BINDING OF NITROVASODILATORS AND BOVINE RETRACTOR PENIS INHIBITORY FACTOR BY HAEMOGLOBIN AND METHAEMOGLOBIN IMMOBILIZED ON AGAROSE

W. Martin and J.A. Smith*, Department of Cardiology, University of Wales College of Medicine, Heath Park, CARDIFF CF4 4XN

Nitrovasodilators such as glyceryl trinitrate (GTN) and azide, and the bovine retractor penis muscle inhibitory factor (IF) relax in vitro smooth muscle preparations by elevation of intracellular cGMP levels (Murad et al, 1978; Bowman and Drummond, 1984). This action of GTN or IF is inhibited by haemoglobin (Hb) but not by its ferric derivative methaemoglobin (MetHb) (Bowman et al, 1982; Martin et al, 1985a, 1985b). We have attempted to clarify the mechanism of this inhibitory action of Hb by examining the ability of Hb and MetHb immobilized on agarose beads (Sigma) to bind or inactivate IF, GTN and azide, as well as nitric oxide (NO) which is believed to be the active metabolite of the nitrovasodilators (Murad et al, 1978).

Glass columns were filled with MetHb bound to agarose (2.5 x 0.5 cm bed volume). When required, the MetHb was reduced to Hb by treating the columns with 3 ml of 2 x 10^{-3}M sodium dithionite, and washed with double distilled water. The ability of Hb or MetHb to bind or inactivate GTN (10^{-5}M), azide (10^{-4}M), IF (1 ml = 2 g wet weight tissue) in both the acid-activated and inactive forms (Gillespie & Martin, 1980), and freshly generated NO was examined at 4°C by passing 4 ml of each solution through the columns. Smooth muscle relaxant activity in the effluent from the columns was assayed on rabbit aortic strips denuded of endothelium and preconstricted with 10^{-7}M phenylephrine. GTN was not bound or inactivated by Hb or MetHb, whereas azide was preferentially bound by MetHb, and NO was preferentially bound by Hb. Neither form of IF interacted with MetHb, but both forms interact with Hb resulting in a destabilisation and a loss of activity.

Guanylate cyclase is a ferrous haemoprotein (like Hb) and in cell free systems its activity is stimulated by NO (Murad et al, 1978). Nitric oxide has a higher affinity for ferrous haemoproteins than for ferric haemoproteins, and this is confirmed by our results. The lack of binding of GTN and the low binding of azide to Hb indicates that such binding is probably not the mechanism by which Hb inhibits nitrovasodilator-induced relaxation. Nitrovasodilators are believed to be metabolised intracellularly to NO (Murad et al, 1978), which then stimulates guanylate cyclase. The molecular size of Hb precludes its entry into cells, but Hb might act as an extracellular 'sink' for NO, maintaining a concentration gradient out of the cell, thus reducing intracellular NO levels. Such a mechanism may explain inhibition of nitrovasodilator-induced relaxation by Hb. Azide is converted intracellularly to NO by the enzyme catalase (Murad et al, 1978), a ferric haemoprotein. This is consistent with our finding of a preferential binding of azide by ferric MetHb. The mechanism of the inhibition of IF-induced relaxation by Hb appears to involve inactivation rather than simple binding. The ability of the IF to interact with ferrous haem moieties suggests that IF, like NO, may stimulate guanylate cyclase by interacting with the ferrous haem moiety of the enzyme.

Bowman et al, (1982) Eur. J. Pharmac., 85, 221-224
Bowman, A. & Drummond, A.H. (1984) Br. J. Pharmac., 81, 665-674
Gillespie, J.S. & Martin, W. (1980) J. Physiol., 309, 55-64
Martin et al, (1985a) J. Pharmac. Exp. Ther., 232, 708-716
Martin et al, (1985b) J. Pharmac. Exp. Ther., 233, 679-685
Murad et al, (1978) Adv. Cyc. Nuc. Res., 9, 145-158

This work was supported by the British Heart Foundation

HISTAMINE-STIMULATED ACCUMULATION OF [^{3}H]-INOSITOL PHOSPHATES IN GUINEA-PIG CEREBELLUM AND ILEAL SMOOTH MUSCLE

J. Donaldson* & S.J.Hill, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

Activation of a wide range of cell surface receptors has been shown to stimulate the hydrolysis of phosphatidylinositol (PI) and the polyphosphoinositides (PPI) to diacylglycerol and the corresponding water soluble products, inositol -1-phosphate (InsP), inositol-1,4-bisphosphate ($InsP_2$), and inositol-1,4,5-trisphosphate ($InsP_3$)(Berridge, 1981). We have previously reported that histamine stimulates the accumulation of InsP in lithium-treated slices of guinea-pig cerebellum and ileal smooth muscle (Donaldson & Hill, 1985). Although the response in cerebellum appears to be mediated exclusively via H_1-receptors, the small H_1-receptor effect in ileum is largely obscured by a second inositol phospholipid response to histamine which is independent of the activation of H_1- and H_2-receptors (Donaldson & Hill, 1985). Since an accumulation of InsP may ultimately result from the breakdown of either PI or PPI, we have investigated the effect of histamine on the accumulation of the more polar products of PPI metabolism ($InsP_2$ & $InsP_3$) in these two tissues and compared the sensitivity of these responses to inhibition by the H_1-receptor antagonist mepyramine.

Slices of guinea-pig cerebellum or longitudinal smooth muscle of guinea-pig ileum (300 x 300 μm) were incubated for 2h at 37°C in Krebs-Henseleit solution containing ^{3}H-myo-inositol (0.5 μM) under an atmosphere of O_2/CO_2 (95:5). The prelabelled slices were subsequently washed in Krebs medium for 1h before being transferred to tubes containing LiCl (10 mM) and, where appropriate, antagonist drug for 30 min. Following stimulation with histamine (45 min), incubations were stopped by addition of chloroform/methanol/10 M HCl (100:200:1 v/v/v) or trichloroacetic acid (15% w/v) and ^{3}H-inositol phosphates were separated by ion exchange chromatography (Berridge et al, 1983). A comparison of different extraction procedures indicated that acid conditions achieved a more efficient extraction of ^{3}H-inositol phosphates (particularly ^{3}H-$InsP_2$ and ^{3}H-$InsP_3$) than the chloroform/methanol (1:2 v/v) employed in our previous study (Donaldson & Hill, 1985).

Incubation of cerebellar slices with histamine (0.1 mM) produced a significant stimulation of ^{3}H-InsP (2835±339 dpm; $p<0.001$), ^{3}H-$InsP_2$ (1419±184 dpm; $p<0.001$) and ^{3}H-$InsP_3$ (742±121 dpm, $p<0.01$) accumulation (expressed as the difference between histamine stimulated and control levels; mean±s.e.mean; 6 experiments). A significant stimulation of the accumulation of ^{3}H-InsP (2334±521 dpm; $p<0.01$), ^{3}H-$InsP_2$ (883±195 dpm; $p<0.01$) and ^{3}H-$InsP_3$ (328±92 dpm; $p<0.05$) was also observed in ileal smooth muscle in response to histamine (0.1 mM; 6 experiments). The responses to histamine (0.1 mM) in cerebellar slices were markedly antagonised by mepyramine (0.1 μM; 84-89% inhibition). In guinea-pig ileum, however, the histamine-induced accumulations of ^{3}H-InsP, ^{3}H-$InsP_2$ and ^{3}H-$InsP_3$ were less sensitive to inhibition by mepyramine (0.1 μM; 27,29 & 36% inhibition respectively). The results of this study suggest that (i) histamine can stimulate PPI hydrolysis in both guinea-pig cerebellum and ileum and (ii) inositol phospholipid responses to histamine in ileal smooth muscle are less sensitive to antagonism by mepyramine than those of cerebellum.

We thank the Smith Kline (1982) Foundation for financial support.

Berridge, M.J. (1981) Mole Cell.Endocr. 24, 115-180.
Berridge, M.J. et al (1983) Biochem.J. 206, 587-595.
Donaldson, J. & Hill, S.J. (1985) Br.J.Pharmac. 85, 499-512.

DIETARY EFFECTS ON GUINEA PIG TRACHEAL REACTIVITY TO HISTAMINE

C. Loesberg*, F.M.A. Woutersen-van Nijnanten and F.P. Nijkamp.
Institute for Veterinary Pharmacology, Pharmacy and Toxicology, State University of Utrecht, P.O.Box 80176, 3508TD Utrecht, The Netherlands.

Histamine has long been regarded as an important mediator in pulmonary hypersensitivity. It has been demonstrated that histamine responsiveness can be influenced by arachidonic acid (AA) metabolites (Johnson, 1982). Furthermore, inhibition of the AA-metabolism has been shown to induce a histamine hyperreactivity (Shore et al.,1985).
Considering that dietary polyunsaturated fatty acids (PUFA's) can influence the synthesis of AA-metabolites by modulating the fatty acid composition of the membranes, we investigated whether dietary PUFA's are able to affect bronchial response to histamine.
80 Male guinea pigs were divided into 4 groups immediately after weaning. During six weeks each group was fed a semi-synthetic diet (35 en%) that differed in the amount of linoleic acid. Dietary group IV received linolenic acid additionally to a diet comparable to dietary group I. Water and food were given ad libitum. The linoleic acid content (% fat) of the diets were 5.85, 11.25, 22.05 and 3.55 in the diets I to IV, respectively. In dietary group IV an amount of 5.30 (% fat) of linolenic acid was also present.
The reactivity to different doses of histamine was determined using isolated tracheal spirals. Fatty acid composition was determined gaschromatographically. Significance was tested using Student's t-test.
Weight gain of all animals (n=80) during the experiment was identical. Histamine reactivity was significantly diminished in dietary group I as compared with the other dietary groups (II, III and IV). The other dietary groups showed a similar response to histamine. Maximal contraction (% $\pm$ SEM) was 82$\pm$3 in dietary group I and 100$\pm$6, 94$\pm$4 and 97$\pm$6 for dietary groups II,III and IV, respectively ($p<.04$).The affinity did not differ between the groups.
PUFA composition of lung membranes differed considerably between the dietary groups. However, when only those fatty acids were selected that were significantly different ($p<.01$) in group I, but did not differ significantly between dietary groups II, III and IV, only two fatty acids could be considered; 22:3 9 and 20:4 6 (AA).The AA-content [% total phospholipid $\pm$ SEM] was 5.6$\pm$.2, 4.9$\pm$.1, 4.6$\pm$.1 and 4.6$\pm$.1 for the dietary groups I to IV, respectively.
In conclusion.
1). A diet relatively low in PUFA's induces a hyporesponsiveness of the trachea to histamine. This hyporeactivity can be abolished by the addition of both linoleic and linolenic acid.
2). It is tempting to speculate upon a role for arachidonic acid in the induction of the histamine hyporeactivity in dietary group I, since the abolishment of the effect coincided with a significant drop in the AA-content of the lung membranes.

The authors wish to thank Unilever Research Laboratory, Vlaardingen, The Netherlands.

Johnson,C.L. (1982) In: The pharmacology of histamine receptors. eds. C.R. Ganellin & M.E. Parsons, Wright PSG, Boston USA. 146p
Shore, S.A. et al. (1985) J.Appl.Physiol. 58, 859p

SK&F 93574 A POTENT AND LONG ACTING HISTAMINE H_2-RECEPTOR ANTAGONIST FOR INTRAVENOUS ADMINISTRATION

R.C.Blakemore, T.H.Brown, R.J.Chenery, G.J.Durant, C.R.Ganellin, M.E.Parsons, A.C.Rasmussen and D.A.Rawlings. Smith Kline & French Research Limited, The Frythe, Welwyn, Hertfordshire, U.K.

In certain clinical conditions, for example, stress related gastrointestinal mucosal damage, a potent long acting histamine H_2-receptor antagonist for parenteral use could offer therapeutic potential.
SK&F 93574 (5-(1,2-dihydro-2-oxopyrid-4-ylmethyl)-2-[2-[5-(dimethylaminomethyl)-furan-2-ylmethylthio]ethylamino]pyrimidin-4(1H)-one, mol.wt.= 415.518) is an analogue of the previously described long acting histamine H_2-receptor antagonist SK&F 93479 (Blakemore et al 1981) in which the basic 5-(6-methylpyrid-3-ylmethyl) substituent is replaced by the neutral 5-(1,2-dihydro-2-oxopyrid-4-ylmethyl) group. The octanol/water partition coefficient for SK&F 93574 (log P = 0.50 at 37°C, pH 8.35) indicates that it is considerably more hydrophilic than SK&F 93479 (log P = 2.06).
On the guinea-pig isolated right atrium, SK&F 93574 antagonised the positive chronotropic action of histamine giving a pA_2 value of 7.57 (7.37-7.82)(60 min. equilibration) with a Schild plot slope of 1.05 ± 0.13 (mean ± 95% confidence limits) indicating competitive antagonism.
SK&F 93574 showed a high degree of selectivity for the histamine H_2-receptor, concentrations up to 486μM failing to inhibit the positive chronotropic response to isoprenaline on the atrium and histamine-induced contractions of the guinea-pig ileum. High concentrations of SK&F 93574 antagonised carbachol-induced contractions of the ileum but the slope of the Schild plot was significantly different from unity (approximate pA_2 value of 3.96).
SK&F 93574 produced a dose-related inhibition of histamine-stimulated gastric acid secretion in the perfused stomach preparation of the anaesthetised rat, 50% peak inhibition being obtained at a dose of 0.006 μmol/kg (0.001-0.024 μmol/kg, n=15, mean + 95% confidence limits) i.v. compared with a value of 1.37 μmol/kg for cimetidine. In the acutely fistulated anaesthetised cat the potency relative to cimetidine was approximately 64, a dose of 0.125 μmol/kg i.v. giving 94% inhibition (n=4)(cf.cimetidine 92% at 8 μmol/kg i.v.). In both the rat and cat, inhibition was prolonged relative to cimetidine. This relatively long duration was also manifest in the conscious Heidenhain pouch dog. Bolus intravenous injection of 0.0625 μmol/kg and 0.125 μmol/kg produced mean peak inhibitory responses of 85% (n=4) & 98% (n=4) respectively, of maximal histamine-stimulated acid secretion (cf. cimetidine 68% at 4 μmol/kg i.v.). Mean peak inhibition was achieved at between 30 and 45 minutes after administration. Three hours after injection of the 0.0625 μmol/kg and 0.125 μmol/kg doses of SK&F 93574, secretion was still inhibited by 63% and 77% respectively.
In vitro SK&F 93574 at 220 μM did not inhibit the binding of dihydrotestosterone to androgen receptors of the rat prostate, indicating a lack of anti-androgenic activity (P.Sivelle, personal communication) and had no significant effect on rat liver cytochrome P450 mixed-function oxidase enzymes in vitro or in vivo.
Thus SK&F 93574 is a selective histamine H_2-receptor antagonist and a potent inhibitor of histamine-stimulated gastric acid secretion after intravenous administration. In all species tested the duration of inhibition was longer lasting than for cimetidine and human studies are now being undertaken.

Ref: Blakemore, R.C., et al. (1981). Br. J. Pharmac. 74, 200P.

