SUBDIVISION OF M_2-RECEPTORS INTO THREE TYPES

H.D. Batink, D. Davidesko, H.N. Doods*, K.J. van Charldorp, A. de Jonge[1] and P.A. van Zwieten, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands. [1]Division of Molecular Pharmacology, Dr. Karl Thomae GmbH, Biberach I, D-7950, F.R.G.

The selectivity profile of pirenzepine was the basis to subclassify the muscarinic receptors in M_1 and M_2, respectively. However, the discovery of compounds like 4-DAMP (4-diphenylacetoxy-N-methylpiperidine methbromide) that were able to discriminate between atrial and ileal muscarinic receptors showed that the M_2-population might be heterogenous. The recent introduction of the cardioselective antagonist AF-DX 116 (11-2[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one) is a further reason to subclassify the muscarinic receptors. This compound has a low affinity for muscarinic binding sites in the submandibular gland and high affinity for those in the heart.

It was the aim of the present study to explore whether the muscarinic binding sites in the submandibular gland are different from the muscarinic receptors on longitudinal muscle of the ileum. Receptor binding studies to membranes obtained from rat atrium and submandibular gland were performed using ^{3}H-N-methylscopolamine (0.4 nM: spec.act.85 Ci/mmol). Experiments were carried out in Hepes-buffer (20 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$; pH=7.5 at 37°C) at 37°C. Specific binding was determined with 1 µM dexetimide. Functional in vitro studies were performed using rat ileal longitudinal muscle strips and paced left atria (3 Hz, 5 ms) at 37°C using a Muralt-Tyrode solution. Oxotremorine was used as the agonist.

Table 1. Comparison of the pA_2 and the pKi values for the antagonists studied.

	pKi heart	pA_2 heart	pKi salivary gland	pA_2 ileum
atropine	8.87±0.04	8.99±0.11	8.78±0.03	9.04±0.05
AF-DX 116	6.71±0.02	6.89±0.07	5.30±0.05	6.21±0.05
AF-DX 237	7.23±0.03	7.13±0.08	5.90±0.02	7.00±0.05
pirenzepine	5.86±0.04	6.15±0.05	6.38±0.03	6.21±0.08
4-DAMP	7.47±0.01	7.70±0.06	8.48±0.04	8.63±0.09

Values are means ± S.E.M., n=4-8. Schild slopes were not significantly different from unity.

Atropine proved to be a non-selective muscarinic antagonist. Pirenzepine and AF-DX 237 (11-2[[2-[(4-hydroxylcyclohexylmethylamino)methyl]-1-piperidinyl]acetyl]-5,11--dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one) made no distinction between the receptors in the heart and ileum. However, pirenzepine possessed a somewhat higher affinity for the binding sites in the submandibular gland compared to those in the heart, whereas AF-DX 237 has a 21-fold preference for cardiac muscarinic binding sites. AF-DX 116 showed a 26-fold higher affinity for the binding sites in the heart than for those in the submandibular gland. Moreover, its affinity for cardiac muscarinic receptors was 5-fold higher than that for ileal muscarinic receptors. 4-DAMP had a high affinity for the binding sites in the submandibular gland and also high affinity for ileal muscarinic receptors. Owing to the non-selectivity of atropine and pirenzepine and the different selectivity profile of AF-DX 116, AF-DX 237 and 4-DAMP for the muscarinic binding sites or receptors in the heart, submandibular gland and ileum we submit that the muscarinic receptors in the three tissues are different. Accordingly, we propose the subclassification into M_2 (heart), M_3 (exocrine glands) and M_4 (smooth muscle).

We would like to thank Dr. Barlow for a gift of 4-DAMP.

$5HT_2$ BINDING SITES IN MICE SUSCEPTIBLE (DBA/2J) AND RESISTANT (C57/B16) TO AUDIOGENIC SEIZURES

R.W. Horton, S.P. Jazrawi* and Y. Yamaguchi, Department of Pharmacology and Clinical Pharmacology, St George's Hospital Medical School, London SW17 ORE.

DBA/2J (DBA) mice have a high genetically determined susceptibility to generalised convulsions induced by auditory stimulation. This susceptibility to audiogenic seizures (AS) is age-related, being maximal at 21-28 days and absent or much reduced at earlier and later ages. The basis of this AS susceptibility is not known. Pharmacological studies suggest an involvement of 5HT function. Enhancement of 5HT function attenuates AS in DBA/2 mice, while 5HT synthesis inhibition increases AS frequency and intensity (Horton et al, 1977; Peters and Lack, 1985; Sparks and Buckholtz, 1985).

In this study we have measured $5HT_2$ binding sites in five brain regions of DBA mice, at ages before (13-15 days) during (21-23 days) and after (40-45 days) the period of maximal susceptibility to AS. Concurrent experiments were performed in age-matched C57/Bl 6 (C57) mice, a strain resistant to AS. [^{3}H] Ketanserin (8 concentrations, 0.05-3nM) was incubated with aliquots (equivalent to 10mg wet wt) of well-washed membranes for 10 min at 37^{o}C in 50mM Tris buffer, pH 7.7. Non-specific binding was defined with 1μM methysergide. The maximal number of binding sites (Bmax) and the equilibrium dissociation constant (K_D) were determined by non-linear regression analysis of saturation plots.

The maximal number of binding sites for 3 brain areas are shown in Table 1.

Table 1 Bmax of [^{3}H] Ketanserin binding

		13-15 days	21-23 days	40-45 days
Cerebral cortex	C57	87.2±5.1	96.3±5.0	113.7±6.9
	DBA	93.4±4.6	122.3±9.3*	115.6±7.2
Hippocampus	C57	33.7±2.3	20.6±2.3	24.2±3.3
	DBA	33.8±4.3	21.0±2.2	24.1±2.4
Pons-Medulla	C57	40.9±7.7	19.9±2.9	18.6±2.8
	DBA	46.1±3.3	23.7±2.3	19.1±4.6

Values are means ± s.e.m. (f moles/mg protein) for 5-6 determinations
* significant difference between strains $p<0.05$ (t-test)

There was a significantly greater number of binding sites in the cerebral cortex of DBA mice than C57 mice at 21-23 days of age, the time of maximal susceptibility of DBA mice to AS, but not at other ages. No differences were found in Bmax between strains in the basal forebrain, hippocampus, mid-brain or pons-medulla and no differences in K_D in any brain region at the ages studied. In hippocampus, mid-brain and pons-medulla the number of binding sites decreased markedly between 13-15 days and 21-23 days, in contrast to the slight increase or no change seen in the cerebral cortex and forebrain. This occurred to a similar extent inboth strains and is thus probably a normal developmental change. The greater number of $5HT_2$ binding sites in the cerebral cortex of DBA mice may relate to the age-related AS susceptibility of these mice.

Horton, R.W. et al. (1977) Euro. J. Pharmacol. 41, 387
Peters, R.I. & Lack, D.B. (1985) Expt. Neurol. 90, 540
Sparks, D.L. & Buckholtz, N.S. (1985) Pharmac. Biochem. Behav. 23, 753.

[^{125}I]-IODO-LSD LABELS A SUBSET OF 5-HT$_2$ RECEPTORS IN HUMAN FRONTAL CORTEX

*J.M. Elliott & Amanda Kent, Department of Pharmacology, St. Mary's Hospital Medical School, London W2 1PG

[^{125}I]-2-iodo-lysergic acid diethylamide (^{125}I-LSD) is reported to be a potent and selective ligand for 5-HT$_2$ receptors in both central and peripheral tissues in the rat[1,2]. However, the closely related compound N1-methyl-^{125}I-LSD is reported to bind with high affinity to 5-HT$_{1c}$ sites in the choroid plexus of the pig[3]. The objective of the present study was to characterize the binding of ^{125}I-LSD in membranes of human frontal cortex tissue, particularly in terms of the 5-HT receptor sub-type labelled.

Human frontal cortex tissue was obtained within 12h. of death, homogenised and resuspended in 15 vol. incubation buffer (5 mM Tris. HCl, 2.5 mM EDTA, 150 mM NaCl, pH 7.4). Tissue was incubated with ^{125}I-LSD at six concentrations in the range 50-500 pM for 90 min at 37°C. Non-specific binding was defined as that observed in the presence of 1 uM ritanserin + 30 uM 5-HT, chosen in order to displace specific binding to both 5-HT$_1$ and 5-HT$_2$ sites. Incubations were terminated by rapid filtration through Whatman GF-B filters.

Analysis of the binding kinetics of ^{125}I-LSD (180 pM) demonstrated half-times for association and dissociation of 12 min and 28 min respectively. The dissociation equilibrium constant (K_d) value derived from the kinetic rate constants was 0.136 nM.

Binding of ^{125}I-LSD (50 pM) was inhibited by a range of serotonergic agonists and antagonists in the following order spiperone > ketanserin > mianserin >> 5-HT > RU 24969 > 8.OH-DPAT. A similar order of potency was observed for the inhibition of ^{3}H-ketanserin binding studied on identical tissue preparations. 5-HT was the most potent of the endogenous biogenic amines in displacing ^{125}I-LSD binding. Comparison of the IC_{50} values obtained for ^{125}I-LSD and ^{3}H-ketanserin showed a significant positive correlation ($r = 0.98$, $p < 0.001$, $n = 9$), suggesting that the site labelled by ^{125}I-LSD corresponded with the 5-HT$_2$ receptor. Similar comparison with affinities at the 5-HT$_{1c}$ site in pig choroid plexus[4] showed no significant correlation ($r = 0.352$, $p > 0.05$, $n = 8$).

Specific binding of ^{125}I-LSD was saturable and of high affinity ($K_d = 0.33 \pm 0.02$ nM, mean $\pm$ sem, n=4). However, in four different tissue samples ^{125}I-LSD consistently labelled 45-51% fewer sites than ^{3}H-ketanserin, resulting in a significantly lower binding capacity for ^{125}I-LSD (136 $\pm$ 11 fmol/mg protein) than ^{3}H-ketanserin (254 $\pm$ 30 fmol/mg protein; $p < 0.01$, Student's unpaired t-test).

We conclude that ^{125}I-LSD labels a site in human frontal cortex which resembles the 5-HT$_2$ receptor, as identified by ^{3}H-ketanserin. However, the difference in binding capacity identified by these two ligands suggests that ^{125}I-LSD labels a subset of the 5-HT$_2$ receptor population. Further studies will be necessary to determine whether this difference in binding capacity may have functional consequences.

1. Kadan *et al* (1984) J. Neurochem. 43, 601-606.
2. Moretti-Rojas *et al* (1983) J. Biol. Chem. 258, 12499-12508.
3. Yagaloff & Hartig (1986) Mol. Pharmac. 29, 120-125.
4. Moyer *et al* (1985) Eur. J. Pharm. 118, 13-23.

SL 81.0385, A NEW SELECTIVE AND POTENT 5-HYDROXYTRYPTAMINE UPTAKE INHIBITOR

S. Arbilla, D. Bigg, Y. Claustre, D. Graham*, E. Habert, S.Z. Langer, C. Pimoule, B. Scatton and H. Schoemaker, Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France

A large number of side-effects induced by tricyclic antidepressants has been attributed to their adrenolytic and anticholinergic activity and to their blockade of NA uptake. This has led to a need for the development of compounds with more selective 5-HT uptake inhibitory properties. In this context, we now present data on the pharmacological profile of 4-[(2-napthalenyl)methoxy]piperidine, SL 81.0385, a novel, non-tricyclic and selective 5-HT uptake inhibitor.

The uptake of [^{3}H]monoamines in vitro into rat brain synaptosomes was measured using the protocol of Ross and Renyi (1975). Human platelet [^{3}H]5-HT uptake and [^{3}H]imipramine binding was determined by the protocol of Segonzac et al. (1985). Inhibition of 5-HT and NA uptake by SL 81.0385 was determined in vivo using the methods of Fuller et al. (1976) and Fuller and Perry (1978). The binding of [^{3}H]paroxetine to rat cerebral cortical membranes was carried out according to Habert et al., 1985. [^{3}H]5-HT uptake into rat brain synaptosomes and human platelets was potently and competitively inhibited by SL 81.0385 with IC_{50} values of 18 nM and 13 nM, respectively. In contrast, SL 81.0385 was 89-fold and 189-fold less potent as an inhibitor of synaptosomal uptake of [^{3}H]NA (IC_{50} = 1.6 µM) and [^{3}H]DA (IC_{50} = 3.4 µM), respectively. Moreover, in vivo, SL 81.0385 inhibited the uptake of 5-HT with an ID_{50} of 2.5 mg/kg i.p., whereas NA uptake was not affected (ID_{50} > 100 mg/kg). The inhibitory activity of SL 81.0385 on cerebral 5-HT uptake, and the selectivity of its action on 5-HT uptake, both in vitro and in vivo, is greater than that of reference compounds. In radioligand binding studies, SL 81.0385 was a very potent inhibitor of [^{3}H]imipramine and [^{3}H]paroxetine binding to rat cerebral cortical membranes, with Ki values of 0.5 nM and 3.2 nM, respectively. Also, [^{3}H]imipramine binding to human platelet membranes was inhibited by SL 81.0385 with a Ki of 2 nM. Binding of [^{3}H]desipramine to membranes from rat vas deferens was inhibited by SL 81.0385 with a Ki value of 350 nM, and [^{3}H]spiroperidol binding to frontal cortex (5-HT_2 receptor index) with a Ki of 6,78 µM. However, SL 81.0385 was inactive (IC_{50} values > 100 µM) on [^{3}H]clonidine, [^{3}H]prazosin, [^{3}H]dihydroalprenolol, [^{3}H]pyrilamine, [^{3}H]diazepam and [^{3}H]QNB binding to cerebral cortical membranes, and [^{3}H]spiroperidol binding to striatal membranes (D2 dopamine receptor index). Dissociation kinetics of [^{3}H]paroxetine binding to rat cerebral cortical membranes from equilibrium conditions showed that citalopram (t1/2 = 98 min), fluoxetine (t1/2 = 94 min), indalpine (t1/2 = 82 min) and SL 81.0385 (t1/2 = 92 min) gave t1/2 values of dissociation which were close to the t1/2 value obtained with unlabelled paroxetine (t1/2 = 104 min). In contrast, imipramine (t1/2 = 202 min) and 5-HT (t1/2 = 185 min) were much less effective in dissociating [^{3}H]paroxetine binding.

The present experiments show that SL 81.0385 is a very selective and potent 5-HT uptake inhibitor, and suggest that the non-tricyclic class of 5-HT uptake inhibitors binds to a different site on the neuronal 5-HT transporter complex than imipramine and 5-HT. The fact that SL 81.0385 is virtually inactive at adrenergic, cholinergic and histaminergic H_1 receptors suggests that the compound should be devoid of most of the side-effects observed clinically with the tricyclic antidepressants.

Fuller R.W. and Perry K.W. (1978) Arch. Int. Pharmacodyn. 235, 229.
Fuller R.W. et al. (1976) Biochem. Pharmacol. 25, 2409.
Habert E. et al. (1985) Eur. J. Pharmacol. 118, 107.
Richelson E. and Nelson A. (1984) J. Pharmacol. Exp. Ther. 230, 94.
Ross S.B. and Renyi A.L. (1975) Acta Pharmacol. Toxicol. 36, 382.
Segonzac A. et al. (1985) J. Neurochem. 44, 349.

5HT RELEASE IN VIVO INCREASED BY NON-SELECTIVE MAO INHIBITION BUT NOT MAO A INHIBITION

C.A. Marsden, M.G. Palfreyman+ and A.J. Sleight*, Department of Physiology & Pharmacology, University of Nottingham Medical School, Nottingham U.K., +Merrell Dow Research Institute, Cincinatti, U.S.A.

Rats treated with a monoamine oxidase inhibitor and L-tryptophan develop a characteristic behavioural syndrome of hyperactivity, reciprocal forepaw treading, lateral head weaving, hindlimb abduction and Straub tail (Grahame-Smith, 1971). Although there is a marked accumulation of brain 5HT, changes in the release of 5HT during this behavioural response have not been studied. Intracranial dialysis (Brazell et al., 1985) offers a method of directly measuring extracellular levels of 5HT and 5HIAA in the freely moving rat which, in turn, can be used as an index of neuronal 5HT release.

Dialysis loops were prepared according to the method of Brazell et al., 1985, perfused with artificial C.S.F. (pH 7.4) at a rate of 1 µl/min and implanted into the ventromedial hypothalami of male Wistar rats (250-300 g) under halothane anaesthetic. The rats were allowed 2-3 hs to recover from the anaesthesia and then 20 min dialysis samples were collected. The samples were then analysed for 5HT and 5HIAA using HPLC-ECD. The rats were given either saline, tranylcypromine (20 mg/kg i.p.), clorgyline (5 mg/kg i.p.), a dose selective for MAO A inhibition, the neuronally selective MAO A inhibitor MDL 72394 (0.5 mg/kg i.p.) (Palfreyman et al., 1985) or MDL 72394 at a non-selective dose (2 mg/kg i.p.). Thirty mins after saline, tranylcypromine or clorgyline and 120 min after MDL 72394 the rats were given L-tryptophan (50 mg/kg i.p.). Extracellular 5HT and 5HIAA levels were measured throughout the experiment and for a further 80 mins following L-tryptophan. Separate groups of 5 rats were treated in exactly the same way as above and the behavioural parameters scored on a scale of 0-3 for 1 h following L-tryptophan (0=absent and 3=continuous). Activity was measured using an infra-red beam system.

Rats treated with either tranylcypromine or MDL 72394 (2 mg/kg i.p.) plus L-tryptophan scored higher behavioural and activity counts than those given saline following by L-tryptophan although this was not significant in the case of MDL 72394 (2 mg/kg i.p.). These treatments also markedly increased 5HT and decreased 5HIAA extracellular levels compared with saline treated rats. Rats treated with either clorgyline, MDL 72394 (0.5 mg/kg i.p.) or saline plus L-tryptophan failed to exhibit the behavioural syndrome and had similar extracellular 5HT and 5HIAA levels as rats treated with saline throughout the experiment.

The results demonstrate that the behavioural response is associated with increased release of 5HT and that this release is not dependent on either neuronal or non-neuronal MAO A. Since rats pretreated with the MAO B selective inhibitor deprenyl, do not exhibit the behavioural syndrome while those given tranylcypromine or clorgyline plus deprenyl do (Squires et al., 1975) it appears that both MAO A and MAO B inhibition is required to evoke the behavioural syndrome and increase extracellular 5HT levels.

AJS is supported by an SERC CASE Studentship. CAM is a Wellcome Senior Lecturer.

Brazell, M.P. et al. (1985) Br. J. Pharmac. 86, pp 209-216.
Grahame-Smith, D.G. (1971) Br. J. Pharmac. 43, pp 856-864.
Palfreyman, M.G. et al. (1985) J. Neurochem. 45, pp 1850-1860.
Squires, R.F. et al. (1975) Psychopharmacologia (Berl.) 41, pp 145-151.

THE EFFECT OF MONOAMINE OXIDASE INHIBITORS ON THE CALCIUM-DEPENDENCY OF 5-HT RELEASE FROM RAT BRAIN SYNAPTOSOMES

K.J. Collard and Suzanne M. Evans*. (Introduced by C.J. Garland).
Department of Physiology, University College, P.O. Box 78, Cardiff CF1 1XL.

It has been repeatedly observed that transmitter release in vivo shows an absolute dependency on extracellular Ca^{2+} (e.g. Katz and Miledi 1969). Consequently, in order to study the physiological processes involved in transmitter release using in vitro preparations, the techniques used should also show a similar complete Ca^{2+}-dependency. This has not always been observed for monoamine transmitters (Hery et al 1983), particularly when monoamine oxidase inhibitors (MAOIs) have been routinely used in the perfusion fluid (Mulder et al 1985). In this study we have examined the Ca^{2+}-dependency of [^{3}H]-5-HT release from preloaded superfused rat brain synaptosomes as a function of stimulus intensity, and examined the effect of MAOI's on this aspect of release. The preparation of synaptosomes, labelling with [^{3}H]-5-HT, perfusion, depolarisation, separation and measurement of released [^{3}H]-5-HT were conducted as previously described (Suter and Collard 1983).

The application of K^+ pulses of low concentration (7.5-15mM) caused an essentially Ca^{2+}-dependent release of [^{3}H]-5-HT. This resembles what might be expected to occur in the physiological in vivo situation. However, at K^+ concentrations of 25 and 50mM, a small non-Ca^{2+}-dependent component appeared. Concentrations of nialamide (1.8×10^{-5}M) and pargyline (7×10^{-6}M), selected to inhibit MAO by similar amounts (80%) significantly increased both the total amount of [^{3}H]-5-HT released and the contribution of the non-Ca^{2+}-dependent component of the evoked release at the K^+ concentrations studied. The effect was particularly marked with 50mM K^+ where the non-Ca^{2+}-dependent contribution increased from about 6% in the absence of nialamide to 41% in its presence, and which accounted for the overall increase in the total amount of [^{3}H]-5-HT released. Qualitatively similar effects were observed with pargyline.

The non-Ca^{2+}-dependent component of [^{3}H]-5-HT release evoked by 50 mM K^+ both in the presence and absence of MAOIs was totally abolished by immobilising the plasma membrane 5-HT carrier with chlorimipramine (5×10^{-7}M) or citalopram (5×10^{-8}M). This suggests that the non-Ca^{2+}-dependent component of the release evoked by high concentrations of K^+ may be mediated by 5-HT leaving the nerve ending via the plasma membrane 5-HT carrier which, from these observations, appears to be activated by depolarisation. Inhibition of MAO would increase the amount of free cytoplasmic 5-HT available to the carrier and consequently the amplitude of the non-Ca^{2+}-dependent component.

These studies imply that in order to conduct meaningful studies of the physiological processes involved in monoamine transmitter release using in vitro techniques, methods should employ mild depolarising stimuli to limit the possible occurrence of Ca^{2+}-independent events, and avoid the use of MAO inhibitors which potentiate these effects.

Hery, M. et al (1983) Eur. J. Pharmac. 89, 9-18.
Katz, B. & Miledi, R. (1969) J. Physiol. 203, 689-706.
Mulder, A.H. et al (1975) Brain Res. 99, 419-424.
Suter, H.A. & Collard, K.J. (1983) Neurochem. Res. 8, 723-730.

