroxyphenylglycol from dogs with pacing-induced heart failure 106P
Forster LA, Katsura M & Änggård EE The oxidative modification of low-density lipoprotein by polymorphonuclear leukocytes 47P
Foster G *see* Ali Z 38P
Foster KA *see* Taylor SG 247P
Foucart S *see* Murphy TV 63P
Foucault F *see* Fardin V 8P
Foulkes R & Shaw S Recombinant human TNF induces a non-constitutive form of nitric oxide-synthase in the endothelium and smooth muscle 292P
Fowler LJ *see* Qume M 170P
Fozard JR *see* Hoyer D 29P
Fuglsang A *see* Aidulus DM 299P
Fuller RW *see* Hans JG 246P

Galzin AM *see* Briand V 82P
Gao B & Cutler MG An etho-pharmacological study of the effects in mice of the ß-adrenoceptor antagonist, propranolol 159P
Gao B & Cutler MG Effects of ritanserin on the behaviour of mice during social encounters 226P
García-Sevilla JA *see* Sastre M 157P
Gardiner SM, Kemp PA, Bennett T, Palmer RMJ & Moncada S Renal vasodilator responses to acetylcholine in Brattleboro rats before, during and after chronic oral ingestion of N^G-monomethyl-L-arginine(L-NMMA) 93P
Gardiner SM, Kemp PA & Bennett T Regional haemodynamic effects of noradrenaline and tyramine in conscious Long-Evans rats 200P
Gardiner SM *see* Widdop RE 86P
Garland LG *see* Uings IJ 204P
Garret C *see* Fardin V 8P
Ge J *see* Cheng CHK 36P
Gerber G *see* Thompson SWN 137P
Gibson A *see* Hobbs AJ 90P
Gibson IC, Spanswick D, Pickering AE & Logan SD Some pharmacological properties of non-NMDA receptors in rat sympathetic preganglionic neurones *in vitro* 19P
Giles H & Lansdell SJ Increased potency of contractile agonists in atheroma-like vascular lesions in the rabbit is not due to endothelial dysfunction 202P
Gill J *see* Jeremy JY 51P
Goudie AJ, Harrison AA, Leathley MJ & Taylor MAI Somatic and affective benzodiazepine withdrawal signs after short term chlordiazepoxide treatment in rats 171P
Gougat J *see* Nisato D 84P
Goulaouic P *see* Advenier C 77P
Gouliaev AH & Nedergaard OA Release of [^{3}H]-5-HT from rabbit isolated aorta evoked by electrical-field stimulation 271P
Gozzard N *see* Hughes B 185P
Graham D, Darles G & Langer SZ The cytoprotective properties of ifenprodil in neuronal cell culture injury models 162P
Graham D *see* Pimoule C 233P
Graham EA *see* Mason GS 251P
Grahame-Smith DG *see* Zetterström T 218P
Grasby P *see* Opacka-Juffry J 224P
Grassi-Kassisse D *see* Withrington PG 306P
Gray AM, Kirkham LJ & Sewell RDE Potentiation of amitryptyline-induced antinociception by acetorphan 143P
Gray JA *see* Mitchell SN 9P
Green AR *see* Cross AJ 20P
Green AR *see* Watson WP 160P
Green M *see* Growcott JW 232P
Greenstein BD *see* Bridges E de F 196P
Gresham GA *see* Hori Y 212P
Gresham GA *see* Hu DE 211P
Grewal SS *see* Kennett GA 173P
Grewal SS *see* Kennett GA 227P
Griffith TM *see* Randall MD 290P
Growcott JW, Holloway B, Wilson C, Mainwaring S & Green M The novel brown adipocyte stimulant, ICI D7114, reveals possible differences in atypical ß-adrenoceptor reserve 232P
Guiraudou P *see* Nisato D 84P
Gupta P A 5-HT_1-like receptor mediates an endothelium-dependent relaxation in guinea-pig isolated jugular vein 98P
Gurden MF & Kennedy I Characterization of the adenosine receptors in dog coronary artery, guinea-pig aorta and guinea-pig fundus 115P
Gurden MF *see* Croning MDR 303P

Hagan RM *see* Birch PJ 134P
Hagan RM *see* Stables JM 250P
Hall IP, Townsend P, Daykin K & Widdop S Control of tissue cyclic AMP content in primary cultures of human airway smooth muscle cells 71P
Hall IP & Widdop S Desensitisation of isoprenaline-induced [^{3}H]-cyclic AMP formation in primary cultures of human airway smooth muscle cells 72P
Hall JA *see* Kaumann AJ 283P
Hamon G, Jouquey S, Chevillard C, Mouren M & Stepniewski JP Evidence that the prodrug trandolapril, a new ACE inhibitor, also displays a direct ACE inhibitory activity 201P
Handley SJ *see* Dursun SM 225P
Handley SL *see* McBlane 221P

Hans JG, O'Shaughnessy KM, Dollery CT, Taylor GW & Fuller RW PAF-induced bronchoconstriction and neutrophil accumulation in the rabbit lung *in vivo* 246P
Hardcastle JE *see* Dewhurst DG 314P
Hardcastle PT *see* Dewhurst DG 314P
Harding SE *see* Brady AJB 96P
Harris GC *see* Eglen RM 105P
Harrison AA *see* Goudie AJ 171P
Harrison SM *see* Birch PJ 134P
Harrison SM *see* Rogers H 81P
Harvey JM *see* Reynolds DJM 165P
Hawcock AB & Barnes JC Characterisation of the contractile responses to angiotensin II analogues in the guinea-pig ileum 260P
Hayes AG *see* Birch PJ 134P
Haynes WG *see* Waugh CJ 114P
Hayward J *see* Wilson C 234P
Headley PM *see* Herrero JF 140P
Heal DJ *see* Davis S 32P
Henderson G *see* Hicks GA 6P
Hendriks MGC, Pfaffendorf M & van Zwieten PA Characterization of muscarinic receptors in isolated perfused mesenteric vascular bed of SHR and normotensive control rats 101P
Hermansen K *see* Andersen LB 281P
Herrero JF & Headley PM Functional evidence for κ receptor heterogeneity between spinal and supraspinal sites 140P
Heuillet E *see* Menager J 214P
Hewitt MJ *see* Watson CD 253P
Hey C *see* Racké K 243P
Hicks GA & Henderson G The effects of dopamine on rat substantia nigra zona compacta neurones studied using the whole cell recording technique 6P
Higgins GA & Sellers EM Behavioural effects of the 5-HT_3 receptor agonists 1-phenylbiguanide and *m*-chlorophenylbiguanide in rats 35P
Higgs G *see* Hughes B 185P
Hilditch A *see* Travers A 197P
Hiley CR, Jones CR & Pelton JT Use of selective inhibitors of [^{125}I]-endothelin-1 binding shows that rat aortic homogenates contain both ET_A and ET_B receptors 264P
Hill JA *see* Rogers H 258P
Hill SJ *see* Dickenson JM 67P
Hill SJ *see* Iredale PA 119P
Hill SJ *see* Marsh KA 66P
Hill SJ *see* Ruck A 10P
Hill SJ *see* White TE 311P
Hindmarsh JG *see* Rose S 3P
Hobbs AJ, Tucker JF & Gibson A A comparison of NANC- and nitroso-thiol-induced relaxations of the mouse anococcygeus muscle 90P
Hoey A, Lehmkuhl A, Wettwer E & Ravens U Differences between DPI 201-106 and BDF 9148 in positive inotropic responses of cardiac preparations from guinea-pig, rat and man 107P
Holloway B *see* Growcott JW 232P
Holloway BR *see* Wilson C 234P
Holman RB *see* Lalies MD 147P
Holtzmann SG *see* Jones DNC 40P
Hope PJ *see* Fleetwood-Walker S 132P
Hori Y, Hu DE, Fan T-PD & Gresham GA Effects of glucocorticoids and angiostatic steroids on sponge-induced angiogenesis in rats 212P
Hoyer D, Schoeffter P, Palacios JM, Kalkman HO, Bruinvels AT, Fozard JR, Siegl H, Seiler MP & Stoll A SDZ 216-525: a selective, potent and silent 5-HT_{1A} receptor antagonist 29P
Hu DE, Smither RL, Fan T-PD & Gresham GA Validation of the rat sponge model for quantitative studies on angiogenesis 211P
Hu DE *see* Hori Y 212P
Huber G *see* Saura J 122P
Hubert JP *see* Pradier L 79P
Hudson AL, Mallard NJ, Wilson J, Tyacke R & Nutt DJ Affinity and selectivity of 6 and/or 7 aromatic substituted analogues of idazoxan for rabbit brain non-adrenoceptor idazoxan binding sites 158P
Hughes B, Gozzard N & Higgs G Differential effects of phosphodiesterase isoenzyme inhibitors (PDEIs) in reversing induced tone in guinea-pig trachea 185P
Hunter AJ *see* Smith CPS 166P
Hunter AJ *see* Wright PW 161P
Hutson PH *see* Patel S 254P

Ibbotson T & Weston AH Interaction of potassium channel openers with [^{3}H]-idazoxan binding at the non-adrenergic idazoxan binding site in the rat liver 62P
Ikeda M, Dewar D & McCulloch J [^{3}H]-PN200-110 binding sites are reduced in discrete regions of the hippocampus in Alzheimer's disease 121P
Ikeda M, Dewar D & McCulloch J Preservation of [^{125}I]-galanin binding sites despite loss of choline acetyltransferase activity in the hippocampus in Alzheimer's disease 255P
Iredale PA, Kendall DA, Martin KF & Hill SJ Comparison of calcium mobilisation responses to different agonists in undifferentiated N1E-115 cells: modulatory effects of phorbol esters 119P
Ireland SJ *see* Birch PJ 134P
Ireland SJ *see* Stables JM 250P
Irlam-Hughes JJ *see* Dennes RP 262P
Irvin CG *see* Coyle AJ 126P
Ismail S, Back DJ & Edwards G The effect of malaria infection on the glucuronidation of AZT and paracetamol *in vitro* 189P