PROPHYLACTIC ANTI-ASTHMA DRUGS IMPAIR THE AIRWAY HYPER-REACTIVITY THAT FOLLOWS EXPOSURE TO PLATELET ACTIVATING FACTOR (PAF)

L. Mazzoni, J. Morley, C.P. Page, S. Sanjar, Preclinical Research, Sandoz Ltd., CH-4002 Basle, Switzerland

PAF has a number of properties appropriate to a mediator of asthma (Morley, Sanjar & Page, 1984), including the capacity to induce non-selective hyper-reactivity of the airways in experimental animals (Mazzoni et al., 1985). During exacerbation of asthma, bronchial hyper-reactivity is more pronounced; hence we have evaluated the effect of prophylactic anti-asthma drugs upon airway hyper-reactivity induced by PAF in the guinea pig.

Airway resistance (R) and dynamic compliance (C_{Dyn}) were recorded in ventilated guinea pigs as described previously (Mazzoni et al, 1985).

Following infusion of PAF (600 ng/kg/hr), substantially larger responses were observed to i.v. injections of bombesin (240 ng/kg), histamine (1.8 µg/kg) and substance P (1.8 µg/kg), doses which in untreated animals produce minimal changes in respiratory parameters. Such hyper-reactivity was reduced, if exposure to PAF was accompanied by infusion of the prophylactic anti-asthma drugs ketotifen, disodium cromoglycate, hydrocortisone or aminophylline (1 mg/kg i.v. bolus followed by 1 mg/kg/hr i.v. infusion). Conversely, the classical histamine (H_1) antagonist (mepyramine, 2 mg/kg), the cyclooxygenase inhibitor (indomethacin, 2 mg/kg) or the beta-stimulant bronchodilator (isoprenaline, 20 µg/kg) failed to inhibit bronchial hyper-reactivity resulting from PAF infusion.

PAF-induced hyper-reactivity in the guinea pig is unaffected by vagal section and is platelet-dependent (Mazzoni et al., 1985); the present study shows that it is independent of histamine or cyclooxygenase metabolites, but is sensitive to inhibition by known prophylactic anti-asthma drugs. For some time, mast cell stabilisation has been recognised as an ineffectual test for selecting prophylactic anti-asthma drugs, PAF-induced hyper-reactivity may prove more rewarding.

Drug	Increase in Airway Resistance ($CmH_2O/l.sec^{-1}$ ± SEM) due to Bombesin (240 ng/kg i.v.)		Increase ($CmH_2O/l.sec^{-1}$ ± SEM)	*Significance 2P
(n=5)	Prior to PAF	After PAF		
No treatment	46 ± 25	231 ± 16	185 ± 14	
Aminophylline	31 ± 6	147 ± 25	116 ± 22	0.033
Cromoglycate	24 ± 10	149 ± 25	125 ± 23	0.067
Hydrocortisone	13 ± 2	104 ± 12	99 ± 20	0.01
Ketotifen	34 ± 11	114 ± 24	80 ± 27	0.01
Indomethacin	38 ± 9	382 ± 24	344 ± 20	<0.001
Mepyramine	59 ± 18	294 ± 43	236 ± 33	0.21
Isoprenaline	54 ± 25	367 ± 47	312 ± 26	0.003

*Students T-test in comparison with no treatment.

Mazzoni, L. et al. (1985). J. Physiol. (in press).
Morley, J., Sanjar, S. & Page, C.P. (1984). Lancet ii: 1142-1144.

INHIBITION OF ALLERGIC INFLAMMATION BY PAF ANTAGONISTS IN THE RABBIT

P.G.Hellewell* & T.J.Williams, Section of Vascular Biology, MRC Clinical Research Centre, Watford Rd., Harrow, Middlesex, HA1 3UJ.

Platelet-activating factor (PAF) induces local oedema when injected intradermally in the rabbit. PAF appears to act directly on venular endothelial cells to produce this effect (Wedmore & Williams, 1981a). We have investigated the possibility that PAF may be involved in allergic inflammation in rabbit skin by testing two putative PAF receptor antagonists on oedema responses induced by exogenous inflammatory mediators and upon responses to endogenous mediators generated in the reversed passive Arthus reaction.

Agonists were mixed with the vasodilator PGE_2 ($3x10^{-10}$moles) and injected intradermally (0.1ml volumes) into the clipped dorsal skin of rabbits. The oedema responses were measured as the 30 min accumulation of intravenously injected ^{125}I-albumin (Wedmore & Williams, 1981b). The PAF antagonists were mixed with agonists prior to injection.

Rhone-Poulenc 48740 (3-(3 pyridyl)-1H,3H pyrollo 1,2-C thiazole-7 carboxamide) and Merck L-652731 (trans-2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrofuran) inhibited oedema responses to intradermal PAF (10^{-9}moles) but not to matching responses induced by the other direct acting mediators, bradykinin ($5x10^{-11}$moles) and histamine (10^{-8}moles). Furthermore, there was no suppression of responses to C5a, f-met-leu-phe and leukotriene B_4 (all at $5x10^{-11}$moles); mediators which induce oedema through an interaction between circulating PMN leukocytes and venular endothelium (Wedmore & Williams, 1981b). A 50% inhibition of the response to PAF + PGE_2 was obtained with $5x10^{-7}$moles 48740RP and $2x10^{-8}$moles L-652731. Using intradermal injections of 10^{-6}moles 48740RP and $5x10^{-8}$moles L-652731 (doses that were used routinely in our studies), no inhibition of cyclooxygenase was observed, as assessed using intradermal bradykinin ($5x10^{-11}$moles) + arachidonic acid ($3x10^{-9}$moles). Similarly, no inhibition of kinin generation was observed, as assessed using intradermal kallikrein (500ng) + PGE_2 ($3x10^{-10}$moles). Neither of the antagonists possessed oedema- producing activitythemselves. Furthermore, L-652731 did not inhibit the generation of C5a in vitro (measured using radioimmunoassay of zymosan activated plasma) or in vivo following intradermal injection of zymosan (Williams & Jose, 1981). However, both compounds were active in an experimental allergic inflammatory reaction. In a reversed passive Arthus reaction, injection of 48740RP or L-652731 together with antibody suppressed local oedema by 49.4% (n=2 rabbits) and 42.8±6.2% (n=4 rabbits) respectively, measured for 2 hours after i.v. antigen challenge (6 replicates per treatment in each rabbit). A similar degree of inhibition was observed in an Arthus reaction that was potentiated by intradermal injection of PGE_2 ($3x10^{-10}$ moles) at 0 and 60 minutes.

These results indicate that 48740RP and L-652731 are selective PAF antagonists in the rabbit. The results also suggest that the secondary release of PAF from PMN leukocytes is not important for the response to C5a, f-met-leu-phe and leukotriene B_4 (see Wedmore & Williams, 1981a, 1981b). Suppression of the Arthus reaction by these antagonists provides evidence that endogenous PAF may have a role in this type of allergic inflammation.

We thank Dr.P.Sedivy of Rhone-Poulenc Sante, Vitry Sur Seine, France and Dr.T.Y.Shen of Merck Sharp & Dohme, Rahway,USA for gifts of 48740RP and L-652731 respectively. We thank the MRC for financial support.

Wedmore,C.V. & Williams,T.J. (1981a) Br.J.Pharmac 74, 916-917P
Wedmore,C.V. & Williams,T.J. (1981b) Nature 289, 646-650
Williams,T.J. & Jose,P.J. (1981) J.Exp.Med. 153,136-153

THE RELEASE OF PAF-ACETHER AND LYSO-PAF FROM SENSITIZED GUINEA-PIG LUNGS

M.F. Fitzgerald*, S. Moncada and L. Parente, Dept. of Mediator Pharmacology, Wellcome Research Laboratories, Beckenham, Kent, U.K.

Increasing experimental evidence suggests that platelet activating factor (PAF-acether, AGEPC) is a potential mediator of inflammation and anaphylaxis. We have recently shown that lyso-PAF but not PAF-acether could be detected in the effluent of isolated lungs when stimulated with Ca^{+} ionophore. In order to explore this further we have now investigated the release of lyso-PAF and PAF-acether during anaphylactic challenge.

Male Dunkin-Hartley guinea-pigs (250-350 g) were sensitized by injecting ovalbumin (Sigma) 50mg s.c. and 50mg i.p. Three weeks later the lungs were removed and perfused with Krebs bicarbonate solution containing 0.25% BSA warmed (37^{0}C) and gassed ($95\%O_2$-$5\%CO_2$) at 5 ml min^{-1}, either through the pulmonary artery or through the trachea as previously described (Parente et al., 1985). The antigen (500 $ng.min^{-1}$) was then infused for five minutes and 1 min (5ml) samples were collected every other minute for 20 minutes. PAF-acether and lyso-PAF were extracted, identified and quantified as previously reported (Parente & Flower, 1985). In the experiments with perfused airways the perfusion pressure as an index of the bronchoconstriction was also recorded.

Sensitized guinea-pig lungs perfused through the pulmonary circulation released substantial amounts of lyso-PAF throughout the collection period with a peak between 6 and 8 minutes after commencing the antigen infusion (lyso-PAF mean release: 4.86 ± 0.25 ng min^{-1}, n=6). In these experiments no PAF-acether was detected in the effluent. Sensitized guinea-pig lungs perfused through the airways released substantial amounts of PAF-acether throughout the collection period with a peak between 4 and 6 minutes after commencing the antigen infusion (PAF-acether mean release: 1.97 ± 0.37 ng min^{-1}, n=5). The release of PAF acether was accompanied by an increase in the perfusion pressure which parallelled the release of the mediator (mean differential increase: 59.0 ± 6.4 mmHg, n=5).

Alveolar macrophages are the most likely cellular source of PAF-acether in the lung. In order to check the capability of these cells to synthetise the mediator, alveolar macrophages collected by bronchial lavage from control guinea-pigs were incubated in vitro and challenged with calcium ionophore A23187 (1 $\mu g.ml^{-1}$, 30 min). In these experimental conditions the cells released 1.73 ± 0.06 ng of PAF-acether and 0.49 ± 0.1 ng of lyso-PAF per 10^{6} cells (n=7).

These results demonstrate that although PAF-acether is not detectable in the effluent of sensitized lungs perfused through the pulmonary circulation it is detectable after antigen challenge when this is carried out through the airways. This observation may be of relevance for the understanding of the role of PAF-acether in lung pathophysiology.

Parente L. et al. (1985) Eur.J.Pharmacol. 112, 281-284.
Parente L. & Flower R.J. (1985) Life Sci. 36, 1225-1231.

PARACETAMOL-INDUCED HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS AFTER IN VITRO ACTIVATION BY MONOOXYGENASES

Sandra Brunelleschi, A. Conti, R. Fantozzi, Maura Lodovici, P.F. Mannaioni & Emanuela Masini*, Department of Preclinical and Clinical Pharmacology "Mario Aiazzi-Mancini", Viale Morgagni 65, 50134 Florence, Italy.

Several lines of evidence suggest that active species of oxygen are involved in histamine release: xanthine oxidase induces the release of histamine from isolated rat mast cells (Ohmori et al., 1979); zymosan or FMLP-activated human neutrophils evoke the release of histamine from isolated rat mast cells, both processes circumstantially involving hydrogen peroxide (Stendhal et al., 1983). When intermediate free radicals are concerned, paracetamol, a commonly used analgesic drug accepted as an aspirin substitute, is believed to exert its cytotoxic effect through a cytochrome P-450 dependent N-hydroxylation reaction, leading to the formation of a transient phenoxyl free radical (N-acetyl-p-benzoquinoneimine: Holme et al., 1984).

Aim of the present experiments is to evaluate whether free radicals intermediates formed during metabolic oxidations may evoke histamine release from rat serosal mast cells.

When a variety of concentrations of paracetamol (10^{-8} -10^{-4}M) are incubated with purified isolated rat peritoneal mast cells, no histamine release was detected. A certain degree of histamine release was observed when high concentrations of paracetamol (10^{-4}M) were incubated with rat mast cells in the presence of liver microsomes (S_9 microsomal fraction) obtained from normal animals. A substantial amount of histamine was released from rat mast cells by low concentrations of paracetamol (10^{-7} -10^{-6}M), after incubation with liver microsomes obtained from animals induced with polychlorinated biphenyls or with phenobarbital. Ascorbate did not inhibit the release of histamine induced by paracetamol, which was significantly reduced by glutathione (10^{-4}M). Further experiments will be carried out evaluating the protection afforded by other free radical scavangers, such as α-tocopherol, superoxide dismutase, catalase and (-) mannitol. The model is consistent with the hypothesis that enzymatic formation of a transient phenoxyl free radical, generated during the in vitro oxidation of paracetamol, causes mast cell injury and histamine release.

Holme, J.A., Dahlin, D.C., Nelson, S.D. & Dybing, E. (1984) Biochem. Pharmacol. 33, 401-406.

Ohmori, H., Komorya, K., Azuma, A., Kurozuma, S. & Hashi, Y. (1979) Biochem. Pharmacol. 28, 333-334.

Stendhal, O., Molin, L. & Lindroth, M. (1983) Int. Archs. Allergy appl. Immun. 70, 277-284.

INHIBITION OF HUMAN NEUTROPHIL ACTIVITY BY CALCIUM ENTRY BLOCKERS

S. Brunelleschi, R. Fantozzi*, A. Giotti, F. Ledda & M. Mian, Department of Pharmacology, University of Florence, Florence, Italy.

Formylated peptides, such as N-formylmethionyl-leucyl-phenylalanine (FMLP), ionophores (e.g., A23187) and an ether-linked phospholipid, PAF-acether (Platelet-activating factor) evoke superoxide anion (O_2^-)production and lysosomal enzyme release from human neutrophils, by activating the cells with different mechanisms (Weissmann et al., 1980). Calcium plays a critical role in stimulus-response coupling of neutrophils and Ca^{2+} movements accompanying neutrophil activation by different stimuli have been described (Korchak et al., 1984). Calcium entry blockers not only affect the contractile properties of cardiac and smooth muscle cells, but also interfere with other Ca^{2+}-requiring processes such as release of inflammatory mediators from different cell types (Chand et al., 1984). To evaluate whether some structurally different Ca^{2+}-entry blockers (diltiazem, verapamil, nifedipine and other dihydropyridines such as nicardipine and nitrendipine) could modulate neutrophil activity, we performed a series of experiments with neutrophils isolated from healthy volunteers and challenged by different stimuli (FMLP, A23187, PAF-acether). O_2^- production was continuously measured as superoxide-dismutase inhibitable cytochrome C reduction. ß-Glucuronidase release was assayed according to Fantozzi et al. (1984). The Ca^{2+} -entry blockers tested dose-dependently inhibited, in the concentration range 10^{-8}-10^{-4}M, both O_2^- production and ß-glucuronidase release. Drug inhibitory effects were antagonized by increasing Ca^{2+} concentrations in the extracellular medium. The inhibitory activities varied considerably depending on the nature of the secretagogue as well as the type of Ca^{2+}-entry blocker tested. Verapamil was an effective inhibitor of O_2^- production and ß-glucuronidase release evoked by either FMLP or A23187. Furthermore, it dose-dependently inhibited chemotaxis induced by zymosan-activated serum, and phagocytosis. Nifedipine did not significantly affect O_2^- production from FMLP-activated neutrophils, but it inhibited ($IC_{50}=4\times10^{-7}$M) production from cells stimulated by A23187. Similar results were obtained with the other dihydropyridines examined. Nifedipine was more active than verapamil on O_2^- production induced by the ionophore. Diltiazem displayed a pattern of behaviour similar to that of nifedipine and it inhibited O_2^- production from neutrophils challenged by PAF-acether. The variable effects of the Ca^{2+} -entry blockers we tested may be related to the different mechanisms of neutrophil activation afforded by the stimuli we used, and to the drug's ability to affect not only Ca^{2+} influx but also intracellular Ca^{2+}-dependent steps involved in neutrophil activation. Differences in the lipid solubility of Ca^{2+} -entry blockers may determine their different abilities to accumulate inside the cells.