GR38032F, A NOVEL SELECTIVE $5HT_3$ RECEPTOR ANTAGONIST

R.T. Brittain, A. Butler, I.H. Coates, D.H. Fortune, R. Hagan, J.M. Hill, D.C. Humber, P.P.A. Humphrey, S.J. Ireland, D. Jack, C.C. Jordan, A. Oxford, D.W. Straughan and M.B Tyers*, Research Division, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 0DJ, U.K.

The classification of 5-hydroxytryptamine (5HT) receptors into 'D' and 'M' types by Gaddum and Picarelli (1957) has been extended by further sub-classification (see Bradley et al., 1986). We report here the pharmacological properties of GR38032F (1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one, $HCl.2H_20$) which has potent and selective antagonist actions at $5HT_3$ receptors.

In the rat isolated vagus nerve (RVN) and superior cervical ganglion (RSCG), 5HT produces concentration-dependent depolarizations (Ireland et al., 1982). GR38032F, 10-300 nM, produced parallel rightward shifts of the log concentration-response curve to 5HT giving a pA_2 value of 8.6 (slope of Schild plot=1.23 [95% conf.lim.=0.93-1.53], n=21) in the RVN and 8.1 (1.13 [0.88-1.38], n=18) in the RSCG. Its selectivity for the $5HT_3$ receptor, compared to $5HT_2$ or $5HT_1$-like receptors, was demonstrated by its lack of effect against 5HT-induced contractions in the rabbit aorta (25uM) and dog saphenous vein (25uM) and against 5HT-induced relaxation in the cat saphenous vein (10uM). Furthermore, GR38032F, 10uM, produced no significant antagonist effects against sub-maximal responses to a wide range of agonists acting on non-5HT receptors in various isolated tissue preparations.

In the anaesthetised cat or rat 2-methyl-5HT, 1-100 µg/kg i.v., induces the Bezold-Jarisch (BJ) reflex by activation of $5HT_3$ receptors on sensory vagal afferents. In the rat, GR38032F caused dose-dependent inhibition of the BJ reflex (mean ED_{50}, [95% conf.lim.]=0.42 [0.18-0.87] µg/kg, i.v.; 7.0 [3-22] µg/kg, orally). In the chloralose-anaesthetized cat, GR38032F was well absorbed from the sub-lingual, intramuscular, sub-cutaneous and rectal routes and had a long duration of action (>3 h at a dose of 30µg/kg i.v.) as measured against the BJ-reflex induced by 2-Me-5HT.

The compound itself, at doses below 3mg/kg i.v. had no significant cardiovascular effects in the anaesthetized cat or in the conscious dog or monkey. Furthermore, no overt behavioural effects were observed in conscious animals (rat, mouse, dog and monkey) except at doses greater than 1mg/kg i.v. or 5mg/kg orally. In the anaesthetized cat or dog, cardiovascular responses to intravenously administered acetylcholine, adrenaline and histamine or to carotid occlusion were unaffected by GR38032F, 1-5mg/kg, i.v.. GR38032F, 1mg/kg i.v., had no effect on vascular dopamine receptors in the mesenteric bed of the anaesthetized dog, presynaptic dopamine receptors in the heart, or on dopamine receptors that mediate contralateral rotation or stereotypy in the rat.

Thus, GR38032F is a potent, highly selective, competitive antagonist at $5HT_3$-receptors. Its selectivity of action is reflected in a wide margin of safety. GR38032F has the clinical potential for markedly improving the treatment of certain psychiatric and gastrointestinal disorders (see Jones et al; Costall et al, this meeting).

Bradley, P.B. et al. (1986). Neuropharmacology, 25, 563-576
Gaddum, J.H. & Picarelli, Z.P. (1957). Br. J. Pharmac. Chemother., 12, 323-328
Ireland, S.J. et al. (1982). Br. J. Pharmac. Proc. Suppl., 75, 16P.

THE ANXIOLYTIC ACTIVITY OF GR38032F, A 5-HT_3 RECEPTOR ANTAGONIST, IN THE RAT AND CYNOMOLGUS MONKEY

B.J. Jones,* N.R. Oakley & M.B. Tyers, Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 0DJ, U.K.

The suggestion that central 5-HT was involved in the anti-punishment and anxiolytic actions of benzodiazepines arose from the observations of Stein et al. (1973). Evidence that 5-HT antagonists are anxiolytic in man has not been forthcoming but the recent emergence of antagonists selective for 5-HT receptor subtypes has opened the way for a re-evaluation. The 5-HT_2 receptor antagonist ritanserin may have some anxiolytic activity in man (Ceulemans et al., 1985) but so far there is no information on the new class of compounds that block the receptor recently classified as 5-HT_3 (Bradley et al., 1986). Accordingly, we have tested the novel 5-HT_3 antagonist GR38032F (Brittain et al, this meeting) for potential anxiolytic activity in rats and cynomolgus monkeys.

In the social interaction (SI) test (File, 1980), pairs of male rats (Hooded Lister, 180-230g) were treated orally with diazepam or GR38032F, 45 min before testing. The experimental conditions of high illumination and unfamiliarity with the test arena gave maximum suppression of SI. GR38032F, 0.0005-0.1mg/kg, caused dose-dependent increases in SI without affecting locomotor activity. Diazepam, 2mg/kg, increased SI to about 2.5 times the control value; an equivalent effect was obtained with 0.01mg/kg GR38032F. The effect of this dose of GR38032F was subsequently shown to decline by about 50% 90 min after treatment but thereafter it declined slowly over the next 6 hours. Under conditions of minimal suppression (low light/familiar) SI was not affected by either diazepam, 2mg/kg, or GR38032F, 0.01 mg/kg. In contrast to its effect in the SI test, GR38032F, 0.0005 - 1.6 mg/kg ip, had no effect in a water-lick conflict test (Vogel et al., 1971), although diazepam, 5mg/kg ip, increased licking 5-fold.

In cynomolgus monkeys (2 male, 2 female, 3.1-5.3 kg), both GR38032F, 0.01 and 0.1 mg/kg, and diazepam, 2.5mg/kg, administered orally, induced behavioural changes suggestive of a potential anxiolytic effect. Thus, both compounds reduced provoked and spontaneous aggressive behaviour as well as agitation and restlessness. In marked contrast to diazepam however, GR38032F did not affect alertness or motor co-ordination.

Thus, in the rat and cynomolgus monkey (as in the mouse and marmoset; Costall et al, this meeting), GR38032F appears to be a highly potent, non-sedative anxiolytic agent. Furthermore, it does not induce any overt behavioural disturbances even at doses 1000 times the minimum effective dose. If GR38032F proves to be effective in man, it will be the first of a new generation of anxiolytic agents that could represent a major advance in the treatment of anxiety.

We would like to thank David Beard, HRC, for help in the monkey experiments.

Bradley, P.B. et al. (1986). Neuropharmac. 25; 563-576
Ceulemans, D.L.S. et al. (1985). Pharmacopsychiat. 18; 303-305
File, S.E. (1980). J. Neurosci. Meth. 2; 219-238
Stein, L., et al. (1973). In: The Benzodiazepines, Eds: S. Garattini, E. Mussini and L.O. Randall, Raven Press, N.Y. pp 299-326
Vogel, J.R. et al. (1971). Psychopharmacologia (Berl.) 21; 1-7

THE ANTIPSYCHOTIC POTENTIAL OF GR38032F, A SELECTIVE ANTAGONIST OF $5HT_3$ RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

Brenda Costall*, Annette M. Domeney, M.E. Kelly, R.J. Naylor & M.B. Tyers[1], Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP, and [1]Neuropharmacology Department, Glaxo Group Research Ltd., Ware, SG12 0DJ.

The aetiology of schizophrenia has been associated, at least in part, with raised mesolimbic dopamine function. Classically, this is suppressed by neuroleptic agents having direct dopamine receptor antagonist action. Here we report that antagonism of mesolimbic receptors with GR38032F (Brittain et al, this meeting) can afford an antagonism of the dopamine response as effectively as neuroleptic agents.

Female Sprague-Dawley rats (Bradford strain, n=6) and common marmosets (Callithrax jacchus, n=4) were subject to standard stereotaxic surgery to implant chronically indwelling guides for injection or infusion (via subcutaneously implanted osmotic minipumps) at the centre of the nucleus accumbens (Costall et al, 1982; Barnes et al, 1985). Dopamine (25µg/24h) infused bilaterally into the nucleus accumbens of rat or marmoset for 13 days caused hyperactivity; in the rat this achieved a maximum intensity of 269±37 counts/60 min (measured in individual photocell cages) and in the marmoset the intensity was in the order of 250-450 counts/60 min (measured in individual cages having 4 infrared units strategically placed). These hyperactivity responses could be antagonised by peripherally administered sulpiride (5-30mg/kg i.p. rat, 0.06-5.0mg/kg i.p. marmoset), fluphenazine (0.1-0.9mg/kg i.p. rat, 0.01-0.1mg/kg i.p. marmoset) and GR38032F (0.0001-0.5mg/kg i.p. rat, 0.001-0.1mg/kg marmoset) given daily for 12 days, the hyperactivity counts being reduced to within the ranges of 25±6 to 89±10 counts/60 min in the rat ($p<0.001$) and 0 to 22±3 counts/60 min in the marmoset ($p<0.001$). In contrast to fluphenazine and sulpiride, GR38032F did not reduce activity levels to below vehicle control values indicating the absence of sedative activity.

Hyperactivity was also induced in the rat following the acute bilateral injection of amphetamine (10µg) into the nucleus accumbens. This response, which was measured in individual photocell cages, could be antagonised by a 30 min pretreatment with sulpiride (0.1-2.5ng), fluphenazine (0.5-10ng) or GR38032F (0.01-1.0ng) given into the nucleus accumbens. Following amphetamine the hyperactivity gained an intensity of 59±6 counts/5 min and this was reduced (at the time of maximum amphetamine responding) to 12±4, 3±1 and 18±2 counts/5 min ($p<0.001$) in the presence of sulpiride, fluphenazine and GR38032F respectively. In contrast to fluphenazine, GR38032F (up to 1.0mg/kg i.p.) failed to induce catalepsy, to antagonise stereotypy induced by amphetamine (5mg/kg i.p.), to initiate circling behaviour on unilateral intrastriatal injection (up to 10µg), to elevate prolactin levels in the rat or (at 10µM) to displace ^{3}H.spiperone in radioligand binding assays.

It is concluded that GR38032F, a selective, potent antagonist at $5HT_3$ receptors, may represent a new class of psychoactive agents having potential to antagonise a raised mesolimbic dopamine activity without apparent interference with 'normal' dopamine function. Such an agent may exert antischizophrenic activity with an absence of side effects normally associated with neuroleptic therapy.

Annette M. Domeney is a Glaxo Fellow.

Barnes et al (1985) Br.J.Pharmac 86, 68p.
Costall et al (1982) Neuropharmacology 21, 327-335.

GR38032F: A POTENT AND NOVEL INHIBITOR OF CISPLATIN-INDUCED EMESIS IN THE FERRET

Brenda Costall, Annette M. Domeney, S.J. Gunning[1], R.J. Naylor*, F.D. Tattersall & M.B. Tyers[1], Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP, and [1]Neuropharmacology Department, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 0DJ.

Cisplatin therapy in man is associated with severe emesis which can be antagonised by high doses of metoclopramide (MCP). This drug, which blocks 5-hydroxytryptamine ($5HT_3$) and dopamine (D_2) receptors also antagonises cisplatin-induced emesis in the ferret (Miner et al, 1986). In this latter model it is now shown that anti-emetic activity can be obtained with a novel drug, GR38032F (Brittain et al, this meeting) which possesses selective $5HT_3$ antagonist activity.

Cisplatin was given to male ferrets (1.2-2.0kg) either intravenously (10mg/kg) as a single dose or intraperitoneally (9mg/kg) once daily for 2 days. In the acute experiments, GR38032F, 0.01-1mg/kg i.v., and metoclopramide, 2-4mg/kg i.v., given 5 min before the cisplatin, reduced or abolished the emetic response and increased the latency to onset of emesis (Table 1). In these experiments GR38032F was some 200 times more potent than metoclopramide. Similarly, in the sub-acute experiments, GR38032F, 1.0mg/kg i.p., and metoclopramide, 1.0mg/kg i.p., given 30 min before and 60 min after cisplatin, 9 mg/kg i.p., reduced or abolished the emesis and delayed its onset on both days of testing. Lower doses have not been tested.

TABLE 1. The effects of GR38032F and MCP to antagonise cisplatin-induced emesis in the ferret

Treatment Dose mg/kg i.v.	n	No. of animals completely protected	No.of emetic episodes (mean ± S.E.M.)	No.of retches (mean ± S.E.M., within 2h period)	Latency period (min) (mean ± S.E.M.)
Cisplatin 10	6	0	9.7±1.3	97.0±10.0	56.0± 6.7
+ GR38032F 1.0	6	6	0*	0*	-
0.1	6	6	0*	0*	-
0.01	6	0	3.5±2.0*	27.5±17.6*	92.0±10.6*
+ MCP 4.0	6	6	0*	0*	-
2.0	6	0	3.6±1.2*	25.3± 8.0*	87.0± 9.1

*$p<0.05$ (Mann Whitney)

In conclusion, GR38032F is a potent inhibitor of cisplatin-induced emesis in the ferret. Since the clinical use of metoclopramide for the prevention of cisplatin-induced emesis may be disadvantaged by dystonic side-effects (initiated by disturbances of extrapyramidal dopamine function), the use of GR38032F, which is devoid of dopamine antagonist properties, may represent an effective, highly selective and potent therapeutic alternative.

Miner et al (1986) Br.J.Pharmac., 88, 615-620.

5-HT_{1B} AGONISTS CAUSE ANOREXIA AT POSTSYNAPTIC RECEPTORS

Curzon, G., Dourish, C.T. and Kennett, G.A.*, Department of Neurochemistry, Institute of Neurology, London WC1., U.K.

The specific 5-HT_{1A} agonist 8-OH-DPAT induces feeding in non-deprived rats by action at somatodendritic autoreceptors (Dourish et al., 1986; Hutson et al., 1986). The present study concerns the effects of two other putative 5-HT_{1A} ligands Buspirone and TVXQ 7821 (ipsapirone) previously found to induce mild hyperphagia (Dourish et al., 1986) and the putative 5-HT_{1B} agonists RU 24969, m-chlorophenylpiperazine (mCPP) and trifluoromethylphenylpiperazine (TFMPP).

Singly housed, normally fed male Sprague Dawley rats (200-250 g) were injected with drugs or saline at 13:00 h, replaced in their home cages and their food intake measured over the next 4 h and 24 h. Both Buspirone and TVXQ 7821 injected s.c. dose dependently increased 4 h food intake, [maximally: Buspirone (8.0 mg/kg), 3.8 ± 0.4 g, n = 13; saline 1.5 ± 0.3 g, n = 15, $p < 0.01$; TVXQ 7821 (20.0 mg/kg), 3.9 ± 0.5 g, n = 7; saline 0.5 ± 0.2 g, n = 8, $p < 0.01$ by Dunnetts test following significant ANOVA]. In contrast i.p. RU 24969, mCPP and TFMPP (Table 1) induced a marked anorexia over 4 h (24 h with RU 24969).

Table 1: Effect of putative 5-HT_{1B} agonists on 4 h and 24 h food intake (g)

Treatment		n	4 h	24 h
Saline		15	1.1 ± 0.2	25.9 ± 0.6
RU 24969	0.2 mg/kg	10	0.85 ± 0.2	23.5 ± 1.6
	1.0 mg/kg	10	0.55 ± 0.1*	21.1 ± 1.4*
	2.0 mg/kg	8	0.30 ± 0.1**	19.4 ± 0.8**
	5.0 mg/kg	8	0.07 ± 0.03**	17.0 ± 0.6**
	10.0 mg/kg	8	0.1 ± 0.1**	14.0 ± 0.9**
Saline		10	1.21 ± 0.16	24.8 ± 0.7
mCPP	0.5 mg/kg	5	0.77 ± 0.5	25.6 ± 0.79
	1.0 mg/kg	5	0.31 ± 0.2*	24.9 ± 0.6
	5.0 mg/kg	10	0.15 ± 0.03**	24.9 ± 0.8
TFMPP	1.0 mg/kg	8	0.52 ± 0.07*	23.8 ± 1.0
	10.0 mg/kg	10	0.26 ± 0.07**	25.3 ± 0.56

Values ± S.E.M. Differences from saline *$p < 0.05$, **$p < 0.01$ (Dunnetts test following ANOVA).

The anorectic effect of RU 24969 (5 mg/kg i.p.) over both 4 h and 24 h was inhibited by pretreatment 30 min earlier with the non-specific 5-HT antagonist methergoline (5 mg/kg s.c.) and cyanopindol (8 mg/kg s.c.) a 5-HT_{1A} and 5-HT_{1B} antagonist. However, the specific 5-HT_{1A} antagonist spiperone (0.05 or 0.1 mg/kg s.c.), the specific 5-HT_2 antagonist ketanserin (2.5 mg/kg i.p.) and the 5-HT depleter PCPA (150 mg/kg daily x3 last dose 24 h before RU 24969) had no effect. Doses of RU 24969, mCPP and TFMPP as low as 0.05, 0.1 and 0.5 mg/kg respectively did not cause the hyperphagia which might have been predicted from in vitro evidence of their action at 5-HT_{1B} terminal autoreceptors (Middlemiss, 1985). The results indicate that the 5-HT_{1A} ligands Buspirone and TVXQ 7821 induce hyperphagia probably (by analogy with 8-OH-DPAT) by acting as presynaptic 5-HT_{1A} agonists, whilst RU 24969, mCPP and TFMPP probably induce anorexia by stimulating 5-HT_{1B} postsynaptic receptors.

Dourish, Hutson, Kennett and Curzon (1986) Appetite, 7, Suppl., 127-140.
Hutson, Dourish and Curzon (1986) Eur.J.Pharmacol., in press.
Middlemiss (1985) J.Pharm.Pharmacol., 37, 434.

INTERACTIONS OF LISURIDE WITH SEROTONIN ($5\text{-}HT_{1A}$) RECEPTORS

D. Hoyer. Preclinical Research. SANDOZ LTD. CH 4002 Basel. Switzerland. (Introduced by B.P. Richardson).

Lisuride, a semi-synthetic ergot, interacts with dopaminergic, serotonergic and adrenergic receptors (Rosenfeld & Makman, 1981, Battaglia & Titeler, 1981). Lisuride behaves as a full agonist to increase 5-HT sensitive adenylate cyclase activity in rat hippocampus. The effect of lisuride was potently inhibited by metitepin and other 5-HT antagonists but not by ketanserin ($5\text{-}HT_2$) or SCH 23390 (dopamine D_1 antagonist). We have demonstrated that this activity correlates very significantly with $5\text{-}HT_{1A}$ binding, $p=.0001$, $r^2=.872$, (Markstein et al, 1986).

In bovine brain membranes [^{3}H]lisuride labels in part a population of sites resembling $5\text{-}HT_1$ recognition sites. Since 3 subtypes of $5\text{-}HT_1$ sites have been described (Pedigo et al, 1981, Pazos et al, 1984, Hoyer et al, 1985a), it was decided to compare the binding profile of a series of drugs to [^{3}H]lisuride binding (Battaglia & Titeler, 1981) with $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, $5\text{-}HT_{1C}$ and $5\text{-}HT_2$ binding performed as described earlier (Hoyer et al, 1985b). Table 1 lists the affinity values (pK_D or pIC_{50}, -log mol/l) obtained.

TABLE 1:	[^{3}H]lisuride	$5\text{-}HT_{1A}$	$5\text{-}HT_{1B}$	$5\text{-}HT_{1C}$	$5\text{-}HT_2$
lisuride	9.00	9.12	6.65	7.71	8.26
5-HT	7.82	8.51	7.63	7.48	7.10
d-LSD	7.70	8.59	6.82	7.93	8.62
bufotenine	7.52	7.88	6.04	7.15	6.42
metergoline	7.26	8.10	7.39	9.19	9.03
spiperone	6.70	7.18	5.27	5.94	8.76
phentolamine	5.75	5.52	5.37	6.05	6.06
haloperidol	5.70	5.32	5.06	4.76	6.61
clonidine	5.41	5.53			
prazosine	5.05	5.00	5.10	4.70	4.96
(-)adrenaline	<5	3.49			
(-)noradrenaline	<5	3.50	4.10	3.52	3.53
dopamine	<5	4.66	4.34	4.43	4.07

A highly significant correlation was found between the affinity of drugs for the $5\text{-}HT_{1A}$ and lisuride binding sites ($p=.0001$, $r^2=.95$) whereas correlations with $5\text{-}HT_{1B}$, $5\text{-}HT_{1C}$ or $5\text{-}HT_2$ binding were much less significant ($p=.012\text{-}.059$, $r^2=.61\text{-}.42$).

Together, the functional and binding data suggests that lisuride is a full agonist at $5\text{-}HT_{1A}$ receptors and that it binds (in addition to dopaminergic and adrenergic sites) to $5\text{-}HT_{1A}$ recognition sites in the mammalian brain.

Battaglia G & Titeler M (1981) Life Sci. 29, 909-916.
Hoyer D et al (1985a) Eur. J. Pharmacol. 118, 1-12.
Hoyer D et al (1985b) Eur. J. Pharmacol. 118, 13-23.
Markstein R et al, (1986) N.S. Arch. Pharmacol. 333, 335-341.
Pazos A et al (1984) Eur. J. Pharmacol. 106, 539-546.
Pedigo NW et al (1981) J. Neurochem. 36, 220-226.
Rosenfeld MR & Makman M (1981) J.Pharmacol. Exp. Ther. 216, 526-531.