Jackson HC & Nutt DJ Opioid receptor involvement in the anticonvulsant action of phenytoin 144P
James IF *see* Arbuckle JB 156P
Jansen JWCM *see* van Giezen JJJ 56P
Jenner P *see* Lange KW 152P
Jenner P *see* Rose S 3P
Jenner P *see* Sian J 178P
Jenner P *see* Smith LA 153P
Jenner P *see* Thomas K 5P
Jennewein HK *see* Patel D 142P
Jeremy JY, Sifakis G, Gill J & Kontoghiorghes G Copper chelators inhibit cyclooxygenase and lipoxygenase activity by washed human platelets, *in vitro* 51P
Jia H *see* Brown MJ 287P
John G *see* Leboeuf J 112P
Johns EJ *see* Munavvar AS 58P
Johnson L *see* Eglen RM 275P
Johnston AR, Dutia MB & McQueen DS Effects of 5-HT on rat medial vestibular nucleus neurones *in vitro* 222P
Johnston HB, Thomas SM & Atterwill CK Are changes in neural metabolism following exposure to aluminium chloride in cell culture associated with free radical production? 168P
Jones CA *see* Davis B 180P
Jones CA *see* Zempléni É 179P
Jones CR *see* Hiley CR 264P
Jones DNC, Carey GJ, Costall B, Domeney AM, Naylor RJ & Tyers MB The 5-HT_3 receptor antagonist, GR68755, prevents a scopolamine-induced cognitive impairment in the common marmoset 228P
Jones DNC & Holtzmann SG Evidence for the involvement of central δ opioid receptors in the interaction between opioid antagonists and amphetamine 40P
Joseph S & MacDermot J Leupeptin identifies multiple pathways for activation of U937 human monocytic cells by thrombin 205P
Joseph S & MacDermot J Thrombin stimulates actin polymerization in U937 human monocytic cells 54P
Joseph S *see* Clesham G 297P
Joseph S *see* Parsaee H 289P
Jouquey S *see* Hamon G 201P

Kalkman HO *see* Hoyer D 29P
Kalso EA *see* Dickenson AH 139P
Karlsson J-A *see* Tomkinson A 70P
Katsura M *see* Forster LA 47P
Kaumann AJ, Sanders L, Hall JA, Murray KJ & Brown MJ Stimulation of $ß_1$- and $ß_2$-adrenoceptors in human ventricular myocardium from failing hearts hastens the onset of relaxation 283P
Kaumann AJ *see* Ferro A 229P
Keen M *see* Krane A 25P
Keen M *see* Malkhani J 26P

Kelly J *see* Kirkham DM 231P
Kelly PAT *see* Philip R 149P
Kelly PAT *see* Sharkey J 269P
Kemp PA *see* Gardiner SM 93P
Kemp PA *see* Gardiner SM 200P
Kemp PA *see* Widdop RE 86P
Kendall DA *see* Iredale PA 119P
Kendall DA *see* Ruck A 10P
Kennedy C Chronic exposure to morphine does not induce dependence at the level of the calcium current in human SH-SY5Y cells 41P
Kennedy I *see* Croning MDR 303P
Kennedy I *see* Gurden MF 115P
Kennett GA, Grewal SS, Upton N & Blackburn TP Reversal of diazepam withdrawal anxiety by BRL 46470A, a novel 5-HT_3 receptor antagonist 173P
Kennett GA & Grewal SS Effect of 5-HT_3 receptor agonists on rat food intake 227P
Kettle CJ *see* Cheetham SC 154P
Khan RM & Zar MA Effect of stimulation-frequency on inhibition by neuropeptide Y of electrically-evoked twitches in rat isolated vas deferens 74P
Kilpatrick GJ *see* Clapham J 15P
Kilpatrick GJ *see* Clapham J 42P
Kirkham DM & Kelly J Direct *in vitro* comparison of the rat atypical ß-adrenoceptors mediating white adipocyte lipolysis and colonic relaxation 231P
Kirkham LJ *see* Gray AM 143P
Kirkman E, Marshall HW & Little RA Modulation by anaesthetic agents of the reflex cardio-inhibitory responses to activation of the baroreflex and the cardiopulmonary C-fibre afferents in the rat 31P
Kontoghioghes G *see* Jeremy JY 51P
Krane A & Keen M Coupling of prostacyclin (IP) receptors to Gs during IP receptor desensitization 25P
Kruk Z *see* Wieczorek W 217P
Krupp J & Feltz P Patch-clamp recording in lateral horn cells of rat spinal cord in a slice preparation: mediation of substance P effects by NK_1 receptors 249P
Kunde K *see* Brodde O-E 108P

Lacour C *see* Nisato D 84P
Lagrasta C *see* Monopoli A 198P
Lalies MD & Holman RB Effects of acute and chronic buprenorphine treatment on dopamine release in the nucleus accumbens of conscious rats 147P
Lamb JF *see* Ameen M 207P
Lambert DG *see* Tobin AB 65P
Lambert JDC *see* Whittington MA 14P
Lambert MB, Omara AAM & Deasy PB Feasibility of non-steroidal anti-inflammatory drugs for transdermal delivery: effect of vehicles *in vitro* 190P
Landon M *see* Sharma A 219P
Lang Ð *see* Evans RA 291P
Lange KW, Löschmann P-A, Wachtel H, Jenner P & Marsden CD Locomotor activity in behaviourally disabled and recovered MPTP-treated marmosets after administration of the dopamine partial agonist terguride 152P
Langer SZ *see* Graham D 162P
Langer SZ *see* Pimoule C 233P
Lansdell SJ *see* Giles H 202P
Laude EA & Morice AH The effect of nedocromil sodium on citric acid and capsaicin cough challenge in the guinea-pig 294P
Lavicky J *see* Steinfath M 282P
Laville M *see* Pradier L 79P
Le Fur G *see* Advenier C 77P
Le Fur G *see* Nisato D 84P
Le Guern S *see* Pradier L 79P
Leathard H *see* Bhupalan A 239P
Leathley MJ *see* Goudie AJ 171P
Leboeuf J, Baissat J, John G & Massingham R Inhibition of veratrine-induced contracture by dopropidil, a new antianginal drug 112P
Lees P *see* Dawson J 206P
Lees P *see* Fairbairn SM 124P
Lehmkuhl A *see* Hoey A 107P
Leideg A *see* Wilhelm D 284P
Leslie RA *see* Reynolds DJM 165P
Leuwin RS *see* Eigenhuis JJ 174P
Levin RJ *see* Przyborski SA 237P
Lewis MJ *see* Evans RA 291P
Libon R *see* Christophe B 259P
Lin Y-SR *see* Quilley CP 193P
Little HJ *see* Watson WP 160P
Little HJ *see* Whittington MA 14P
Little RA *see* Kirkman E 31P
Liu K-L *see* Zhang B-L 191P
Liu YJ, Shankley NP, Welsh NJ & Black JW Development and application of a model of angiotensin-II receptor ligand interactions in isolated smooth muscle assays 266P
Liu YJ, Shankley NP, Welsh NJ & Black JW Pharmacological analysis of angiotensin-II receptor ligand interactions in the isolated rabbit aorta assay 87P
Löffler J *see* Saura J 122P
Logan SD *see* Gibson IC 19P
Long SK & Cramer WCM Cholecystokinin-induced depolarization of neonate rat spinal cord *in vitro* 256P
Long SK *see* Roberts MHT 28P
Lopez-Belmonte J, Whittle BJR & Moncada S Effects of close-arterial infusion of nitrovasodilators on rat gastric mucosal integrity 95P
Long SK *see* Roberts MHT 28P
Lopez-Garcia JA *see* Roberts MHT 28P
Löschmann P-A *see* Lange KW 152P
Loury DN *see* Eglen RM 275P
Lowe KC & Armstrong FH Effects of perfluorochemicals on liver microsomal enzymes in rats 188P
Lowenstein PR *see* Reynolds DJM 165P
Loxley HD *see* Taylor AD 57P
Luscombe GP *see* Davis S 32P
Lynn B & Cotsell B Action of topical capsaicin on blood flow and C-fibre nociceptors in the skin of anaesthetized rats 131P

McBlane JW, Critchley MAE & Handley SL Light intensity influences the response to 8-OH-DPAT in the elevated x-maze model of anxiety 221P
McCarthy AL, Raju KS, Woolfson RG & Poston L Endothelial function and sensitivity to vasoconstrictor agents in isolated small human feto-placental arteries 94P
McCreary AC & Marsden CA Antagonism of cocaine-induced hyperactivity and stereotypy by the D_1 antagonist SCH 23390 150P
McCulloch J *see* Ikeda M 255P
McCulloch J *see* Kieda M 121P
MacDermot J, Donnelly LE & Boyd RS ADP-ribosyltransferase activity of NG108-15 cells modifies the structure, activity and abundance of $G_s\alpha$ 27P
MacDermot J *see* Boyd RS 215P
MacDermot J *see* Clesham G 297P
MacDermot J *see* Joseph S 54P
MacDermot J *see* Joseph S 205P
MacDermot J *see* Parsaee H 289P
McDermott AM, Watson CD & Nutt DJ High relative affinity of RX77368 for the rat retrosplenial cortex 252P
MacDonald AE *see* Asghar AUR 309P
McEwan JR *see* Clesham G 297P
McEwan JR *see* Parsaee H 289P
McGiff JC *see* Carroll MA 194P
McGiff JC *see* Quilley CP 193P
McGrath JC *see* Shaw AM 92P
Maclagan J *see* Watson N 78P
Maclagan J *see* Wessler I 69P
MacLean MR & Nally J Hypoxia-induced vasoconstriction in the isolated rabbit pulmonary artery: total inositol phosphate accumulation and protein kinase C activation 308P
McLees A *see* Sneddon P 238P
McQuare HJ *see* Dickenson AH 139P
McQueen DS *see* Asghar AUR 309P
McQueen DS *see* Johnston AR 222P
Mainwaring S *see* Growcott JW 232P
Majewski H *see* Murphy TV 63P
Major JS *see* Oldham AA 83P
Malherbe P *see* Richards JG 8P
Malkhandi J & Keen M The influence of GTP concentration on the dose/response curves for prostacyclin receptor agonists 26P
Mallard NJ *see* Hudson AL 158P
Mann GE *see* Bogle RG 91P
Manning AS *see* Christophe B 259P