Chand, N., Diamantis, W., Pillar, J. & Sofia, R.D. (1984) Br. J. Pharmac. 83, 899-902.
Fantozzi, R., Brunelleschi, S., Cambi, S., Blandina, P., Masini, E. & Mannaioni, P.F. (1984) Agents and Actions 14, 441-450.
Korchak, H.M., Rutherford, L.E. & Weissmann, G. (1984) J. Biol. Chem. 259, 4070-4075.
Weissmann, G., Smolen, J.E. & Korchak, H.M. (1980) N. Engl. J. Med. 303, 27-34.

NEUTROPHIL-MEDIATED HISTAMINE RELEASE FROM MAST CELLS: A ROLE FOR OXYGEN RADICALS

S. Brunelleschi*, R. Fantozzi, P.F. Mannaioni & E. Masini, Department of Pharmacology, University of Florence, Florence, Italy.

Human neutrophils produce oxygen radicals, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical, and release lysosomal enzymes when are activated during inflammatory processes or are exposed to different stimuli,in vitro. Exogenous histamine and mast cell secretory products inhibit both enzyme release and O_2^- production from human neutrophils stimulated by the chemotactic peptide, N-formylmethionyl-leucyl-phenylalanine (FMLP), (Fantozzi et al.,1984;1985). Furthermore, H_2O_2 produced by the reaction between hypoxanthine and xanthine oxidase,causes histamine release from rat peritoneal mast cells (Ohmori et al.,1979). These results suggest both that neutrophils and mast cells interact during inflammatory and immune processes and that neutrophil-derived oxygen radicals can release histamine from mast cells. To further investigate the ability of neutrophil-derived products to evoke the release of histamine, we set up an experimental model in vitro where human neutrophils and rat serosal mast cells were mixed together. Neutrophils were isolated from healthy volunteers and serosal mast cells were isolated from Wistar albino rats, according to Fantozzi et al.,(1983). Histamine was determined fluorimetrically; O_2^- production was continuously measured as superoxide-dismutase inhibitable cytochrome C reduction; H_2O_2 release was measured by the fluorescent scopoletin assay. FMLP stimulated human neutrophils in the concentration range 10^{-9}-10^{-6}M and the maximum effects on oxygen radical production and enzyme release were achieved at 10^{-7}-10^{-6}M. FMLP has been previously demonstrated not to affect rat mast cells at the same concentrations (Fantozzi et al.,1983). When neutrophils were challenged by FMLP in the presence of mast cells, we measured a release of histamine, which increased by increasing the concentrations of FMLP in the medium, with a maximum release (30-40%) at peptide concentrations of 10^{-7}-10^{-6}M. The dose-response curve of histamine release from mast cells paralleled those of O_2^- and H_2O_2 production from FMLP-stimulated neutrophils. The ability of FMLP-activated neutrophils to elicit histamine release from mast cells could be affected by drugs. Dose-dependent inhibitory effects were observed by treating the cell suspensions with disodium cromoglycate (10^{-6}-10^{-4}M) or with flavonoids, such as silymarin (10^{-7}-10^{-4}M).The model described appears to be a suitable approach for investigating the relationship between neutrophils and mast cells; for evaluating the role of oxygen radicals in causing histamine release; and for studying the interactions between drugs and cells that are involved in inflammatory and immune processes.

Fantozzi, R., Brunelleschi, S., Banchelli Soldaini, G., Blandina, P., Masini, E., Raimondi, L. & Mannaioni, P.F. (1983) Agents and Actions 13, 218-221.

Fantozzi, R., Brunelleschi, S., Cambi, S., Blandina, P., Masini, E. & Mannaioni, P.F. (1984) Agents and Actions 14, 441-450.

Fantozzi, R., Brunelleschi, S., Giuliattini, L., Blandina, P., Masini, E., Cavallo, G. & Mannaioni, P.F. (1985) Agents and Actions 16, in press.

Ohmori, H., Komoriya, K., Azuma, A., Kurozumi, S. & Hashi Oto, Y. (1979) Biochem. Pharmac. 28, 333-334.

DIFFERENTIAL INHIBITION OF PLATELET RECEPTOR-ACTIVATED FUNCTIONAL RESPONSES AND TRANSDUCTION PROCESSES BY cAMP

M. Bushfield*, A. McNicol & D.E. MacIntyre, Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, Scotland

Agonist (e.g. platelet-activating factor, PAF)-induced platelet activation is mediated, at least in part, by processes regulated by the stimulatory second messenger molecules 1,2-Diacylglycerol (DAG) and cytosolic free Ca^{2+} ($[Ca^{2+}]i$). Formation of DAG and elevation of $[Ca^{2+}]i$ are initiated by PAF-induced hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) (Michell, 1983; O'Rourke et al, 1985). In contrast, elevation of platelet cAMP content ([cAMP]) by agents (e.g. PGD_2, PGI_2, adenosine) results in inhibition of platelet activation. Such inhibitory effects may be a consequence of suppression of agonist-induced phosphoinositide hydrolysis and elevation of $[Ca^{2+}]i$ (Feinstein et al, 1983; Lapetina, 1984); of stimulation of Ca^{2+} sequestration (Kaser-Glanzmann et al, 1977) and/or of abrogation of DAG- and/or Ca^{2+}-dependent biochemical reactions (e.g. myosin light chain kinase activity) (Feinstein et al, 1981).

To investigate the possible mechanism(s) by which cAMP inhibits platelet reactivity we examined the effects of PGD_2 on [cAMP] and on aggregation, ATP-secretion, elevation of $[Ca^{2+}]i$ and phosphoinositide hydrolysis induced by sub-maximal concentrations of PAF (18-180nM). All studies were performed using plasma-free suspensions of human platelets. Platelet aggregation was monitored photometrically and ATP secretion by luciferin-luciferase luminescence, $[Ca^{2+}]i$ was measured by using quin 2 and phosphoinositide hydrolysis was monitored as $[^{32}P]$-phosphatidate (-PtdA) formation (Pollock et al, 1984). [cAMP] was measured by radio-immunoassay of ethanol-extracted samples (Harper & Brooker, 1975).

PAF (1-180nM) induced concentration-dependent platelet aggregation, ATP-secretion (up to 80% of maximum), elevation of $[Ca^{2+}]i$ (from 82±7nM to around 700nM) and formation of $[^{32}P]$-PtdA (2-10 fold above basal). PGD_2 (10-3000nM) elicited a concentration-dependent elevation of platelet [cAMP](from 6.9±0.4 to around 140 pmol/10^8 cells) and inhibited PAF-induced aggregation (I_{50}=3nM), ATP-secretion (I_{50}=2nM), elevation of $[Ca^{2+}]i$ (I_{50}=30nM) and $[^{32}P]$-PtdA formation (I_{50}=20nM). Aggregation and ATP-secretion were abolished by a 2 fold increase in [cAMP] whereas maximal inhibition of $[^{32}P]$-PtdA formation and elevation of $[Ca^{2+}]i$ required a greater than 10 fold elevation of [cAMP].

Hence, modest increments of platelet [cAMP] abolish PAF-induced platelet functional responses (aggregation, ATP secretion) with little effect on phosphoinositide hydrolysis or on elevation of $[Ca^{2+}]i$. This suggests that inhibition of platelet activation by elevated [cAMP] is mediated by suppression of the biochemical reactions modulated by DAG or $[Ca^{2+}]i$, rather than by inhibition of the transduction processes that lead to DAG formation and elevation of $[Ca^{2+}]i$.

This study was supported by the M.R.C., S.E.R.C. and by the Medical Research Funds of the University of Glasgow.

Feinstein, M.B. et al (1981) in "Platelets in Biology and Pathology", Gordon, J.L. Ed. (pp.437-472) Elsevier, Amsterdam
Feinstein, M.B. et al (1983) Biochem. Biophys.Res.Comm. 113, 598-604
Harper, J.F. & Brooker, G. (1975) J.Cyclic Nucleotide Res. 1, 207-218
Kaser-Glanzmann, R. et al (1977) Biochim.Biophys.Acta 466, 429-440
Lapetina, E.G. (1984) Biochim.Biophys.Res.Comm. 120, 37-44
Michell, R.H. (1983) Trends Biochem.Sci. 8, 263-265
O'Rourke, F.A. et al (1985) J.Biol.Chem. 260, 956-962
Pollock, W.K. et al (1984) Biochem.J. 299, 833-842

THE ROLE OF PRODUCTS OF PLATELET ACTIVATION IN COLLAGEN-INDUCED DEATH IN RABBITS

Deirdre Robertson* & G M Smith, School of Pharmacy, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen, AB9 1FR.

Mallarkey and Smith (1985) have shown that i.v. administration of Collagen (40μg/kg) causes sudden death in rabbits. Since death could be prevented by indomethacin and calcium channel blocking drugs, these workers concluded that death was due to coronary vasoconstriction and myocardial ischaemia caused by TXA_2 released from platelets. However, further studies of the role of TXA_2 in this model suggested that other mediators may be involved (Robertson & Smith, 1984). This study investigates the possible roles of TXA_2, 5HT and PAF in the mediation of the lethal effects of collagen in the rabbits.

Rabbits were anaesthetized with Diazepam and fentanyl-fluanisone (Hypnorm) and allowed to breathe spontaneously. Arterial blood pressure, circulating platelet count (Smith & Freuler, 1973) and lead III ECG were continuously recorded. Drugs or vehicle were injected i.v. 5 mins prior to i.v. injection of collagen (type 1 Diamed). In survivors, further doses of collagen were given at 15 min intervals. Blood samples (2ml) were withdrawn before and 1 min after each collagen injection and assayed for TXB_2 by radioimmunoassay.

Collagen (40μg/kg) causes a severe hypotension, fall in platelet count, increase in plasma levels of TXB_2 and ST-segment elevation, indicating the presence of myocardial ischaemia. In the control groups 100% and 80% of rabbits die within 2-5 min of collagen administration of either the first or second dose of collagen. Table 1 shows the effect of pretreatment of rabbits with the cyclo-oxygenase inhibitors indomethacin and piroxicam, the TX synthetase inhibitor dazoxiben, the TX receptor antagonist EPO92, the $5HT_2$ antagonist ketanserin, the 5HT M-receptor antagonist ICS 205-930 and the PAF receptor antagonist CV3988. The results suggest that 5HT and possibly PAF also have a role as well as TXA_2 in the lethal effects of collagen.

Table 1

Treatment	Dose mg/kg	%Survival > 2 collagens	%Inhibition of fall in platelet count	%Inhibition of Plasma TXB_2 levels	n =
Indomethacin	0.125	100	45.7	100	5
Piroxicam	2	100	29.6	100	5
Dazoxiben	2	50	39.2	100	6
EPO92	2.5+*	67	15.2 (NS)	20.6 (NS)	9
Ketanserin	1	60	20.6 (NS)	63	5
ICS 205-930	1	20 (NS)	15.5 (NS)	0.3 (NS)	5
CV3988	5	40 (NS)	62	43.7 (NS)	5

*10 mg/kg/hr infusion n = number of rabbits NS = not significant

Mallarkey G. & Smith G.M. (1985) Thromb. Haemostas. 53, 70-74
Robertson D. & Smith G.M. (1984) IUPHAR 9th Int. Cong. Pharmac. 1497P
Smith G.M. & Freuler F. (1973) Bibl. Anat. 12, 229-234

DIFFERENTIAL EFFECT OF PERTUSSIS TOXIN ON THE INHIBITORY ACTIONS OF ADENOSINE IN GUINEA-PIG ISOLATED ILEUM AND TRACHEA

J.F.Tucker & A. von Uexküll*, Department of Pharmacology, Chelsea College, Manresa Road, London, SW3 6LX.

The functional involvement of adenylate cyclase in signal transduction from receptors can be studied using Pertussis toxin which has been shown to selectively abolish receptor mediated inhibition of adenylate cyclase by inactivation of an inhibitory GTP binding regulatory protein (Ni) (Murayama & Ui, 1983).

Using Pertussis toxin we have explored the link between purinoceptors and adenylate cyclase in two isolated guinea-pig tissues, myenteric neurones of the ileum and tracheal smooth muscle.

Segments of ileum and trachea obtained from guinea-pigs 2 or 3 days after intraperitoneal injection of 100 to 125 µg/Kg purified Pertussis toxin (Irons & Maclennan, 1979) or vehicle were set up in Kreb's solution at 36°C and gassed with 95% O_2 + 5% CO_2. Contractions were recorded isometrically under 1g tension. In the ileum the contractile effects of acetylcholine (ACh) and the presynaptic inhibitory effects of adenosine and its stable derivative 2-chloroadenosine (2-CA) were determined as described previously (Tucker, 1984). Tracheal zig-zags comprising three cartilagenous rings were prepared by the method of Emmerson & Mackay (1979). Prostaglandin generation in the trachea was prevented by the inclusion of indomethacin (10^{-5}M)in the bathing medium. After a 30-60 min. equilibration period cumulative dose response curves were constructed for pilocarpine. The tone of the tissue was then raised with 2 x 10^{-6}M pilocarpine and the inhibitory effects of cumulative doses of adenosine and 2-CA determined.

Pertussis toxin had no effect on the responses of the ileum to ACh but markedly attenuated the presynaptic inhibitory effects of adenosine and 2-CA. The maximum responses to adenosine and 2-CA were reduced to 62.3 ± 3.1% (mean ± s.e. mean: n = 26) and 47.2 ± 5.2% (n = 6) respectively compared to control. The slopes of the dose response curves were reduced from 77.6 ± 3.4 to 38.0 ± 2.3 and 65.4 ± 5.7 to 20.5 ± 1.7 respectively.

In the trachea, Pertussis treatment had no effect on the contractile action of pilocarpine nor any effect on the relaxant actions of adenosine and 2-CA.

The contrasting effects of Pertussis toxin reported here suggest that in myenteric neurones of the guinea-pig ileum, but not in tracheal smooth muscle, the actions of adenosine and 2-CA are mediated by way of a GTP binding protein which in several other tissues (Murayama & Ui, 1983) has been shown to be linked to adenylate cyclase inhibition.

Emmerson, J. & Mackay, D. (1979) J.Pharm.Pharmac. 31, 798.
Irons, L.I. & Maclennan, A.P. (1979) Biochim.biophys.Acta, 580, 175-185.
Murayama, T. & Ui, M. (1983) J.biol.Chem. 258, 3319-3326.
Tucker, J.F. (1984) Br.J.Pharmac. 83, 326-328.

ACTIVATION AND DESENSITIZATION OF PRESYNAPTIC α_2-ADRENOCEPTORS AFTER INHIBITION OF NEURONAL UPTAKE

J.A. García-Sevilla* & J.K. Zubieta, Department of Pharmacology, Faculty of Medicine, University of the Basque Country, Leioa, Spain.