NEUROCHEMICAL AND BEHAVIOURAL EFFECTS OF LY165163: A PUTATIVE 5-HT_{1A} CENTRALLY ACTING AGONIST

G.Curzon, T.P.Donohoe and P.H.Hutson*, Department of Neurochemistry, Institute of Neurology, Queen Square, London WC1

1-[2-(4-aminophenyl)ethyl]-4-(trifluoromethylphenyl) piperazine (LY165163) binds selectively and with high affinity to 5-HT_{1A} recognition sites in rat brain in vitro (Ransom et al., 1986). We now present neurochemical and behavioural evidence that LY165163 is a centrally active 5-HT agonist.
The effects of the drug on regional brain 5-HT and catecholamine synthesis were determined as follows. Male Sprague-Dawley rats (Charles-River U.K. Ltd) (250-300 g) maintained on a 12 h light-dark cycle (lights on 06.00h) were given either 0.9% NaCl (1 ml/kg s.c.) or LY165163 (1 mg/kg s.c.) 30 min before injection with the decarboxylase inhibitor NSD 1015 (100 mg/kg i.p.) and killed 30 min later. Brains were removed, dissected into regions and stored at -70°C until analysis for 5-HTP and DOPA by HPLC with electrochemical detection. Consistent with 5-HT agonist action LY165163 significantly decreased 5-HTP accumulation in the striatum (-45%), hippocampus (-26%), pons medulla (-36%), septum (-29%), midbrain (-40%) and cortex (-52%) but not in hypothalamus (-25%) DOPA accumulation was significantly increased in striatum (119%) and cortex (77%) but not in hypothalamus (20%), midbrain (7%), pons + medulla (8%) or septum (8%).

LY165163 (0.06-4 mg/kg s.c.) dose-dependently increased food intake of satiated rats in the home cage over 2,4 and 24 h. The increase at 2 and 4 h was prevented by depletion of brain 5-HT with p-chlorophenylalanine (150 mg/kg i.p., 72, 48 and 24 h) before LY165163 (1 mg/kg s.c.).

The hyperphagic effect is somewhat similar to that of another 5-HT_{1A} agonist, 8-OH-DPAT and is therefore probably similarly mediated i.e. by action at somatodendritic 5-HT_{1A} receptors (Dourish et al., 1985; Hutson et al., 1986).

Unlike 8-OH-DPAT, LY165163 (1, 2, 4 and 10 mg/kg s.c.) did not induce the postsynaptically mediated 5-HT behavioural syndrome but at the 3 highest doses rats appeared behaviourally quiet and performance on the rotorod was impaired. When LY165163 (1 mg/kg s.c.) was given 30 min before 5-methoxy-N,N-dimethyltryptamine (5 mg/kg i.p.), the components of the 5-HT behavioural syndrome were not inhibited, with the exception of head weaving and locomotion.

While 8-OH-DPAT causes a marked hypothermia in rats (Hjorth, 1985; Goodwin and Green, 1985), LY165163 caused a less pronounced hypothermia 30 min after s.c. injection (1 mg/kg: -0.4°C; 2 mg/kg: -0.57°C; 4 mg/kg: -0.9°C) compared with that of 8-OH-DPAT (1 mg/kg s.c. -2.5°C).

Thus LY165163 like 8-OH-DPAT decreased central 5-HT synthesis and caused hyperphagia, but unlike 8-OH-DPAT it had a less pronounced hypothermic effect and did not induce the 5-HT behavioural syndrome. Taken together these data indicate that LY165163 is a centrally active 5-HT agonist but with more pronounced effects on presynaptic than postsynaptically mediated 5-HT behaviour.

Dourish, C.T. et al. (1985) Psychopharmacol., 86, 197-204.
Goodwin, G.M. and Green, A.R. (1985) Br.J.Pharmac., 84, 743-753.
Hjorth, S. (1985) J.Neural.Transm., 61, 131-135.
Hutson, P.H. et al. (1986) Eur.J.Pharm., in press
Ransom, R.W. et al. (1986) J.Neurochem., 46, 68-75.

THE PUTATIVE ANTIDEPRESSANT BTS 54 524 RAPIDLY AND POTENTLY DOWN REGULATES CORTICAL β-ADRENOCEPTORS IN THE RAT

W.R. Buckett, G.P. Luscombe and P.C. Thomas, Research Department, The Boots Company PLC., Nottingham NG2 3AA

BTS 54 524 (N-1-(1-(4-chlorophenyl)cyclobutyl)-3-methylbutyl-N,N-dimethylamine hydrochloride monohydrate) has a pharmacological profile consistent with an antidepressant effect (Buckett et al, this meeting). Repeated administration of most clinically effective antidepressants decreases the sensitivity of the noradrenaline-receptor coupled adenylate cyclase in rat brain (Sulser, 1978), although only some also induce subsensitivity of central β-adrenoceptors as measured by ligand binding techniques (Banerjee et al, 1977). We now report that repeated administration of BTS 54 524 rapidly and potently decreases specific ^{3}H-dihydroalprenolol (^{3}H-DHA) binding to cortical β-adrenoceptors in rat brain.

Male Sprague-Dawley (Charles River) rats (150-250g) were orally administered either BTS 54 524, antidepressant drugs (Table 1) or distilled water for 1 or 3 or 7 days. The cortex was rapidly dissected out 24h after the last drug treatment and stored at -20°C until required. Tissue preparation, the ^{3}H-DHA binding assay, and analyses of the number of cortical β-adrenoceptors (Bmax) and their affinity (KD) were performed as previously described (Buckett & Thomas, 1982). The ability of these compounds to displace specific ^{3}H-DHA binding from an *in vivo* preparation of rat cortex was also studied.

The results show that BTS 54 524 decreased Bmax following oral administration of only 1 mg/kg for 3 days (Table 1). Of the other compounds tested, imipramine, nomifensine (both 10 mg/kg/day) and dothiepin (100 mg/kg/day) induced β-adrenoceptor subsensitivity over this short treatment period; KD values were unaffected. The potency of BTS 54 524 was emphasised in the 7-day study where it was the only compound to reduce Bmax (Table 1). A single oral 30 mg/kg dose of BTS 54 524, imipramine or nomifensine each failed to alter Bmax as assessed 24h after treatment. In addition, BTS 54 524 (up to 10^{-4}M) did not displace *in vitro* specific ^{3}H-DHA binding from cortical preparations. The remaining compounds were also inactive or had IC_{50} values greater than 10^{-4}M (imipramine, dothiepin).

Table 1 Effect of 3 day or 7 day oral administration of BTS 54 524 or other antidepressants on cortical β-adrenoceptors (Bmax)

	3-day treatment				7-day treatment	
Compound	Dose (mg/kg)	Effect on Bmax	Dose (mg/kg)	Effect on Bmax	Dose (mg/kg)	Effect on Bmax
BTS 54 524	1	-22%*	3	-29%*	4	-34%**
Imipramine	3	+ 1%	10	-19%*	4	-11%
Nomifensine	3	+10%	10	-26%*	4	- 9%
Dothiepin	10	- 2%	100	-15%*	10	- 7%
Mianserin	10	- 2%	50	-14%	10	0%
Citalopram	10	- 9%	30	-13%	Not tested	

**p<0.01; *p<0.05 vs Bmax determined in cortex obtained from distilled water treated control rats (Student's t-test; n=8-10 rats/treatment).

BTS 54 524 is therefore a putative antidepressant which rapidly and potently induces β-adrenoceptor subsensitivity, probably as a consequence of monoamine uptake inhibition (Buckett et al, this meeting), and may thus exhibit a rapid clinical effect.

Banerjee, S.P. et al (1977). Nature 268, 455
Buckett, W.R. et al (1987). This meeting
Buckett, W.R. & Thomas, P.C. (1982). Br. J. Pharmac. 75, 82P
Sulser, F. (1978). Pharmakopsychiatry 11, 43

NEUROLEPTIC INDUCED DOPAMINE RECEPTOR SUPERSENSITIVITY AND TARDIVE DYSKINESIA MAY INVOLVE ALTERED BRAIN IRON METABOLISM

D. Ben-Shachar and M.B.H. Youdim*, Rappaport Family Research Institute and Department of Pharmacology, Faculty of Medicine, Technion, Haifa, Israel.

Nutritional iron-deficiency (ID) in rats induces biochemical and behavioural D_2 receptor subsensitivity which can be reversed by iron-therapy (Youdim, 1985; Ben-Shachar et al., 1986). The dyskinesia in Haller-Vorden Spatz disease is thought to be associated with extensive accumulation of iron and neuronal degeneration in globus pallidus and s. nigra (Youdim, 1985). More recently similar findings have been reported by Campbell et al. (1985) in brains from subjects with haloperidol-induced tardive dyskinesia (TD). The reports that neuroleptics are avid chelators of iron (Rajan et al., 1974) prompted us to investigate the relationship between neuroleptic induced dopamine D_2 receptor supersensitivity and brain non-haem iron metabolism as a basis for TD.

Sprague-Dawley male rats (21 days old) fed an iron-deficient diet for 4 weeks show progressive decreases in ^{3}H-spiperone binding (-30%, $P<0.001$) in caudate nucleus, behavioural response (-60%, $P<0.005$) to apomorphine (2mg/kg) and brain non-haem iron content (-35%, $P<0.01$). Chronic daily treatment of rats with haloperidol (5mg/kg, 21 days) either a week after the start or at the end of 4 weeks of ID prevented and reversed the effects of ID on ^{3}H-spiperone binding and apomorphine induced locomotor activity). In these animals ^{3}H-spiperone binding and apomorphine behavioural responses are similar to control-haloperidol treated rats. These data are matched by the ability of haloperidol also to prevent and reverse the decrease in caudate nucleus non-haem iron noted due to 4 weeks of ID. Both haloperidol (5mg/kg) and chlorpromazine (10mg/kg) treatments (21 days, 21 days old rats) respectively cause significant reductions of liver non-haem iron 161± 13µg/g and 172± 5µg/g, when compared to non-neuroleptic treated control 220±12µg/g (n=6-10). Since iron normally cannot pass the blood brain barrier, $FeCl_3$ (5µl/100mM) was injected intraventricularly into rats. Three weeks later dopamine receptor supersensitivity was noted by the increases in ^{3}H-spiperone binding (50± 10%, n=6, $P<0.001$) and apomorphine induced behavioural responses (62± 15%, n=6, $P<0.001$), confirming the data of Czernansky et al. (1983).

The present study indicates that an alteration in brain iron can profoundly affect dopamine D_2 receptor function. Furthermore, the iron-chelating activities of neuroleptics may accord special properties to these drugs, namely, their ability to either mobilize iron from peripheral tissue into the brain, or decrease iron turnover in the brain with the resultant increase of this element. Such a phenomena would be consistent with the increased deposition of iron and neuronal degenerative processes noted in the globus pallidus and substantia nigra of TD (Campbell et al., 1985).

Ben-Shachar, D. et al (1986) Int.J.Devl.Neurosci. 4, 81-88.
Campbell, W.D. et al (1985) Am.J.Psychiat. 142, 364-366.
Czernansky, J.C. et al (1983) Life Sci. 32, 385-391.
Rajan, K.S. et al. (1974) Adv.Biochem.Psychopharmac. 9, 571-591.
Youdim, M.B.H. (1985) in Handbook of Neurochemistry, Vol. 10. Lajath A. ed. New York, Plenum pp. 731-755.

ANTAGONISM BY KETOTIFEN OF CHANGES IN DOPAMINE RECEPTOR SENSITIVITY IN THE RAT BRAIN

Julie C. Barnes*, Brenda Costall and R.J. Naylor, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP

Ketotifen can prevent or reverse tachyphylaxis of the β-adrenergic system following chronic treatment with β_2-adrenoceptor agonists (Bretz et al, 1983). Here we show that ketotifen also prevents changed dopamine (DA) receptor function following chronic DA agonist/antagonist treatment.

Ketotifen was given in 2 situations of DA receptor 'supersensitivity': (1) enhanced sensitivity to the locomotor stimulant actions of (-)N-n-propylnorapomorphine [(-)NPA, 0.05mg/kg s.c.] caused by 13 days infusion of DA into the nucleus accumbens (ACB) or (-)sulpiride i.p. and (2) enhanced spontaneous locomotion following co-administration of DA (intra-ACB) and haloperidol (i.p.) for 13 days. Male Sprague-Dawley rats were subject to standard stereotaxic surgery for the implantation of chronically indwelling bilateral guide cannulae for infusions of DA (25μg/24h) into the ACB via subcutaneously implanted osmotic minipumps. For peripheral infusions, pumps were implanted in the peritoneal cavity. Locomotor activity was measured in individual photocell cages. In all experiments n = 5.

DA infused into the ACB for 13 days caused enhanced (to 229.6-239% of vehicle control values, $P<0.001$) responsiveness to the locomotor stimulant effects of (-)NPA which developed 7 to 14 days after discontinuing the infusion. Ketotifen (1mg/kg/24h, by i.p. infusion) administered at the same time as DA prevented the enhanced responding to (-)NPA post-infusion (responses indistinguishable from those of vehicle control animals, $P>0.05$). Similarly, 13 days i.p. infusion of (-)sulpiride (5mg/kg/24h) caused enhanced locomotor responding to (-)NPA, manifest during infusion on day 13 (to 233% of control values, $P<0.001$) and for up to 14 days post-infusion (increased to 58-70% of controls, $P<0.01$-$P<0.001$). Ketotifen (1mg/kg/24h i.p.) administered throughout the sulpiride treatment prevented the increased responding to (-)NPA (values indistinguishable from those of vehicle control animals, $P>0.05$). Haloperidol (0.15mg/kg i.p. b.d.) given at the same time as a 13 day intra-ACB infusion of DA caused marked elevations of spontaneous locomotion when the drug regime was withdrawn after 13 days, activity counts reaching 212-260/60 min compared to 74-124/60 min for vehicle-treated animals, and lasting for 70+ days). Such enhanced activity was not manifest following 13 days treatment with DA or haloperidol alone. Ketotifen (1mg/kg/24h i.p.) administered concomitantly with the DA/haloperidol treatment delayed the development of enhanced spontaneous activity for up to 8 days post-infusion ($P<0.01$). When administered acutely, ketotifen (0.1-1.0mg/kg i.p. 1h pretreatment) dose-dependently reversed hyperactivity established following withdrawal of DA/haloperidol treatment, the effects of a single dose persisting for up to 6 days (1hr after ketotifen, 1mg/kg i.p., counts were reduced to 91±11/60 min, $P<0.001$, reaching 127±18/60 min after 6 days, $P<0.01$).

It is concluded that whilst ketotifen does not block DA receptors directly (Barnes et al, 1986) it can attenuate or antagonise the functional consequences attributed to changes in DA receptor sensitivity. Such an action may be important in psychiatric disease states in which changes in DA receptor sensitivity are hypothesised to occur either as a result of the disease process itself or drug intervention.

This work was supported by the MRC.

Barnes, J.C. et al (1986) Neuropharmacology, submitted
Bretz, U. et al (1983) Eur. J. Pharmac. 86, 321-328

DIFFERENTIAL LINKAGE OF DOPAMINE D_2 TO D_1 STIMULATED CYCLIC AMP ACCUMULATION IN RAT STRIATUM AND LIMBIC FOREBRAIN

M Keen, E Kelly* and S R Nahorski, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, Leicester, LE1 7RH

Although the actions of dopamine agonists and antagonists can differ in the dopamine-rich areas of striatum and limbic forebrain, there is no evidence for pharmacological heterogeneity of the D-1 and D-2 receptors between these regions. In the striatum, however, postsynaptic D-1 and D-2 receptors are linked in opposite ways to adenylate cyclase (Stoof and Kebabian, 1981) and behavioural studies have suggested the possibility of interplay between these sites (Molloy and Waddington, 1985). We report here that the linkage of D-2 sites to D-1 stimulated cyclic AMP accumulation is not present in limbic forebrain.

The cyclic AMP content of striatal or limbic (constituted by the nucleus accumbens and olfactory tubercle) slices (0.3 x 0.3 mm) was estimated in the presence of 1 mM 3-iso-butyl-1-methyl xanthine using a protein binding assay. In striatal slices, the selective D-1 agonist SKF 38393 (1 μM) enhanced cyclic AMP accumulation, which was in turn inhibited by > 50% by the selective D-2 agonist LY 171555 (10 μM; Table 1). This inhibition was reversed by the selective D-2 antagonist (-)-sulpiride (10 μM; Table 1). On the other hand in limbic slices LY 171555 did not inhibit SKF 38393 (1 μM) stimulated cyclic AMP accumulation (Table 1). Conversely, in both striatal and limbic slices the muscarinic agonist carbachol (10 μM) inhibited D-1 stimulated cyclic AMP accumulation by >65%. In other experiments (-)-sulpiride (30 μM) enhanced the ability of dopamine (3-50 μM) to elevate cyclic AMP accumulation in striatal but not limbic slices. Similar results were obtained when these experiments were performed in the presence of (-)-propranolol (1 μM), to negate any effect of dopamine on the beta-adrenoceptor.

Table 1

	Basal	SKF38393 (1μM)	SKF38393 (1μM) +LY171555 (10μM)	SKF38393 (1μM) +LY171555 (10μM) +(-)-sulpiride (10μM)
Striatum	14.2 ± 1.1	26.7 ± 1.2	19.9 ± 0.9*	29.6 ± 2.3
Limbic	21.6 ± 0.8	32.6 ± 3.0	32.6 ± 0.9	30.5 ± 2.5

Cyclic AMP accumulation (pM/mg protein) in striatal and limbic slices in response to D-1 and D-2 selective drugs. The data was obtained in a single experiment. * $p < 0.05$ compared to SKF 38393 alone, Student's 't' test.

These results indicate that, unlike striatum, D-2 receptors in the limbic forebrain do not appear to be coupled to D-1 stimulated cyclic AMP accumulation. This could underlie some of the different consequences of dopaminergic drug interactions in different regions of the brain.

This work was supported by the Wellcome Trust.

Stoof JF and Kebabian JW (1981) Nature, 294, 366
Molloy AG and Waddington JL (1985) Eur J Pharmacol, 108, 305

INDOMETHACIN INCREASES THE KILLING OF MALIGNANT CELLS BY METHOTREXATE

A. Bennett and J.D. Gaffen*, Dept. Surgery, King's College School of Medicine and Dentistry, The Rayne Institute, 123 Coldharbour Lane, London SE5 9NU.

Mice with NC carcinoma given the cytotoxic drugs methotrexate (MTX) and melphalan survived longer when indomethacin or flurbiprofen were also given (Bennett et al, 1982). The interaction may have been with just the MTX since in isolated NC cells indomethacin increased both the killing by MTX and the cellular accumulation of radiolabel when the cells were incubated with tritiated MTX (Gaffen et al 1985, 1986). We now report further studies on the mechanism of this interaction, and we describe a similar increased killing of a human breast cancer cell line.

NC cells were grown as a suspension in liquid medium (Gaffen et al 1985). DU4475 cells derived from a metastatic cutaneous nodule of human breast carcinoma (Langlois et al, 1979) were grown as a suspension in RPMI 1640 supplemented with 5% newborn bovine serum and antibiotics. Epithelial cells from human normal embryonic intestine (No. 407, Flow Laboratories) were grown as monolayers in BME supplemented with 15% serum and antibiotics ± the drugs described . Measurements were made of growth (clonogenic or microturbidimetric assays, Gaffen et al, 1985), tritium accumulation after incubation with labelled MTX (Gaffen et al, 1986; or with slight modification), and tritiated MTX binding (Dixon et al, 1965).

In the normal intestinal cells MTX 5-10ng/ml reduced the clonal growth by 17-71%, but indomethacin 1µg/ml had no additional effect. Neither indomethacin nor flurbiprofen affected their accumulation of label during incubation with tritiated MTX.

With the NC cancer cells, indomethacin increased the accumulation of tritium (uptake and/or retention) during incubation with tritiated MTX (63% increase at 60 min, $P<0.001$). However, flurbiprofen 1µg/ml appeared to decrease the cytotoxicity of MTX 2-40 ng/ml, and it did not alter the accumulation of label when the NC cells were incubated with tritiated MTX. Thus indomethacin may not interact with MTX by inhibiting prostaglandin synthesis. The interaction was probably not due to displacment of the cytotoxic drug from binding to serum proteins, since the percent of labelled MTX bound to newborn bovine serum was 51 ± 3% with or without indomethacin 1 µg/ml. Inhibition of cAMP phosphodiesterase by indomethacin was unlikely to be a factor since theophylline 9 µg/ml had little or no effect on the killing of NC cells by 8 ng/ml MTX (35% killing with MTX + theophylline, 33% with MTX alone).

The finding that indomethacin 1µg/ml increased by 6.8-20.5% the killing of human breast cancer DU4475 cells by MTX 2.5-10ng/ml, strengthens the possibility that indomethacin may be useful in selectively increasing the cytotoxicity of MTX in human cancer.

We thank the CRC and MRC for support.

Bennett, A. et al (1982) Br. J. Cancer 45, 762-768
Dixon, R.L. et al (1965) Fed. Proc. 24, 454
Gaffen, J.D. et al (1985) J. Pharm. Pharmacol. 37, 261-263
Gaffen, J.D. et al (1986) Progr. Lipid Res. in press

EARLY EVENTS IN THE PATHOGENETIC PROCESSES UNDERLYING IDOMETHACIN INDUCED INTESTINAL LESIONS IN RATS

G. Benoni[2], L. Cuzzolin[2], P. Del Soldato*, D. Foschi[1], E. Fracasso[2] and G.P. Velo
Pierrel S.p.A. Via Bisceglie, 96 Milan, Italy, [1]Inst.Biom.Sci., University of Milan, Milan, Italy and [2]Inst.Pharmacology, University of Verona, Verona, Italy

Non steroidal-anti-inflammatory drugs are known to cause intestinal ulcers in both animals and humans. This aspect has been dramatically highlightened by the withdrawal from the market of some drugs which provoked severe intestinal toxicity in animals and even caused some deaths in humans. Therefore it seems mandatory to elucidate the mechanism of their intestinal toxicity. Although it has been emphasized that kinetic factors are important for the formation of these reactions, there is evidence that ulcerative process is still active when plasma or tissue levels of the compounds are almost undectable. The aim of the present study was to evaluate the early phases of the process, in which tissue concentration is of primary importance. For this purpose a new animal model was developed in our laboratories.By continuous i.a. injection of indomethacin into urethane-anesthetized rats at doses from 0.05 to 0.5 mg/kg/min it was possible to induce intestinal mucosal erosions within 1-2 hours. Under these experimental conditions, the effects of such factors as flora, antibiotics, prostaglandins, free radicals, which have been held to be of primary importance in the development of intestinal lesions (Del Soldato, 1984), have been evaluated. Unlike fasting, clindamycin (1 mg/kg p.o. and i.p.) or 4 hr bile duct ligation, PGE_2 (20-100 µg/kg i.p. or p.o.) and cysteamine (50-200 mg/kg i.p.), were effective in inhibiting intestinal mucosal damage. This is consistent with the evidences that: 1) Indomethacin as well as other non steroidal-anti-inflammatory drugs react with oxygen free radical species (Hiller and Wilson, 1983) with the formation of a spectroscopically defined chemical structure (Duchstein, 1985). 2) The earliest modifications of bacterial flora caused by indomethacin are detectable not before 17 hours following its administration (Benoni et al.,1986). To further substantiate the hypothesis the oxygen free radicals, but not intestinal bacteria, are involved in the early phase of the ulcerogenic process, the influence of 10 k rad irradiated indomethacin (8 mg/kg p.o. or 0.05 mg/kg/min i.a.)on aerobic and anaerobic bacteria populations of intestinal content or intestinal lesions was determined according to procedures previously described (Del Soldato et al., 1985; Benoni et al., 1986). A marked decrease of bacteria (Bacteroides, E.coli, Streptococcus faecalis) was observed, although the intestinal lesions were present in a greater extent as compared to animals treated with non-irradiated indomethacin ($P < 0.01$). These findings allow to suggest that unlike food, flora or bile excretion, prostaglandins and free radicals, through an opposite role, markedly influence the early phase of the ulcerogenic process.