Manning AS *see* Christophe B 304P
Margiotta P *see* Carroll MA 194P
Marsden CA *see* Aspley S 43P
Marsden CA *see* Beckett SRG 177P
Marsden CA *see* McCreary AC 150P
Marsden CD *see* Lange KW 152P
Marsden CD *see* Rose S 3P
Marsden CD *see* Sian J 178P
Marsden CD *see* Smith LA 153P
Marsden CD *see* Thomas K 5P
Marsh KA & Hill SJ Bradykinin-induced phosphoinositide hydrolysis and calcium mobilisation in cultured bovine tracheal smooth muscle cells 66P
Marshall FH, Barnes JC & Michel AD Characterization of angiotensin II stimulation of phosphoinositide metabolism in rat hepatocytes 183P
Marshall HW *see* Kirkman E 31P
Marshall K *see* Yeardley HL 241P
Marshall PW *see* Beckett SRG 177P
Marshall PW *see* Curwen JO 245P
Martin GR *see* Anderson IK 30P
Martin KF *see* Cheetham SC 154P
Martin KF *see* Iredale PA 119P
Martin PL & Cusack NJ Halogen substituted N^6-endonorbornyl-9-methyladenines are potent and selective A_1-adenosine receptor antagonists 116P
Martin SW & Broadley KJ Comparison of the vascular responses to dopexamine and fenoldopam in noradrenaline- and U46619-constricted rat kidney 192P
Mason GS, Graham EA & Elliott PJ NK_1 receptors in the guinea-pig mediate locomotor hyperactivity 251P
Mason R *see* Scott G 16P
Massingham R *see* Leboeuf J 112P
Mathy MJ *see* Pfaffendorf M 293P
Mazzoni J *see* Dewhurst DG 313P
Meldrum BS *see* Smith SE 21P
Menager J & Heuillet E NK_1 tachykinin receptors on the U373MG cell line: coupling to inositol phosphate formation 214P
Mertens MJF, Batink HD, Pfaffendorf M & van Zwieten PA Impaired response of SHR hearts to α_1-adrenoceptor stimulation caused by changes in post-receptor events 109P
Michel AD *see* Dennes RP 88P
Michel AD *see* Marshall FH 183P
Michel MC, Broede A, Michel-Reher M, Zerkowski H-R & Brodde O-E Chronic β_1-adrenoceptor antagonist treatment does not affect activity of G_s-protein or adenylate cyclase in human right atrium 64P
Michel MC *see* Büscher R 184P
Michel MC *see* Erdbrügger W 230P
Michel-Reher M *see* Michel MC 64P
Miller KW *see* Tonner PH 187P
Millican SA, Webster J & Wallace HM Hydrogen peroxide-mediated damage in human vascular endothelial cells 302P
Millican SA *see* Wallace HM 301P
Millns PJ *see* Ruck A 10P
Minisclou C *see* Carter C 18P
Miranda L *see* Cirino G 104P
Misra A *see* Cross AJ 20P
Misra A *see* Watson WP 160P
Mitchell R *see* Fleetwood-Walker S 132P
Mitchell SN & Gray JA Systemic administration of nicotine increases hippocampal noradrenaline release *in vivo* largely by an action at the locus coeruleus 9P
Mo N & Wallis DI Properties of rat stellate ganglion cells: comparison of putative cardiac-projecting and non cardiac neurones 248P
Mocada S *see* Gardiner SM 93P
Mollace V *see* Salvemini D 97P
Molony V *see* Fleetwood-Walker S 132P
Moncada S *see* Lopez-Belmonte J 95P
Monopoli A, Cigola E, Ricci R & Ongini E Effects of repeated administration of SCH 34826, a neutral endopeptidase inhibitor, on myocardial hypertrophy in the spontaneously hypertensive rat 199P
Monopoli A, Ongini E, Lagrasta C & Olivetti G Effects of long-term administration of the ACE inhibitor, spirapril, on development of hypertension and myocardial hypertrophy in spontaneously hypertensive rats 198P
Morgan PF *see* Durcan MJ 169P
Morice AH *see* Laude EA 294P
Morley J *see* Dewhurst DG 313P
Motheith S *see* Brown MJ 287P
Mouren M *see* Hamon G 201P
Munavvar AS & Johns EJ Evaluation of the α_1-adrenoceptor subtypes in the renal vasculature of the stroke-prone spontaneously hypertensive rat 58P
Munro F *see* Fleetwood-Walker S 132P
Murphy CT & Westwick J Modulation of the activation of platelets by genistein, an inhibitor of tyrosine kinase(s) 52P
Murphy TV, Foucart S & Majewski H Prejunctional α_2-adrenoceptors in mouse atria function through G-proteins which are sensitive to *N*-ethylmaleimide, but not pertussis toxin 63P
Murray KJ *see* Kaumann AJ 283P
Murray TK *see* Cross AJ 20P
Murray-Lyon IML *see* Bhupalan A 239P

Nahorski SR *see* Challiss RAJ 24P
Nahorski SR *see* Tobin AB 65P
Naik G *see* Forster C 106P
Naline E *see* Advenier C 77P
Nally J *see* MacLean MR 308P
Nandha KA *see* Benito-Orfila MA 267P
Naylor RJ *see* Cheng CHK 36P
Naylor RJ *see* Jones DNC 228P
Naylor RJ *see* Rudd JA 274P
Naylor RJ *see* Tuladhar BR 278P
Nebel U *see* Wermelskirchen D 103P
Nedergaard OA *see* Gouliaev AH 271P
Neliat G *see* Advenier C 77P
Nelson H *see* Aceto JF 110P
Nelson N *see* Aceto JF 110P
Newberry NR, Watkins CJ & Sprosen TS BRL 46470A antagonises 5-HT_3 receptor-mediated responses on the rat vagus and on NG108-15 cells 276P
Ng LL *see* Davies JE 203P
Nicholson CD *see* Shahid M 129P
Nicoll D *see* Aceto JF 110P
Nisato D, Cazaubon C, Lacour C, Gougat J, Guiraudou P, Bernhart C, Perreaut P, Brelière JC & Le Fur G Pharmacological properties of SR 47436, a non-peptidic angiotensin II receptor antagonist 84P
Norman KE *see* Rossi AG 49P
Nourshargh S *see* van Uexkull 209P
Nutt DJ *see* Hudson AL 158P
Nutt DJ *see* Jackson HC 144P
Nutt DJ *see* McDermott AM 252P

Oblin A, Bidet S & Schoemaker H Polyamines modulate the binding of the NMDA antagonist [^{3}H]CGP 39653 via the glycine site 182P
Oldham AA, Allott CP, Major JS, Pearce RJ, Roberts DA & Russell ST ICI D8731: a novel, potent and orally effective angiotensin II antagonist 83P
Oldham AA *see* Allott CP 261P
Oliveira JC, Sebastião AM & Ribeiro JA The frog brain possesses one binding site for [^{3}H]-DPCPX different from the A_1 or the A_2 adenosine receptors 176P
Olivetti G *see* Monopoli A 198P
Olverman HJ *see* Waugh CJ 114P
Omara AAM *see* Lambert MN 190P
O'Neill MF *see* Banks RJA 89P
Ongini E *see* Monopoli A 198P
Ongini E *see* Monopoli A 199P
Opacka-Juffry J, Ahier RG, Grasby P, Cunningham V, Cremer JE & Sarna GS Effect of buspirone on regional cerebral blood flow determined in conscious rats 224P
O'Shaughnessy CT *see* Waldron GJ 272P
O'Shaughnessy KM *see* Hans JG 246P

Page C *see* Dewhurst DG 313P
Page CP *see* Fairbairn SM 124P
Palacios JM *see* Hoyer D 29P
Palmer RMJ *see* Gardiner SM 93P
Parker RMC *see* Fleetwood-Walker S 132P
Parratt JR *see* Wainwright CL 117P