Inhibition of neuronal uptake is an early and major biochemical effect by which most tricyclic antidepressant drugs initiate their therapeutic actions. Desensitization of presynaptic alpha-2 adrenoceptors also has been involved in the mechanism of action of these drugs (Crews & Smith, 1978). Since inhibition of noradrenaline (NA) neuronal uptake results in its accumulation in the synaptic cleft, the sensitivity of presynaptic alpha-2 adrenoceptors could be indirectly modulated by antidepressant drugs. This hypothesis was tested in the rat isolated field-stimulated vas deferens (0.1 Hz, 0.3 ms, 20-40 V), a preparation which possess a powerful reuptake system for NA and presynaptic alpha-2 adrenoceptors which mediate the inhibition of the electrically-induced twitch responses.

Basal twitch responses elicited by field stimulation developed a tension of 850±69 mg (n=10). The in vivo administration of various antidepressant drugs (10 mg/kg; i.p.; 2 h) resulted in marked different degrees of inhibition of the basal twitch responses which were rapidly reversed to control values by idazoxan (10^{-5}M). The rank order of potency in inhibiting the basal twitch was found to be (in % maximal): desipramine (6±1%)>protriptyline (13±2%)> nortriptyline (42±7%)>maprotiline (57±4%)=imipramine (57±4%)>amitriptyline (60±3%)> viloxazine (63±3%)>iprindole (69±4%)≫zimelidine (94±4%). There was a positive and significant correlation (r=0.914; P<0.001) between the potencies (log K_i) of these drugs for blockade of $|^3H|$NA uptake into rat brain synaptosomes (Richelson & Pfenning, 1984) and the reductions (% maximal) of the basal twitch responses induced by the same drugs. These data suggest that the acute inhibition of NA neuronal uptake activates (being NA the agonist) presynaptic inhibitory alpha-2 adrenoceptors which results in inhibition of the twitch responses.

In control preparations, clonidine inhibited in a concentration-dependent manner (10^{-9}M-10^{-7}M) the twitch responses (pD_2=8.19±0.17; n=8). The long-term administration (10 mg/kg; i.p.; 14 days) of desipramine decreased by 14 times the effecttiveness of clonidine (pD_2=7.04±0.10; n=4; P<0.001); maprotiline decreased it by 10 times (pD_2=7.21±0.27; n=3; P<0.001) and zimelidine by 7 times (pD_2=7.35± 0.06; n=3; P<0.005). These data indicate that prolonged inhibition of NA reuptake desensitizes (being NA the responsible agonist) presynaptic inhibitory alpha-2 adrenoceptors which results in a reduction of clonidine sensitivity.

It is concluded that in vivo inhibition of NA neuronal uptake by antidepressant drugs modulates presynaptic inhibitory alpha-2 adrenoceptors through an early activation followed by a desensitization process which might explained, if similar changes operate inthe CNS, the delayed onset of action of antidepressant drugs.

Supported by CAICYT Grant No. 1018/81.

Crews, F.T. & Smith, C.B. (1978) Science 202, 322.
Richelson, E. & Pfenning, M. (1984) Eur. J. Pharmac. 104, 277.

RAT PLATELETS AND CULTURED PROMEGAKARYOBLASTS SHARE A COMMON PURINORECEPTOR

D.E. MacIntyre & Linda J. MacMillan*, Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ.

Platelet activation is initiated when stimulatory agonists (e.g. Thrombin, ADP) combine with distinct receptors that are coupled to phosphoinositide hydrolysis and consequent formation of 1,2-Diacylglycerol (DAG) and elevation of the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$). In contrast, platelet inhibitory agonists (e.g. PGI_2, adenosine) combine with distinct receptors that are coupled to adenylate cyclase and consequent cAMP formation (Feinstein et al, 1981; Nishizuka, 1984). ADP-induced human platelet activation is antagonised competitively by ATP and AMP, and non-competitively by adenosine (Cusack et al, 1982). This spectrum of action of adenine nucleotides and nucleosides indicates that the ADP purinoreceptor on platelets differs from purinoreceptors in other tissues (Burnstock, 1976). Using rat platelets and an eternal line of rat promegakaryoblasts (RPM) capable of maturing in culture to megakaryocytes (Weinstein et al, 1981) we have shown that the transduction processes activated by stimulatory and inhibitory platelet agonists are also evident in the progenitor cell (MacIntyre et al, 1983; MacMillan et al, 1985). In the present study we compared the effects of ATP, ADP, AMP and adenosine on phosphoinositide hydrolysis and Ca^{2+} flux in rat platelets and RPM to investigate whether the apparently unique purinoreceptor for ADP on platelets is also expressed in the megakaryocyte.

Rat platelets and RPM were prepared essentially as described previously (Weinstein et al, 1981; MacIntyre & MacMillan, 1985). $[Ca^{2+}]i$ was monitored by using quin 2 (Tsien et al, 1982) and phosphoinositide hydrolysis was monitored as $[^{32}P]$-PO_4 (Pollock et al, 1984).

Resting $[Ca^{2+}]i$ in rat platelets = 87±14nM (mean ±S.E., n=17) and in RPM = 154±32nM (mean ±S.E., n=10). In both cell types ADP>ATP but not AMP or adenosine elicited a concentration dependent elevation of $[Ca^{2+}]i$. Mean EC_{50} values (±S.E., n=3-4) for ADP and ATP respectively were 1.63±1μM and 20±10μM in platelets, and 0.2±0.1μM and 2.7±1.7μM in RPM. When tested at equimolar concentrations (100μM) ADP and ATP but not AMP or adenosine elicited a significant increase in $[^{32}P]$-PtdA in both platelets and RPM.

These results indicate that ADP and ATP, but not AMP or Adenosine, can elicit phosphoinositide metabolism and Ca^{2+} flux in rat platelets and RPM , and that, in terms of elevation of $[Ca^{2+}]i$, ADP is a more potent agonist than ATP. Thus rat platelets and their progenitor cells share a common purinoreceptor that is coupled to inositol phospholipid hydrolysis and elevation of $[Ca^{2+}]i$. The effects of ATP would suggest that this purinoreceptor on the rat platelet and RPM differs from that on the human platelet.

This study was supported by the MRC and by the Medical Research Funds of the University of Glasgow. LJMM is an MRC scholar.

Burnstock, G. (1976) J.Theor.Biol. 62, 491-503
Cusack, N.J. et al (1982) Br.J.Pharmac. 75, 397-400
Feinstein, M.B. et al (1981) in "Platelets in Biology and Pathology 2" Ed. J.L. Gordon, North Holland, Amsterdam, pp. 437-472
MacIntyre, D.E. et al (1983) Br.J.Pharmac. 79, 361P
MacIntyre, D.E. & MacMillan, L.J. (1985) Br.J.Pharmac. 84, 25P
MacMillan, L.J. et al (1985) Thrombosis and Haemostasis, in press
Nishizuka, Y. (1984) Nature 308, 693-698
Pollock, W.K. et al (1984) Biochem.J. 219, 833-842
Tsien, R.Y. et al (1982) J.cell Biol. 94, 325-334
Weinstein, R. et al (1981) Blood 58, 110-121

582P

ADENOSINE MODULATION OF THE DOPAMINERGIC TRANSMISSION IN STRIATUM

M.P. Abbracchio*, F. Cattabeni and G. Lombardelli, Inst. Pharmacology & Pharmacognosy, Via A. del Sarto 21 - 20129 Milan, Italy.

Adenosine (A) might be involved in the modulation of the dopaminergic function in striatum (Green et al., 1982), where both A_1 and A_2 receptors are present. Activation of A_1 high affinity receptors by A or its analogs inhibits striatal adenylate cyclase, whereas A_2 low affinity receptors are functionally linked to adenylate cyclase in a stimulatory manner. On the bases of results suggesting that imbalances between striatal A and dopamine (DA) might be involved in neurological syndromes associated with movement disorders, we focussed our attention on rat striatal adenylate cyclase as a possible site of interaction between A and DA, and we studied the effects on striatal DA dependent adenylate cyclase of two A analogs L-phenyl-isopropyl-A (L-PIA) and 5'-N-Ethylcarboxamide-A (NECA). According to previous results (Wojcik and Neff, 1983), nM L-PIA concentrations slightly inhibited adenylate cyclase activity, whereas stimulation of enzyme activity was obtained at uM concentrations. In contrast, NECA only showed a stimulatory effect on adenylate cyclase activity, with a percent of maximal stimulation quantitatively higher with respect to L-PIA suggesting that NECA is devoid of an action on A_1 receptors and behaves as a "full agonist" on A_2 receptors. The effects of the two analogs on DA dependent adenylate cyclase were then studied. In the presence of increasing L-PIA concentrations, DA dependent adenylate cyclase was inhibited, and even at higher L-PIA concentrations which by themselves activated the enzyme, no addition between L-PIA and DA stimulatory effects was observed. In contrast, NECA did not inhibit DA sensitive adenylate cyclase, but potentiated it even at low concentrations. We then examined the effects of a surgical or a pharmacological alteration of the striatal DAergic system on A dependent adenylate cyclase activity and on the modulation of DA sensitive adenylate cyclase by A analogs. After destruction of the DAergic nigro-striatal pathway with 6-OHDA, upregulation of striatal DA receptors was accompanied by supersensitivity of both L-PIA and NECA stimulated adenylate cyclase activities. Furthermore, modulation of striatal DA sensitive adenylate cyclase by L-PIA was greatly modified by denervation. In fact, L-PIA lost its inhibitory activity on DA dependent adenylate cyclase, with an effect which more closely resembled that normally exerted by NECA. It thus seems that a supersensitivity of A receptors also implies an alteration of the modulatory action of A on the DAergic system. Subchronic treatment with reserpine also seemed to bring about supersensitivity of both DA and A sensitive adenylate cyclase activities in striatum. To verify whether these effects are simply due to alterations of neurotransmitter release at the level of DAergic presynaptic terminals in striatum, or whether they can be observed after any condition that renders DA postsynaptic receptors supersensitive, we are now evaluating the effects of subchronic neuroleptic treatments on A dependent adenylate cyclase and on A modulation of the DAergic transmission in striatum.

Green, R.D., Proudfit, H.K. and Yeung, S.M.H. (1982) Science, 218, 58.
Wojcik, W.J. and Neff, N.H. (1983) Neurosci. Lett. 41, 55.

FREQUENCY DEPENDENT INTERACTIONS BETWEEN ADENOSINE AND TRANSMITTER RELEASES ELECTRICALLY EVOKED, FROM RAT BRAIN SLICES

F. Caciagli; R. Ciccarelli; M. Del Coco; P. Di Iorio*; O.N. Longhi. Department of Pharmacology. University of Chieti, Medical School. Chieti. Italy.

Evidence has been put forward that Adenosine(A), in the CNS may play a double role as cotransmitter and metabotropic regulator. Probably, its activity on the cAMP system largely contributes to activate metabolic events and to inhibit nervous spontaneous firing and several synaptically evoked responses. Among these mutual interactions between A and transmitter releases could be a key step to be carefully evaluated.

To study these interactions, slices (250 μ thick) were obtained from two different rat brain areas (cortex and hypothalamus). A field electrical stimulation (trains of 100 pulses of alternating polarity of 30 mA/cm^2 and 5 msec duration at different frequencies) was delivered (for 5 min) to the slices, transferred in suitable microchambers and superfused with oxygenated Krebs solution(0.9 ml/min). This experimental model permits to simultaneously measure A, NE and Ach releases both at rest and electrically evoked. ^{3}H-A and ^{14}C-NE incubations and calculation of fractional and total release were performed according to '(Lloyd & Stone, 1981; Taube et al., 1977; James & Cubeddu, 1984)' methods respectively. Endogenous Ach release was assayed on the guinea pig ileum in vitro.

The amount of ^{3}H-A outflow electrically evoked was frequency dependent both in cortical and in hypothalamic slices. This A release was partially Na^+(TTX 5x10^{-7} M) and Ca^{2+} dependent. Furthermore the basal and evoked A release could be totally prevented when the slices, before and during incubation,were pretreated with Dipyridamole(5x10^{-4} M) to block A uptake. Table 1 shows that a frequency dependent release was found even for endogenous Ach and for ^{14}C-NE.

Table 1 ^{3}H-A, ^{14}C-NE (as % of total tissue radioactivity) and endogenous Ach (as ng/g/min) releases electrically evoked from rat cortical slices.

	0 Hz	0.5 Hz	1 Hz	2 Hz	5 Hz	10 Hz	20 Hz
Adenosine	2.1±0.1	2.2±0.2	4.8±0.3	7.5±0.4	13.3±0.7	17.9±1.1	33.3±2.6
Norepinephrine	1.0±0.1	----	2.6±0.3	5.1±0.5	10.6±0.4	----	16.7±1.1
Acetylcholine	5.1±0.3	12.5±0.5	22.0±1.0	56.4±2.6	65.0±3.8	88.7±4.8	----

Different stimulation thresholds to obtain an appreciable evoked release could be identified: for A a frequency stimulation higher than those found for both transmitters was necessary. At lower frequencies, able to induce an evoked A release, Dypiridamole,added tp the superfusion medium after incubation, significantly influences only NE release. Beginning from the frequency of 20 Hz, Dipyridamole was also effective in inhibiting endogenous Ach release. A greater inhibition appeared detectable at higher frequencies. Thus, it is possible to suggest that trigger mechanisms, frequency dependent, regulate A inhibition on transmitter release.

References:

Lloyd, H.G.E. & Stone, T.W. (1981) in Biochem. Pharm. 30, 11, pp 1239-1243.
Taube, D.H. et al. (1977) in Arch. of Pharm. 299, pp 123-141.
James, M.K. & Cubeddu, L.X. (1983) in J. Pharm. Exp. Ther. 229, 1, pp 98-104.

(-)N^6-PHENYLISOPROPYLADENOSINE COMPETITIVELY INHIBITS BAY K 8644, CALCIUM AGONIST, ON ISOLATED GUINEA-PIG ATRIA

L. Caparrotta, G. Fassina*, G. Froldi & R. Poja, Department of Pharmacology, Padua University, Largo E. Meneghetti 2 - 35131 Padova (Italy)

Adenosine inhibits calcium influx into the cells by a yet unknown mechanism. (-)N^6-phenylisopropyladenosine (PIA), a stable analogue of adenosine, decreases spontaneous contractile tension and frequency in isolated guinea-pig atria at nanomolar concentrations (Evans & Schenden, 1982). Bay K 8644, a dihydropyridine derivative, is capable of stimulating the contractility of cardiac muscle acting as an accelerator of the influx of Ca^{2+} through the slow Ca^{2+} channels (Schramm *et al.*, 1983).