Benoni G. et al. in Side-effects of Anti-inflammatory Drugs Rainsford, KD, and Velo GP, Eds, Part 2, Lancaster MPT Press. In press
Del Soldato P, (1984) in Advances in inflammatory research Rainsford KD, and Velo GP, Eds, 6, 89-102, Raven Press
Del Soldato P, et al. (1985) Agents and Actions 16, 393-396
Duchstein HJ, (1985) Arch.Pharm. 318, 127-134
Hiller KD, and Wilson RL, (1983) Biochem.Pharmacol. 32, 109

100P

CUTANEOUS EFFECTS OF NEUROPEPTIDES ARE DEPENDENT UPON BOTH HISTAMINE AND CYCLOOXYGENASE PRODUCTS

P.J. Barnes[1], D.C. Crossman & R.W. Fuller*, Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W12 OHS and [1]Cardiothoracic Institute, Fulham Road, London SW3.

Neuropeptides, substance P (SP), calcitonin gene related peptide (CGRP) and neurokinins A and B (NKA, B) produce wheal and flare following intradermal injection. SP induced flare is dependent upon histamine release as it is inhibited by antihistamine drugs (Barnes et al, 1986). In vitro CGRP cause the production of prostacyclin by cultured endothelial cells and, as this is a potent vasodilator substance, some of the effects of these neuropeptides may be via cyclo- oxygenase products. We have, therefore, investigated the effects of aspirin and terfenidine on the cutaneous response to injected SP, CGRP, NKA and B.

Twelve subjects (6 female) age 24-35 years took part in the study. Six of the subjects took either placebo, terfenidine 60 mg or aspirin 600 mg before intradermal injections of CGRP (250 pmol), NKA (1000 pmol) or histamine (5 nmol) in 50 µl via a 27 G needle into the skin of the forearm. The other subjects took either placebo or aspirin 600 mg before intradermal injections of SP (250 pmol) or NKB (1000 pmol). Flare was measured by planimetry at 5 and 30 min and wheal at 10 minutes following the injection.

The results (mean ± SD) are summarised in the table which shows the area of wheal and flare in square centimetres and analysed by two-way analysis of variance.

	Terfenidine			Aspirin			Placebo		
	5'F	30'F	W	5'F	30'F	W	5'F	30'F	W
SP	nd	nd	nd	9.2± 4.1*	6.6± 3.3	0.8± 0.2	11.9± 4.8	7.9± 4.0	0.9± 0.2
CGRP	1.0± 0.6*	1.5± 0.3	0.5± 0.2	0.8± 0.2*	1.7± 1.4	0.4± 0.2	3.6± 4.0	2.3± 1.3	0.5± 0.1
NKA	1.6± 0.6*	0.0± 0.6*	0.9± 0.4*	3.4± 2.4*	0.2± 2.4*	1.1± 0.4*	5.5± 3.0	3.1± 3.0	1.4± 0.4
NKB	nd	nd	nd	0.9± 0.8	0.0± 0.0	0.9± 0.6	1.1± 0.8	0.0± 0.9	1.0± 0.6
HIST	1.4± 1.0*	0.6± 1.0*	0.4± 0.1*	8.4± 2.2	5.4± 2.1	0.8± 0.2	7.5± 2.1	4.7± 2.0	0.7± 0.2

nd = not done; * = significantly different from placebo ($P < 0.05$)

In conclusion, the release of both histamine and cyclooxygenase products is required for some of the cutaneous responses of tachykinins, and the early responses to CGRP. However, the late flare to CGRP is not inhibited by either compound, and aspirin does not inhibit the effect of NKB or the wheal of SP. This further supports the existence of multiple tachykinin receptors.

We wish to acknowledge the support of the Medical Research Council.

Barnes, P.J. et al (1986) Br.J.Pharmac. 88, 741

RELEASE OF HISTAMINE AND 5-HYDROXYTRYPTAMINE FROM RAT PERITONEAL MAST CELLS

D.L. Cohen, T.H.P. Hanahoe and Wendy M. Purcell*, Division of Biological and Environmental Sciences, The Hatfield Polytechnic, College Lane, Hatfield, Herts. AL10 9AB.

Rat mast cells contain histamine and 5-hydroxytryptamine (5HT) within their secretory granules and both amines may be released when the cells are actively stimulated. In the rat, 5HT is considerably more potent than histamine in contracting smooth muscle and increasing vascular permeability. Nevertheless, histamine release has been the traditional measure of mast cell secretion and relatively little is known concerning the output of 5HT from these cells. Theoharides et al (1982) have shown that secretion of histamine, but not that of 5HT may be inhibited by the antidepressant drug amitriptyline, results which have been confirmed by Carraway et al (1984). These observations suggest separate secretory mechanisms within the mast cell for these two amines and we have examined this possibility by measuring their output in response to a variety of agonists.

Mixed peritoneal cells were obtained by lavage from female Wistar rats weighing between 275 and 350g. After differential counting and disruption by sonication, fluorimetric assay of histamine (Anton & Sayre 1969) and 5HT (Snyder et al 1965) showed an average of 15.56 $\pm$ 1.34 pg histamine and 4.18 $\pm$ 0.48 pg 5HT per mast cell (n=9). Further analysis showed that the ratio of histamine to 5HT in these cells was 4.43 $\pm$ 0.93 (n=9). In other experiments cells incubated in Tyrode solution at 37°C with varying concentrations of compound 48/80, the calcium ionophone A23187, a mixture of specific antigen (N.braziliensis) and phosphatidylserine, or a mixture of dextran and phosphatidylserine, for a period of 15 min. secreted both amines in a concentration dependent fashion.

Comparison of the histamine and 5HT dose-response curves to these agonists indicates that the secretion of these amines does not always occur in parallel. For example, with compound 48/80 a maximum secretion of histamine (80%) occurred at a concentration of 3 µg/ml: however, this concentration released only 30% of the 5HT and the 5HT curve had not reached a maximum at a concentration of 100 µg/ml. The slope of the histamine curve was greater than that for 5HT, and the ratio of histamine to 5HT altered, dependent upon the agonist concentration. Similar results were obtained with both specific antigen and A23187. However, secretion of the amines in response to dextran was parallel.

Anton, A.H. and Sayre, D.F. (1969). J. Pharmac, exp. ther. 166, 285.
Carraway, R.E. et al (1984). Br. J. Pharmac, 81, 227.
Snyder, S.H. et al (1965). Biochem. Pharm. 14, 831.
Theoharides, T.C. et al (1982). Nature 297, 229.

*Wendy Purcell is a SERC scholar.

HISTAMINE RELEASE FROM HUMAN DISPERSED SKIN MAST CELLS INDUCED BY SUBSTANCE P

R.C. Benyon, M.K. Church and M.A. Lowman*, Clinical Pharmacology, Centre Block, Southampton General Hospital, Southampton SO9 4XY.

Substance P (SP) released from peripheral afferent nerve fibres is thought to play an important role in antidromic vasodilatation and neurogenic inflammation of the skin. Evidence that SP-induced histamine release from dermal mast cells may be involved in these responses has been gained from studies of human skin *in vivo* and rat peritoneal mast cells *in vitro* (Foreman et al, 1983). We have assessed the ability of SP and related substances to release histamine from human dispersed skin mast cells *in vitro*.

Mast cells were dispersed by enzymatic digestion of infant foreskin (Benyon et al, 1986). Except for time course studies, histamine release reactions were allowed to proceed for 15 min at 37°C. In inhibition studies cells were preincubated with antagonists for 10 min prior to challenge with secretagogues. Histamine was measured spectrofluorimetrically.

SP released histamine in a concentration-dependent manner from 7.0±2.3% (mean ± SEM) at 1 μM to 17.2±2.9% at 30 μM. In contrast, neither the N-terminal basic amino acid sequence SP_{1-4} (Arg-Pro-Lys-Pro) nor the fragment SP_{4-11} induced effective release. SP-induced release was rapid, being complete within 20 s, was energy-dependent, as shown by blockade with 2-deoxy-D-glucose and antimycin A, but was only partially dependent on extracellular calcium, calcium deprivation inhibiting release induced by SP (10 μM) by 55.2±17.8% ($p<0.05$).

The nature of the cell surface activation site of skin mast cells is distinct from E or P type receptors described in smooth muscle preparations in that physalaemin and eledoisin were inactive and [D-Pro^2, D-Trp^{7,9}]-SP was an agonist rather than an antagonist.

The SP analogue [D-Pro^4, D-Trp^{7,9,10}]-SP_{4-11} (SPA) inhibited SP-induced histamine release in a concentration-related manner over the range 1-30 μM SPA, 30 μM SPA inhibiting release induced by 10 and 30 μM SP by 85.7±5.1% ($p<0.01$) and 74.0±6.9% ($p<0.01$) respectively. In addition to inhibiting SP-induced release, SPA also inhibited histamine release induced by compound 48/80 (10 μg/ml), poly-L-lysine (10 μM) and morphine (300 μM) by 82.4±6.7% ($p<0.01$), 80.1±8.6% ($p<0.02$) and 79.5±11.5% ($p<0.05$) respectively.

Our studies demonstrate that the characteristics of SP-induced histamine release from human skin mast cells are similar to those described for rat peritoneal mast cells. Our observations that high concentrations of SP are required for mast cell activation and that SPA inhibits histamine release induced by secretagogues other than SP suggest the presence of a low affinity activation site with poor specificity.

Foreman, J.C., Jordan, C.C., Oehme, P., Renner, H. (1983). J. Physiol. 335, 449-465.
Benyon, R.C., Church, M.K., Clegg, L.S., Holgate, S.T. (1986). Int. Archs. Allergy appl. Immun. 79, 332-334.

COMPARISON OF THE RECEPTORS FOR TACHYKININS IN THE VAS DEFERENS AND PORTAL VEIN OF THE RAT

A.T. McKnight and J.J. Maguire*, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Harlow, England.

The original proposal for the subdivision of tachykinin receptors into 'SP-P' (= NK1) and 'SP-E' (= NK2) types was based on pharmacological data, where the rank order of potencies of agonist peptides in guinea-pig ileum and rat vas deferens were compared (Lee et al., 1982); from similar considerations the existence of a third type of receptor, 'SP-N' (= NK3) has been proposed (Laufer et al., 1985). Recently we have enlarged upon earlier work on the classification of the receptors for tachykinins in the vas deferens (NK2), and have attempted to identify other bioassay preparations that display the pattern of agonist potencies characteristic of the NK3 receptor. We here report that the portal vein of the rat contains a population of tachykinin receptors that is distinguishable from that of the vas deferens.

Single vasa deferentia or longitudinal strips of portal vein (2-3cm) from 200-300g Sprague-Dawley rats were mounted in 3ml organ baths containing Krebs solution at 37°C or 30°C respectively. For the vasa, field stimulation was with single, biphasic pulses delivered at 0.15Hz. In either preparation the potencies of 12 natural and synthetic tachykinins were compared with reference to eledoisin.

Our results from the vas deferens were in agreement with those by other groups, and in terms of the naturally occurring tachykinins the order of potency was: neurokinin A > kassinin > eledoisin = neurokinin B >> substance P > physalaemin.

In the portal vein eledoisin produced an increase in the frequency and amplitude of the myogenic contractions at low concentrations ($< 3 \times 10^{-9}$M), and a frank contraction at higher concentrations. A measurable response was seen in most preparations in the dose range 10^{-9} - 10^{-6}M, with a geometric mean value for EC_{50} of 2.04×10^{-8}M ($pEC_{50} \pm$ s.e.mean 7.69 $\pm$ 0.09, n=20); substance P was more than 10 times less potent, and neurokinin B 10 times more potent than eledoisin. For the natural tachykinins the order of potency was: neurokinin B > eledoisin > kassinin > neurokinin A > physalaemin > substance P

This order is similar to that reported at the putative SP-N receptor in the myenteric plexus (Laufer et al., 1985) or for the inhibition of the binding of [^{125}I-BH]eledoisin in rat cortex (Cascieri et al., 1985). We have found that an excellent correlation exists ($r = 0.977$) between our values for EC_{50} in portal vein, and corresponding values for IC_{50} (pooled from several sources) for the inhibition of [^{125}I-BH]eledoisin binding in rat cortex, while no such correlation exists for our values of EC_{50} from the rat vas deferens.

We conclude that the receptors for tachykinins in the portal vein are distinct from those in the vas deferens of the rat, and are close pharmacological correlates for cortical eledoisin binding sites. In terms of the available criteria, the receptors of the portal vein may be classified as being of the NK3 (SP-N) type.

Cascieri, M.A., Chicchi, G.C. & Liang, T. (1985) J. biol. Chem. 260, 1501-1507
Laufer, R., Wormser, U., Friedman, Z.Y., Gilon, C., Chorev, M. & Selinger, Z. (1985) Proc. natn. Acad. Sci. U.S.A. 82, 7444-7448
Lee, C.-M., Iversen, L.L., Hanley, M.R. & Sandberg, B.E.B. (1982) Naunyn-Schmiedebergs Arch. Pharmac. 318, 281-287.

BUSPIRONE POTENTIATES CONTRACTILE RESPONSE OF VAS DEFERENS TO FIELD STIMULATION

J.P.M. Finberg & M.B.H. Youdim*, Rappaport Family Research Institute and Department of Pharmacology, Faculty of Medicine, Technion, Haifa, Israel.

Buspirone is a novel anxiolytic, non-benzodiazepine drug which is known to affect release and metabolism of dopamine, noradrenaline and 5-HT in the CNS (Skolnick et al., 1984). Since buspirone increases firing rate of the rat locus coeruleus both in vivo (Sanghera et al., 1983) and in vitro (Trulson and Henderson, 1984) we decided to investigate its peripheral noradrenergic pharmacology using the isolated vas deferens as a model system.

In both rat and guinea pig vas deferens, buspirone (10^{-6}) caused marked potentiation of contractile response to electrical field stimulation (1ms, 0.1 Hz and 0.5ms, 5Hz for 1 sec every 10 sec respectively, at 280 mA). In rat vas deferens, contractile responses were increased to 279±29 (SEM, n=4-5) % of control at 10^{-6}M buspirone, and 321±69% control at 10^{-5}M. The IC_{50} for inhibition of field stimulation responses by clonidine was increased to 150±13% control at 10^{-6}M buspirone, and 404±15% control at 10^{-5}M buspirone. By comparison, yohimbine (0.26μM) increased contractile responses to 174±49% control and increased clonidine IC_{50} to 531±99% control. Thus the potentiation of field stimulation induced contractions by buspirone cannot be explained solely by α_2-adrenoceptor blockade. In the unstimulated rat vas deferens, buspirone antagonised contractile responses to a submaximal dose of noradrenaline.

Noradrenaline stores in the isolated rat vas deferens were labelled by incubation with ^{3}H-noradrenaline (10^{-6}M) and efflux induced by electrical field stimulation (1Hz, 1ms, 280 mA for 90 sec) in Krebs solution containing desmethylimipramine (1μM) and corticosterone (28μM). Buspirone (10^{-5}M) caused an increase in spontaneous efflux of ^{3}H (1489±185 to 3080±467 dpm per 2 min, n=4) as opposed to a decrease in time-matched control (1937±57 to 1554±51 dpm per 2 min, n=6), and caused a slight increase in fractional release induced by field stimulation (0.093±0.008 to 0.115±0.019% as opposed to reduction from 0.084±0.008 to 0.079±0.01% in control).

These results indicate that buspirone possesses α_1- and α_2-adrenoceptor blocking activity in the isolated vas deferens, and also stimulates release of noradrenaline, however its ability to potentiate twitch responses may be mediated by a different action (e.g. <u>via</u> purinergic receptors) since noradrenaline may not be the neurotransmitter involved in the twitch response of the vas deferens.

Sanghera, M.K., McMillen, B.A. & German, D.C. (1983) Eur.J.Pharmac. 86, 107-110.
Skolnick, P., Paul, S.M. & Weissman, B.A. (1984) Pharmacology 4, 308-314.
Trulson, M.E. & Henderson, L.J. (1985) Eur.J.Pharmac. 106, 195-197.

POSSIBLE INVOLVEMENT OF NORADRENERGIC AND TRYPTAMINERGIC NEURONES IN THE INTESTINAL ANTISECRETORY ACTION OF MORPHINE

I.M. Coupar* and D.A. Taylor, School of Pharmacology, Victorian College of Pharmacy, 381 Royal Parade, Parkville 3052, Australia

It is now recognized that the antidiarrhoeal action of opiates and related compounds is due to their ability to inhibit intestinal fluid secretion stimulated by a variety of ingested and endogenous diarrhoea producing substances (Awouters et al., 1983). We report the results of experiments designed to determine whether the antisecretory effect of morphine is mediated by an action on enteric nerves.

Water transport rates were measured by a previously described method whereby the jejunum of anaesthetised rats is perfused with a saline solution containing phenol red as a non-absorbable marker and intestinal fluid secretion is stimulated by infusion of vasoactive intestinal peptide (VIP) into the left common carotid artery at the rate of 0.8 µg/min (Coupar 1985).

Amine levels of the jejunum were determined spectrofluorometrically following tissue extraction. Control amine levels were 0.33 ± 0.03 µg/g of noradrenaline and 1.86 ± 0.2 µg/g of 5-HT. Pretreatment of animals with 6-hydroxydopamine (6-OHDA, three daily i.p. injections of 50 mg/kg) resulted in a 92% reduction in the level of noradrenaline without altering the level of 5-HT. Pretreatment of a separate group with parachlorophenylalanine (PCPA, 200 mg/kg, i.p.) resulted in a 69.5% reduction in the level of 5-HT without altering the level of noradrenaline. In saline pretreated rats, morphine (10 mg/kg, i.v.) did not alter the amine levels in the jejunum during the 30 min following injection.

Intra-arterial infusion of VIP starting 5 mins before and continuing during the perfusion of the jejunum totally reversed normal water transport from absorption of 216 ± 27 to induce a large net secretion of 392 ± 26 µl/g in 20 mins. Intravenous injection of morphine (10 mg/kg) 10 min before commencing perfusion of the jejunum blocked the VIP-induced secretion to the extent that no significant net fluid transport occurred across the mucosa of the jejunum (-6 ± 38 µl/g in 20 mins). This antisecretory effect of morphine, equivalent to a 64% reversal of the VIP-induced change in fluid transport, was unmodified in animals injected with atropine (0.25 mg/kg, i.v.), hexamethonium (20 mg/kg, i.v.) and propranolol (1 mg/kg, i.v.).

In contrast other pretreatments did modify the antisecretory effect of morphine. Methysergide (30 µg/kg, i.v.), ketanserin (30 µg/kg, i.v.) and PCPA caused an inhibition of the antisecretory effect of morphine. After methysergide, ketanserin or PCPA, morphine reversed VIP-induced secretion by 6%, 19% or 22% respectively compared to the 64% reversal observed in control animals. After 6-OHDA or phentolamine (2 mg/kg, i.v.) pretreatment the antisecretory effect of morphine was also inhibited. In 6-OHDA-pretreated animals, morphine produced an 11% reversal of the VIP-induced secretion. Similarly, morphine produced only 23% reversal of VIP-induced secretion in animals injected with phentolamine. None of the above drug pretreatments altered the level of fluid absorption ($P > 0.1$) or the magnitude of the VIP-induced secretion ($P > 0.1$, analysis of variance).

It is concluded that morphine produces its antisecretory effect in the jejunum, at least in part, by activation of noradrenergic and tryptaminergic systems.

Awouters, F. et al. (1983) Ann. Rev. Pharmacol. Toxicol. 23, 279-301.
Coupar, I.M. (1985) J. Pharmacol. Methods 13, 331-338.

INHIBITION OF ELECTRICALLY-EVOKED CONTRACTIONS OF THE RAT ANOCOCCYGEUS BY SOMATOSTATIN AND TWO CYCLIC ANALOGUES

T Priestley* and G N Woodruff, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex

The naturally occurring cyclic tetradecapeptide, somatostatin-14 (SS-14), has previously been shown to inhibit electrically evoked contractions of the rat vas deferens, guinea-pig ileum and rabbit ear artery (Cohen et al, 1978). We have investigated the inhibition of electrically-evoked contractions of the rat anococcygeus muscle by SS-14, the N-terminally extended molecule SS-28 and two synthetic cyclic-hexapeptide analogues (Pro-Phe-DTrp-Lys-Thr-Phe) (I) and (N.Me.Ala-Tyr-D-Trp-Lys-Val-Phe) (II), which have potent inhibitory effects on growth hormone, insulin and glucagon-release *in vivo* (Veber et al, 1984).

Anococcygeus muscles were removed from male Sprague-Dawley rats (250-350g) as described by Gillespie (1972). Muscle pairs were separated, mounted in 3ml organ baths and arranged for isometric recordings. Preparations were continuously perfused ($1ml.min^{-1}$) with a gassed ($95\%O_2$, $5\%CO_2$) modified, Krebs-Henseleit solution at 37°C (composition in mM: NaCl 118; KCl 4.74; $CaCl_2$ 2.54; KH_2PO_4 1.19; $MgSO_4$ 1.20; Glucose 11). Transmural field stimulation was applied at 10Hz for 1 s every 20 s, unless otherwise stated.