Parsaee H, Joseph S, MacDermot J & McEwan JR The role of calcium in the independent regulation of the release of EDRF and prostacyclin from bovine aortic endothelial cells 289P
Parsaee H *see* Clesham G 297P
Patel D, Jennewein HK, Schmidhammer H & Traynor JR *In vitro* evaluation of the opioid antagonist 3-hydroxycyprodime 142P
Patel IA, Rueff A, Urban L & Dray A Concanavalin-A prevents cyclic-GMP-induced desensitization of bradykinin on peripheral nociceptors in the neonatal rat *in vitro* 136P
Patel N *see* Challiss RAJ 24P
Patel S & Hutson PH Effect of galanin on hypothermia in mice induced by RS86 and 8-OH DPAT: involvement of ATP-sensitive potassium channels 254P
Paterson SJ & Cunningham JR Opioid binding sites in bovine retina 145P
Patten C & Bull DR Apamin differentiates between quinpirole- and BHT920-induced inhibition of dopamine overflow *in vitro* 146P
Peachell PT *see* Peirce MJ 123P
Pearce RJ *see* Allott CP 261P
Pearce RJ *see* Oldham AA 83P
Pearson JD *see* Bogle RG 91P
Pearson S *see* Zempléni É 179P
Peek AR *see* Ameen M 207P
Peers SH, Duncan GS, Bolton C & Flower RJ Importance of endogenous corticosteroids in the control of the cellular immune response in the Brown Norway rat 48P
Pei Q *see* Zetterström T 218P
Peirce MJ, Warner JA & Peachell PT Effect of okadaic acid on histamine release from activated human lung mast cells and basophils 123P
Pelton JT *see* Hiley CR 264P
Perkins LA *see* Eglen RM 275P
Perreaut P *see* Nisato D 84P
Perretti M & Flower RJ Lipocortin-1 inhibits the neutrophil infiltration elicited by interleukin-1 in the mouse air-pouch 44P
Peters T *see* Wermelskirchen D 103P
Peters Th *see* Wilhelm D 284P
Pettinger SJ *see* Wilson C 234P
Pfaffendorf M, Mathy MJ & van Zwieten PA Differential effects of various dihydropyridine calcium antagonists on the contraction pattern of rat coronary artery stimulated with high potassium 293P
Pfaffendorf M & van Zwieten PA Characteristics of muscarinic receptors in the rat isolated portal vein 100P
Pfaffendorf M *see* Hendriks MGC 101P
Pfaffendorf M *see* Mertens MJF 109P
Pfaffendorf M *see* Pijl AJ 111P
Philip R, Sharkey J & Kelly PAT Acute cocaine produces focal uncoupling of blood flow from metabolic demand in sub-regions of the rat striatum 149P
Philipp T *see* Büscher R 184P
Phillips JA & Steward A The hypocalcaemic response to intravenous calcitonin in juvenile rats: the importance of age and other factors 270P
Phillipson KD *see* Aceto JF 110P
Philpott AJ *see* Shahid M 129P
Phipps S *see* Elliott JM 223P
Pickering AE *see* Gibson IC 19P
Piek T *see* Eigenhuis JJ 174P
Pijl AJ, Pfaffendorf M & van Zwieten PA Nifedipine, lacidipine and nisoldipine in the rat isolated working heart: a comparative study 111P
Pile AJ & Smaje LH The H_3 antagonist thioperamide inhibits the permeability effect of histamine in rat microvessels via an H_1 receptor effect 210P
Pimoule C, Graham D & Langer SZ Characterization of the α_{1B}-adrenoceptor gene product expressed in a stably transfected HeLa cell-line 233P
Pinto A *see* Cirino G 104P
Pistelli A *see* Salvemini D 97P
Pleuvry BJ *see* Rodger C 120P
Plumpton C *see* Sun B 263P
Pohan D *see* Wessler I 69P
Pollock D *see* Aidulus DM 299P
Pollock D *see* Shaw AM 92P
Poole-Wilson PA *see* Brady AJB 96P
Poppers D *see* Tonner PH 187P
Poston L *see* McCarthy AL 94P
Pradier L, Laville M, Le Guern S, Hubert JP, Doble A & Blanchard JC NK_1 tachykinin receptors on the U373MG cell line: antagonist effects of RP 67580 79P
Pratt JA *see* Brett RR 172P
Prince RJ & Simmonds MA Antagonism of the steroid recognition site of the $GABA_A$ receptor by epipregnanolone 12P
Prioetto V *see* Advenier C 77P
Przyborski SA, Levin RJ & Young A Evidence for M_3 muscarinic cholinoceptors on enterocytes which induce electrogenic secretion in rat small intestine 237P
Puttick RM Excitatory action of prostaglandin E_2 on rat neonatal cultured dorsal root ganglion cells 133P

Quilley CP, Lin Y-SR & McGiff JC Chloride anion concentration as a determinant of renal vascular responsiveness to vasoconstrictor agents 193P
Quinn NP *see* Rose S 3P
Qume M, Davies J & Fowler LJ Chronic treatment with the GABA-transaminase inhibitors EOS and GVG enhance basal GABA release from cross-chopped hippocampal slices 170P

Racké K, Bäring J, Brunn G, Hey C, Reimann A & Wessler I Characterization of muscarinic-paracrine interactions in the regulation of noradrenaline release from rat isolated trachea 243P
Racké K *see* Wessler I 69P
Raeburn D *see* Tomkinson A 70P
Raiteri M *see* Bonanno G 11P
Raju KS *see* McCarthy AL 94P
Ramage AG *see* Anderson IK 30P
Ramsey S *see* Eglen RM 275P
Randall MD & Griffith TM A role for EDRF in collateral perfusion following arterial occlusion 290P
Randall RW *see* Uings IJ 204P
Ravens U *see* Hoey A 107P
Rees H, Terenzi MG & Roberts MHT The involvement of ACh on antinociception evoked by electrical stimulation of the anterior pretectal nucleus 130P
Reeves JP *see* Aceto JF 110P
Reimann A *see* Racké K 243P
Reynolds DJM, Lowenstein PR, Harvey JM & Leslie RA Cholinergic vagal afferent fibres and presynaptic muscarinic receptor binding sites are present in the dorsal vagal complex of the ferret 165P
Reynolds GP *see* Davis B 180P
Reynolds GP *see* Zempléni É 179P
Ribeiro JA *see* Correia-de-Sá P 312P
Ribeiro JA *see* Oliveira JC 176P
Ricci R *see* Monopoli A 199P
Richards JG, Bertocci B, Da Prada M & Malherbe P Cellular expression of catechol-*O*-methyltransferase (COMT) in rat CNS and peripheral organs revealed by *in situ* hybridization histochemistry 8P
Richards JG *see* Saura J 122P
Ritchie IM *see* Sharkey J 269P
Riva L *see* Briand V 82P
Rivy JP *see* Carter C 18P
Roberts DA *see* Allott CP 261P
Roberts DA *see* Oldham AA 83P
Roberts JC *see* Wright PW 161P
Roberts MHT, Lopez-Garcia JA & Long SK Modulatory and depolarising actions of 5-HT in rat neocortex *in vitro* 28P
Roberts MHT *see* Rees H 130P
Robertson MJ *see* Wragg A 265P
Rodger C & Pleuvry BJ Anticonvulsant effects of calcium channel antagonists in combination with established anti-epileptic drugs 120P
Rogers DC *see* Wright PW 161P
Rogers H, Harrison SM & Birch PJ Behavioural effects of direct intrathecal administration of neurokinins in the weanling rat 81P
Rogers H, Hill JA & Birch PJ Calcitonin gene related peptide (CGRP) potentiates NK_1 receptor-mediated paw oedema, but not intrathecal NK_1 agonist-induced behaviour in the rat 258P

Rogers H *see* Birch PJ 134P
Rose S, Hindmarsh JG, Steiger MJ, Quinn NP, Jenner P & Marsden CD Plasma HVA levels following debrisoquine administration do not reflect brain dopamine loss in Parkinson's disease 3P
Rossi AG, Donigi-Gale D, Shoupe TS, Edwards R, Norman KE & Williams TJ The role of complement, PAF and leukotriene B_4 in the reversed passive Arthus reaction in rabbit skin 49P
Rothaul AL *see* Wright PW 161P
Ruck A, Millns PJ, Hill SJ & Kendall DA Iprindole down-regulates β_1-adrenoceptors in cultured rat C_6 glioma cells 10P
Rudd JA, Bunce KT, Cheng CHK & Naylor RJ The effect of cisplatin on plasma 5-HT, 5-HIAA and 5-HTP levels during emesis in the ferret 274P
Rueff A & Dray A Pharmacological characterization of 5-HT-induced effects on peripheral nociceptors of the neonatal rat *in vitro* 37P
Rueff A *see* Patel IA 136P
Rump LC & Bohmann C Evidence that sympathetic nerve endings of rat kidney possess α_2-autoreceptors of the α_{2A}-subtype 60P
Russell ST *see* Allott CP 261P
Russell ST *see* Oldham AA 83P
Russell VP, Thompson AM, Sturton RG & Fitzgerald MF Extent of antigen-induced eosinophilia and enhanced production of reactive oxygen species in aerosol and parenterally sensitized guinea-pigs 125P