The effect of Bay K 8644 was studied in the presence of PIA on isolated guinea-pig atria, spontaneously beating or electrically driven. In our experimental conditions, PIA had a negative inotropic and chronotropic effect at concentrations from 1 nM to 100 nM (IC_{50} = 20 nM). PIA inhibited the positive inotropic effect of Bay K 8644 in a concentration-dependent manner, producing a parallel shift of the dose-response curve to the right, indicative of a possible competitive antagonism (Fig. 1). IC_{50} for PIA was 2±1 nM and 0.7±0.3 nM in atria from normal and reserpi-

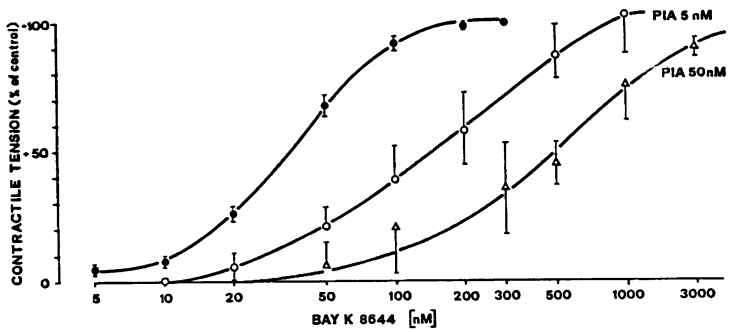

Figure 1 Effect of PIA 5 nM (o) and 50 nM (Δ) on the concentration-effect curve to Bay K 8644 (●) in guinea-pig isolated atria. Each point represents the mean ± s.e. value of 15 (●), 7 (o) and 6 (Δ) preparations.

nized guinea-pigs, respectively. The same antagonism was shown by PIA versus Bay K 8644 in electrically driven atria. IC_{50} of PIA was ≃ 5 nM. These data suggest that PIA is able to interact with the dihydropyridine derivative Bay K 8644 at the level of a common receptor in the slow Ca^{2+} channels. Our data are in accordance with adenosine ability to depress the Ca^{2+}-mediated action potentials of guinea-pig atrial muscle (Schrader *et al.*, 1975) and give experimental support to the proposed interference of purines with Ca^{2+} influx in cardiac muscle (Hughes & Stone, 1983). A role of adenosine in modulating Ca^{2+} channels is possible and of physiopathological interest.

Evans, D.B. & Schenden, J.H. (1982) Life Sci. 31, 2425-2432.
Hughes, P.R. & Stone, T.W. (1983) Br.J.Pharmac. 80, 149-153.
Schrader, J., Rubio, R. & Berne, R.M. (1975) J.Mol.Cell.Cardiol. 7, 427-433.
Schramm, M., Thomas, G., Towart, R. & Franckowiak, G. (1983) Nature, Lond. 303, 535-537.

CHLORAMPHENICOL SIMULTANEOUSLY PRODUCES ENDPLATE ION CHANNEL BLOCK AND SLOWS CHANNEL CLOSING RATE

C.Prior* , J.Dempster, F.Henderson & I.G.Marshall. Department of Physiology & Pharmacology, University of Strathclyde, Glasgow.

We have shown that, like other antibiotic compounds (Fiekers et al, 1979; 1983), the four diastereoisomers of chloramphenicol convert single exponential decays of endplate currents (EPCs) and miniature endplate currents (MEPCs) into two components, one faster and one slower than control. Experiments were performed on the voltage-clamped costocutaneous muscle of the garter snake. The drugs used were D- and L- threo and D- and L- erythro chloramphenicol (0.2 to 1.0mM). Concentration-dependent splitting of EPC decay, as shown by the four chloramphenicols, is usually explained in terms of block of open endplate ion channels. The kinetics of this type of block can be described by the sequential model (Ruff, 1977; Adler et al, 1978) in which the blocked channel momentarily reverts to the open state before closing or becoming reblocked. Kinetic analysis of both our EPC and MEPC data suggested that the chloramphenicols did not obey the predictions of this model. In particular, the slow phase of decay appeared to make an extraordinarily large contribution to overall current. Integration of EPCs and MEPCs indicated that the charge passed in the presence of the drugs was greater than in control currents. This increase was concentration-dependent but not voltage-dependent.

By an extension of Ruff's analysis (1977) we calculated channel closing rate in the presence and absence of drug. D-threo chloramphenicol (0.5mM at -90mV), for example, produced a 2.05 ± 0.13 (mean $\pm$ S.E., n=8) times decrease in closing rate during MEPCs. The decrease in closing rate was concentration-dependent and we suggest that this effect is due to the high lipid solubility of the chloramphenicols (log P = 1.14, Leo et al, 1971), leading to a change in dielectric constant within the membrane and hence a hindering of the voltage-dependent conformational change which results in channel closing.

Taking the decrease in closing rate into account leads to marked changes in the measured rate constants and affinity constant of the drugs. Failure to take account of, for example, a two-fold decrease in closing rate leads to a two-fold underestimate of unblocking rate constant and an approximately two fold overestimate of affinity.

Supported by grants from the Wellcome trust, Organon Ltd., M.R.C. We thank Warner Lambert-Parke Davis for the gift of drugs.

Adler, M. et al. (1978). Mol. Pharmac. 14, 514.
Fiekers, J.F. et al. (1979). Nature. 281, 680.
Fiekers, J.F. et al. (1983). J. Pharmac. exp. Ther. 227, 308.
Leo, A. et al. (1971). Chem. Rev. 71, 525.
Ruff, R.L. et al (1971). J. Physiol. 264, 89.

LACK OF ANTAGONISM BY TAURINE ON CHLORIDE CHANNEL BLOCK BY ANTHRACENE-9-CARBOXYLIC ACID IN RAT SKELETAL MUSCLE

S.H. Bryant, Diana Conte-Camerino*[1], Flavia Franconi[2], A. Giotti[2] and Mercede Mambrini[1]. Department of Pharmacology and Cell Biophisics, University of Cincinnati, USA. [1]Cattedra di Farmacologia, Dipartimento Farmacobiologico, Università di Bari, Italia. [2]Dipartimento di Farmacologia Preclinica e Clinica "M. Aiazzi Mancini", Università di Firenze, Italia.

A common feature of the action of taurine on excitable cells is to induce a hyperpolarization and a stabilization of the membranes (Oja and Kontro, 1983). In previous studies we gave evidence that the stabilizing action of taurine on mammalian skeletal muscle is due specifically to an increase of membrane chloride conductance (G_{Cl}) that in conseguence determines a reduction of fibers excitability (Conte-Camerino et al., 1983). In the present study experiments were designed to clarify the mechanisms of taurine action on membrane G_{Cl}. To this aim the possible antagonism between taurine and anthracene-9-carboxylic acid (9AC), a selective inhibitor of membrane G_{Cl} in mammalian muscle (Bryant and Morales-Aguilera, 1971), was studied on the electrical parameters of rat extensor digitorum longus (EDL) muscles, in vitro, with intracellular microelectrodes (Conte-Camerino et al., 1982).

In our experiments taurine was unable to antagonize the block of G_{Cl} induced by 9AC in EDL fibers (Table). So also the sulfonic aminoacid did not antagonize the increased excitability by 9AC in consequence of G_{Cl} block.

TREATMENT	N° of fibers	G_m ($\mu S/cm^2$)	N° of fibers	G_{Cl} ($\mu S/cm^2$)	G_{Cl} ($\mu S/cm^2$)
Controls	26	2320 ± 150	20	291 ± 61	2029 ± 120
TAU (60mM)	22	3643 ± 366	16	351 ± 88	3292 ± 284
TAU and 9AC (60mM and 5μM)	23	955 ± 101	15	315 ± 72	640 ± 91
9AC (5μM)	19	1190 ± 109	16	370 ± 95	820 ± 103

Values are mean ± s.e.m. from 4-5 muscles. For conductances calculation (G_m,G_K and G_{Cl}, respectively total, potassium and chloride membrane conductances), measurements were made in Cl^- containing and Cl^- free medium. (TAU for taurine).

It has been suggested that 9AC blocks membrane G_{Cl} by occluding the channels (Bryant and Morales-Aguilera, 1971). The lack of antagonism by taurine on 9AC shown in our experiments,lead us to hypotezize that the sulfonic aminoacid operates by affecting the gating of Cl^- channel and therefore is unable to show any effect when 9AC has blocked the channel.

Acknowledgements: This research was supported by Italian CNR grants 83.0037204 and 84.0230804.

Bryant, SH. & Morales-Aguilera, A. (1971) J. Physiol. (Lond.) 219, 367 - 383.
Conte-Camerino,D., Bryant,S.H. & Mitolo-Chieppa,D. (1982) Exp.Neurol.77, 1-11.
Conte-Camerino,D., Franconi,F., Mitolo-Chieppa,D. & Giotti,A. (1983) Acta Pharmac. Tox. 53/1, 109.
Oja,S.S. & Kontro,P. (1983) in "Handbook of Neurochemistry": Laitha,A. (ed.), vol. 3, pp; 501 - 533. Plenum Plubishing Corp..

THE EFFECTS OF TWO ERYTHRINA ALKALOIDS ON ENDPLATE CURRENTS

S.V.P. Jones*, A.L. Harvey & I.G. Marshall. Department of Physiology & Pharmacology, University of Strathclyde, Glasgow.

Neuromuscular blockade is usually associated with charged quaternary compounds, however two tertiary amines, beta-erythroidine and dihydro-beta-erythroidine have previously been shown to be nicotinic antagonists (Unna et al, 1944; Van Maanen, 1950). In this study we attempt to assess if mechanisms other than receptor block play a part in the neuromuscular blocking action of these compounds. The effects of the drugs have been tested on endplate current (EPC) amplitude and decay and on acetylcholine-induced current fluctuations in the voltage-clamped costocutaneous muscle of the garter snake.

Low concentrations of beta-erythroidine (10^{-5}M) and dihydro-beta-erythroidine (10^{-6}M) reduced EPC amplitudes without altering their time courses. Higher concentrations (5×10^{-5}M to 2×10^{-4}M beta-erythroidine and 5×10^{-6}M to 5×10^{-5}M dihydro-beta-erythroidine) reduced EPC amplitude by more than 75%. At these concentrations, the normally single exponential decay of the EPC was converted into two components, one faster and one slower than control decay. For both compounds the time constant of the slow phase of decay (T_s) was both voltage- and concentration-dependent, whereas that of the fast phase (T_f) was concentration- but not voltage-dependent. The results for beta-erythroidine are illustrated in Table 1.

Table 1. Effects of beta-erythroidine on EPC decay at -70 and -110mV

	T_f (msec)				T_s (msec)			
Conc.	-70mV		-110mV		-70mV		-110mV	
5x10-5M	0.73±0.03	(10)	0.71±0.04	(10)	3.91±0.29	(8)	8.35±0.84	(7)
2x10-4M	0.36±0.04	(6)	0.44±0.03	(4)	5.99±0.25	(6)	13.38±0.76	(4)
	mean±sem	(n)		(n)		(n)		(n)

The above results were supported by data obtained from noise analysis in which two time constants were obtained from a double Lorentzian fit. The results at high concentrations were interpreted in terms of the compounds blocking endplate ion channels. Calculated rate constants were shown to be independent of membrane voltage, indicating that the compounds act as uncharged molecules. Unblocking rate constants were 480±10 and 850±100 sec^{-1} (n=15); and blocking rate constants were 7.6±0.6 and 36.3±6.4 x $10^6 M^{-1} sec^{-1}$ (n=15) for beta-erythroidine and dihydro-beta-erythroidine respectively. We conclude that low concentrations of the erythroidines reduce EPC amplitude largely by receptor block, but that higher concentrations produce endplate ion channel block.

We thank Dr. R.C. Hider and Merck Sharp and Dohme Inc. for the supply of the erythroidines. SVPJ is an SERC postgraduate student.

Unna, K. et al. (1944). J. Pharmac. exp. Ther. 80, 39.
Van Maanen, E.F. (1950). J. Pharmac. exp. Ther. 99,255

588P

ELECTROPHYSIOLOGICAL ACTIONS OF FACILITATORY TOXINS FROM MAMBA VENOMS ON MAMMALIAN NEUROMUSCULAR JUNCTIONS

A.J. Anderson* & A.L. Harvey, Department of Physiology & Pharmacology, University of Strathclyde, Glasgow, G1 1XW.

The venoms of green and black mambas (Dendroaspis angusticeps and D. polylepis) contain toxins that facilitate the release of neurotransmitters at peripheral and central synapses (Harvey et al, 1984). The mechanism of action of the facilitatory toxins is unknown. We have investigated their effects on neuromuscular transmission by using intracellular recording from mouse phrenic nerve-hemidiaphragm preparations and by using focal extracellular recording from inside perineural sheaths of mouse triangularis sterni nerve-muscle preparations (Brigant & Mallart, 1982).

Dendrotoxin (1.4 - 5.6 μM) and toxin I (3.9 - 5.2 μM) produced two time-dependent effects on mouse diaphragm preparations at 37°C. In muscles paralysed by tubocurarine (2.3 - 3.5 μM) or by altering the Ca^{2+}:Mg^{2+} ratio (0.5 - 1.25 mMCa^{2+}; 1.6 - 2.2 mMMg^{2+}), the toxins increased the amplitude of endplate potentials (e.p.p.s.) by up to 10 fold. This effect could be detected within 5 min of adding the toxin. As the toxins did not alter the amplitude of miniature e.p.p.s., the duration of e.p.p.s. or m.e.p.p.s., or the input resistance of the muscle fibres, the increase in e.p.p. amplitude is presumably due to an increase in the number of quanta released by each nerve impulse.

After 25 to 60 min exposure to the toxins, single stimulation of the phrenic nerve resulted in 2 or 3 e.p.p.s. At this time, spontaneously occurring e.p.p.s. could also be recorded, especially in solutions with reduced Ca^{2+} concentrations. Both the repetitive and the spontaneous firing of e.p.p.s. were abolished by tetrodotoxin (6.3 - 15.6 nM).

Several approaches were taken in attempts to determine the site of action of dendrotoxin on motor nerve terminals. The toxin did not enhance the increase in frequency of m.e.p.p.s. produced by the Ca^{2+} ionophore A23187 (20 μM), suggesting that it did not directly stimulate Ca^{2+}-mediated release processes. Also, dendrotoxin had no effect on transmitter release induced by KCl (10-20 mM), indicating that it was not influencing depolarisation-activated Ca^{2+} channels. Extracellular recording of pre-synaptic currents from motor nerve terminals revealed that 60 min exposure to dendrotoxin (5.6 μM) produced multiple firing of nerve terminal action potentials in response to single nerve impulses. There were no major changes in the individual ionic currents, and repetitive firing was still induced by dendrotoxin after the main K^+ current had been blocked by 3,4-diamino-pyridine (100 μM).

The mechanism by which the facilitatory toxins increase motor nerve excitability and transmitter release remains to be established. It is possible that the toxins act directly on Na^+ channels or on a type of K^+ channel that is responsible for preventing repetitive firing. Blockade of a transient K^+ current by dendrotoxin has recently been demonstrated to occur in some neural cell bodies (Dolly et al 1984).

This work was supported by a grant from the Wellcome Trust. We thank Dr. A. Mallart for his help with the extracellular recording experiments.

Brigant, J.L. & Mallart, A. (1982) J. Physiol. (Lond.) 333, 619
Dolly, J.O. et al (1984) J. Physiol. (Paris) 79, 280
Harvey, A.L. et al (1984) Toxin Reviews 3, 91

LEUPEPTIN DOES NOT BLOCK THE INDUCTION OF LONG-LASTING POTENTIATION IN HIPPOCAMPAL CA_1 NEURONES

B. R. Sastry, Neuroscience Research Laboratory, Department of Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, B. C., V6T 1W5, Canada.

The long-lasting potentiation (LLP) of synaptic transmission, that occurs in neurones in the CA_1 area of the hippocampus after a brief tetanic stimulation of an afferent input, has been suggested to be due to a Ca^{2+} activated effect of calpain I on fodrin, leading to (1) the uncovering of glutamate receptors and (2) a structural change in the shape of CA_1 cell dendrites (Burns, 1985, Lynch and Baudry, 1984, Siman et al., 1985). Leupeptin (acetyl-L-leucyl-L-leucyl-L-argininal), which inhibits the activity of enzymes like calpain I, was shown to counteract the effect of Ca^{2+} to enhance the number of glutamate binding sites (presumed to be receptors) in hippocampal membranes (Baudry et al., 1981). In the present investigation on transversely sectioned rat hippocampal slices, I have examined whether leupeptin can interfere with the development of LLP.