Electrically-evoked contractions of the anococcygeus were inhibited in a concentration-related manner by SS-14, SS-28, and the two cyclic hexapeptides. Concentration-response curves to each agonist were simultaneously analysed using a four parameter logistic equation as described by DeLean et al (1978). Using this protocol the following pIC_{50} values (mean $\pm$ s.e.mean) were obtained: II = 8.34 $\pm$ 0.04 (n=4); SS-28 = 7.50 $\pm$ 0.06 (n=4); SS-14 = 7.41 $\pm$ 0.10 (n=4); I = 7.21 $\pm$ 0.05 (n=3). The pIC_{50} values for SS-28, SS-14 and I were found not to differ significantly ($P>0.05$), however, II was significantly ($P<0.001$) more potent than SS-14, mean 8.6-fold (6.76-10.96, log-normally distributed data). Maximum inhibitions did not differ significantly between any of the agonists, mean = 73.9% (n=15). Both maximum response and the potency of SS-14 varied inversely with external calcium concentration and stimulation frequency. None of the analogues produced any direct increases in muscle tone, in concentrations up to $10^{-5}M$ and neither SS-14 ($3x10^{-7}M$) nor I ($3x10^{-7}M$) had any effect on increases in tone induced by bath application of noradrenaline ($3x10^{-8}$-$10^{-6}M$). When guanethidine ($3x10^{-5}M$) and carbachol ($10^{-5}M$) were added to the perfusate to produce an increase in muscle tone, field stimulation (5Hz, 1 s trains every 50 s) elicited a relaxation (Gillespie, 1972). SS-14 ($10^{-6}M$) weakly but consistently inhibited the relaxation response (mean inhibition, 18%, n=4).

Inhibition of the contractile response in the anococcygeus by somatostatin presumably involves the presynaptic regulation of noradrenaline release. However, the mechanism underlying the inhibition of electrically-evoked relaxations is at present obscure. These experiments have provided the first data on the autonomic pharmacology of compounds I and II and the first evidence of a presynaptic role for somatostatin in the rat anococcygeus.

Cohen M L, Rosing E, Wiley K S & Slater I H (1978) Life Sci 23, 1659-1664
DeLean A, Munson P J & Rodbard D (1978) Am J Physiol 235, E97-E102
Gillespie J S (1972) Br J Pharmac 45, 404-416
Veber D F (1984) Life Sci 34, 1371-1378

CONTRACTIONS TO 5-HYDROXYTRYPTAMINE (5-HT) RECEPTOR AGONISTS IN THE HUMAN SAPHENOUS VEIN

James R. Docherty, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2.

We have recently reported that contractions to 5-HT in human saphenous vein involve two components: high concentrations of 5-HT act at 5-HT-2 receptors to produce a contraction which is antagonised by ketanserin (1 uM); low concentrations of 5-HT act at receptors at which ketanserin (1 uM) is not an antagonist (Docherty & Hyland, 1986). Yohimbine was an antagonist at both of these 5-HT receptors. In the present study, we have investigated the ketanserin-resistant 5-HT receptor further using the 5-HT-1 receptor agonist 5-carboxamidotryptamine (5-CT) and the 5-HT-1A receptor ligand 8-OH-DPAT (see Middlemiss & Fozard, 1983).

Human saphenous veins were obtained from coronary artery bypass grafts of predominantly male patients, were cut into spiral strips, and placed in organ baths in Krebs-Henseleit solution. Agonists were administered cumulatively until a maximum response was obtained, and agonist concentration-response curves were repeated 2 hrs later in the presence of antagonist or vehicle.

5-HT contracted the human saphenous vein with an EC50 (concentration producing 50% of maximum contraction) of 0.40 uM (95% confidence limits of 0.27-0.59 uM), and a maximum contraction of 1.28±0.25 g (n=13). 8-OH-DPAT and 5-CT produced maximum contractions of 24.9± 12.5% (n=3) and 57.3±4.5% (n=10) of the maximum contraction to 5-HT, respectively. The EC50 of 5-CT was 2.0 uM (0.85-4.7 uM)(n=7). Yohimbine (10 uM) antagonised the effects of 5-CT with an apparent pA_2 of 5.59±0.13 (n=3). This compares with a pA_2 for yohimbine against 5-HT obtained from a Schild plot of 5.48 (see Docherty & Hyland, 1986).

Like ketanserin (1 uM), cyproheptadine (0.1 uM) shifted only the upper part of the 5-HT concentration-response curve, while higher concentrations of cyproheptadine reduced the maximum response to 5-HT, perhaps by action as a calcium entry blocker. Cyanopindolol (1-10 uM) had little effect on the concentration-response curves to either 5-HT or 5-CT.

In conclusion, contractions to 5-HT in the human saphenous vein are mediated by two types of 5-HT receptor: the 5-HT-2 receptor at which ketanserin is an antagonist, and a 5-HT-1-like receptor at which 5-CT is an agonist. 5-CT is probably a full agonist at the 5-HT-1-like receptors, whereas 8-OH-DPAT was a partial agonist. Yohimbine has equal potency as an antagonist of both these 5-HT receptor mediated responses. The lack of effect of cyanopindolol against 5-CT makes it difficult to positively identify the 5-Ht-1-like receptor with any binding site.

Supported by the Royal College of Surgeons in Ireland and by the Medical Research Council of Ireland. We gratefully acknowledge Drs Neligan, Shaw and Wood of the Mater Hospital, Dublin for generously supplying tissues.

Docherty, J.R. & Hyland, L. (1986). Br. J. Pharmacol. 89, 77-81.
Middlemiss, D.N. & Fozard, J. (1983). Eur. J. Pharmacol. 90, 151-153

DIFFERENT MUSCARINIC RECEPTOR PROFILES IN THE GUINEA PIG TRACHEA AND OESPHAGEAL MUSCULARIS MUCOSAE

R.M. Eglen, M.M. Huff and R.L. Whiting, Institute of Pharmacology, Syntex Research, 3401 Hillview Ave., Palo Alto, CA 94304 U.S.A.

It has been shown (Kamikawa et al., 1985) that muscarinic receptors in the guinea-pig oesophageal muscularis mucosae (MM) exhibit a higher affinity (pA2 = 7.4) for pirenzepine in comparison to the ileum (pA2 = 6.8, Mitchelson, 1985). The aim of the present study was to assess the muscarinic receptor profile of the guinea-pig MM in comparison to the trachea (T), using a range of selective muscarinic agonists and antagonists.

The guinea-pig MM and T were prepared as described previously (Kamikawa et al., 1985; Emmerson & MacKay, 1979). Hexamethonium (0.28 mM) was present in all experiments. Cumulative concentration-response curves were constructed in each tissue, and antagonist affinities (pA2), were determined, allowing 60 min. equilibration at each antagonist concentration. All values are mean, sem <5%, n = 4-8.

Agonists. The potencies (-log EC50) for the majority of agonists examined were not significantly ($p < 0.5$) different to the two tissues (MM - carbachol, 6.8, bethanechol, 5.7, and pilocarpine, 6.0; T - carbachol, 6.9, bethanechol, 5.6 and pilocarpine, 5.9). RS 86 (Freedman, 1986) was 10 fold more potent in the MM (6.0) in comparison to the trachea (5.0). All compounds were full agonists with the exception of pilocarpine (intrinsic activities, MM - 0.33, T - 0.61) and RS 86 (intrinsic activities, MM - 0.83, T - 0.85).

Antagonists Similar (pA2) values were observed, in both tissues, for the following antagonists: atropine (MM = 9.3, T = 9.1), 4 diphenyl acetoxy N methyl piperidine (MM = 9.0, T = 9.1), silaprocyclidine(MM = 8.4, T = 8.7), AF-DX 116 (Giachetti et al., 1986; MM = 6.1, T = 6.2) and gallamine (MM = <4.0, T = <4.0). In contrast, the pA2 value for pirenzepine was significantliy ($p<0.05$) greater in the MM (7.4) in comparison to the trachea (6.8). These pirenzipine pA2 values were also observed using RS 86 as the agonist in the MM (7.4) and pilocarpine in the trachea (6.8). All Schild slopes, with the exception of gallamine, were not significantly different ($p<0.05$) from unity.

In summary, these data suggest that, with the exception of pirenzepine, the receptor profile in the MM is similar to that in the trachea. It is interesting to note that the receptor profile in the MM, including the high pirenzipine value, is similar, to that reported for the aortic endothelium of the rabbit (Eglen & Whiting, 1985).

Eglen, R.M. & Whiting, R.L. (1985) Br.J.Pharmac. 84 003-005.
Emmerson, J. & MacKay, D. (1979) J.Pharm.Pharmacol., 31, 798.
Freedman, S.B. (1986) Br.J.Pharmac. 87, 29P
Giachetti, A. et al., (1986) Gastroenterol.88, 261P.
Kamikawa, Y et al., (1985) Gastroenterol. 88, 706-716.
Mitchelson, F. (1984) Trend Phar. Sci. 5 (sup.) 12-16.

DECREASE IN SMOOTH MUSCLE α_1-ADRENOCEPTORS AFTER REVERSIBLE NORADRENERGIC DENERVATION

T. Bennett (1), P.G. Strange (2) and K. Williams (3)*, Departments of Biochemistry (2,3) and Physiology & Pharmacology (1), The Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

The expansor secundariorum (ESM), a discrete smooth muscle in the chick wing, contains α_1-adrenoceptors which mediate contraction of the muscle, and which can be selectively labeled with [3H]-prazosin (Bennett et al, 1986a). We have previously reported a decrease in the apparent number of α_1-adrenoceptors in the ESM, measured at a single concentration of [3H]-prazosin, after reversible noradrenergic denervation with 6-hydroxydopamine (6-OHDA)(Bennett et al, 1986b). We have now examined the maximum binding capacity (B_{max}) and apparent equilibrium dissociation constant (K_D) of [3H]-prazosin binding in ESM after 6-OHDA treatment, and have measured methoxamine- and 5-HT-stimulated contraction of the ESM in vitro. Chicks were injected i.v. with 6-OHDA (100 mg/kg in 0.9% NaCl + 0.1% ascorbate) or with vehicle only (controls). Seven days after injection, ESMs were removed and used for for organ bath experiments in which non-cumulative dose-response curves to methoxamine or 5-HT were measured via isometric transducers, and EC_{50} and maximum response (Rmax) values calculated. Other ESMs were used for [3H]-prazosin binding assays; ESMs (from 4 chicks in each experiment) were homogenized in HEPES-buffered medium (Bennett et al 1986b), centrifuged (100,000 g, 60 min), the pellet resuspended, and aliquots (200-300 µg protein) of this were incubated with increasing concenrations of [3H]-prazosin. Non-specific binding was defined with 3µM phentolamine and assays were terminated by filtration over glass fibre filters. Noradrenaline (NA) was extracted by a modification of the method of Anton & Sayre (1962) and measured by HPLC with electrochemical detection (Macdonald and Lake, 1985). Results are summarised in the table.

Table Effects of 6-OHDA on the ESM (means ± sem)

		CONTROL	6-OHDA
Specific [3H]-prazosin binding			
	B_{max} (fmoles/mg protein)	78.9 ± 4.1	55.7 ± 5.7 ***
	K_D (nM)	1.5 ± 0.5	1.4 ± 0.2
Methoxamine	Rmax(g)	2.83 ± 0.26	1.83 ± 0.23 **
	-log EC_{50} (M)	5.69 ± 0.03	5.78 ± 0.03 *
5-HT	Rmax (g)	3.24 ± 0.35	2.86 ± 0.28
	-log EC_{50} (M)	5.33 ± 0.07	5.60 ± 0.13
NA (pmoles/mg wet weight)		8.57 ± 0.44	3.95 ± 0.78 ***

*p<0.05, **p<0.02, ***p<0.01 (Student's unpaired t-test), n = 6 to 8.

Seven days after 6-OHDA treatment, the ESM was partially re-innervated (assessed by NA content). The number of α_1-adrenoceptors was reduced by 30%, but the affinity for [3H]-prazosin was unaltered. There were small increases in sensitivity to methoxamine and 5-HT, and the methoxamine Rmax (but not that of 5-HT) was reduced after 6-OHDA. The latter effect may be a consequence of the decrease in α_1-adrenoceptor number.

This work was supported by the Medical Research Council.

Anton, A.H. & Sayre, D.F. (1962) J. Pharmacol. Exp. Ther. 138, 360-375.
Bennett T. et al, (1986a) Br. J. Pharmac. proc. suppl. (in press, July 1986 meeting).
Bennett T. et al, (1986b) Br. J. Pharmac. 87, 87P.
Macdonald, I.A. & Lake, D.M. (1985) J. Neurosci. Meth. 13, 239-248.

NICOTINE AND MUSCARINE AUTORECEPTORS ON THE RAT PHRENIC NERVE

H. Kilbinger & I. Wessler* (introduced by E. Muscholl), Dept. of Pharmacology, University of Mainz, Obere Zahlbacher Str. 67, D-6500 Mainz, F.R.G.

The literature concerning possible nicotine or muscarine autoreceptors on the motor nerve is controversial (Gundersen & Jenden, 1980; Wilson, 1982; Bowman et al., 1984). So far, release studies have failed to present clear evidence for the existence of these autoreceptors. It was previously reported (Wessler & Kilbinger, in press) that transmitter release from the rat phrenic nerve preincubated with [^{3}H]-choline can be measured in the absence of cholinesterase inhibitors. Using this radiolabelling technique evidence was presented (Wessler et al., in press) that the motor nerve is endowed with nicotine autoreceptors which mediate a positive feedback mechanism. The present study was undertaken to examine the inhibitory effect of tubocurarine (TC) on evoked [^{3}H]-acetylcholine (ACh) release at different stimulation frequencies, and to investigate possible effects of muscarinic agonists and antagonists on evoked [^{3}H]-ACh release from the phrenic nerve.

Endplate preparations of the left hemidiaphragm were incubated (40 min) with 1 µM [^{3}H]-choline (5 µCi) in Tyrode's solution, and labelling of neuronal transmitter stores was carried out during a nerve stimulation (0.5 ms impulse duration, 8 volts) at 1 Hz. After the labelling and a subsequent 60 min washout period (superfusion rate 15 ml/min) tritium efflux was measured as 2, 3 or 5 min samples. Hemicholinium-3 (10 µM) was present from the washout period onwards. Release of [^{3}H]-ACh was evoked by two periods of electrical nerve stimulation (S1, S2; 100 pulses at 0.5, 1, 5, 25 or 50 Hz; 1500 pulses at 5 or 25 Hz). Stimulation frequency and train length were identical during both stimulation periods. S1 was regarded as control and the substances were added before (15 or 26 min) S2.

TC (1-10 µM) significantly ($P < 0.05$) inhibited [^{3}H]-ACh release evoked by a train of 100 pulses at 5, 25 or 50 Hz maximally by 60%. At 1 Hz TC (1 µM) caused only a 30% inhibition ($P < 0.05$). When 100 pulses were applied at 0.5 Hz, TC did not affect the release. The muscarinic agonist oxotremorine had two effects on evoked (100 pulses at 5 Hz) [^{3}H]-ACh release. 10 nM oxotremorine enhanced the release nearly 2-fold, whereas higher concentrations (1 and 10 µM) inhibited the release by about 50%. Both effects were antagonized by 0.1 µM scopolamine added 26 min before S1. Scopolamine (0.1 µM) alone, produced a significant ($P < 0.05$) increase in [^{3}H]-ACh release evoked by a train of 100 pulses at 5 Hz, whereas the release evoked by a longer train (1500 pulses at 5 or 25 Hz) was significantly ($P < 0.05$) reduced by the antagonist.

It is concluded that the motor nerve is endowed with two populations of muscarine autoreceptors. Scopolamine enhanced the release following a short train which suggests a muscarinic autoinhibition during short stimulation periods (seconds). Scopolamine reduced the release following a longer train which suggests a muscarinic autofacilitation during longer stimulation periods (minutes). Moreover, transmitter release from the motor nerve is regulated by a nicotinic autofacilitation, especially at higher stimulation rates.

Supported by the Deutsche Forschungsgemeinschaft.

Bowman, W.C. et al. (1984) Seminars in Anesthesia III, 275-283.
Gundersen, C.B. & Jenden, D.J. (1980) Br. J. Pharmac. 79, 8-10.
Wessler, I. & Kilbinger, H. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol., in press.
Wessler, I. et al. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol., in press.
Wilson, D.F. (1982) Am. J. Physiol. 242, C366-C372.

MCN-A-343 ACTIVATION OF NEURONAL MUSCARINIC RECEPTORS INDUCES RELAXATION OF THE ISOLATED RAT DUODENUM

R. Micheletti* and A. Schiavone (introduced by A. Giachetti), Department of Pharmacology, Istituto De Angeli, Via Serio 15, I-20139 Milano.

The parasympathetic system controls gastrointestinal motility through muscarinic receptors located on intramural neural structures and on smooth muscle cells. Since compound McN-A-343 has proved able to discriminate among different muscarinic responses, either by virtue of subtype selectivity or partial agonist behaviour, we investigated its effect on a segment of whole intestine, containing both the neural and smooth muscle receptors.

Segments of duodenum obtained from SD rats (250-300 g) were suspended under 2 g tension in 10 ml organ baths, containing Tyrode solution at 37° C.
Motility was measured isotonically.

McN-A-343 evoked a biphasic response, consisting of a relaxation followed by a tonic contraction or by an increase in amplitude of phasic activity.
This response pattern was peculiar to McN-A-343, as it was not observed with a number of muscarinic agonists (acetylcholine, carbachol, oxotremorine, bethanechol, pilocarpine), which all induced only concentration-dependent tonic contractions. While the excitatory component of McN-A-343 effect did not appear related to the concentration, the relaxation was dose-dependent in the range 0.1 -10 μM with maximum effect occuring between 3 and 10 μM. The pD_2 was 6.51 $\pm$ 0.055 (n=12). The magnitude of maximal relaxation was comparable to that induced by 30μM papaverine.
McN-A-343 relaxation was not affected by animal pretreatment with reserpine (5 mg/kg i.m., 48h previously) (n=4). It was competitively antagonized by atropine, with a pA_2 of 8.93 $\pm$ 0.24, and by 10μM bicuculline (mean DR=4.3, n=3). Blockade by 1μM tetrodotoxin or apamine completely abolished the relaxant effect; in the presence of apamine, dose-dependent contractions were observed with McN-A-343, 3 to 30 μM (n=4).

Our findings evidentiate a muscarinic excitation of a neuronal inhibitory pathway in the rat duodenum, and suggest that the neurotransmitter released might be GABA. The muscarinic receptor appeared sensitive only to McN-A-343, among a number of muscarinic agonists.
Further investigation are needed to clarify the subtype of the muscarinic receptor, and the precise nature of the neuromediator released.

112P

EXPOSURE TO PAF-ACETHER AEROSOL INDUCES AIRWAY HYPERESPONSIVNESS TO 5-HT IN GUINEA-PIGS

M.F. Fitzgerald*, I.W. Lees, L. Parente & A.N. Payne. Department of Mediator Pharmacology, Wellcome Research Laboratories, Beckenham Kent. BR3 3BS.

Inhaled PAF-acether has been demonstrated to cause a prolonged increase in non-specific airway responsiveness in normal volunteers for up to two weeks (Cuss et al., 1986). In dogs, inhalation of PAF-acether also induces airway hyperresponsiveness which may be due to the release of thromboxane A_2 (Chung et al., 1986). In contrast, PAF-acether given as an intravenous infusion in anaesthetized guinea-pigs causes a non-specific airway hyperresponsiveness which is cyclo-oxygenase independent but platelet dependent (Mazzoni et al., 1985).

We have now investigated the bronchoconstrictor responses of anaesthetized guinea-pigs to aerosols of PAF-acether and subsequent airway reactivity to aerosols of 5-hydroxytryptamine (5-HT). Male Dunkin-Hartley guinea-pigs (350-500g) were anaesthetized with sodium pentobarbitone (60mg/kg/i.p.) and mechanically ventilated (54 strokes/min,1 ml air/100g body wt). Both cervical vagi were cut, a carotid artery cannulated for the recording of blood pressure and a jugular vein cannulated for the administration of drugs. Aerosols were administered using an ultrasonic nebuliser (Lees and Payne, 1986). Pulmonary inflation pressure (PIP), an index of intrathoracic airway calibre, was measured from a lateral port in the afferent limb of the ventilator circuit.

In individual animals, administration of aerosols of PAF-acether (0.1 to 100µg/ml in saline/0.25% bovine serum albumin 30 sec.) induced a concentration-related increase in PIP of up to a maximum of 20.5±2.7 cm/H_2O accompanied by a fall in mean blood pressure of up to 12.43±3 mm/Hg (mean±s.e.mean n=6). Aerosols of 5-HT (30µg/ml) were administered for sequentially-increasing time periods from 2-60 seconds, at 15 minute intervals, beginning 15 minutes after pre-exposure to saline aerosol (0.9%, 30 secs: a hydration control), and provoked dose-related increases in PIP of up to 35.1±0.9 cm/H_2O (n=5). The bronchoconstrictor effect of aerosolised 5-HT was not modified by prior exposure to an aerosol of lyso-PAF (10µg/ml, 30 sec) given in place of saline. However, 15 minutes after PAF-acether aerosol (10µg/ml, 30 sec) there was a marked increase in bronchial reactivity, 2,5 and 10 sec administration of 5-HT aerosol provoking a rise in PIP of 7.8±1.9, 18.9±1.7 and 27.6±2.0 cm/H_2O respectively as opposed to 1.6±0.5, 4.0±0.5 and 6.4±0.8 cm H_2O after saline aerosol (mean ± se. mean, $p<0.05$ in all cases, n=5). PAF-acether aerosol, given at 10µg/ml for 30 seconds itself caused an increase in PIP of 8.8±0.8 cm/H_2O (n=5). To exclude the possibility that the hyperresponsiveness seen was due to a non-specific contractile effect, an aerosol of histamine was administered (10µg/ml, 30 sec),to a separate group of animals 15 minutes before the bronchial reactivity to 5-HT was assessed. Histamine, although causing an increase in PIP of 6.0±0.5 cm/H_2O (n=3), did not significantly modify bronchial reactivity to aerosolised 5-HT (30µg/ml, 2-60 sec).

These results demonstrate that in guinea-pigs prior exposure to an aerosol of PAF-acether causes airway hyperresponsiveness to aerosolised 5-HT.

Cuss, F.M., Dixon, C.M. & Barnes, P.J.(1986). Am.Rev.Respir. Dis. 132, A212.
Chung, K.F., Aizawa, H., Leitauf, G.D., Ueki, I.F., Evans, T.N. & Nadel, J.A. (1986) J. Pharmacol. Exp. Ther. 235, 580-584.
Mazzoni, L., J., Page, C.P. & Sanjar, S. (1985). Brit. J. Pharmac. 86, 571P.
Lees, I.W. & Payne, A.N. (1986) Brit. J. Pharmac, 87, 225P.