Sacquet J *see* Zhang B-L 191P
Sakamoto T, Elwood W, Barnes PJ & Chung KF Inhaled lyso-PAF causes plasma exudation in guinea-pig airways 244P
Salt TE *see* Eaton SA 15P
Salvemini D, Mollace V, Pistelli A, Änggård E & Vane J Metabolism of glyceryl trinitrate to nitric oxide in endothelial and smooth muscle cells and the effect of *E. coli* lipopolysaccharide 97P
Sanders L *see* Kaumann AJ 283P
Sanger G *see* Wardle K 279P
Sarna GS *see* Opacka-Juffry J 224P
Sassard J *see* Zhang B-L 191P
Sastre M & García-Sevilla JA α_2-Adrenoceptors in the human brain postmortem: ratio of high affinity agonist sites to antagonist sites and effect of age 157P
Saura J, Shih JC, Da Prada M, Chan-Palay V, Ulrich J, Huber G, Löffler J & Richards JG Differential cellular expression of MAO-A and MAO-B in control versus Alzheimer brains 122P
Saxena PR *see* Bax WA 99P
Saxena PR *see* Den Boer MO 280P
Saxena PR *see* Tadipatri S 34P
Saxena PR *see* van Gelderen EM 288P
Schachter M *see* Calder JA 307P
Schäfer E *see* Brodde O-E 108P
Schmidhammer H *see* Patel D 142P
Schmitz W *see* Steinfath M 282P
Schoeffter P *see* Hoyer D 29P
Schoemaker H Further characterization of polyamine-sensitive [^{3}H]-ifenprodil binding to the NMDA receptor 17P
Schoemaker H *see* Oblin A 182P
Scholz H *see* Steinfath M 282P
Schwietert HR, Wilhelm D, Wilffert B & van Zwieten PA The contractile effect of some α-adrenoceptor antagonists in the rat portal vein and the putative involvement of ATP-sensitive K^+ channels 61P
Scott G & Mason R The effect of excitatory amino acid antagonists on retinogeniculate neurotransmission in the rat 16P
Scott LC *see* Souness JE 186P
Scott RH, Stapleton SR & Currie KPM Ca^{2+}-dependent actions of palmitoyl-DL-carnitine on cultured rat DRG neurones 118P
Sebastião AM *see* Cascalheira JF 175P
Sebastião AM *see* Oliveira JC 176P
Sedgwick AD *see* Dawson J 206P
Seigl H *see* Hoyer D 29P
Seiler MP *see* Hoyer D 29P
Sellers EM *see* Higgins GA 35P
Senior J *see* Yeardley HL 241P
Sever PS *see* Calder JA 307P
Sewell RDE *see* Gray AM 143P
Shahid M, Philpott AJ, de Boer J, Zaagsma J & Nicholson CD Cyclic nucleotide phosphodiesterase (PDE) isoenzyme activities in human peripheral bronchi 129P
Shankley NP *see* Liu YJ 87P
Shankley NP *see* Liu YJ 266P
Shapiro G *see* Boddeke HWGM 164P
Sharkey J, Ritchie IM & Kelly PAT Focal increases in cerebrovascular permeability following MK801 administration in the rat 269P
Sharkey J *see* Philip R 149P
Sharma A, Landon M & Fone KCF Regional distribution of 5-HT_{1C} and 5-HT_2 receptor peptides on the rat brain measured using polyclonal antisera 219P
Shaw AM, Pollock D, McGrath JC & Thomson JE L-N^{ω}-nitro arginine methyl ester and haemoglobin expose or enhance pressor response to 5-HT in the rat isolated perfused lung 92P
Shaw DJ *see* Taylor SG 242P
Shaw DJ *see* Taylor SG 247P
Shaw S *see* Foulkes R 292P
Sheehan MJ *see* Cammack C 148P
Shih JC *see* Saura J 122P
Shoaib M & Stolerman IP Locomotor activity after nicotine infusions into the fourth ventricle of rats 167P
Shoupe TS *see* Rossi AG 49P
Sian J, Dexter DT, Jenner P, Marsden CD & Cohen G Comparison of HPLC and enzymatic recycling assays for the measurement of oxidised glutathione levels in brain 178P
Siepmann F *see* Büscher R 184P
Sifakis G *see* Jeremy JY 51P
Simmonds MA *see* Prince RJ 12P
Simoons ML *see* Bax WA 99P
Sinclair J *see* Sun B 263P
Sivilotti L *see* Thompson SWN 137P
Smaje LH *see* Pile AJ 210P
Smith CPS, Chape A, Hunter AJ & Bennett GW Effects of chronic administration of atropine and scopolamine on elevated plus-maze behaviour in the rat 166P
Smith D, Watson ML, Bourne AD, Thompson RC & Westwick J An interleukin-1 receptor antagonist inhibits antigen-induced eosinophil accumulation and bronchial hyperreactivity in guinea-pigs 128P
Smith D *see* Bourne AD 53P
Smith K & Docherty JR Evidence for an alpha$_{2A}$-ligand binding site in rat submandibular gland 59P
Smith LA, Jenner P & Marsden CD Reversal of MPTP-induced motor deficits in common marmosets following oral or transdermal administration of piribedil 153P
Smith RD & Weston AH Studies on the inhibitory effects of guanabenz on the field-stimulated rat isolated anococcygeus muscle 236P
Smith SE & Meldrum BS Cerebroprotective effects of GYKI52466 (1-4-aminophenyl-4-methyl-7,8-methylen-dioxy-5H-2,3-benzodiazepine) after focal ischaemia in the rat 21P
Smither RL *see* Hu DE 211P
Snape MF *see* Cross AJ 20P
Sneddon P & McLees A P_{2X}-purinoceptor and muscarinic cholinoceptor mediated contractions of isolated urinary bladder smooth muscle from adult and neonatal rabbits 238P
Soares-da-Silva P Role of actin cytoskeleton in the regulation of the renal synthesis of dopamine 1P
Sorrentino R *see* Cirino G 104P
Souness JE & Scott LC Stimulation of eosinophil cyclic AMP phosphodiesterase (PDE) by vanadate/glutathione 186P
Spacey GD *see* Uings IJ 204P
Spanswick D *see* Gibson IC 19P
Sprosen TS *see* Newberry NR 276P
Stables JM, Hagan RM & Ireland SJ Tachykinins can depolarize the rat isolated superior cervical ganglion via NK_1 receptors 250P
Stamford JA, Hafizi S & Palij P Actions of the nomifensine metabolite

8-amino-2-methyl-4-(3-methoxy-4-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline on striatal dopamine efflux and uptake 4P
Stanford SC *see* Davis S 32P
Stapleton SR *see* Scott RH 118P
Steel JA *see* Allott CP 261P
Stefanich E *see* Eglen RM 105P
Steiger MJ *see* Rose S 3P
Steinfath M, Lavicky J, Schmitz W & Scholz H Distribution of β_1- and β_2-adrenoceptors in right and left atria and ventricles obtained post-mortem from nonfailing and failing human hearts 282P
Stepniewski JP *see* Hamon G 201P
Stevens EJ, Willars GB & Tomlinson DR Basal and angiotensin II-stimulated prostacyclin release by perfused rat lung in experimental diabetes: effects of insulin and aldose reductase inhibition 46P
Steward A *see* Phillips JA 270P
Stolerman IP *see* Shoaib M 167P
Stolls A *see* Hoyer D 29P
Sturton RG *see* Russell VP 125P
Sugden D Luzindole (2-benzyl *N*-acetyltryptamine) is a competitive melatonin receptor antagonist in *Xenopus laevis* melanophores 195P
Sullivan A *see* Eglen RM 105P
Sullivan AF *see* Dickenson AH 139P
Sumner MJ *see* White DG 300P
Sun B, Plumpton C, Sinclair J & Brown MJ Expression of CGRP in cultured endothelial cells with plasmid DNA 263P

Tadipatri S & Saxena PR Is 2-methyl-5-HT a selective 5-HT_3 receptor agonist? 34P
Taneja DT *see* Ford APDW 235P
Tang H-M *see* Eglen RM 275P
Taylor AD, Loxley HD, Cowell A-M, Flower RJ & Buckingham JC Evidence that lipocortin 1 mediates the inhibitory effect of dexamethasone on the secretion of prolactin by the rat anterior pituitary gland 57P
Taylor GW *see* Hans JG 246P
Taylor JF *see* Bowring NE 73P
Taylor MAI *see* Goudie AJ 171P
Taylor SG, Buckle DR, Foster KA, Shaw DJ, Ward JS & Arch JRS Evidence that the novel, airways selective benzopyranol, BRL 55834, is a potassium channel activator 247P
Taylor SG, Buckle DR, Foster KA, Shaw DJ, Ward JS & Arch JRS An investigation of the selectivities of BRL 55834 and BRL 38227 as relaxants of guinea-pig tracheal spirals relative to portal vein 242P
Teoh TG *see* Breslin E 295P
Terenzi MG *see* Rees H 130P
Thomas K, Jenner P & Marsden CD Striatal D_2 receptors are uncoupled from adenylyl cyclase following 6-hydroxydopamine lesion of the nigrostriatal pathway 5P
Thomas SM *see* Johnston HB 168P
Thompson AM *see* Russell VP 125P
Thompson NT *see* Uings IJ 204P
Thompson RC *see* Smith D 128P
Thompson SWN, Gerber G, Sivilotti L & Woolf CJ NMDA and non-NMDA receptor-mediated slow ventral root potentials recorded from the neonatal rat hemisected spinal cord *in vitro* 137P
Thomson JE *see* Shaw AM 92P
Timóteo MA *see* Correia-de-Sá P 312P
Tobin AB, Lambert DG & Nahorski SR Desensitisation of recombinant muscarinic M_3 receptor responses following short term exposure to agonist 65P
Tomkinson A, Dupuy A, Karlsson J-A & Raeburn D Comparison of the *in vitro* airway smooth muscle relaxant properties of rolipram, isoprenaline and salbutamol 70P
Tomlinson AE *see* Curwen JO 245P
Tomlinson DR & Ettlinger CB Protein kinase C and modulation of the sodium pump of peripheral nerve 68P
Tomlinson DR *see* Stevens EJ 46P
Tonner PH, Poppers D & Miller KW Anaesthetic potency of propofol is reduced by hydrostatic pressure 187P
Townsend P *see* Hall IP 71P
Travers A & Hilditch A Temporal involvement of the renin-angiotensin system in hypertension produced by left renal artery ligation in conscious rats 197P
Traynor JR *see* Bardaji E 141P
Traynor JR *see* Patel D 142P
Trezise DJ & Weston AH Comparison of the relaxant and hyperpolarising actions of calcitonin-gene related peptide (CGRP) and BRL38227 in rabbit isolated basilar artery: studies using glibenclamide 75P
Trimble ER *see* Ennis M 55P
Tucker JF *see* Hobbs AJ 90P
Tuladhar BR, Costall B & Naylor RJ Variation in the relaxation response to 5-HT in different regions of the rat small intestine 278P
Tyacke R *see* Hudson AL 158P
Tyers MB *see* Dennes RP 88P
Tyers MB *see* Jones DNC 228P

Uings IJ, Thompson NT, Bonser RW, Spacey GD, Randall RW & Garland LG The role of tyrosine phosphorylation in receptor coupling to phospholipases in the human neutrophil 204P
Ullyott RT *see* Dewhurst DG 313P
Ulrich J *see* Saura J 122P
Upton N *see* Kennett GA 173P
Urban L *see* Patel IA 136P

Van Broeck D *see* Advenier C 77P
van den Buuse M & Buisson-Defferier S Effect of MDL 73005EF on 5-HT_{1A} receptor-mediated blood pressure responses in conscious spontaneously hypertensive rats 277P
van den Ende R, Batink HD & van Zwieten PA Influence of ischaemia on binding behaviour of muscarinic agonists and antagonists in rat ventricular preparations 285P
van Gelderen EM & Saxena PR 5-HT-induced responses after N^G-nitro-L-arginine methyl ester in pithed rats 288P
van Giezen JJJ & Jansen JWCM Dexamethasone inhibits fibrinolysis *in vivo* by modulation of t-PA and PAI-1 levels 56P
van Heuven-Nolsen D *see* Bax WA 99P
van Meel JCA, Wienen W & Entzeroth M Hypotensive effects of the angiotensin II receptor antagonist DuP 753 in renal hypertensive rats 257P
van Wilgenburg H *see* Eigenhuis JJ 174P
van Zwieten PA *see* Hendriks MGC 101P
van Zwieten PA *see* Mertens MJF 109P
van Zwieten PA *see* Pfaffendorf M 100P
van Zwieten PA *see* Pfaffendorf M 293P
van Zwieten PA *see* Pijl AJ 111P
van Zwieten PA *see* Schwietert HR 61P
van Zwieten PA *see* van den Ende R 285P
Van-Zwieten PA *see* Zhang JS 85P
Vane J *see* Salvemini D 97P
Vilain P *see* Advenier C 77P
Villalón CM *see* Den Boer MO 280P
Vohra MM & Willams B Determining 5-HT by reverse phase h.p.l.c. and electrochemical detection after precolumn derivatization with OPA-2ME 216P
von Uexkull C, Nourshargh S & Williams TJ Vasodilator mediators induced by live *E. coli* in the skin 209P