In controls, the population spike recorded from the cell body area of CA_1 neurones induced by the stimulation of stratum radiatum was potentiated following a 400 Hz tetanus (200 pulses). If the tetanus was given during the last min of a 10 min application of leupeptin hemisulphate (50 or 100 μM) LLP could still be induced. The results are summarized in table 1 below.

Table 1. Effects of leupeptin on CA_1 population spike.

Drug conc.	n	Population spike as a % of predrug control	
		During drug applic.	30 min after 400 Hz tetanus
0.0 μM	7	100	243.0 ± 56.00
50 μM	5	87.0 ± 7.86	300.4 ± 17.69
100 μM	5	65.2 ± 4.21	348.4 ± 13.38

The effects of intracellular injection of leupeptin in CA_1 neurones was also examined on the induction of LLP. The amount of current required in stimulating the stratum radiatum to elicit an action potential in the CA_1 neurone in 50 % of attempts was taken as the 'threshold'. LLP is associated with a reduction of this 'threshold'. Leupeptin was injected by applying square wave pulses (200 ms, 0.1 nA, 2 Hz) for 5 min and during the last 1 min the test tetanus was applied in the stratum radiatum. Ten min after the tetanus, the 'threshold' was reduced to 64.30 ± 2.19% of the control (n = 6). In controls (no leupeptin), the corresponding value was 66.65 ± 3.86% (n = 6). There was, therefore, no significant difference in LLP.

These results indicate that the effects of leupeptin in CA_1 neurones do not lead to an interference with the induction of LLP.

This work was supported by the Medical Research Council of Canada.

Baudry, M. et al. (1981) Science 212, 937-938.
Burns, N. R. (1985) Nature 313, 178-179.
Lynch, G. and Baudry, M. (1984) Science 224, 1057-1063.
Siman, R. et al. (1985) Nature 313, 225-228.

590P

ANOMALOUS "DEPRESSANT" ACTIVITY OF TRAZODONE AND RELATED HALOGENATED PHENYLPIPERAZINES IN A PUTATIVE MODEL OF DEPRESSION

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

The clinical efficacy of the antidepressant trazodone (Gershon, 1981) may partly reside in its major metabolite, m-chlorophenylpiperazine (Caccia et al, 1981). Unlike other atypical antidepressants, however, trazodone is inactive in a putative model of depression in rats (Porsolt, 1981). Using a semi-automated Porsolt test method we have investigated the effect on mice of treatment with trazodone, m-chlorophenylpiperazine and other halogenated phenylpiperazines.

Male CD1 mice (20-24g; Charles River) were orally administered either the test compound or deionised water 1h prior to their mobility being measured by a Doppler recording system during a 4 min period in the Porsolt test. Compounds were dissolved in deionised water and where necessary neutralised to pH 7 prior to oral treatment over at least a 1.0-100 mg/kg dose range.

Trazodone reduced the mobility of mice, an effect potently elicited by its metabolite m-chlorophenylpiperazine (Table 1). However, p-chlorophenylpiperazine and p-fluorophenylpiperazine did not decrease mobility and at the highest dose (100 mg/kg) both increased it in a manner similar to antidepressants (cf. imipramine; Table 1). Compared to trazodone and m-chlorophenylpiperazine, relatively few pharmacological properties have been reported for the latter two compounds, making it of interest that the 5-hydroxytryptamine agonist 1-(m-trifluoromethylphenyl)piperazine also reduced the mobiltiy of mice (Table 1). Only the high doses of trazodone induced overt sedation prior to the Porsolt test.

Table 1: Effect of trazodone, halogenated phenylpiperazines, imipramine and trifluoperazine on the mobility of mice in the Porsolt test.

Treatment	Dose (mg/kg p.o., 1h previously): percentage change in mobility compared to vehicle treated controls.			
Trazodone	100: -22	30: -35**	10: -8	3: -18
m-ChloroPP	10: -38**	3: -34**	1: -46**	0.3: -15
p-ChloroPP	100: +40**	30: -1	10: -10	3: -15
p-FluoroPP	100: +57*	30: 0	10: -31	3: -12
m-TrifluoroPP	100: -27*	30: -47*	10: -20	3: -2
Imipramine	100: +121**	30: +55*	10: +18	3: +8
Trifluoperazine	10: -38*	3: -60**	1: -24	0.3: -14

PP: phenylpiperazine. n = 10-15 mice/treatment.
*p<0.05; **p<0.01 vs vehicle treated controls (Student's t-test).

Therefore trazodone and pharmacologically active m-halogenated phenylpiperazines reduced the mobility of mice in the Porsolt test which may be interpreted as a "depressant" action in contrast to the antidepressant response of mice to imipramine. This anomalous "depressant" response may be due to the known action of these compounds on central 5-hydroxytryptamine mechanisms. However an interaction with cerebral dopamine systems cannot be excluded as neuroleptics are the only other class of drugs to induce a similar depressant response in this test system (cf. trifluoperazine; Table 1).

Caccia, S. et al (1981) J. Pharm. Pharmac. 33, 477
Gershon, S. (1981) Royal Society of Medicine: International Congress and Symposium Series. No. 46, p49
Porsolt, R.D. (1981) in "Antidepressants : Neurochemical, Behavioural and Clinical Perspectives". Ed. S.J. Enna et al. Raven Press. p.121.

A COMPARISON OF THE EFFECTS OF NICOTINE AND DIAZEPAM ON RAT BEHAVIOUR IN AN ELEVATED X-MAZE

D.J.K. Balfour* & C.A. Graham, Department of Pharmacology and Clinical Pharmacology, University Medical School, Ninewells Hospital, Dundee, DD1 9SY.

There is evidence to suggest that nicotine dependence may be related to its ability to attenuate behavioural responses to aversive stimuli (Balfour, 1982). In the present study an elevated x-maze, incorporating open platforms as an aversive stimulus, has been used to compare the effects of nicotine with those of a conventional anxiolytic, diazepam.

Male Sprague-Dawley rats, weighing approximately 250 g, were pretreated with daily subcutaneous injections of nicotine (0.4 mg/kg) or saline or intragastric injections of diazepam (25 mg/kg) or vehicle (40 percent (v/v) propylene glycol in water) for 6 days. On day 7 the nicotine-treated rats and half the saline-treated rats were given nicotine, the remainder of the saline-treated animals being given saline as before. Three minutes after the injection each rat was placed in the centre of a symmetrical x-maze, raised 1 m from the laboratory floor and composed of two enclosed runways (43 cm x 9 cm with 9 cm sides) and two open runways (43 cm x 9 cm with 3 cm sides) and the number of entries into each runway was recorded for 20 minutes. The diazepam-treated rats and half the vehicle-treated rats were given diazepam on day 7, the remainder being given vehicle. Thirty minutes after the injections their activity in the x-maze was recorded for 20 minutes. All the rats were killed at the end of the test session and blood samples taken for the estimation of plasma corticosterone (Mattingly, 1962).

Acute injections of nicotine and diazepam increased plasma corticosterone ($P < 0.05$) from 22 $\pm$ 4 μg/100 ml to 36 $\pm$ 5 μg/100 ml ($n = 7$) and from 25 $\pm$ 2 μg/100 ml to 32 $\pm$ 2 μg/100 ml ($n = 5$) respectively and decreased the total number of entries made although only the effect of diazepam, a reduction from 35 $\pm$ 2 to 16 $\pm$ 2 entries per 20 minutes, reached statistical significance ($P < 0.01$). The administration of diazepam to rats pretreated with the drug reduced plasma corticosterone ($P < 0.05$) to 11 $\pm$ 5 μg/100 ml ($n = 5$) and increased ($P < 0.05$) total activity to 44 $\pm$ 3 entries/20 min. Injections of nicotine to rats pretreated with nicotine also increased ($P < 0.01$) total entries from 35 $\pm$ 3 to 57 $\pm$ 5 entries/20 min ($n = 7$) although in these rats, plasma corticosterone levels were not significantly different to those found in saline-treated rats. For the trial as a whole neither diazepam nor nicotine had any significant effects on the ratio of open:closed runway entries. However, pretreatment with diazepam increased ($P < 0.05$) the ratio from 0.69 $\pm$ 0.13 to 0.96 $\pm$ 0.09 during the first 12 minutes of the trial whereas it decreased ($P < 0.05$) the ratio from 0.95 $\pm$ 0.26 to 0.32 $\pm$ 0.11 during the last 8 minutes. These changes were not observed with nicotine. It is concluded that the effects of nicotine in this apparatus cannot be attributed to an anxiolytic property of the drug.

This study was supported by a grant from the Medical Research Council.

Balfour, D.J.K. (1982) Pharmac. Ther., 15: 239-250.
Mattingly, D. (1962) J. Clin. Path., 15: 375-379.

PROCONVULSANT ACTION OF Ro 15-3505, THE 7-CHLORO ANALOGUE OF Ro 15-1788, ON ISONIAZID CONVULSIONS IN RATS

L. Pieri*, P. Biry and G. Wdonwicki (introduced by J.G. Richards), Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel, Switzerland.

The selective benzodiazepine antagonist Ro 15-1788 is devoid of any proconvulsant activity in mice and rats both p.o. and i.v. (Bonetti et al., 1982 ; Pieri & Biry, 1985). Its 7-chloro analogue, Ro 15-3505 (Figure 1), is 5 to 15 times more potent as a benzodiazepine antagonist than Ro 15-1788 in animals and in man. In contrast to Ro 15-1788, Ro 15-3505 shows a slight proconvulsant effect on pentylenetetrazol-induced seizures in mice (Haefely, 1983). Therefore we have studied the effect of Ro 15-3505 on isoniazid-induced clonic-tonic convulsions in rats, following the protocols already used with β-CCE (ethyl-β-carboline-3-carboxylate) and Ro 15-1788 (Pieri & Biry, 1985).

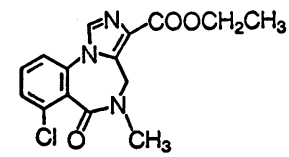

Figure 1 Ro 15-3505

Male SPF-Fü albino rats were injected s.c. with different doses of isoniazid, followed 15 min later by 1 mg/kg Ro 15-3505 i.v. Ro 15-3505 produced a statistically significant ($p < 0.01$) shift to the left of the isoniazid dose-response curve for clonic-tonic convulsions ; the shift was about the same as that previously observed with 10 mg/kg β-CCE i.v. (Pieri & Biry, 1985). Additionally, we studied the effect of different doses of Ro 15-3505, given either i.v. or orally 15 min after a dose of isoniazid (320 mg/kg s.c.) eliciting by itself clonic-tonic convulsions in 10-20 % of the rats (Table 1). The rats were observed for 2 h after isoniazid. Statistical significance ($p < 0.001$) (*) was calculated by Chi^2 test.

Table 1 Number of rats with clonic-tonic convulsions

Vehicle i.v.	Ro 15-3505 i.v. (mg/kg)	Vehicle p.o.	Ro 15-3505 p.o. (mg/kg)
1/20	0.01 : 4/20 (n.s.)	2/20	0.3 : 7/20 (n.s.)
1/20	0.03 : 13/20 (*)	4/20	3.0 : 19/20 (*)
1/20	0.1 : 16/20 (*)	3/20	10.0 : 18/20 (*)
1/20	0.3 : 17/20 (*)		

The proconvulsant effect of Ro 15-3505 (10 times more potent by the i.v. route) seemed to be not strictly dose-dependent and reached a submaximal plateau ; this was also found in mice using other convulsive challenges (Pieri et al., unpublished observations). Ro 15-3505 by itself, up to the highest dose tested, had no noticeable effect in our rats.

The marked proconvulsant effect of 3 mg/kg Ro 15-3505 i.v. was dose-dependently antagonized by Ro 15-1788 (10,30 mg/kg p.o.) given either before or after Ro 15-3505.

The inverse agonistic component of Ro 15-3505 is probably, on the whole, weak ; for instance this compound was unable up to high doses to counteract some effects of meprobamate or phenobarbitone in mice (Pieri et al., unpublished observations). Preliminary human volunteer studies indicated the appearance of a transient spike-and wave activity in the EEG and possibly the prevention of day-time somnolence (Haefely, 1983). Ro 15-3505 is an interesting pharmacological tool, differing from Ro 15-1788 by the absence of an agonistic component ; its therapeutic potential is being investigated.

Bonetti, E.P. et al. (1982) Psychopharmacology 78, 8-18.
Haefely, W. (1983) In Benzodiazepine Recognition Site Ligands : Biochemistry and Pharmacology (Eds. G. Biggio & E. Costa), 73-93, Raven Press, New York.
Pieri, L. and Biry, P. (1985) Eur.J.Pharmacol., in press.

$GABA_A$ AND BENZODIAZEPINE BINDING SITES IN THE CORTEX OF DEPRESSED SUICIDE VICTIMS

S.C. Cheetham*[1], M.R. Crompton[2], C.L.E. Katona[3], R.W. Horton[1], S.J. Parker[1] & G.P. Reynolds[4]. Departments of Pharmacology[1], Forensic Medicine[2] and Psychiatry[3], St. George's Hospital Medical School, London SW17 ORE and Department of Pathology[4], Queen's Medical Centre, Nottingham, NG7 2UH.

Repeat administration of antidepressant drugs to animals induces changes in the numbers of monoamine receptors. GABA receptors are also influenced by such treatment e.g. increases in $GABA_B$ and decreases in $GABA_A$ and benzodiazepine (BZ) receptors have recently been reported (Pilc & Lloyd, 1984; Suzdak & Gianutsos, 1985; Suranyi-Cadotte et al, 1984). Thus changes in GABA receptors may be involved in the mechanism of antidepressant actions and in the neurochemical disturbances underlying affective disorders.

In this study BZ and $GABA_A$ binding sites have been quantitated in frontal (Brodman area 10) and temporal (Brodman area 38) cortical brain regions of a group of suicide victims (8 M, 3 F, mean age ± s.e.m. 40.5±3.0 years) and a control group (8 M, 3F, 42.9±3.2 years) dying suddenly of non-neurological causes. The suicide victims studied included only those in whom a firm diagnosis of depression could be made; subjects in whom the diagnosis was unclear or there was a history of schizophrenia, epilepsy, alcoholism or drug abuse were excluded.

^3H Flunitrazepam binding (8 concentrations, 0.05-15nM) was performed on well-washed membrane preparations. $GABA_A$ binding sites were determined by the ability of GABA (8 concentrations 0.1-500μM) to increase BZ binding. The results are presented as maximal % stimulation over baseline (max stim) and the concentration of GABA producing half maximal stimulation (EC_{50}). Assays were performed blind on coded samples.

Table 1 $GABA_A$ binding sites in frontal cortex of suicides and controls.

	K_D	Bmax	EC_{50}	Max stim
Controls (n=11)	1.73±0.12	1802±101	1.54±0.44	76.5±5.7
Depressed Suicides (n=11)	1.66±0.09	2138±107*	1.53±0.21	70.2±4.2

Values are means ± s.e.m.
K_D = nM Bmax = f moles/mg protein EC_{50} = μM * P<0.05 (Students t test).

The number of BZ binding sites was significantly greater (19%), but the K_D unaltered, in the frontal cortex of the suicide victims compared to controls (Table 1). There was no difference in either Bmax or K_D in the temporal cortex (not shown). EC_{50} and maximal stimulation of BZ binding by GABA did not differ between suicides and controls.