PLATELET ACTIVATING FACTOR INDUCES INTERLEUKIN-1 PRODUCTION FROM HUMAN ADHERENT MACROPHAGES

M.L. Barrett, G.P. Lewis, S. Ward* and J. Westwick, Department of Pharmacology, Royal College of Surgeons, Lincolns Inn Fields, London WC2A 3PN

Interleukin-1 (IL-1) is implicated in both the inflammatory and the immunological responses that are thought to result in rheumatoid joint destruction (Oppenheim et al, 1986). Although many inducers of IL-1 production also activate phospholipase A_2 and generate platelet activating factor (PAF, 1-O-octadecyl-2-O-acetyl-sn-glycero-3-phosphoryl-choline), the role of PAF in IL-1 production is not known. Therefore we have determined the effect of R-PAF, S-PAF (unnatural enantiomer) and two non-hydrolysable PAF agonists, PR1501 and PR1502 (Rasmussen, personal communication) on resting and lipopolysaccharide (LPS)-induced IL-1 production from human adherent macrophages.

Human peripheral blood mononuclear cells were prepared from fresh human heparinised blood (Gordon et al, 1979). Aliquots of cell suspensions (0.5ml) in RPMI 1640 + 10% heat-inactivated foetal calf serum containing $6.2x10^5$ viable cells were added to multiwell plates. Following a 2-hr incubation at 37°C, non-adherent cells were removed by suction, adherent cells were washed in situ and supplied with 0.4 ml RPMI 1640 per well. Duplicate aliquots were treated with 50 µl drug vehicle, PAF agonists (0.003-300nM) followed 10 min later by the addition of 50µl vehicle or LPS (3µg/ml). Cells were incubated at 37°C for 48-hr. Serial dilutions of harvested cell-free supernatants from macrophages incubated with either PR1501, PR1502 or R-PAF with or without LPS, gave a dose-related generation of IL-1-like activity, as assayed in the mouse thymocyte (LAF) assay (Mizel,1984) and the rheumatoid adherent synovial cells (RASC) assay (Dayer et al, 1979). An example of the results obtained in the LAF assay with 1 in 16 dilutions of supernatants from PAF-treated macrophages is shown below.

Macrophage treatment	(-)LPS	^{3}H-TdR incorporation (dpm, mean ± s.e.mean)	(+)LPS(3µg/ml)
Vehicle	1150±136		2802±217
0.003 nM PAF	2409±700		4510±483
0.3 nM PAF	2653±665		5043±526
3 nM PAF	3550±180		4811±507

Similar results were seen for PR1501 and PR1502.

Similarly, in the RASC assay, 1 in 25 dilutions of the above supernatants from macrophages stimulated with either vehicle, 0.3, 3, 30 nM PR1501 or LPS plus the same doses of PR1501 produced 7.1±2, 8.5±2, 23±6, 48±6 or 75±12, 80±5, 93±6, 123±14 ng PGE_2/ml respectively. The addition of authentic IL-1, 0.3, 1.2, 2.5 and 5 u/ml to the RASCs produced 21±5, 110±15, 160±5 and 120±3 ng PGE_2/ml (n=4) respectively. These observations, together with our earlier findings that PAF receptor antagonists can inhibit T-lymphoblast proliferation (Barrett et al, 1986) support the concept that PAF may have an important immunomodulatory role.

We are grateful to Dr Rasmussen and Dr Hadvary for the supply of non-hydrolysable PAF agonists and S-PAF respectively. We are also grateful to CIBA-GEIGY Inc for financial support. S. Ward is a MRC scholar.

Barrett, M.L. et al. Br.J.Pharmacol. (in press)
Dayer, J.M. et al. (1979) J.Clin.Invest. 64, 1386-1392
Gordon, D. et al. (1979) Br.J.Pharmacol, 67, 17-22
Mizel, S.B. (1981) Manual of Macrophage Methodology, ed.Herscowitz et al, 407-416
Oppenheim et al. (1986) Immunology Today, 7(2) 45-56

114P

ROLE OF PAF AS A MEDIATOR OF ENDOTOXIN-INDUCED GASTROINTESTINAL DAMAGE

V. Lagente†, P. Lidbury, G. Steel, B.B. Vargaftig†, J.L. Wallace* & B.J.R. Whittle, Department of Mediator Pharmacology, Wellcome Research Laboratories, Beckenham, Kent, and † Unite des venins, Unite associee Institut Pasteur, Paris, France.

Platelet-activating factor (PAF) is a potent pro-ulcerogenic agent in the stomach and small intestine (Rosam et al., 1986; Wallace & Whittle, 1986a,b) and may play a role as a mediator of gastrointestinal ulceration in septic shock. We tested this hypothesis in the rat by examining the effects of three PAF receptor antagonists on endotoxin-induced gastrointestinal damage. Furthermore, the ability of the jejunum to release PAF under control and endotoxic shock conditions was also assessed.

Male, Wistar rats (n=4 to 9 per group) were anaesthetized with sodium pentobarbitone and a 25 gauge needle was inserted into a tail vein for administration of drugs. Shock was induced by the i.v. administration of E. coli lipopolysaccharide 0111:B4 (25 mg/kg). Some rats were pretreated i.v. 5 min before endotoxin with one of the PAF antagonists (CV3988, 10 mg/kg; BN52021, 10 or 20 mg/kg; Ro-193704, 1 or 2 mg/kg). Gastric and small intestinal damage were assessed, blindly, 20 min after endotoxin administration, using both macroscopical and histological scoring (0 to 3 scale). Systemic arterial blood pressure was measured throughout the experiment via a carotid cannula.

For determination of PAF release, 300 mg of jejunum was excised 5 min after administration of endotoxin or vehicle (n=6 per group), as above. The tissue samples were placed in 3.0 ml of 0.25% bovine serum albumin/0.9% saline and finely minced for 15 sec. After mixing on a vortex for 30 sec, the sample was centrifuged (30 sec; 9000 xg) and the supernatant extracted according to the method of Parente & Flower (1985). PAF-like bioactivity was measured by the ability of a sample to induce aggregation of indomethacin-treated washed platelets from rabbit. The aggregating agent was verified as PAF by thin layer chromatography and by susceptibility to inhibition by two PAF antagonists (CV3988, 10 μM; L-652731, 1 μM).

Endotoxin administration resulted in a biphasic hypotension and extensive haemorrhagic damage to the stomach and small intestine (mean macroscopic damage scores of 2.4 ± 0.3 and 3.0 ± 0, respectively). All three PAF antagonists significantly ($p<0.05$) reduced such damage. For example, Ro193704 (2 mg/kg) reduced the macroscopic damage scores for the stomach and small intestine to 0.7 ± 0.4 and 0.7 ± 0.2, respectively ($p<0.001$). The second phase of endotoxin-induced hypotension was also significantly ($p<0.001$) reduced by all three PAF antagonists. PAF release from control jejunum was 70 ± 31 pg/g tissue. PAF release from jejunum of endotoxin-treated rats was highly variable, but was still significantly increased over controls (1213 ± 489 pg/g; $p<0.001$).

These results support the hypothesis that endogenous release of PAF contributes to the hypotension and gastrointestinal damage associated with endotoxin shock in the rat.

Rosam, A-C., Wallace, J.L. & Whittle, B.J.R. 1986. Nature 319, 54-56
Wallace, J.L. & Whittle, B.J.R. 1986a. Prostaglandins 31, 989-998
Wallace, J.L. & Whittle, B.J.R. 1986b. Br. J. Pharmacol., 89, 415-422.
Parente, L. & Flower, R.J. 1985. Life Sci. 36, 1225-1231

ACTIONS OF PLATELET-ACTIVATING FACTOR (PAF) ON THE RAT GASTRIC MICROCIRCULATION

P.H. Guth, T. Morishita, Y. Ohya and B.J.R. Whittle*
Center for Ulcer Research and Education, V.A. Wadsworth Medical Center, L.A., U.S.A. and *Dept. Mediator Pharmacology, Wellcome Research Laboratories, Beckenham, Kent.

Platelet-activating factor (PAF) induces substantial gastric mucosal vasocongestion and haemorrhage following intravenous infusion in the rat (Rosam et al., 1986). Changes in the local microcirculation leading to stasis may contribute to such actions. In the present study therefore, the effects of intravenously - infused PAF on the microcirculation of the rat gastric mucosa and submucosa have been investigated, using in vivo gastric microscopy techniques.

A fibre-optic light carrier rod was inserted into the gastric lumen of the pentobarbital-anaesthetized rat via an incision in the forestomach to transilluminate the stomach wall. Removal of the serosal and muscle layers allowed direct microscopic visualization and diameter measurement of the submucosal vasculature, which were video-recorded via a T.V. camera (Whittle et al., 1985). To determine mucosal blood flow, a portion of the corpus mucosa was exposed through an incision in the forestomach, and red blood cell velocity (RCV) was measured in the capillaries using a computerized tracking correlator.

Intravenous infusion of PAF (25-100 ng/kg/min) for 20 min, induced extensive dose-related vasocongestion in the gastric mucosa on macroscopic observation. Vasoconstriction in the submucosal vessels was not observed, the diameter of the venules (56±1μm, mean ± s.e. mean; n=20) and arterioles (26±0.2μm) being not significantly ($P>0.05$) reduced during PAF administration at any of the doses studied. However, dose-related stasis of blood flow was noted with 58% and 50% (n=12) incidence of stasis in the venules and arterioles respectively during administration of the highest dose of PAF. Intravenous infusion of lyso-PAF (200 ng/kg/min) for 20 min, which did not induce vasocongestion or damage in the mucosa, had no significant action or arteriolar or venular diameter, nor did it cause stasis.

Administration of PAF (25ng/kg/min) significantly reduced RCV after 20 min of infusion (from 0.7±0.1 to 0.5±0.04mm/sec, n=5; $P<0.05$). PAF (50 ng/kg/min) significantly reduced RCV within 5 min, declining to 0.1±0.03 mm/sec ($P<0.001$) after 20 min, whereas with PAF (100ng/kg/min) RCV fell to non-detectable levels (>0.05 mm/min; $P<0.001$) within 5 min of commencing the infusion.

The present findings indicate that in ulcerogenic doses, PAF induces sluggish flow in the gastric microcirculation, which was not the result of vasoconstriction in submucosal venules and arterioles. The fall in gastric perfusion pressure resulting from the hypotension during PAF infusion could contribute to the decreased blood flow. However, the substantial haemoconcentration induced by PAF (Doebber et al., 1984) leading to rheological changes in the blood, along with occulsion of smaller capillaries by aggregates of white cells may contribute to the vasocongestion, vascular stasis and damage in the gastric mucosa.

Doebber, J.W., Wu, M.S. & Shen, T.Y. (1984) Biochem. Biophys. Res. Comm. 125, 980-987.
Rosam, A-C. R., Wallace, J.L. & Whittle, B.J.R. (1986) Nature 319, 54-56.
Whittle, B.J.R. Oren-Wolman, N. & Guth, P.H. (1985) Am. J. Physiol. 248, G580-580.

[^{3}H]-52770 RP: A NOVEL LIGAND FOR PAF-RECEPTOR SITES IN RABBIT PLATELET PREPARATIONS

I. Cavero, D. Lavé, O. Marquis and C. Robaut *, Rhône-Poulenc Santé, Centre de Recherche de Vitry, 13 quai Jules Guesde, B.P.14, 94400 Vitry-sur-Seine, France.

The platelet activating factor (PAF-acether) is an endogenous substance which can play a significant role in various physiological and pathological processes. In fact, this phospholipid is a potent aggregant of platelets and leukocytes, and can produce hypotension, myocardial depression and bronchoconstriction. The aim of this communication is to present the characterization of N-(2-^{3}H-3-chlorophenyl)-3-(3-pyridinyl)-1H,3H-pyrrolo-[1,2-c]-thiazole-7-carboxamide, named [^{3}H]52770 RP, which is a potent and specific antagonist ligand of PAF recognition sites on rabbit platelet preparations.

Washed rabbit platelets (10^8/ml) or crude platelet membrane preparations (0.12 mg protein/ml) were suspended in a buffer solution (composition in mM : NaCl 140; KCl 2.7; NaH_2PO_4 0.4; $MgCl_2$ 2; $NaHCO_3$ 12; Tris-HCl 10; dextrose 6.2; BSA 0.25 % w/w) and incubated at 20°C for 30 min with 0.5- 30 nM of [^{3}H]52770 RP (1.036 TBq/mmol) synthesized in our laboratory or 0.05-5 nM [^{3}H]PAF(C18) (3TBq/mmol, Amersham). The non-specific binding of these ligands was determined in presence of 10 µM 52629 RP (same structure as 52770 RP except for the replacement of the 3-chlorophenyl by a 3-methoxyphenyl moiety). Incubations were stopped by vacuum filtration over Whatman GF/C glass fibre filters to separate bound from free ligand. The filters were then dried and placed into vials containing 4-5 ml of scintillation fluid to determine radioactivity counts.

52770 RP displaced in a potent, specific and competitive manner [^{3}H]PAF with a Ki of 7 nM, from its binding sites in intact platelets. [^{3}H]52770 RP displayed high affinity, specificity and saturable binding to a single class of recognition sites in both intact platelets and crude platelet membranes. In these preparations (n=5), the values of binding parameters were, respectively, 8.5 ± 2.9 and 7.6 ± 0.8 nM for Kd; 0.20 ± 0.05 pmole/5.10^7 platelets and 3.66 ± 0.62 pmole/mg protein for Bmax and 0.96 and 0.91 for nH. The maximum number of PAF binding sites as determined using [^{3}H]PAF (2336 ± 163 sites/platelet; Bmax 1.940 ± 0.014 pmole/5.10^7 platelets; Kd 1.21 ± 0.44 nM; n=10) was of the same magnitude as that obtained with [^{3}H]52770 RP (2420 ± 269 sites/platelet). The (+)-52770 RP was 300-fold more potent than the (-)-isomer as a displacer of [^{3}H]52770 RP in intact platelets. A variety of pharmacological agents, including pro- and anti-aggregating compounds, did not affect [^{3}H]52770 RP binding, in contrast to PAF-acether and its antagonists, such as L-652,731 and triazolam, which competitively antagonized the binding of the new ligand. The Ki values for PAF-acether were 2.7 ± 0.7 and 10.4 ± 2.7 nM (n=3) in intact and disrupted platelet preparations, respectively. Finally, in intact platelets the rank order of potency (Ki) for 36 analogs of 52770 RP to displace [^{3}H]PAF from its binding sites was highly correlated ($r=0.96$, $p<0.001$) to that observed for their ability to antagonize [^{3}H]52770 RP binding.

These results indicate that [^{3}H]52770 RP binds to a single class of recognition sites, which appear to be located on cellular membrane structures. Furthermore, this binding site displays stereospecific discrimination and the same pharmacological requirements as the PAF binding sites. On the basis of these results and due to the ability of reciprocal displacement between 52770 RP and PAF, it is reasonable to conclude that [^{3}H]52770 RP labels PAF-receptor sites in rabbit platelets. Thus, the new radioligand may represent a novel useful tool for furthering our insights into the possible physiological and pathological roles played by PAF.

THE PRODUCTION OF PLATELET ACTIVATING FACTOR (PAF) BY HUMAN EPIDERMAL CELLS

*F.M. Cunningham, [1]I. Leigh and A.I. Mallet. Institute of Dermatology, Homerton Grove, London E9 and [1]London Hospital, Mile End Road, London E1.

Platelet activating factor (PAF) has been identified in the lesional skin of patients with the skin disease psoriasis (Mallet & Cunningham, 1985) and is released into chambers placed over abraded lesional psoriatic skin (Mallet et al., in press). Since the resident skin cell population is a possible source of the PAF found in chamber fluid, production of PAF by mixed human epidermal cell suspensions, obtained from non-psoriatic subjects, has now been examined.

Freshly trypsinised adult human epidermal cells (10^7 ml^{-1} in HEPES buffered minimal essential medium) were incubated at 37°C with 2 μM ionophore, A23187. After 15 min, cell suspensions were centrifuged at 10,000 g and 1.1 vol methanol added to supernatant and cell pellet fractions. Lipids were extracted by ultrasonication, addition of 1.1 vol of chloroform and centrifugation. The lower layers were collected, evaporated and applied to silica gel TLC plates eluted with chloroform:methanol:acetic acid:water (50:50:10:4). The position of PAF was determined by reference to authentic standards, the silica gel from the appropriate region scraped off, and the PAF recovered by extraction into chloroform:methanol:water (2:2:1.8) to eliminate carry over of the silica gel. The evaporated residues were then resuspended in saline containing 0.25% fatty acid free bovine serum albumin and the biological activity of each sample determined in an aggregation assay using washed guinea pig platelets preincubated with aspirin and phosphocreatine/creatine phosphokinase. The amount of PAF in each sample was estimated from a calibration curve to synthetic PAF (Mallet & Cunningham, 1985).

Table 1.

Sample	PAF (pg / 10^7 cells) cell pellet	supernatant
1	25	*
2	45	*
3	70	38
4	243	95

* = below detection limit of 7 pg

Mixed epidermal cells stimulated with ionophore produced picogram quantities of PAF (Table 1). Previous experiments (Mallet & Cunningham, 1985) have shown more than 78% recovery of radiolabelled PAF after TLC, suggesting that the low levels of PAF measured in the present study do not result from losses during sample preparation. Incorporation and rapid metabolism of the PAF produced by epidermal cells may take place, as has been reported for neutrophils (O'Flaherty et al., 1986). Whether epidermal cells from psoriatic patients produce altered amounts of PAF has yet to be determined.

Mallet, A.I. & Cunningham, F.M. (1985). Biochem. Biophys. Res. Commun. 126, 192-198.
Mallet, A.I., Cunningham, F.M., Wong, E. & Greaves, M.W. Clin. exp. Dermatol. (in press)
O'Flaherty, J.T., Surles, J.R., Redman, J., Jacobson, D., Piantadosi, C. & Wykle, R.L. (1986). J. Clin. Invest. 78, 381-388.

THE INVOLVEMENT OF PAF ENDOTOXIN-INDUCED PULMONARY PLATELET RECRUITMENT

L. Beijer, J. Botting[1], A.O. Oyekan[1], C.P. Page[1]* and R. Rylander, Dept. of Environmental Hygeine, University of Gothenburg, Sweden and [1]Dept. of Pharmacology King's College, Chelsea Camps, London University, Manresa Road, London, SW3.

Airborne endotoxin has been implicated in a number of occupational lung diseases including byssinosis and certain types of occupational asthma. Clinical studies have revealed platelet activation as a feature of byssinosis (Bomski, et al 1971) and exposure of experimental animals to endotoxin has been observed to be associated with a pulmonary recruitment of platelets (Hinton, et al 1983). The cardiovascular effects resulting from exposure to endotoxin have recently been suggested to be secondary to the release of the ether-linked phospholipid platelet activating factor (PAF) (Terashita, et al 1985). As PAF has a number of biological properties appropriate to a mediator of pulmonary inflammation including the recruitment of platelets into the lung, we have investigated the effect of the selective PAF antagonists, CV-3988, BN 52021 and brotizolam on endotoxin-induced pulmonary platelet recruitment.

Guinea-pigs (Male, Dunkin-Hartley) were injected with 111-Indium oxine labelled homologous platelets and exposed (40 min) to an aerosol containing endotoxin (Sigma, derived from E.Coli 026:B6) in a continuous flow exposure chamber. The guinea-pigs were then anaesthetised (urethane 1.5g/kg i.p.) and platelet accumulation in the lung monitored for 2.5h by collimated scintillation detectors linked to an automated isotope monitoring system (Page, et al 1982). Endotoxin (25-100 µg/ml) induced a progressive, dose-related accumulation of platelets into the thoracic region over the 2.5h period following the exposure (peaking at 1.5h). A submaximal dose of endotoxin (50µg/ml) induced a 13.0 ± 2.6 % increase in thoracic counts at 1.5h which was significantly inhibited by pretreatment of animals with brotizolam (0.5 and 1.0mg/kg i.v. in 20 % 2-propylene glycol; 8.7 ± 1.6 and -0.5 ± 1.4 % increase respectively), BN 52021 (2 and 5mg/kg i.v. in 2 % DMSO; -0.1 ± 1.3 and -0.9 ± 1.2 % increase respectively), and CV-3988 (0.1 and 1.0mg/kg i.v. in distilled water; 5.8 ± 1.6 and 3.0 ± 0.9 % increase respectively). Pretreatment with solvents for the drugs had no effect except for DMSO (2%) which produced inhibition of platelet accumulation in the lung that was significant but less than that obtained with BN 52021. These results suggest that PAF may be involved in the platelet activation and subsequent pulmonary accumulation associated with exposure to endotoxin.

Bomski, H. et al (1971) Int. Arch. Arbeitsmed. 27, 309.
Hinton, D.E. et al (1983) In 'Proceedings from 7th Cotton Dust Research Conference National Cotton Council', Eds. Wakelyn, P.J. and Jacobs, R.R pp 39.
Page, C.P. et al (1982) Thrombos. Haemostas. 47, 210.
Terashita, Z.I. et al (1985) Eur. J. Pharmacol. 109, 257.

CLENBUTEROL/CHLORDIAZEPOXIDE INDUCED BACKWARD WALKING IN MICE IS MODIFIED BY MILD STRESS

Christina Davies[1,2], Clare Stanford*[3], Hannah Steinberg[1], Elizabeth A. Sykes[1,4] & P. Terry[1], [1]Psychopharmacology Group, Department of Psychology, University College London, Gower Street, London WC1E 6BT [2]Cambridgeshire College of Arts and Technology, Cambridge [3]Department of Pharmacology, Middlesex Hospital Medical School, London W1 [4]School of Psychology, Middlesex Polytechnic, Enfield, Middx.

We have recently shown that clenbuterol and conventional anti-depressants can induce pronounced and sustained backward walking in mice, but only if co-administered with a single moderate dose (5 mg/kg i.p.) of chlordiazepoxide (CDZP) (Davies *et al*, in press). We now report that both the incidence and the intensity of this backward walking can be changed by some kinds of mild stress.