Wachtel H *see* Lange KW 152P
Waddington JL *see* Daly SA 7P
Wainwright CL & Parratt JR The anti-arrhythmic effects of the A_1-adenosine agonist R-PIA are mediated by brady-cardia 117P
Waldron GJ, O'Shaughnessy CT & Connor H Sumatriptan does not modify capsaicin-induced relexation of guinea-pig isolated basilar artery 272P
Wallace A *see* Allen MJ 240P
Wallace HM, Millican SA & Aung T A possible role for polyamines in the growth of endothelial cells 301P

Wallace HM *see* Millican SA 302P
Wallace S *see* Eglen RM 275P
Wallis DI *see* Mo N 248P
Ward JS *see* Taylor SG 242P
Ward JS *see* Taylor SG 247P
Wardle K & Sanger G Guinea-pig distal colon: a sensitive model of the putative 5-HT_4 receptor 279P
Warner JA *see* Peirce MJ 123P
Warren JB *see* Brady AJB 96P
Watkins CJ *see* Newberry NR 276P
Watson CD, Hewitt MJ, Fone KCF & Bennett GW Behavioural and biochemical effects of the TRH analogue RX77368 and scopolamine on radial arm maze performance in the rat 253P
Watson CD *see* McDermott AM 252P
Watson ML *see* Bourne AD 53P
Watson ML *see* Smith D 128P
Watson ML *see* Weg VB 127P
Watson N, Maclagan J & Barnes PJ Demonstration of a facilitatory role of endogenous tachykinins in cholinergic neurotransmission in guinea-pig trachea 78P
Watson WP, Misra A, Little HJ & Green AR Felodipine antagonises cerebral [^{3}H]-PN 200-110 binding, but not the ethanol withdrawal syndrome 160P
Watts IS *see* White DG 300P
Waugh CJ, Dockrell MEC, Haynes WG, Olverman HJ, Williams BC & Webb DJ Inhibition of ^{125}I-endothelin-1 binding to rat cardiac membranes by BRL 38227 114P
Webb DJ *see* Waugh CJ 114P
Webster J *see* Millican SA 302P
Webster RA *see* Dalley JW 2P
Webster RA *see* Dalley JW 151P
Weg VB, Watson ML, Faccioli LH & Williams TJ [^{111}In]-eosinophil accumulation during passive cutaneous anaphylaxis in the guinea-pig 127P
Weinen W *see* van Meel JCA 257P
Welsh NJ *see* Liu YJ 87P
Welsh NJ *see* Liu YJ 266P
Wermelskirchen D, Wilffert B, Nebel U, Wirth A & Peters T Veratridine-induced [$^{45}Ca^{2+}$] uptake as an indication of electrically silent Na^+ channels in isolated rat aorta 103P
Wessler I, Pohan D, Maclagan J & Racké K Cromakalim inhibits [^{3}H]acetylcholine release from parasympathetic nerves of the isolated rat trachea via an epithelium-dependent mechanism 69P
Wessler I *see* Racké K 243P
Weston AH *see* Ibbotson T 62P
Weston AH *see* Smith RD 236P
Weston AH *see* Trezise DJ 75P
Westwick J *see* Bourne AD 53P
Westwick J *see* Murphy CT 52P
Westwick J *see* Smith D 128P
Wettwer E *see* Hoey A 107P
Wheeler-Aceto H & Cowan A Studies with CI-977 and ICI 204448 support a role for peripheral receptors in κ-mediated antinociception and diuresis 39P
White DG, Sumner MJ & Watts IS The endothelin isopeptides do not release EDRF or prostacyclin (PGI_2) from human umbilical vein endothelial cells (HUVECS) 300P
White TE & Hill SJ Adenosine-stimulated accumulation of total [^{3}H]-inositol phosphates in hamster vas deferens DDT_1MF-2 cells 311P
Whiting RL *see* Eglen RM 105P
Whiting RL *see* Eglen RM 275P
Whiting RL *see* Wong EHF 33P
Whittington MA, Lambert JDC & Little HJ Ethanol withdrawal hyperexcitability in isolated hippocampal slices is not due to decreases in GABA-mediated inhibition 14P
Whittle BJR *see* Lopez-Belmonte J 95P
Widdop RE, Gardiner SM, Kemp PA & Bennett T PD 123177-evoked inhibition of the haemodynamic effects of angiotensin II in conscious rats after exposure to EXP 3174 86P
Widdop S *see* Hall IP 71P
Widdop S *see* Hall IP 72P
Wieczorek W & Kruk Z Electrically stimulated release of 5-HT from the rat prefrontal cortex measured by fast cyclic voltammetry in brain slices 217P
Wiernsperger N *see* Zhang B-L 191P
Wilffert B *see* Schwietert HR 61P
Wilffert B *see* Wilhelm D 284P
Wilhelm D, Ebbert M, Leidig A, Wilffert B & Peters Th The phosphodiesterase IV inhibitor rolipram enhances mechanical and electrical effects of the phosphodiesterase III inhibitor R 80122 284P
Wilhelm D *see* Schwietert HR 61P
Willars GB *see* Stevens EJ 46P
Willfert B *see* Wermelskirchen D 103P
Williams AD *see* Dewhurst DG 314P
Williams B *see* Vohra MM 216P
Williams BC *see* Waugh CJ 114P
Williams TJ *see* Rossi AG 49P
Williams TJ *see* van Uexkull 209P
Williams TJ *see* Weg VB 127P
Wilson C, Pettinger SJ, Hayward J, Birtles S & Holloway BR Cardiovascular effects of the thermogenic ß-adrenoceptor agonist, ICI D7114, in conscious telemetred dogs 234P
Wilson C *see* Growcott JW 232P
Wilson J *see* Hudson AL 158P
Wirth A *see* Wermelskirchen D 103P
Withrington PG, Antunes E, Grassi-Kassisse D & de Nucci G U46619, a stable thromboxane mimetic, can constrict canine splenic arterial vasculature without significant effect on capsular smooth muscle 306P
Wong EHF, Wu I, Eglen RM & Whiting RL Labelling of species variants of 5-HT_3 receptors by a novel 5-HT_3 receptor ligand, [^{3}H]RS-42358-197 33P
Wong EHF *see* Eglen RM 275P
Wood IC, Caulfield MP, Buckley NJ & Brown DA Pharmacological profile of the muscarinic receptor in a mouse neuroblastoma (N18TG2) which expresses the m_4 muscarinic receptor gene 163P
Woolf CJ *see* Thompson SWN 137P
Woolfson RG *see* McCarthy AL 94P
Worrall V *see* Allott CP 261P
Worton S *see* Butler A 273P
Wragg A, Robertson MJ & Clark KL Prevention and reversal of angiotensin II-induced tachyphylaxis in the rabbit isolated aorta 265P
Wright PW, Rogers DC, Roberts JC, Rothaul AL & Hunter AJ Photothrombic lesions of the frontal cortex impair the acquisition of a spatial memory task in rats 161P
Wu G *see* Ali Z 38P
Wu I *see* Wong EHF 33P

Yeardley HL, Coleman RA, Marshall K & Senior J The effects of PGE_2, sulprostone and AH13205 on hamster uterus *in vitro* 241P
Young JM see Arias-Montaño JA 23P

Zaagsma J *see* Shahid M 129P
Zar MA *see* Khan RM 74P
Zempléni É, Jones CA, Pearson S & Reynolds GP Glutamate modulates endogenous dopamine release from superfused minislices of both olfactory tubercle and striatum of rat 179P
Zerkowski H-R *see* Brodde O-E 108P
Zerkowski H-R *see* Michel MC 64P
Zetterström T, Finn D, Pei Q & Grahame-Smith DG Effects of the sulphonylurea gliquidone on the efflux of radiolabelled 5-HT and dopamine from rat brain slices 218P
Zhang B-L, Liu K-L, Sacquet J, Benzoni D, Wiernsperger N & Sassard J Effects of naftidrofuryl on the renal function and the synthesis of prostanoids in isolated perfused kidneys of spontaneously hypertensive rats 191P
Zhang JS & Van-Zwieten PA Characterization of two novel nonpeptide angiotensin II antagonists 85P