Three of the suicide subjects had BZs present in their blood at post-mortem. After excluding these, the increase in the number of BZ binding sites in the suicide group was similar (17.5%) but now failed to reach statistical significance (n=8).

The present results suggest that a more extensive study of BZ binding in depressed suicide victims is desirable.

We thank the Wellcome Trust for financial support. S.C.C. is an M.R.C. scholar.

Pilc, A. & Lloyd, K.G. (1984) Life Sci. 35, 2149-2154
Suranyi-Cadotte, B.E. et al (1984) Eur. J. Pharmac. 106, 673-675
Suzdak, P.D. & Gianutsos, G. (1985) Neuropharmac. 24, 217-222.

THE EFFECTS OF THE 5-HT$_{1A}$ LIGAND, 8-OH-DPAT, APPLIED IONTOPHORETICALLY TO RAT BRAINSTEM NEURONES

M. Davies* and M.H.T. Roberts, Department of Physiology, University College, Cardiff. CF1 1XL

8-Hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) is a centrally active 5HT agonist (Hjorth et al, 1982) which is highly selective for the 5HT 1A binding site in the rat brain (Middlemiss and Fozard 1983). Its effects upon cells excited by 5HT are not known. 8-OH-DPAT and 5HT were applied microiontophoretically to spontaneously active neurones in the medullary brainstem of the rat anaesthetised with halothane. Recording sites were marked by ejection of pontamine sky blue dye. Both compounds were predominantly depressant and as shown by the table, many of the cells excited by 5HT were depressed by 8-OH-DPAT. The converse was not observed.

Table 1

		5HT +	5HT -
8-OH-DPAT	+	5	0
	-	7	21

The drugs were always applied with similar iontophoretic currents to a cell and it was observed that 8-OH-DPAT caused smaller excitatory responses than 5HT but larger depressant responses than 5HT. Depressant responses to 8-OH-DPAT were often very prolonged, continuing for up to 12 min following a 50 nA, 1 min application whereas depressant responses to 5HT rarely exceeded 3 min duration. These responses were not associated with reduction in spike amplitude nor changes in spike duration.

It may be concluded that the putative 5HT 1A ligand 8-OH-DPAT tends to depress cells which respond to 5HT in the rat brainstem although occasionally excitatory responses occur. It has been reported previously (Davies et al, 1985) that the excitation of cells by 5HT may be blocked selectively by intravenous application of the selective 5HT 2 antagonists ketanserin (300 ug/kg) or methysergide (1 mg/kg) but depressant responses are resistant to these doses. One explanation of these observations is that the excitation of cells is due to the action of 5HT on a receptor resembling the 5HT 2 binding site and depressant responses to an action upon a 5HT 1A-like receptor.

M.D. is an SERC scholar.

Davies, M. et al (1985) Br. J. Pharmac. 85, 255P
Hjorth, S. et al (1982) J. Neural. Transm. 55, 169-188
Middlemiss, D.N. and Fozard J.R. (1983) Eur. J. Pharmacol. 90, 151-153

AN ANALYSIS OF THE CARDIOVASCULAR EFFECTS OF 5-HYDROXYTRYPTAMINE IN CONSCIOUS DOCA-SALT HYPERTENSIVE RATS

D.W. Dalton*, W. Feniuk and P.P.A. Humphrey, Department of Cardiovascular Pharmacology, Glaxo Group Research Ltd, Ware, Herts. SG12 ODJ.

Intravenous administration of 5-hydroxytryptamine (5-HT) produces variable and complex effects on blood pressure and heart rate in the cardiovascular system, due to its interaction with different types of 5-HT receptor (Kalkman et al., 1984; Lawang and Saxena, 1985). We have further characterised the 5-HT receptors involved in conscious DOCA-salt hypertensive rats where we have found that the classical triphasic effect of 5-HT on blood pressure to be more pronounced.

Male rats, CD Charles River, were made hypertensive by unilateral nephrectomy and treatment with DOCA (40 mg/kg s.c. twice weekly for 3 weeks). In these rats (mean diastolic blood pressure 137 $\pm$ 4 mmHg and heart rate 389 $\pm$ 9 b/min, n=22) weighing 280-320g, drugs were administered intravenously (i.v.) into a cannulated jugular vein and arterial blood pressure was measured directly from a cannulated carotid artery. Heart rate was derived using an instantaneous ratemeter. All values shown are mean $\pm$ s.e.m.

5-Hydroxytyptamine (3 and 10 µg i.v. n=6) predominantly produced dose-related decreases in blood pressure (43 $\pm$ 11 and 99 $\pm$ 12 mmHg) and profound bradycardia (163 $\pm$ 50 and 304 $\pm$ 45 b/min) which resulted from activation of the Bezold-Jarisch reflex (Fozard, 1984). In separate animals (n=6), these effects were mimicked by similar doses of the M-receptor agonist, 2-methyl 5-HT and were greatly attenuated by the M-receptor antagonist MDL 72222 (0.03 and 0.1 mg/kg i.v.) (Fozard, 1984; Donatsch et al., 1984). After MDL 72222 (0.3 mg/kg i.v.), 5-HT 1-30 µg, now produced dose-related increases (15 $\pm$ 5 to 42 $\pm$ 7 mmHg) followed by decreases in blood pressure (8 $\pm$ 2 to 25 $\pm$ 4 mmHg) and tachycardia. The pressor responses could be mimicked by the 5-HT_2 receptor agonist, α-methyl 5-HT, (Feniuk et al., 1981) at 3-30 µg i.v., n=6. The selective 5-HT_2 receptor antagonist ketanserin (0.3 mg/kg i.v.) antagonised the pressor effects of α-methyl 5-HT and abolished the pressor component of the biphasic response of 5-HT in animals already treated with MDL 72222. In these animals, 5-HT now produced dose-related depressor responses (24 $\pm$ 3 to 45 $\pm$ 6 mmHg) and tachycardia which could be blocked by subsequent treatment with methiothepin 1 mg/kg i.v. The selective 5-HT agonist, 5-carboxamidotryptamine (5-CT) (Feniuk et al., 1984) at doses of 0.1-3 µg i.v., n=4, produced only dose-related depressor responses and tachycardia, which were blocked by methiothepin 0.3 and 1 mg/kg i.v. Treatment with all 5-HT antagonists did not affect the depressor responses produced by isoprenaline (0.01 and 0.03 µg/kg i.v).

This study indicates that the complex cardiovascular actions of 5-HT in conscious rats involves stimulation of at least three different 5-HT receptors. The initial bradycardia involves activation of "M-like" 5-HT receptors since it can be selectively stimulated by 2-methyl 5-HT and antagonised by MDL 72222. The secondary vasopressor effect appears to involve stimulation of 5-HT_2 receptor as the response can be mimicked by α-methyl 5-HT and antagonised by ketanserin. Finally, the vasodepressor response appears due to vasodilation via "5-HT_1-like" receptors, since this effect of 5-HT is selectively mimicked by 5-CT and antagonised by methiothepin (metitepine), two ligands with high affinity for 5-HT_1 binding sites in the brain (see Engel et al., 1983; Leysen et al., 1982).

Donatsch et al. (1984). Br. J. Pharmac. Proc. Suppl., 81, 33P.
Engel, G. et al. (1983). N.S. Arch. Pharmac., 324, 116.
Fozard, J.R. (1984). N.S. Arch. Pharmac. 326, 36.
Feniuk, W. et al. (1981). Br. J. Pharmac. 73, 191P.
Feniuk, W. et al. (1984). Br. J. Pharmac. 82, 209P.
Kalkman, H. et al. (1984). J. Hypertension, 2 (suppl. 3), 143.
Lawang, A. and Saxena, P.R. (1985). Br. J. Pharmac., Proc. Suppl., 85, 316P.
Leysen, J. et al. (1982). Molec. Pharmac. 21, 301-314.

5-HYDROXYTRYPTAMINE CAUSES TACHYCARDIA IN PIGS BY ACTING ON RECEPTORS UNRELATED TO 5-HT_1-, 5-HT_2- OR M-TYPE

D.J. Duncker, P.R. Saxena & P.D. Verdouw
Depts. Pharmacol. & Cardiol., Erasmus University, Rotterdam, The Netherlands.

5-Hydroxytryptamine (5-HT) can either decrease or increase heart rate. Though bradycardia, via Bezold-Jarisch reflex, is mediated by M-type 5-HT receptors (Fozard, 1984), tachycardic response may involve 5-HT_1 (in cats; Saxena *et al.*, 1985), 5-HT_2 (in rats; Saxena & Lawang, 1985), or M (in rabbits; Fozard, 1984) receptors. Because of this species difference we studied the nature of heart rate responses in 18 pentobarbital anaesthetized pigs.

The baseline values of heart rate and mean blood pressure were 81±3 beats.min^{-1} and 80±3 mmHg, respectively. Intravenous administration of 5-HT (3, 10 and 30 $\mu g.kg^{-1}$) elicited a dose dependent tachycardia (Table 1). Bradycardia was not observed and blood pressure showed moderate biphasic responses with the three doses of 5-HT; pressor effect, 1±1, 7±2 and 14±3 mmHg; depressor effect, -16±5, -8±1 and -10±2 mmHg, respectively.

Table 1: Effect of various antagonists on the tachycardic response (beats.min^{-1}) to 5-HT in the pig

Antagonist (affected receptor type)	Dose mg. kg^{-1}	n	Tachycardia (beats.min^{-1}) by 5-HT ($\mu g.kg^{-1}$) 3 Before	3 After	10 Before	10 After	30 Before	30 After
Phentolamine (α_1,α_2)	1.0	5	26±4	24±6	42±4	38±6	58±5	51±12
Propranolol (β_1,β_2)	0.5	5	24±4	27±4	43±3	43±4	60±4	60±3
Mepyramine (H_1)	1.0	4	31±5	35±3	45±7	47±7	55±7	53±5
Cimetidine (H_2)	1.0	4	38±5	33±3	48±5	47±4	58±5	56±6
Verapamil (Ca^{2+} channel)	0.1Ψ	3	23±4	23±2	44±2	34±7	58±2	45±5
Methiothepin (5-HT_1,5-HT_2)	0.5	6	22±3	21±4	40±5	39±4	53±5	53±3
Methysergide (5-HT_1,5-HT_2)	0.5	6	26±3	28±3	50±4	49±3	63±5	63±3
Metergoline (5-HT_1,5-HT_2)	0.5	5	29±2	22±3	49±3	46±6	63±4	63±6
Mesulergine (5-HT_1,5-HT_2)	0.3	3	25±1	26±1	40±2	45±2	52±4	56±6
Ketanserin (α_1,5-HT_2)	0.5	4	20±4	18±4	42±8	41±7	58±7	57±7
Cyproheptadine (5-HT_2)	0.5	5	17±3	23±3*	40±3	45±5*	54±3	60±4*
Pizotifen (5-HT_2)	0.5	5	29±2	26±2	49±3	45±2	61±5	56±4
Mianserin (5-HT_2)	0.5	5	24±2	23±2	45±1	44±3	59±3	54±4
ICS 205-930 (M)	0.3	3	29±3	21±3	43±4	41±4	54±6	56±0.3
MDL 72222 (M)	0.3	4	28±4	22±5*	45±4	45±5	60±5	58±6

*Ψ, bolus injection followed by infusion of 0.01 mg.kg^{-1}.min^{-1}; *, Significantly different from the corresponding response before injection of the antagonist. Several antagonists were used in a given experiment, but each drug (except verapamil) was at least once given first.*

As shown in Table 1 none of the drugs, which included antagonists of both adrenoceptors, both histamine receptors, Ca^{2+} channels and the three 5-HT receptors, blocked tachycardic responses to 5-HT. It is therefore concluded that 5-HT elicits tachycardia in the pig via a new receptor-type which is different from the 3-types (5-HT_1, 5-HT_2 and M) characterized so far.

FOZARD, J.R. (1984). *N.S. Arch. Pharmacol.*, 326, 36-44.
SAXENA, P.R. & LAWANG, A. (1985). *Arch. Int. Pharmacodyn.*, In press.
SAXENA, P.R. *et. al.* (1985). *N.S. Arch. Pharmacol.*, 330, 121-129.

AMPLIFICATION BY 5-HT OF CONTRACTIONS INDUCED WITH NORADRENALINE IN THE ISOLATED KIDNEY OF THE RAT IS INCREASED WITH AGE

W.J. Janssens* and J.M. Van Nueten, Department of Pharmacodynamics, Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Isolated kidneys of 2 and 6-month old Wistar rats (W.R.) and spontaneously hypertensive rats (S.H.R.) were perfused with tyrode solution of the following millimolar composition: KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.04; $NaHCO_3$ 11.9; NaH_2PO_4 0.42; NaCl 136.9; glucose 11.1; Ca EDTA 0.026. The solution was gassed with a 95 % O_2 - 5 % CO_2 mixture and kept at 37° C (Collis and Vanhoutte, 1977). Flow was set at 6 ml/min. In kidneys taken from both strains at both ages, serotonin (infused to reach a final concentration of 1.4×10^{-8} - 2.3×10^{-7} M) amplified vasoconstrictor responses (measured as increases of perfusion pressure) to noradrenaline (injected as a bolus of 0.062 or 0.25 µg). This amplification was larger in the 6-month old animals than in the 2-month old:

Increase in perfusion pressure (mm Hg):	W.R. 2 months	W.R. 6 months	S.H.R. 2 months	S.H.R. 6 months
Noradrenaline control	20.2 ± 3.4	29.6 ± 5.4	29.9 ± 4.9	26.2 ± 5.0
Noradrenaline in presence of serotonin	34.2 ± 3.8[a]	60.9 ± 8.9[a]	45.0 ± 5.7[a]	52.6 ± 8.6[a]
Amplification	14.2 ± 2.3	31.7 ± 5.6[b]	13.4 ± 2.7	26.3 ± 3.9[b]

[a] Increase in perfusion pressure significantly larger than in control conditions.
[b] Amplification significantly larger than in 2-month old animals.

The younger animals became rapidly tachyphylactic to this amplifying effect of serotonin which could not be repeated a second time. Tachyphylaxis was much less pronounced in the older animals:

Amplification	W.R. 2 months	W.R. 6 months	S.H.R. 2 months	S.H.R. 6 months
1st response mmHg	11.4 ± 2.5[a]	29.3 ± 11.4[a]	13.8 ± 5.0[a]	30.2 ± 6.8[a]
2nd response mmHg	3.3 ± 3.3[b]	12.2 ± 4.5[a,b]	4.0 ± 5.1[b]	16.7 ± 5.3[a,b]

[a] Significant amplification.
[b] Second amplification significantly smaller than first amplification.

The 5-HT_2 serotonergic antagonist ketanserin inhibited the amplifying effect at a concentration (6.3×10^{-9} M) which did not (S.H.R.) or only slightly (30 % inhibition W.R.) affect the response to noradrenaline alone.

These experiments indicate that in the perfused kidney of the rat the amplifying effect of serotonin becomes more pronounced at older age, and is mediated by 5-HT_2 serotonergic receptors. Possibly the difference between young and old animals can be explained by decreased tachyphylaxis in the older rats.

Collis, M.G. and Vanhoutte, P.M. (1977) Circ. Res. 41: 759-767.

EFFECTS OF RITANSERIN AND CINANSERIN ON THE CARDIOVASCULAR SYSTEM OF THE CAT

A.G. Ramage, Department of Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill Street, Hampstead, London, NW3 2PF.