While making a film, we found that bright lights, noise, heat, etc. did not induce 'freezing' in mice, but, on the contrary, caused conspicuous backward walking, not only in clenbuterol/CDZP mixture-treated mice but also in mice treated with CDZP alone. Filming conditions were therefore reinstated, and female adult 129SV experimentally naive mice were tested under the influence of CDZP 5mg/kg alone: 5/6 walked backwards, compared with 0/5 of a CDZP 5mg/kg-treated group tested in normal lighting conditions.

Since acute 'filming' stress had apparently exacerbated backward walking (cf Kennett *et al*, 1985), we investigated whether backward walking was attenuated in stress-adapted animals. 17 mice were habituated to another, simpler form of stress (14 saline injections and handling over 3½ days), which had been shown in rats to result in β-adrenoceptor down-regulation (Stanford *et al*, 1984). When these stress-adapted mice were tested with a clenbuterol 2.5mg/kg/CDZP 5 mg/kg combination, only 2/9 walked backwards, compared with 5/5 among experimentally naive, unhandled drug mixture-treated mice. Among undrugged controls, 1/8 stress-adapted and 0/5 experimentally naive mice walked backwards. However, when the stress-adapted drug-mixture treated mice were later re-tested in another room, 7/9 walked backwards, suggesting that the unaccustomed stress of moving rooms was still able to elicit this behaviour.

Finally, 1.5, 3 or 6mg/kg buspirone, a non-benzodiazepine anxiolytic with putative anti-depressant actions (e.g. Kennett *et al*, 1986), which affects serotonergic and dopaminergic transmission, failed to substitute for either constituent drug in the clenbuterol/CDZP mixture, though it appeared to confer some 'protection' from backward walking if co-administered with the clenbuterol/CDZP combination.

We conclude that, in the conditions of our experiments: 1) a single mild 'filming' stress can take the place of clenbuterol in CDZP-treated mice and induce backward walking, presumably through β-receptor activation, 2) a repeated mild stress can attenuate clenbuterol/CDZP induced backward walking, probably by adapting mice to the handling and injections involved in the test procedure, 3) the GABA receptor seems vital for backward walking, since buspirone did not substitute for CDZP in the clenbuterol/CDZP mixture, 4) our findings also reinforce evidence for a link between $GABA_A$ and β-adrenoceptors shown after administration of the benzodiazepine inverse agonist FG 7142 (Stanford *et al*, in press).

Help from Mr. M. Shapero and Boehringer Ingelheim Ltd. is gratefully acknowledged.

Davies, C. et al (1986) Br. J. Pharmac. 89, Proc. Suppl. 531P.
Kennett, G.A. et al (1986) Br. Assoc. Psychopharm. [July] (Abstracts).
Kennett, G.A. et al (1985) Brain Research, 330, 253-263.
Stanford, S.C. et al (1984) Neurosci. Letters, 45, 163-167.
Stanford, S.C. et al (1986) Europ. J. Pharmac. (in press).

THE ACTIONS OF THE STEROIDAL CONVULSANT RU 5135 ON GLYCINE AND $GABA_A$ RECEPTORS

H. Callachan, J.J. Lambert* & J.A. Peters, (introduced by G.A. Lyles). Neurosciences Research Group, Department of Pharmacology & Clinical Pharmacology, Ninewells Hospital, University of Dundee, Dundee. DD1 9SY.

The amidine steroid RU 5135 (3α-hydroxy-16-imino-5β-17-aza-androstan-11-one) is a potent convulsant which has been reported to bind to both GABA and glycine recognition sites in radioligand binding studies (Hunt and Clements-Jewery, 1981; Olsen, 1984) and to block extracellularly recorded responses to GABA and glycine in rat brain slices (Simmonds & Turner, 1985). However, the electrophysiological actions of this agent at the single cell level have not been examined. The present report describes the effects of RU 5135 upon GABA- and glycine-induced currents, recorded under voltage-clamp, from bovine chromaffin cells and murine spinal neurones in culture.

Bovine chromaffin cells were isolated and maintained in cell culture for 1 to 6 days prior to use in experiments. Murine spinal neurones dissociated from day 12 mouse foetuses and maintained in vitro for 2 - 4 weeks before use. Transmembrane currents activated by locally applied agonists were recorded under voltage-clamp conditions using the 'whole cell' configuration of the patch-clamp technique (Hamill et al 1981).

Currents evoked by GABA (100μM) on bovine chromaffin cells (cf Cottrell et al, 1985) were reversibly and dose-dependently suppressed by bath applied RU 5135 (0.1-10nM). At a concentration of 1nM the steroid depressed the GABA-induced current to 43 ± 2% (mean ± S.E., n = 12) of control. By comparison, the $GABA_A$ receptor antagonist bicuculline (3 μM) reduced GABA-evoked responses to 15 ± 6% (n = 3) of control. The RU 5135-induced depression of responses to GABA occurred in the absence of a significant change in their reversal potential, was voltage independent, and was associated with a reduction in the underlying membrane conductance increase.

In murine spinal neurones GABA-induced currents were reduced to 45 ± 7% (n = 3) of control in the presence of 1nM RU 5135. Similarly, responses to glycine were depressed to 43 ± 3% (n = 3) of control by 3nM RU 5135. Bovine chromaffin cells possess nicotinic receptors which are pharmacologically similar to those of autonomic ganglia (Lambert et al, 1986). In contrast to its potent antagonism of GABA and glycine-evoked responses RU 5135 (< 1μM) had no effect upon ACh-induced currents recorded from bovine chromaffin cells.

In summary, RU 5135 is a potent antagonist of responses mediated both by GABA and glycine receptors, and its GABA antagonist properties contrast with the GABA potentiating actions of some steroids (Callachan et al, 1986; Harrison & Simmonds, 1984).

We thank the MRC and the Wellcome Foundation for support; Roussel for the gift of RU 5135 and Professor Cottrell for supplying the spinal neurones.

Callachan, H. et al (1986) J. Physiol. (In Press).
Cottrell, G.A., Lambert, J.J. & Peters, J.A. (1985) J. Physiol. 365, 90p.
Hamill, O.P. et al (1981) Pflügers Arch. 391, 85-100.
Harrison, N.L. & Simmonds, M.A. (1984) Brain Res. 323, 287-292.
Hunt, P. & Clements-Jewery, S. (1981) Neuropharmac. 20, 357-361.
Lambert, J.J., Nooney, J.M. & Peters, J.A. (1986) Br. J. Pharmac., 87, 28P.
Olsen, R.W. (1984) Eur. J. Pharmac. 103, 333-337.
Simmonds, M.A. & Turner, J.P. (1985). Br. J. Pharmac., 84, 631-635.

IN VIVO BINDING CHARACTERISTICS OF BENZODIAZEPINE RECEPTOR LIGANDS IN MOUSE BRAIN: QUANTITATIVE AUTORADIOGRAPHY AND IMAGE ANALYSIS

R. Glinz, H. Möhler, J.G. Richards* & J.M. Sequier, Pharmaceutical Research Department, F. Hoffmann-La Roche & Co. Ltd., CH-4002 Basel, Switzerland

The therapeutic effects of benzodiazepine minor tranquillizers are triggered by their selective interaction with binding sites on $GABA_A$ receptors which regulate the gating function of chloride channels in the CNS. The distribution and density of benzodiazepine binding sites in the CNS have been demonstrated by in vitro radiolabelling techniques and recently by immunohistochemical techniques using specific radioligands and monoclonal antibodies, respectively (Richards et al, 1986a,b,c).

In order to further characterize drug-receptor interactions in discrete brain regions under physiological conditions, it is essential to develop an in vivo binding assay with anatomical resolution. For investigations of benzodiazepine receptor ligands this has been achieved by radiolabelling CNS binding sites with a bolus i.v. injection of the antagonist ^{3}H-Ro 15-1788(1mCi = 3.5 µg/kg) to mice 5 min before decapitation. The distribution and density of binding sites were evaluated by quantitative autoradiography and image analysis using calibrated tritium standards on LKB Ultrofilm^R.

As found in vitro, there was a discrete localization of specific binding sites; 80-85% of total binding was inhibited by diazepam, 100mg/kg p.o. 15 min prior to radiolabelling. Of the 13 brain regions investigated, those with the highest and lowest binding densities were the external plexiform layer of the olfactory bulb and in the granular layer of the cerebellum (1028 and 180 fmol/mg prot respectively). In competition binding experiments to determine regional ID_{50} values, the agonists diazepam & midazolam and the partial agonist Ro 23-0364 were administered in the doses 1,3,10,30,100mg/kg p.o. 15 min before radiolabelling to produce dose-response curves. The mean ID_{50} values for these ligands(3.9, 7.0 & 2.6 mg/kg p.o. respectively) correspond well with those obtained biochemically. No obvious differences in the regional affinites between the ligands were observed. The affinities of other partial agonists and so-called BZ_1-specific ligands in vivo are currently being studied. The regional time-course of binding inhibition by benzodiazepine receptor ligands has also been examined with this assay.

Future applications of this high resolution binding method will include studies of the GABA modulation of benzodiazepine receptors and their regulation during acute and chronic drug tolerance.

Richards, J.G. et al (1986a) Experientia 42, 121-126
Richards, J.G. et al (1986b) J. Neurosci. (submitted)
Möhler, H. et al (1986c) This meeting

TRIAZOLAM DISPLACEMENT OF [^{3}H]-RO 15-1788 AND [^{3}H]-ETHYL-β-CARBOLINE-3-CARBOXYLATE IN DIFFERENT REGIONS OF RAT BRAIN

Angela Clow*, Vivette Glover and M. Sandler

Bernhard Baron Memorial Research Laboratories and Institute of Obstetrics and Gynaecology, Queen Charlotte's Hospital, London W6 OXG

Triazolam, a powerful short acting hypnotic, when tested in parallel with alprazolam (both triazolobenzodiazepines) showed anomalous *in vitro* benzodiazepine (BZ) receptor binding properties to rat whole brain homogenates in the displacement of ^{3}H-Ro 15-1788 and ^{3}H-ethyl-β-carboline-3-carboxylate (β-CCE) (Clow et al., 1985). Unlike other clinically used benzodiazepines, and alprazolam, it had lower IC_{50} values at 37°C than 0°C, showed no GABA shift at 0°C and gave Hill coefficients of less than 1 at 37°C. It seems unlikely that these results can be explained entirely by changes in affinity of the ^{3}H-ligands at 37°C as alprazolam was tested in parallel and did not show these anomalies.

To discover if triazolam was acting selectively on a subclass of BZ receptor, perhaps specifically responsible for its potent hypnotic effects or even its unusual and distressing side effects (Van Der Kroef, 1979), we have studied its displacement of ^{3}H-Ro 15-1788 and ^{3}H-β-CCE in 4 regions of rat brain: striatum (STM); hippocampus (HIP); frontal cortex (FC); cerebellum (CEB), and whole brain (WB), (see Table 1) reported to contain different distributions of BZ receptor types (Dubnick et al., 1983). BZ receptors were assayed as described (Clow et al., 1985) in duplicate on 2-5 separate occasions and results pooled for comparison by Hill plot analysis.

Table 1

Hill coefficients (± SEM) and IC_{50} values for triazolam (1-30nM) inhibition of 1nM ^{3}H-Ro 15-1788 and 0.5nM ^{3}H-β-CCE binding to rat brain homogenates after incubation at 0°C and 37°C for 50 min.

	1nM ^{3}H-Ro 15-1788				0.5nM ^{3}H-β-CCE			
	0°C		37°C		0°C		37°C	
	Slope	IC_{50}	Slope	IC_{50}	Slope	IC_{50}	Slope	IC_{50}
WB	1.10 ± 0.04	4.6	0.84 ± 0.04*	2.1×	1.00 ± 0.06	6.0	0.59 ± 0.06*	2.5×
STM	0.94 ± 0.06	6.6	0.91 ± 0.06	2.7×	0.86 ± 0.07	4.1	0.69 ± 0.05	2.8×
HIP	1.04 ± 0.04	4.7	0.86 ± 0.04**	2.6×	1.07 ± 0.05	5.3	0.75 ± 0.08*	2.6×
FC	1.02 ± 0.03	4.9	0.94 ± 0.05	2.2×	1.00 ± 0.03	5.0	0.88 ± 0.04*	1.8×
CEB	0.99 ± 0.07	4.6	0.91 ± 0.08	2.1×	0.94 ± 0.05	4.9	0.55 ± 0.07**	0.9×

Different from 0°C × $p < 0.01$ comparison of elevation of regression lines from Hill plots.
Different from 0°C * $p < 0.05$) comparison of slopes of regression lines from Hill
** $p < 0.005$) plots.

The results are shown in Table 1. There was no striking difference in IC_{50} values between the regions. A decrease in IC_{50} at 37°C, like that observed in whole brain, was seen in all regions. Similarly all regions showed a trend of reduced Hill coefficients at 37°C, which was most marked with ^{3}H-β-CCE.

The previously described anomalous properties of triazolam thus appeared equally apparent in all the brain regions studied and it is not possible to interpret this in terms of BZ 1 and BZ 2 receptor distribution.

Clow,A., Glover,V., Sandler,M. (1985) J.Neurochem. 45, 621-625.
Dubnick,B., Lippa,A.S., Klepner,C.A., Coupet,J., Greenblatt,E.N., Beer,B. (1983) Pharmac.Biochem.Behav. 18, 311-318.
Van Der Kroef,C. (1979) Lancet ii, 526.

INTERACTION OF THE SUBSTITUTED BENZAMIDE, CLEBOPRIDE WITH BRAIN BENZODIAZEPINE BINDING SITES

J.K. Chivers[1], R.W. Horton[*], P.G. Jenner[1], Sandra Lowther, C.D. Marsden[1] and B. Testa[2]. Department of Pharmacology and Clinical Pharmacology, St George's Hospital Medical School, London SW17 ORE, [1] University Department of Neurology and Parkinson's Disease Society Research Centre, Institute of Psychiatry and King's College Hospital Medical School, London SE5 8AF and [2]The School of Pharmacy, University of Lausanne, CH-1005 Lausanne, Switzerland.

Substituted benzamide drugs are cerebral dopamine receptor antagonists. The prototype drug, metoclopramide is widely used as an anti-emetic, while sulpiride has clinically useful antipsychotic properties. Recent animal behavioural studies suggest that sulpiride and tiapride may possess anxiolytic properties (Costall et al, 1986). Since brain benzodiazepine (BZ) binding sites are thought to be an important site of the anxiolytic drug action, we have studied the effects of some substituted benzamide drugs on brain BZ binding in vitro.

Bovine cortical membranes were prepared and (^{3}H) flunitrazepam (^{3}H FNM) binding performed as for rat membranes (Horton et al, 1982). Sulpiride, tiapride, sultopride and metoclopramide in concentrations up to 10^{-5}M produced no, or only minimal, displacement of (^{3}H)FNM (1nM) binding. Clebopride inhibited (^{3}H)FNM binding with an IC_{50} of 74±11 nM (n=3). Clebopride inhibition of (^{3}H)FNM binding was competitive (increased K_D but not Bmax) and was readily reversed by removal of the cleopride by washing and centrifugation.

The nature of the interaction with BZ binding sites was further investigated by determining the ability of clebopride to displace (^{3}H)Ro 15-1788 (1.3nM) binding in the presence (100μM) and absence of GABA and to displace (^{3}H)Ro 15-1788 binding to photoaffinity labelled (PAL) membranes (Möhler, 1982). Diazepam and ethyl ß-carboline-3-carboxylate (ßCCE, a BZ inverse agonist) were used as reference compounds.

Diazepam displaced (^{3}H)Ro 15-1788 in control membranes with a 3.6-fold greater potency in the presence than in the absence of GABA. ßCCE and clebopride were less potent in the present of GABA (Table 1). Diazepam was 30-fold less potent in PAL membranes than control membranes. The potency of ßCCE and clebopride did not differ significantly in PAL and control membranes (Table 1).

Table 1. Diazepam, ßCCE and clebopride displacement of (^{3}H)Ro 15-1788

	Diazepam	ßCCE	Clebopride
Ratio IC_{50} $\frac{-GABA}{+GABA}$	3.6±0.5	0.80±0.02	0.73±0.05
Ratio IC_{50} $\frac{PAL}{Control}$	29.9±3.5	0.98±0.02	1.46±0.09

Values are mean ± s.e.m for at least 3 observations

The interaction of clebopride with BZ binding sites is quite different to BZ agonists such as diazepam but is similar to the inverse agonsits such as ßCCE. Since clebopride is chemically dissimilar to the benzodiazepines, and ß-carboline esters it may represent another distinct chemical structure to study the molecular interactions of the BZ binding site.

Costall, B (1986) Br.J.Pharmac 88, Proc Suppl. 332P
Horton, R.W. et al (1982) J.Neurochem. 39, 864
Möhler, H (1982) Eur.J.Pharmac. 80,435

GABA RECEPTOR-MEDIATED INHIBITION OF RELEASE OF 5-HYDROXYTRYPTAMINE (5-HT) FROM THE INTERMEDIATE LOBE OF THE RAT HYPOPHYSIS

Margarethe Holzbauer, K. Racké*# & D.F. Sharman, AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, U.K. #Department of Pharmacology, University of Mainz, F.R.G.

Hormone release from the intermediate lobe (IL) of the rat hypophysis is regulated by dopaminergic, GABAergic and serotonergic nerves which arise from the central nervous system and synapse upon the glandular cells. In addition, an interaction between these neuronal systems may also occur at presynaptic sites. Thus, endogenous dopamine released in the IL can inhibit the release of 5-HT in this tissue (Racké et al., 1986). In the present experiments it was tested whether the release of 5-HT in the IL can be modulated via GABA receptors.

Isolated neurointermediate lobes (NIL) or neural lobes (NL) with their stalks held in a platinum wire electrode were incubated in Krebs-HEPES solution which contained pargyline (10 µM) and imipramine (1 µM). In most experiments (-)sulpiride (1 µM) was also added to the medium in order to block the inhibitory effect of endogenous dopamine on 5-HT release. The medium was changed every 10 min and 5-HT determined by HPLC with electrochemical detection (Holzbauer et al., 1985). The pituitary stalks were stimulated electrically after 50 min (S1) and after 80 min (S2) of incubation with pulses of 0.2 ms, 10 V, 4 mA, 5 Hz, 5 times for 1 min with 1 min intervals. Muscimol and baclofen were added 10 min before S2, bicuculline 20 min before S2.

Under the present incubation and stimulation conditions the evoked release of 5-HT from the NIL reflects 5-HT release from the IL almost exclusively because the evoked release of 5-HT from the isolated NL in the presence of (-)sulpiride was only 1.3 ± 0.5 pg/lobe (mean ± S.E.M., n=3) whereas that from the NIL was 105 ± 10 pg/lobe (n=37). In the absence of (-)sulpiride, the evoked release of 5-HT from the NIL was 20 ± 4.6 pg/lobe. The effect on 5-HT release from the NIL of muscimol which stimulates $GABA_A$ receptors preferentially, and its interaction with bicuculline, a selective $GABA_A$ receptor antagonist, are shown in Table 1.

Table 1: Effects of muscimol and bicuculline on the evoked release of 5-HT (S2/S1)

Muscimol:	0	100 nM	1 µM	10 µM
No bicuculline	1.08 ± 0.08 (7)	1.04 ± 0.11 (3)	0.65 ± 0.05** (3)	0.55 ± 0.02** (4)
Bicuculline 1 µM	1.01 ± 0.02 (4)	-	1.14 ± 0.12# (4)	0.85 ± 0.03#* (4)

Mean values ± S.E.M. of (n) experiments. Significance of differences from the controls (absence of drugs): *P < 0.05; **P < 0.01; from the corresponding value in the absence of bicuculline #P < 0.01.

The selective $GABA_B$ receptor agonist (-)baclofen also caused a decrease of the evoked release of 5-HT from the NIL by about 40 %, with a maximal effect already at 1 µM. However, the inhibitory effect of (-)baclofen remained unaffected in the presence of bicuculline 10 µM.

In conclusion, the release of endogenous 5-HT from the intermediate lobe of the rat pituitary gland can be inhibited via $GABA_A$ and $GABA_B$ receptors.

Holzbauer, M., Racké, K. and Sharman D.F. (1985) Neuroscience 15, 723-728.
Racké, K., Holzbauer, M. and Sharman, D.F. (1986) Neurosci. Lett. 67, 324-328.

M.H. is supported by a grant from Beecham Pharmaceuticals.
K.R. is in receipt of a British Council Travel Grant.

CORRELATION BETWEEN THE BEHAVIOURAL EFFECT OF DESIPRAMINE AND $GABA_B$ RECEPTOR REGULATION IN THE OLFACTORY BULBECTOMIZED RAT.

D. Joly, K.G. Lloyd*, Ph. Pichat and D.J. Sanger, Laboratoires d'Etudes et de Recherches Synthelabo (L.E.R.S.) 31, Av. P.V. Couturier, 92220- Bagneux, France.

Although antidepressant drugs (ADs) induce various behavioural and biochemical effects there are few correlations between the two. A model for behavioural effects of ADs is the olfactory bulbectomized(OLB) rat (Garrigou et al,1981).As $GABA_B$ receptors are decreased in the frontal cortex of OLB rats(Lloyd and Pichat,1986) and as ADs increase $GABA_B$ binding in the rat frontal cortex (Lloyd et al,1985), we investigated a possible correlation between the behavioural effects of desipramine and $GABA_B$ binding in the frontal cortex of OLB.
Male Wistar rats (250-300 g) were submitted to olfactory bulbectomy or sham-operated under chloral hydrate anesthesia. Fourteen days later daily treatment was commenced with DMI(5mg/kg/d) or saline for 13 days. Forty eight hr after the last treatment the animals were tested for passive avoidance learning (Garrigou et al,1981). Immediately after the behavioural testing the animals were decapitated and the frontal cortex removed, carefully examined for damage and then frozen in dry ice. Membranes were prepared within 48hrs and $GABA_B$ binding performed at a concentration of 10nM ^{3}H-GABA according to Lloyd et al,(1985).

Table. $GABA_B$ Receptor Binding (fmol/mg protein) in frontal cortex of OLB rats responding, or failing to respond, to repeated DMI.