Subject Index

A-68930 7P
ACE inhibition 201P
ACE inhibitors 198P, 262P
Acetorphan 143P
Acetylcholine 43P, 69P, 93P, 130P
Acetylcholine release 42P
Actin 54P
Adenine analogues 116P
Adenine nucleotides' extracellular metabolism 175P
Adenosine 213P, 309P, 311P
Adenosine receptors 176P
Adenosine A_1 receptors 117P
Adenosine A_{2a} receptors 115P, 303P
Adenosine A_{2b} receptors 115P, 303P
Adenylate cyclase 27P, 64P
Adenylate cyclase/cyclic AMP system 312P
Adenylyl cyclase 5P, 26P
Adjuvant arthritis 309P
Adomorphine 151P
ADP-ribosyltransferase 27P, 215P
α_2-Adrenergic receptors 139P
α-Adrenoceptor antagonists 61P
$ß_1$-Adrenoceptor antagonists 64P
ß-Adrenoceptor down regulation 282P
ß-Adrenoceptor reserve 232P
ß-Adrenoceptor subtypes 282P
α-Adrenoceptor-mediated auto-inhibition 310P
Adrenoceptors 286P
α-Adrenoceptors 58P, 63P, 70P, 229P, 230P
α_1-Adrenoceptors 184P, 296P
α_2-Adrenoceptors 59P, 157P, 158P
α_{2A}-Adrenoceptors 59P
ß-Adrenoceptors 72P, 231P
$ß_1$-Adrenoceptors 10P, 283P
$ß_2$-Adrenoceptors 283P
Agonist potencies 115P
α_1-Agonists 109P
AII receptor antagonists 257P
AII receptors 82P
Airway hyperresponsiveness 126P
Airway microvascular leakage 244P
Airway smooth muscle relaxation 70P
Airways 77P, 242P, 243P, 247P
Airways function 124P
Aldose reductase 46P
Aluminium 168P
Alzheimer's disease 121P, 122P, 255P
α-Aminobutyric acid 13P
Amitryptyline 143P
AMP extracellular formation 175P
Amphetamines 40P, 150P
Anaesthesia 31P, 187P
Analgesia 132P, 138P
Anaphylaxis 127P
Angiogenesis 211P, 212P
Angiotensin 89P, 183P, 193P, 260P, 261P, 265P
Angiotensin antagonists 83P
Angiotensin II 87P, 88P, 266P
Angiotensin II antagonists 85P
Angiotensin AT_2 receptors 86P
Anococcygeus 90P
Antagonism 87P, 266P
Antagonist potencies 303P
Antagonists 29P, 250P, 261P
α_1-Antagonists 116P
Anti-angiogenesis 212P
Anticonvulsants 20P, 120P
Antidepressants 10P
Anti-epileptic drugs 120P
Anti-inflammatory drugs 190P
Antinociception 38P, 39P, 130P, 143P
Anti-oxidants 208P
Anxiety 224P
Anxiolytics 226P
Aorta 102P, 230P
Apamin 146P
Aphetamine 239P
Apomorphine 2P
Arginine 215P
Arrhythmias 117P
Artery ligated rat 197P
Arthus 49P
Articular mechanoreceptors 309P
Assay 5-HT in blood platelets 216P
Assay techniques 178P
Asthma 128P, 129P
Astrocytes 122P
Atherogenesis 47P
Atherosclerosis 202P
ATP 310P
ATP-sensitive K^+-channels 6P, 61P

Barbiturates 12P
Baroreflex 31P
Behaviour 7P, 35P, 81P, 88P, 150P, 225P, 226P, 253P, 258P
Benzodiazepine receptors 172P
Benzodiazepine site 50P
Benzodiazepines 171P
Binding 230P, 240P
Binding site 176P
Binding study 85P
Blood-brain barrier 151P
Blood-brain barrier permeability 269P
Blood pressure 30P, 277P, 298P
Blood vessels 272P, 310P
Bovine retina 145P
Bradykinin 66P, 135P
Brain slices 217P
BRL55834 73P, 242P, 247P
Bronchoconstriction 246P
Bronchodilators 73P
Buprenorphine 147P
BW 737C 7P
BW 755C 208P

Caffeine 169P
Calcitonin 270P
Calcitonin gene-related peptide 258P
Calcium 53P, 67P, 289P
Calcium channel antagonists 120P
Calcium channels 121P
Calcium currents 41P, 156P
Capsaicin 131P, 272P
Carbachol 23P
Cardiac 110P, 114P, 286P
Cardiac contractility 107P
Cardiac myocyte 96P
Cardioactive drugs 281P
Cardiopulmonary afferents 31P
Cardiovascular 83P, 261P
Catecholamine 286P
Catecholamine mechanisms 225P
Cathinone 239P
Cell culture 162P
Cell proliferation 82P
Cellular expression of catechol-*O*-methyltransferase *see* COMT
Cellular immune response 48P
Central $5\text{-}HT_2/5\text{-}HT_{1C}$ receptors 30P
Central nervous system *see* CNS
Cerebral anti-ischaemics 17P
Cerebral blood flow 149P, 224P
Cerebral cortex 24P
CGRP 76P, 263P
Chemotaxis 45P
Chlordiazepoxide 171P
Chloride 193P
Chloromethiazole 13P, 20P
Cholecystokinin 256P
Choline acetyltransferase 255P
Chronic diazepam 172P
Chronic inflammation 206P
Cisplatin 274P
Clone 110P
CNS 6P, 9P, 17P, 29P, 31P, 33P, 36P, 41P, 121P, 146P, 151P, 153P, 162P, 164P, 165P, 176P, 180P, 181P, 186P
Cocaine 149P, 150P
Cognition 88P, 166P, 228P, 253P
Cognitive function 161P
Collateral perfusion 290P
Complement 49P
Computer simulation 313P, 314P
COMT mRNA 8P
Corticosteroids 48P
Co-transmission 310P
Cough 294P
Culture 168P
Cultured cells 10P
Cultured endothelial cells 263P
Cultured human airway smooth muscle 71P
Cultured neurones 118P
Cyclic AMP 71P, 108P, 213P
Cyclic nucleotide phosphodiesterase isoenzymes 129P
Cyclooxygenase 50P, 194P
l-Cysteine sulphinate 15P
Cytokine 292P
Cytoprotection 95P

D1 binding 154P
D-1/D-2 receptor interactions 5P
Debrisoquine 3P
Defence response 177P
Delta receptors 141P
Dependence 171P
Desensitization 25P, 65P, 72P
Diabetes 46P
Dialysis 147P
Diazepam 173P
Dihydropyridine calcium antagonists 111P
Dihydropyridines 160P
Dihydropyriding calcium antagonists 293P
Dihydroxyphenylglycol 106P
Dipsogenicity 262P
Disease 189P
Diuresis 39P
Dopamine 6P, 89P, 149P, 179P, 180P
Dopamine agonists 153P
Dopamine antagonists 151P
Dopamine D-1 receptors 7P
Dopamine D-2 receptors 6P
Dopamine receptors 150P
Dopamine release 146P, 147P
Dopamine synthesis 1P
Dopaminergic drugs 154P
Dopexamine 192P
Dopropidil 112P
Dorsal root ganglion cells 133P
DPDPE 141P
Drinking 155P
DuP 753 197P, 257P, 265P

E. coli 209P
Ecto-adenylate kinase inhibition 175P
EDRF 290P, 300P
EGF 301P
Electrophysiology 107P, 250P, 256P
Elevated plus-maze 166P
Elevated X-maze 221P
Emesis 165P, 274P
Enalapril 197P
[^{125}]-Endothelin-1 binding 264P
Endothelin receptor subtypes 264P
Endothelins 114P, 295P, 298P, 300P
Endothelium 94P, 98P, 301P, 302P
Endothelium-derived relaxing factor 289P, 291P, 297P
[^{3}H]-[D-Ala^{2},MePhe^{4},Gly-ol^{5}]-Enkephalin 145P
[DPen^{2},DPen^{5}]-Enkephalin *see* DPDPE
Entorhinal cortex 42P
Eosinophil 53P, 127P, 128P
Epilepsy 170P
Ethanol withdrawal 14P, 160P
Excitatory amino acid receptors 19P
Excitatory amino acids 16P, 21P, 22P, 213P

Felodipine 160P
Fenoldopam 192P
Fibrinolysis 56P
Fibroblasts 203P
Food intake 220P, 227P
Frog brain 176P
Fura-2 119P

GABA 12P, 14P, 170P, 172P, 173P
$GABA_B$ receptor subtypes 11P
Galanin 43P, 254P, 255P
Gastric damage 95P
Geto-placental arteries 94P
Glucocorticoids 57P
L-Glutamate 15P, 22P
Glutamate 179P, 180P
Glutathione 178P
Glutathione reductase 178P
Glyceryl trinitrate 97P
Glycine receptor 182P
G-proteins 54P, 27P, 63P, 64P, 27P, 287P
GR82334-NK_1 antagonist 134P
Gs 25P
Gsα 215P
GTP sensitivity 223P
Guinea-pig distal colon 279P
Guinea-pig ileum 232P, 260P
Guinea-pig pulmonary artery 273P
Guinea-pig trachea 185P
Guinea-pigs 251P, 284P, 313P
GVG 170P

H_2O_2 302P
H_3 receptors 155P
Haemodynamics 86P, 200P, 298P
Haemoglobin 211P
Head-shake 225P
Heart 113P, 248P, 284P
Heart failure 106P
Hepatocytes 183P
High affinity state 157P
Hippocampus 9P, 14P, 121P
Histamine 23P, 67P, 123P, 155P, 210P
Histamine H_3 receptors 42P
Histamine liberation 55P
L-Homocysteate 15P
HPLC 178P
HPLC-EC detection 216P
5-HT 28P, 34P, 36P, 38P, 92P, 202P, 216P, 217P, 220P, 222P, 224P, 271P, 273P, 274P, 278P, 288P, 295P, 305P
5-HT receptors 99P
5-HT_1-like receptors 98P, 280P
5-HT_{1A} antagonist 277P
5-HT_{1A} receptor binding 223P
5-HT_{1A} receptors 29P, 277P
5-HT_{1C} receptor antagonists 226P
5-HT_{1C} receptors 219P, 225P
5-HT_2 191P
5-HT_2 receptor antagonists 226P
5-HT_2 receptors 32P, 219P, 222P, 225P
5-HT_3 antagonists 228P
5-HT_3 receptor agonists 35P, 227P
5-HT_3 receptors 33P, 34P, 35P, 276P
5-HT_3 release 36P
5-HT_4 receptors 275P, 279P
Human 99P
Human airway smooth muscle 72P
Human atrium 287P
Human brain 157P
Human bronchial smooth muscle 129P
Human heart 108P
Human heart failure 283P
Human internal mammary artery 229P
Human saphenous vein 229P, 305P
Human umbilical vein endothelial cells 300P
Hybridization histochemistry 8P
Hydrogen peroxide 53P
3-Hydroxycyorodime 142P
6-Hydroxydopamine 5P
Hypercholesterolaemic agents 203P
Hypertension 58P
Hypocalcaemic assay 270P
Hypothermia 169P, 254P
Hypoxia-induced pulmonary vasoconstriction 308P