Experiment	OLB Saline	OLB-Desipramine	
		Responders (2-4 Trials)	Non-Responders (7-10 trials)
1	46.5±4.8(7)	83.3±13.3(5)*	68.2±7.4(4)
2	38.2±1.3(5)	153±17.6(3)**	110.7±7.1(4)**
3	98.3±8.9(7)	101.0±11.6(6)	66 (2)
Combined data	62.6±6.9(20)	105.8±10.2(14)**	84.8±8.5(10)

Data expressed as means ± S.E.M. Number of animals in parentheses.
* $p<0.05$; ** $p<0.01$ vs saline-treated rats.
Olfactory bulbectomy was associated with a decreased passive avoidance learning (6.1±0.7 trials in all bulbectomized rats vs 2.8±0.3 in sham-operated animals) and a decrease in $GABA_B$ binding in the frontal cortex (84.8±7.7 fmol/mg protein in sham-operated rats; $p<0.05$). A relatively low dose of desipramine (5mg/kg/d; 13 d) was associated with a variable response: some animals "responded" to treatment (ie. learned within2-3 trials) and others retained their behavioural deficit (7-10 trials). In 2 of 3 experiments a positive behavioural response to desipramine was associated with significantly greater $GABA_B$ binding in the frontal cortex than in saline treated animals. When the data from all experiments are combined, the desipramine responders had significantly greater $GABA_B$ binding than the saline treated OLB rats.$GABA_B$ binding in the occipital cortex of animals from experiment 2 was not different between saline-treated (23.3 fmol/mg protein), DMI responding (16.2 fmol/mg protein) and DMI non responding (20.2 fmol/mg prot) OLB rats. $GABA_B$ binding in the frontal cortex of non-responders tended to be lower than that of responders, and was not significantly different than that of saline-treated OLB rats(combined data).
These data suggest that the upregulation of $GABA_B$ receptors in the frontal cortex is associated with a positive behavioural response to an antidepressant in the OLB model, and is consistent with a GABAergic mechanism of ADs.

Garrigou, D. et al (1981) Psychopharmacology 74, 66-77.
Lloyd, K.G. and Pichat, P. (1986) Brit. J. Pharmac. 87, 36P
Lloyd, K.G. et al (1985) J. Pharmac. Exp. Therap. 235, 191-199.

AN INVESTIGATION INTO THE MECHANISM OF ACTION OF PINACIDIL IN RAT BLOOD VESSELS

J.S. Southerton, S.G. Taylor*, Sheila W. Weir & A.H. Weston, Smooth Muscle Research Group, Department of Physiological Sciences, Medical School, University of Manchester, Manchester M13 9PT.

Pinacidil [(±)N-cyano-N-4-pyridyl-N-1,2,2-trimethylpropylguanidine monohydrate] is one of a group of cyanoguanidine derivatives with antihypertensive properties. In vitro experiments in a variety of vascular tissues have indicated that pinacidil has a direct arterial vasodilatory effect, but attempts to localise its mode of action have been unsuccessful (Kaergaard Nielsen & Arrigoni-Martelli, 1981; Arigoni-Martelli & Finucane, 1985; Cohen & Colbert, 1986). In the present study, the effects of pinacidil have been investigated using whole portal veins and endothelium-free segments of aorta removed from male Wistar rats (300-500 g).

When these tissues were mounted for isometric tension recording in a MOPS-buffered physiological salt solution at 37°C, a 30 min pre-incubation with pinacidil (0.1-10 μM) produced a concentration-dependent reduction in mechanical responses to noradrenaline (0.001-100 μM) and to K^+ (5-20 mM). Responses to higher concentrations of K^+ (40-80 mM) were little affected by pinacidil (0.1-10 μM) although at higher concentrations (>30 μM), some inhibition of responses to K^+ (40-80 mM) was observed. When tone was induced in aortic segments using noradrenaline (100 nM) or K^+ (20 mM), cumulative application of pinacidil (0.1-30 μM) produced a concentration-dependent relaxation. When spasms were induced with higher concentrations of K^+ (40-80 mM), pinacidil had little relaxant effect. Similar observations were made using portal vein.

Aortic segments and portal veins were loaded with ^{86}Rb (1 μCi/ml) for 90 min after which the ^{86}Rb was allowed to efflux into normal physiological salt solution for 14 min. Subsequent challenge with pinacidil (10 μM) for 8 min produced a significant and sustained increase in the ^{86}Rb efflux rate coefficient in both tissues. In portal vein no change in cyclic GMP concentrations measured by radio-immunoassay was observed after 1, 2, or 4 min exposure to pinacidil, 10 μM.

Using microelectrodes the membrane potential of rat aorta was -47±0.5mV (mean ± s.e.mean, n=20). On exposure to pinacidil 10 μM, an increase in membrane potential to -76±1 mV (n=10) was observed. This increase was maximal at about 3 min and was maintained in the continuing presence of pinacidil.

These results show that the inhibitory actions of pinacidil in isolated blood vessels are associated with the opening of Rb-permeable K^+-channels in the smooth muscle membrane. This allows the membrane potential to approach the potassium equilibrium potential, an effect which may form the basis of the antihypertensive action of pinacidil.

Supported by the Mason Medical Foundation, the SmithKline Foundation and by SERC Case Awards to J.S.S., S.G.T. and S.W.W. The advice of Professors Kuriyama and Tomita and their colleagues during a study visit to Japan by A.H. W. (supported by the Royal Society) and the help of Dr Keith Willan (Leo) is gratefully acknowledged.

Arrigoni-Martelli, E. & Finucane, J.(1985) In: New Drugs Annual: Cardiovascular Drugs. Vol 3, 133, New York, Raven Press
Cohen, M.L. & Colbert, W.E.(1986) Drug Development Research, 7, 111
Kaergaard Nielsen, C. & Arrigoni-Martelli, E. (1981) Acta. Pharmacol. Toxicol. 49, 427

AGE INFLUENCES RESPONSES OF RAT AORTA AND PULMONARY ARTERY TO BAY K 8644, POTASSIUM, CALCIUM AND NORADRENALINE

S.R. O'Donnell & J.C. Wanstall*, Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Brisbane, 4067, Australia.

In vascular smooth muscle, one or more of the mechanisms whereby contractile or relaxant drugs can alter free intracellular calcium may be influenced by the age of the animal. This study has compared the responses of aorta and pulmonary artery from young (2 months) and aged (⩾20 months) male Wistar rats to various contractile agents which increase intracellular calcium by different mechanisms.

Single ring preparations of aorta or pulmonary artery (endothelium removed) were set up at an optimal resting tension of 10 mN, in physiological salt solution (PSS; 37°C; 95% O_2/5% CO_2). Cumulative concentration-response (contraction) curves were obtained to KCl, $CaCl_2$, noradrenaline (Nor) and the calcium channel agonist, Bay K 8644. For $CaCl_2$, Ca^{++}-free PSS, in which 80 mM NaCl was replaced with 80 mM KCl, was used. For Bay K 8644, PSS with an additional 6 mM KCl was used, because in normal PSS responses to this drug, except on aorta from young rats, were very variable. Mean negative log EC50 values, and maximum contractile responses, expressed as tension/cross-sectional area (mN/mm^2), were obtained.

Preparations of aorta and pulmonary artery from young rats were significantly more sensitive to Bay K 8644, KCl and $CaCl_2$ than were those from aged rats, and, on aorta, preparations from young rats were also significantly more sensitive to Nor (Table 1). The above differences between young and aged rats were greater on aorta than on pulmonary artery.

Table 1 Mean negative log EC50 values (s.e. mean in parentheses)

	n	Aorta			Pulmonary Artery		
		Young	Aged	Relative potency (Aged=1)	Young	Aged	Relative potency (Aged=1)
Bay K 8644 (+ KCl 6 mM)	4-6	8.12 (0.18)	7.40** (0.11)	5.2	7.42 (0.06)	6.92** (0.12)	3.2
KCl	5-7	2.33 (0.10)	1.58*** (0.03)	5.6	1.86 (0.04)	1.60** (0.05)	1.8
$CaCl_2$	7-8	3.66 (0.06)	3.09*** (0.10)	3.7	3.35 (0.08)	3.12* (0.06)	1.7
Nor	5	8.44 (0.08)	7.82** (0.10)	4.2	7.97 (0.14)	7.79 (0.06)	1.5

* 0.05>P>0.01; ** 0.01>P>0.001; *** P<0.001 (Student's *t* test; Aged v. Young).

No difference between young and aged rats was seen (a) in the maximum responses to the above contractile agents, with the exception of $CaCl_2$ on aorta (young, 11.8±0.95 mN/mm^2, significantly less than aged, 14.4±0.71; 0.05>P>0.01), or (b) in responses to caffeine (50 mM) or the calcium ionophore, A23187 (0.1 to 10 μM).

These results show that, when compared with young rats, preparations from aged rats have a reduced sensitivity to those contractile agents which depend, either in whole or in part, on the influx of calcium through calcium channels (voltage- or receptor-operated). This reduced sensitivity, which was more marked on aorta than pulmonary artery, was not accompanied by a reduction in the contractile ability of the vessels *per se*. Thus it is possible, though yet to be established, that there are age-related changes in the calcium channels and/or the membrane potential in these blood vessels.

Supported by the National Health and Medical Research Council of Australia.

THE LAEVO ENANTIOMER OF BAY K 8644 CAN CONTRACT THE RAT AORTA IN THE ABSENCE OF EXTRACELLULAR POTASSIUM IONS

I. Cavero and K. Lawson*, Rhône-Poulenc Santé, Centre de Recherches de Vitry, 13 quai Jules Guesde, B.P. 14, 94403 Vitry-sur-Seine, France.

The Bay k 8644 (racemic form)-induced contraction of the rat aorta has been suggested to occur only when Ca^{++} channels are already partially activated (i.e. following K^+-depolarisation) (Spedding et al., 1986). In this investigation, the role of potassium ions (K^+) in the contractile activity of (-)-Bay k 8644, an extracellular calcium ion mobiliser, was assessed on rat isolated aorta.

Aortic rings obtained from male Sprague-Dawley rats (300-350g) were suspended in either a physiological salt solution (PSS) containing 1 mM K^+ (composition in mM: NaCl 117; $CaCl_2$ 1.25; KH_2PO_4 1.0; $MgSO_4.7H_2O$ 1.2; Glucose 11.5; $NaHCO_3$ 25) or K^+-free PSS (isotonicity being maintained by the addition of NaH_2PO_4). A load of 2g tension was applied to each tissue.The presence of a functional endothelium was indicated by a relaxation induced by acetylcholine (2 μM) following noradrenaline (20 nM) contraction; in contrast, aortic rings that had been rubbed to remove the endothelium, failed to respond to acetylcholine challenge. Cumulative concentration-response curves to 5-hydroxytryptamine (5-HT) were constructed after 10 min exposure to either ethanol (0.1% : control) or (-)-Bay k 8644 (10-100 nM). Furthermore, the contractile effects of (-)-Bay k 8644 were assessed on aortic rings with a functional endothelium, suspended in K^+-free solution. The concentrations producing 50% of the maximal response to 5-HT (EC_{50}) were calculated for each preparation and reported as means ± s.e. mean.

In preparations bathed with the solution containing 1 mM K^+, (-)-Bay k 8644 displaced the 5-HT concentration-response curves to the left of the control. This effect was concentration-related in tissues with a functional endothelial structure (EC_{50}: Control: 6.47 ± 1.67 μM; 10 nM : 2.95 ± 0.36 μM; 30 nM : 1.82 ± 0.2 μM; 100nM : 1.7 ± 0.52 μM, n=5/group); while, there was no change in the maximum response to 5-HT. After removal of the endothelium an increased responsiveness of this preparation to 5-HT (EC_{50}: 2.36 ± 0.61 μM, n=6) was observed, consequently the shift of the 5-HT concentration-response curve was maximal with 10 nM (-)-Bay k 8644 (EC_{50}: 0.98 ± 0.55 μM, n=6). (-)-Bay K 8644 (10-100nM) did not change the concentration-response curve to 5-HT on the rat aorta suspended in K^+-free PSS.

In K^+-free PSS, (-)-Bay k 8644 (10-3000 nM) caused contractions of rat aortic rings with a functional endothelium (maximum response : 528 ± 118 mg occurring at 300 nM, n=5). The concentration-response curve to (-)-Bay k 8644 (10-3000 nM) was bell-shaped suggesting that the higher concentrations used may exert antagonistic activity. When the rat aorta was bathed in K^+-free and Ca^{++}-free PSS (containing 1 mM EGTA), (-)-Bay K 8644 failed to produce a contractile response.

In conclusion, Bay k 8644 can induce a contractile response, which is extracellular Ca^{++}-dependent, in aortic rings with endothelium not subjected to K^+ depolarisation. Thus, this extracellular calcium ion mobiliser does not appear to require, as suggested by Spedding et al. (1986), a partial K^+ activation of voltage-operated channels to translocate the calcium from extracellular sources into the cytosol. However, extracellular K^+ is necessary for obtaining an enhancement of 5-HT contractile responses by (-)-Bay k 8644, in aortic rings with or deprived of endothelium. The greater responsiveness of the latter preparation to 5-HT suggests that the endothelium liberates a relaxant factor which functionally antagonises the 5-HT contraction on vascular smooth muscle.

Spedding M. et al. (1986). J. Cardiovasc. Pharmacol., in press.

RELAXATION OF RAT UTERUS BY BRL34915

Diane Edwards, M. Hollingsworth*, R.C. Small & A.H. Weston, Smooth Muscle Research Group, Department of Physiological Sciences, Medical School, University of Manchester, Manchester M13 9PT.

BRL34915 produces relaxation of bronchial, gastro-intestinal and vascular smooth muscles, effects which are associated with the opening of Rb-permeable K^+-channels (Allen et al., 1986; Hamilton et al., 1986; Southerton et al., 1986; Weir & Weston, 1986 a,b). In the present study, the effects of BRL34915 on rat uterus have been investigated.

Using strips of uterus from the day 22 pregnant rat, BRL34915 (0.04-1.3 μM) produced a concentration-dependent inhibition of spontaneous mechanical activity which was antagonised by procaine (1 mM) but was unaffected by propranolol (0.1 μM). BRL34915 (1-10 μM) abolished the spasm to KCl (10 mM), reduced that to KCl (20 mM) but had no effect on responses to KCl (40-80 mM). The phasic component of spasms produced by oxytocin (0.2-20 nM) was selectively abolished by BRL34915 (10 μM) whilst the tonic component of oxytocin contractions was largely unaffected.

Using extracellular recording the inhibitory effects of BRL34915 on spontaneous and on oxytocin-induced activity comprised abolition of electrical spikes and phasic tension waves. Oxytocin-induced tonic tension was unaffected. Intracellular microelectrode recordings were made from uterine strips in the presence of oxytocin (0.2 nM) to ensure regular spike firing. Under these conditions BRL34915 (10 μM) abolished spike production, an effect which was followed by a hyperpolarisation of 5 ± 1 mV (mean ± sem, n=4).

In endometrium-free myometrial strips loaded with ^{86}Rb for 90 min, oxytocin (0.2-20 nM) produced a significant increase in ^{86}Rb efflux. However, exposure to BRL34915 (1-10 μM) had no effect on the ^{86}Rb efflux rate coefficient in these tissues.

The effects of BRL34915 against spontaneous and KCl-induced spasms are identical to those observed in other smooth muscles and are consistent with BRL34915 acting to open K^+-channels in the plasma membrane. The inhibitory effects of BRL34915 against the voltage-dependent component of oxytocin spasms (Edwards et al., 1986) are also consistent with such an action. However, the failure of BRL34915 to evoke marked uterine hyperpolarisation or to promote ^{86}Rb efflux was not anticipated. It is possible that the uterine K^+-channels opened by BRL34915 are impermeable to ^{86}Rb. Alternatively, a reduced ^{86}Rb efflux through K^+-channels involved in spike repolarisation may have offset any increased ^{86}Rb efflux via K^+-channels opened directly by BRL34915. These possibilities are being further investigated.

Supported by the National Fund for Research into Crippling Diseases, the North Western Regional Health Authority and the Smith Kline (1982) Foundation. The help of Dr Tom Hamilton (Beecham) and Miss N. Natt is gratefully acknowledged.

Allen, S.L. et al (1986) Br.J.Pharmac. 89, 395-405.
Edwards, D. et al (1986) Br.J.Pharmac. 88, 899-908.
Hamilton, T.C. et al (1986) Br.J.Pharmac. 88, 103-111.
Southerton, J.S. et al (1986) Oxford Meeting, Physiological Society
Weir, S.W. & Weston, A.H. (1986a) Br.J.Pharmac. 88, 113-120.
Weir, S.W. & Weston, A.H. (1986b) Br.J.Pharmac. 88, 121-128.

THE EFFECTS OF SOME K^+ CHANNEL BLOCKERS AND K^+ CHANNEL ACTIVATOR BRL 34915 UPON THE RABBIT AORTA

N.S. Cook*, H.L. Griffiths and R.P. Hof, Cardiovascular Department, Sandoz Ltd., CH-4002 Basel, Switzerland

Isometric tension was measured from rabbit aortic rings contracted either by KCl-depolarization or by receptor activation with angiotensin II (AII) as previously described (Hof et al., 1982). Contractions in the presence of various test substances were expressed as a percentage of the previous tissue maximum response to agonist in the absence of substance. Tetraethylammonium (TEA, 3 and 10 mM) and 3,4-diaminopyridine (DAP, 0.3 and 1 mM), two K^+ channel blockers, potentiated both KCl and AII contractions of the aorta. In the case of depolarization this was manifest as a leftwards shift of the KCl log concentration-response curves whereas for AII contractions the maximal tissue response was also increased. In addition, DAP (1 mM) but not TEA increased the baseline tension of the rings (suspended in a medium containing 4.7 mM KCl). The Ca^{2+} antagonist PN 200-110 (10^{-8} M) caused only small (10%) reductions in AII responses, but greatly reduced the potentiation of AII responses by TEA, suggesting the influx of Ca^{2+} through voltage-sensitive Ca^{2+} channels was necessary for this potentiation by TEA.

Quinine (30-300 µM) showed effects resembling a Ca^{2+} channel blocker in the rabbit aorta; KCl contractions were non-competitively inhibited by quinine with a pD'_2 of 4.1, whereas AII contractions were not affected by concentrations up to 100 µM. Apamin (10^{-6} M) and Toxin I (10^{-6} M), neurotoxins known to block K^+ channels, had no effect on either KCl or AII contractions of the aorta.

The K^+ channel activator BRL 34915 (BRL, 0.1-10 µM) caused a rightwards shift of the log concentration-response curve to KCl, as reported previously by Weir and Weston (1986) in the rat aorta, and non-competitively antagonized AII responses. At 100 µM, BRL also reduced the maximum tissue response to KCl. Apamin (10^{-6} M) and Toxin I (10^{-6} M) failed to modify the vasorelaxant activity of BRL, but TEA (1 and 10 mM) antagonized the effects of BRL. In all of the above experiments, aortic rings denuded of endothelium gave essentially the same results as those rings with an intact endothelium.

In conclusion, it appears K^+ channels sensitive to TEA and DAP, but not to apamin or Toxin I, are present in the rabbit aorta. The potentiated contractions seen when these channels are blocked suggest they may play an important physiological role in regulating vascular tone.

We are grateful for the following gifts; Beecham Pharmaceuticals (BRL 34915), Dr. A.L. Harvey (Toxin I), Dr. P.N. Strong (Apamin).

Hof R.P. et al. (1982) J. Cardiovasc. Pharmac. 4, 344-351.
Weir S.W. and Weston A.H. (1986) Br. J. Pharmac. 88, 121-128.

A STUDY ON THE MECHANISM OF NEGATIVE INOTROPY OF SOME CALCIUM ENTRY BLOCKERS IN ISOLATED GUINEA-PIG HEARTS

H.W.G.M. Boddeke*, J.B. Heynis, B. Wilffert[1] and P.A. van Zwieten, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, the netherlands.[1]Institut fur experimentelle Medizin, Janssen, Raiffeissen Strasse 8, D-4040 Neuss 21, F.R.G.

In the present study we investigated the interaction of the calcium entry blockers nifedipine, verapamil, diltiazem, flunarizine and bepridil with the positive inotropic effects induced by calcium, by the dihydropyridine calcium entry promoter Bay k 8644 and by the calcium ionophore A 23187 in isolated guinea-pig hearts. Hearts were perfused with a Tyrode solution at a calcium concentration of 1.3 or 0.9 mM and at 37°C (frequency 4 Hz). LVP was measured by means of a latex balloon placed in the left ventricle. The concentration response curves of the positive inotropic effect of calcium and A 23187, respectively, were constructed at an initial calcium concentration of 0.9 mM, whereas the experiments with Bay k 8644 were performed at 1.3 mM. In the experiments with calcium as well as A 23187 the concentration of the calcium entry blockers equalled IC-50 values for negative inotropy of these compounds. (nifedipine $2.6\pm0.04\times10^{-8}$M, verapamil $8.4\pm0.05\times10^{-8}$M, diltiazem $1.3\pm0.06\times10^{-6}$M, flunarizine $5.1\pm0.08\times10^{-7}$M, bepridil $5.4\pm0.09\times10^{-7}$M). The negative inotropic effects of all calcium entry blockers investigated are reversed by calcium in a similar manner, the same maximum of 243±8.3% (n=6) as for the control calcium being achieved. Pretreatment with Bay k 8644 (at concentrations of 10^{-8}, 10^{-7}, 10^{-6}M) differentially influenced the negative inotropic action of the calcium entry blockers. For nifedipine a parallel shift of the negative inotropic response curve after pretreatment with Bay k 8644 was observed. For verapamil the concentration response curve shifted in a non-parallel fashion, whereas for diltiazem a decrease of the maximum was observed after pretreatment with Bay k 8644. The negative inotropic response to flunarizine and bepridil was only slightly antagonized after pretreatment with 10^{-6}M bay k 8644. Similarly, a different pattern of antagonistic activity of the calcium entry blockers towards the positive inotropic action of A 23187 (3×10^{-7}M), which amounted to 60.4% of the initial value (n=6) was observed. Nifedipine as such did not reduce the A 23187 induced rise in contractile force, whereas this effect was partly supressed by verapamil and diltiazem. Complete supression of the A 23187 effect was achieved, however, by both flunarizine and bepridil. In conclusion, the calcium entry blockers studied displayed comparable calcium antagonistic effects, but their mechanism of action is dicriminated by the calcium entry promoter Bay k 8644. However these results cannot directly be translated into differential interactions of the calcium entry blockers with calcium channels, since intracellular sites of action may also be involved as suggested by the experiments with the calcium ionophore A 23187.