ICI 07114 232P
Idazoxan 158P
Idazoxan binding sites 158P
Ifenprodil 162P
Immunopharmacology 123P
Imperialine 105P
In vitro studies 100P
Inflammation 49P, 208P, 209P
Inhibitors 71P, 185P
Inositol triphosphate 240P
Inositol 1,4,5-trisphosphate 65P
Inositol phosphate receptors 25P, 26P
Inositol phosphates 23P, 66P, 184P, 308P, 311P
Interleukin 44P
Interleukin 1 128P
Intestinal absorption 314P
Intestinal innervation 239P
Intracellular Ca^{2+} 118P
Intracranial 272P
In vivo 9P
Iprindole 10P
Isolated atria 281P
Isolated coronary artery 293P
Isolated kidney 191P

Joint 76P
Jugular vein 98P
Juvenile rats 270P

K^+ channel opener 304P
Kappa opioid 140P
Kidney 184P, 193P, 194P

Leupeptin 205P
Light intensity 221P
Lipocortin 57P
Liver enzymes 188P
Locomotion 89P
Locomotor activity 167P, 251P
Losartan 88P
Luzindole 195P
Lymphoblasts 207P
Lymphocyte migration 206P
Lyso-PAF 244P
Lytic damage 392P

Macrophages 91P
Malaria 189P
MAO-B 122P

Marmosets 152P, 153P, 228P
Mast cells 55P, 123P
Medial prefrontal cortex 2P
Medial vestibular nucleus 222P
MEL-14 206P
Melanophores 195P
Melatonin receptor 195P
Membrane potential 102P
Metabolism 189P
Metabotropic glutamate receptors 181P
2-Methyl-5-HT 34P
N-Methyl-D-aspartate 20P, 28P
1-Methyl-4-phenyl-1,2,3,6-tetra-hydropyridine *see* MPTP
Mice 159P, 226P, 281P
Microcirculation 210P
Microdialysis 36P
MK801 269P
Monocytes 205P
Morphine 138P
Motor nerve endings 312P
MPTP 152P, 153P
Muscarinic antagonists 166P
Muscarinic m3 receptors 65P
Muscarinic receptor subtypes 100P, 101P, 105P
Muscarinic receptors 163P, 165P
Muscarinine receptor agonists 164P
Myocardial hypertrophy 198P
Myocardial hypertrophy and re-modelling 199P
Myocardial ischaemia 117P

N1E-115 cells 119P
Na^+ channel 103P
Na^+ channel modulators 107P
Na^+/Ca^{++} exchange 110P
Na/K pumps 207P
Nalaxone 169P
Nedocromil 294P
Negative inotropy 111P
Neocortex 28P
Neuroblastoma cells 13P, 276P
Neurokinin receptors 250P
Neurokinin-1 receptors 249P
Neurokinin-A 77P
Neurokinins 78P, 81P, 251P
Neuroleptics 2P, 151P
Neuromedin U 267P
Neuromodulation 74P
Neurones 168P
Neuropeptide Y 74P
Neurophysiology 16P
Neurotixicity 162P
Neutral endopeptidase inhibitor 199P
Neutrophil accumulation 124P
Neutrophil migration 44P
Neutrophils 45P, 246P
NG108-15 cell 156P
N^G-monomethyl-L-arginine 93P
N^G-nitro-L-arginine 288P
Nicotine 167P
Nicotinic 9P
Nitric oxide 90P, 92P, 93P, 94P, 95P, 96P, 97P, 292P
Nitric oxide production 91P
Nitro-L-arginine methyl ester 290P
Nitrosothiols 90P
Nitrovasodilator 96P
NMDA 18P, 137P
NMDA antagonist 182P
NMDA receptors 17P, 138P, 181P
NO *see* nitric oxide
Nociception 131P, 132P, 134P, 135P, 136P, 137P, 140P
Nociceptors 131P, 136P
Nomifensine 4P
Non-adrenergic non-cholinergic 90P
Non-peptide angiotensin II antagonists 84P, 259P, 262P
Non-peptide antagonists 77P, 86P, 260P
Noradrenaline 9P, 38P, 200P, 296P, 305P
Noradrenaline metabolism 106P
Noradrenaline release 60P, 63P, 234P
Novel $5HT_3$ receptor agonist 173P
NPY-receptor 268P
Nucleus accumbens 147P

Oedema 76P, 258P
Oestrogen receptors 196P
8-OH-DPAT 220P, 221P
Okadaic acid 123P
Olfactory cortex 181P
Olfactory tubercle 179P
Opioid antagonists 40P, 142P
Opioid binding 145P
Opioid receptors 142P, 144P
Opioids 41P, 139P
$[^3H]$-ouabain 207P

PAF 52P, 244P, 246P
in horses 124P
Pain 37P, 38P, 39P, 134P
Parkinson's disease 3P
Patch-clamp recording 249P
PDE-inhibitors 284P
Peptides 74P, 75P, 79P, 80P, 114P, 245P, 256P
Perfluorochemicals 188P
Perfused mesenteric vessel bed 101P
Periaqueductal grey 177P
Permeabiity 210P
PGE_2 45P
$PGF_{2\alpha}$ 295P
pH 203P
Pharmacology 113P
Pharmocodynamics 281P
Phenytoin 144P
Phorbol 119P
Phorbol ester 68P
Phosphodiesterase 70P, 71P, 185P
Phosphoinositide turnover 22P, 183P
Phosphoinositides 24P
Physical dependence 41P
(-)-pinacidil 102P
Piribedil 153P
Pithed rat 259P
Pituitary gland 57P
Plasma HVA levels 3P
Platelets 50P, 52P, 97P
Polyamines 17P, 18P, 182P, 301P
Polymorphonuclear leukocytes 47P
Porcine carotid circulation 280P
Positive inotropic effects 108P
Post-mortem human heart 282P
Post-receptor events 109P
Potassium channel activator 73P, 242P, 247P
Potassium channel blockers 291P
Potassium channel openers 62P, 69P
Potassium channels 113P, 114P, 254P
Precolumn derivation and assay of 5HT 216P
Prejunctional α-adrenoceptor subtypes 60P
Pressure reversal 187P
A_2-presynaptic adenosine receptors 312P
Pretectum 130P
Propofol 187P
Propranolol 159P
Prostacyclin 46P, 289P, 297P
Prostaglandin E_2 133P
Prostaglandins 243P
Prostanoids 191P
Prostration 167P
Protein kinase C 68P, 308P
Protein phosphatases 123P

Rabbit aorta 271P, 291P
Radial maze 253P
Radioimmunoassey 219P
Radiolabelled 5HT 218P
Radioligand binding 141P
Radioligand binding studies 80P
Rat aorta 102P, 193P, 264P, 292P, 296P
Rat atria 112P
Rat brain 219P, 223P
Rat CNS 8P
Rat everted sac 314P
Rat frontal cortex *in vitro* 180P
Rat fundus 267P
Rat intestine 278P
Rat kidney 60P, 192P
Rat liver 62P
Rat portal vein 61P, 100P
Rat striatum 154P
Rats 48P, 88P, 188P, 281P, 293P
inbred 55P
$^{86}Rb^+$ efflux 304P
Receptor autoradiography 172P
$ß_2$-Receptor antagonists 135P
Receptors 87P, 202P, 266P
α-Receptors 192P
$α_2$-Receptors 156P
ß-Receptors 287P
Relaxation responses 278P
Release 11P, 43P
Renal artery 197P
Renal blood flow 58P
Renal hypertensive rat 85P, 257P
Renal vasodilatation 93P, 192P
Respiratory pharmacology 313P
Restrosplenial cortex 252P
Ritanserin 226P
Rose-bengal 161P
RS-42358-197 33P
RX77368 252P

Saffan 177P
Saphenous vein 99P
Second messengers 52P, 53P, 63P, 64P, 66P, 67P, 119P, 175P, 183P, 184P, 186P, 204P, 213P, 214P, 215P, 240P, 284P, 308P
Seizure threshold 144P
Sensory nerves 273P
Sensory neurones 133P
Serotonin determination 216P
SHR 101P, 109P, 199P
Sibutramine 32P
Skin 131P
Smooth muscle 67P, 311P
Social behaviour 159P
Sodium 203P, 294P
Sodium pump 68P
Somatostatin 132P
Spinal 81P
Spinal antinociception 139P
Spinal cord 137P, 256P
Spinal reflexes 140P
Spirapril 198P
Spleen volume 306P
Splenic circulation 306P
SR 47063 304P
SR 47436 259P
Stellate ganglia 248P
Steroids 12P, 212P
Stress 32P
Striatum 4P
Stroke 161P
Submandibular gland 59P
Substance P antagonist 80P
Subtype 33P
Sumatriptan 272P, 273P
Superfused synaptosomes 11P
Sympathetic nerve activity 30P
Sympathetic neurones 19P, 248P
Sympathetic preganglionic neurones 249P
SZS 205,557 275P

Tachykinin receptors 80P
Tachyphylaxis 265P
Terguride 152P
Thrombin 54P, 205P
Thromboxane 297P, 306P
Thyrotropin-releasing hormone 252P, 253P
Tolerance 139P
Trachea 69P
Transdermal application 153P
Transdermal drug delivery 190P
Transmitter release 271P
Transolapril 201P
Transolaprilate 201P
Tyramine 200P
Tyrosine kinase 52P

Vagus nerve 165P, 276P
Vanadium 249P
Vas deferens 74P
Vascular density 211P
Vasoactivity 194P
Vasodilators 209P
Vehicles 190P
Ventricular relaxation 283P
Veratridine 103P
Vision 16P
Voltammetry 4P, 146P

Working heart 111P

Y1-receptors 268P
Y2-receptors 268P

Zacopride 36P