The lack of selectivity of antagonists for 5-HT_2 receptors makes it difficult to identify a role for these receptors in cardiovascular control. Recently, a reputedly highly selective 5-HT_2 antagonist, LY 53857 (Cohen et al, 1983), has been shown to increase femoral arterial conductance and central sympathetic tone without affecting blood pressure (Ramage, 1985). To investigate whether any of these actions could be attributed to 5-HT_2 blockade alone, the effects of two other 5-HT_2 antagonists, ritanserin (Janssen, 1985) and cinanserin (Rubin et al, 1964), have been studied on the cardiovascular system of the cat.

Cats were anaesthetised with α-chloralose (70 mg kg^{-1}) and pentobarbitone sodium (12 mg). Recordings of brachial arterial pressure, heart rate, femoral arterial conductance and preganglionic sympathetic nerve activity were made simultaneously as described previously (Ramage, 1984). Skeletal muscle paralysis was produced by decamethonium (0.25 mg kg^{-1}). A cumulative dose response curve (0.03 - 3 mg kg^{-1}) was produced for each drug with injections given into the jugular vein every 10 min. Control injections of vehicle, DMSO (n=5) for ritanserin and saline (n=5) for cinanserin, were also carried out in separate experiments.

Ritanserin (n=5) and cinanserin (n=5) caused dose related increases in femoral arterial conductance reaching maxima of 61±11 and 55±12 (x 10^{-3}) ml $mmHg^{-1}$ min^{-1} respectively at 3 mg kg^{-1}. These increases in femoral arterial conductance were significantly ($p < 0.05$) different from control values over the dose range 0.1 - 3mg kg^{-1}. For cinanserin, the increase in femoral arterial conductance was not associated with any significant change in blood pressure, heart rate or preganglionic sympathetic nerve activity. However, the highest dose (3 mg kg^{-1}) of cinanserin caused a transient fall in mean blood pressure of 26±5 mmHg which was associated with an increase in preganglionic sympathetic nerve activity and a slight rise in heart rate. For ritanserin, the increase in femoral arterial conductance produced by high doses (1 and 3 mg kg^{-1}) was associated with significant decreases in mean blood pressure of 10±2 and 24±6 mmHg respectively. These later falls in blood pressure did not cause the expected increases in preganglionic sympathetic nerve activity and heart rate. In fact, at a dosage of 3 mg kg^{-1} ritanserin caused a significant fall in heart rate and a decline in preganglionic sympathetic nerve activity. This indicates that ritanserin causes inhibition of central sympathetic tone when given in high doses.

The different effects of high doses of these antagonists probably reflects their loss of selectivity. The ability of low doses of these drugs to cause an increase in femoral arterial conductance like that of LY 53857, suggests that 5-HT_2 receptors may play a role in controlling skeletal muscle blood flow. The failure of ritanserin and cinanserin to cause sympathoexcitation suggests that this action of LY 53857 is not attributable to 5-HT_2 receptor blockade.

I wish to thank Mr. S. Wilkinson for technical assistance and Janssen Pharmaceutical Ltd. for support.

Cohen, M.L. et al (1983)	J. Pharmac.Exp.Ther. 227, 327-332
Janssen, P.A.J. (1985)	J. Cardiovasc.Pharmac. 7, Suppl 7. S2-S11
Ramage, A.G.(1984)	Neuropharmac. 23, 43-48
Ramage, A.G.(1985)	Eur.J.Pharmac. 113, 295-303
Rubin, B. et al (1964)	Arch.Int.Pharmacodyn.Ther. 152, 132-143

HEART RATE RESPONSES TO SELECTIVE AGONISTS OF 5-HYDROXYTRYPTAMINE$_1$ RECEPTORS IN THE SPINAL CAT

P.R. Saxena
Department of Pharmacology, Erasmus University, Rotterdam, The Netherlands.

We recently reported that 5-hydroxytryptamine (5-HT) causes tachycardia in the spinal cat via 5-HT$_1$-like receptors since its response was antagonized by methysergide but not by ketanserin or cyproheptadine (Saxena *et al.*, 1985). Furthermore, 5-carboxamidotryptamine (5-CT), a compound with high selectivity for 5-HT$_1$-like receptors (Feniuk *et al.*, 1981; Engel *et al.*, 1983; Saxena & Lawang, 1985), has similar effects as 5-HT (Connor *et al.*, 1985; Saxena *et al.*, 1985). In this investigation the effects of 5-CT and three other 5-HT$_1$ agonists, BEA 1654 (Verdouw *et al*, 1985), 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT) and 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1H indole succinate (RU 24969), have been compared on heart rate in pentobarbital anaesthetized spinal cats.

The results presented in Table 1 show that 5-CT was above 1000 fold more potent and considerably more effective than BEA 1654 in increasing heart rate; RU 24969 and 8-OH-DPAT were not much effective. Methiothepin (0.5 mg.kg^{-1}), but not ketanserin (0.5 mg.kg^{-1}), antagonized the responses to both BEA 1654 and 5-CT

Table 1. Effect of 5-HT$_1$ agonists on heart rate (beats.min^{-1}) in spinal cats

Compound	Pretretment	Dose (mg.kg^{-1})				
		0.1	0.3	1.0	3.0	10.0
8-OH-DPAT	-	0.5±0.5	2±1	9±3	22	-
RU 24969	-	0±0	3±1	8±3	8	-
BEA 1654	-	4±1	11±4	25±5	36±6	49±5
BEA 1654	Ketanserin	5±1	9±2	25±6	40±6	53±5
BEA 1654	Methiothepin	0±0*	0±0*	0.5±0.5*	5±2*	21±8*
5-CT$^{\Psi}$	-	26±4	50±4	63±4	68±4	70±5
5-CT$^{\Psi}$	Ketanserin	33±2	55±8	65±11	69±9	67±10
5-CT$^{\Psi}$	Methiothepin	0±0*	0±0*	0±5*	2±1*	7±4*

*n=4-7, except where no is given, n=1. *, Significantly different from the respective value without pretreatment. Ψ, Doses in μg.kg^{-1}.*

suggesting thereby that these drugs act via 5-HT$_1$-like receptors. However, agonist potencies in eliciting functional effects did not correlate with the affinities (Middlemiss, unpublished) for either the 5-HT$_{1A}$ or 5-HT$_{1B}$ binding subsites. It is, therefore, concluded that the 5-HT$_1$ receptor in feline heart is either unrelated to these subsites, or receptor occupation by these agonists (except 5-CT) is not effectively transducted into functional response.

CONNOR, H.E. (1985). *Br. J. Pharmacol.*, 85, 222P.
ENGEL, G. (1983). *N.S. Arch. Pharmacol.*, 324, 116-124.
FENIUK, W. *et al.* (1981). *Br. J. Pharmacol.*, 73, 191P-192P.
SAXENA, P.R. & LAWANG, A. (1985). *Arch. Int. Pharmacodyn.*, In press.
SAXENA, P.R. *et. al.* (1985). *N.S. Arch. Pharmacol.*, 330, 121-129.
VERDOUW, P.D. *et al.* (1985). *Eur. J. Pharmacol.*, 107, 337-346.

600P

SODIUM ALTERS THE KINETICS ASSOCIATED WITH NEURONAL UPTAKE OF 5-HYDROXYTRYPTAMINE

C.M. Brown, A.D. Dye, K. Goodwin, A.T. Kilpatrick*, and R.L. Whiting, Department of Pharmacology, Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS.

The tricyclic antidepressant imipramine (IMI), a potent inhibitor of 5-hydroxytryptamine (5HT) uptake, binds with high affinity to specific sites in brain (Langer & Raisman, 1983). In rat brain tricyclic antidepressants show classical competitive inhibition of IMI binding whereas non-tricyclic inhibitors of 5HT uptake and 5HT itself inhibit in a complex manner (Sette et al, 1983). This has led to the suggestion that IMI modulates 5HT uptake through an allosteric mechanism (Langer & Raisman, 1983). In contrast, Briley & Moret (1985), suggested that complex inhibition of IMI binding occurred due to the existance of two [^{3}H]-IMI binding sites; a sodium-dependent and sodium-independent site. This study examined the effect of sodium on the kinetics of 5HT uptake to gain further insight into the molecular relationship that exists between IMI and the 5HT uptake pump.

Isolation of crude synaptosomes (P_2) from the corpus striatum of 27 female Dunkin Hartley guinea-pigs and the subsequent uptake of 5HT was carried out as described by Wood & Wyllie (1983). Experiments were carried out at various sodium concentrations, over the 5HT range 60-200 nmol.litre^{-1}, in the presence or absence of 50 nmol.litre^{-1} IMI. Each determination was carried out in triplicate and each value represents the mean ± S.E. of 3 experiments.

Kinetic analysis indicated that the affinity constant (Ku) of the uptake system, its transport capacity (Vu) and the IMI inhibitory constant (Ki) were dependent upon the concentration of sodium (see Table 1).

Table 1 Effect of sodium on 5HT uptake kinetics

Sodium (mmol.litre^{-1})	Kinetic Constants Vu (pmol.min^{-1} mg. protein^{-1})	Ku (nmol.litre^{-1})	$-pK_i$
130	3.67 ± 0.4	137.8 ± 17.5	7.55 ± 0.06
100	2.50 ± 0.2*	59.4 ± 6.2**	7.76 ± 0.06*
65	2.11 ± 0.4*	74.7 ± 4.1**	7.56 ± 0.04

Statistical significance: * $p < 0.05$ relative to 130 mmol.litre^{-1} constant
** $p < 0.02$ relative to 130 mmol.litre^{-1} constant

In the absence of IMI, 5HT uptake at 100 mmol.litre^{-1} sodium was consistant with a double displacement reaction, indicating a requirement for sodium. Kinetic analysis indicated that competitive inhibition by IMI occurred at each sodium concentration. The high affinity of IMI and the sodium dependence displayed by 5HT uptake, suggests that the 5HT uptake pump represents the sodium-dependent, high affinity IMI site observed in binding studies.

Briley, M. & Moret, C. (1985) Br.J.Pharmac. (Cardiff meeting), in press.
Langer, S.Z. & Raisman, R. (1983) Neuropharmacology, 22, 407-413.
Sette, et al (1983) J.Neurochemistry, 40, 622-628.
Wood, M. & Wyllie, M.G. (1983) Naunyn-Schmiedeberg's Arch.Pharmacol., 322, 129-135.

8-OH-DPAT INHIBITS TRANSMITTER RELEASE FROM GUINEA-PIG ENTERIC CHOLINERGIC NEURONES BY ACTIVATING 5-HT_{1A} RECEPTORS

J.R. Fozard[1*] and H. Kilbinger[2], [1]Merrell-Dow Research Institute, 16 rue d'Ankara, 67084 Strasbourg-Cedex, France and [2]Department of Pharmacology, University of Mainz, Obere Zahlbacher Str. 67, D-6500 Mainz, F.R.G.

8-Hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) is a putative central 5-HT receptor agonist (Hjorth et al. 1982) which shows selectivity for the 5-HT_{1A} subtype of the 5-HT_1 recognition site (Middlemiss and Fozard, 1983). In this report we demonstrate that 8-OH-DPAT inhibits electrically evoked transmitter release from the enteric cholinergic neurones of guinea-pig ileum and provide evidence that the response is mediated by the putative 5-HT_{1A} receptor.

Proximal ileum was removed from guinea-pigs of either sex weighing 250-400g. Whole ileum preparations were set up in Tyrode's solution and tension changes recorded isometrically. Transmural stimulation (2ms ; supramaximal voltage) was applied at 0.05Hz. Longitudinal muscle-myenteric plexus preparations were incubated with ^{3}H-choline and tension changes and the outflow of tritium in response to field stimulation (0.1Hz) measured as previously described (Kilbinger and Pfeuffer-Friederich, 1985).

8-OH-DPAT, 0.005-20.5 µmol/l, caused concentration-dependent inhibition of the contraction of transmurally stimulated whole ileum. The concentration-response curve was biphasic with a clear inflexion at approximately 1.28 µmol/l. The shallow, first phase of the curve reached a maximum inhibition of 35-40% ; the steeper, second phase reached complete inhibition associated with a relaxation of the basal tone of the ileum at 20.5 µmol/l. Responses to carbachol and histamine (in each case, 0.01-3.2 µmol/l) were unaffected by 8-OH-DPAT at concentrations up to 1.28 µmol/l. The first (but not the second) phase of the concentration-response curve to 8-OH-DPAT was shifted to the right in the presence of metergoline, 0.1 µmol/l (apparent pA_2 = 7.71), methiothepin 0.03 µmol/l (7.58), (-)-pindolol, 1 µmol/l (7.20), spiperone, 0.5 µmol/l (7.58) and buspirone, 0.5 µmol/l (7.39). (+)-Pindolol, 1 µmol/l, ketanserin, 1 µmol/l, MDL 72222, 1 µmol/l, prazosin, 0.01 µmol/l and idazoxan, 0.5 µmol/l were inactive.

In the longitudinal muscle-myenteric plexus preparation, 8-OH-DPAT, 0.01-1 µmol/l, did not change the spontaneous outflow of tritium but caused inhibition of tritium outflow evoked by electrical field stimulation. The EC50 was 0.02 µmol/l and a maximum inhibition of 31% was obtained. The response to 8-OH-DPAT was not affected by tolazoline, 3 µmol/l, but was abolished in the presence of buspirone, 1 µmol/l, or methiothepin 0.1 µmol/l.

Thus, low concentrations of 8-OH-DPAT cause inhibition of stimulation-evoked transmitter release from the enteric cholinergic neurones of the guinea-pig ileum. Blockade of the response by metergoline or methiothepin suggests the involvement of a 5-HT receptor ; the stereoselectivity of action of pindolol and antagonism by both spiperone and buspirone implicates the putative 5-HT_{1A} receptor in the response.

Hjorth, S. et al. (1982) J.Neural.Trans. 55, 169-188.
Kilbinger, H. & Pfeuffer-Friederich, I. (1985) Br.J.Pharmac. in press.
Middlemiss, D.N. & Fozard, J.R. (1983) Eur.J.Pharmac. 90, 151-153.

602P

THE EFFECT OF NICARDIPINE, A CALCIUM ENTRY BLOCKER, ON PARACETAMOL INDUCED INJURY IN MICE

A. CHITRANUKROH, P. COOK and I. JAMES. Academic Department of Medicine, Royal Free Hospital, London NW3 2QG

Current treatment of paracetamol overdose by the administration of sulfhydryl nucleophiles such as N-acetylcysteine and cysteamine is only effective when given early, that is before maximal hepatic gluthathione depletion or covalent binding of the toxic metabolite to vital cellular macromolecules occur (Mitchell et al., 1974). These occur at approximately 1-2 hours post paracetamol in the mouse and 12-15 hours in man. We have investigated a possible late treatment of paracetamol overdose. This treatment is based on the assumption that high cytosolic concentration of calcium plays a major role in the sequence of events leading to hepatocyte injury and death (Farber, 1984). It is preposed that the accumulation of calcium ions in the cytosol occurs as a consequence of and subsequent to the binding of toxic metabolite of paracetamol to vital macromolecules. Thus, treatment which prevents the accumulation of calcium should also prevent or reduce hepatocellular injury. We tested the effect of nicardipine, a calcium channel blocker, on paracetamol induced hepatic injury in mice.

Female TO white mice were injected i.p. with 500mg/Kg paracetamol. It was estimated that this dose would produce hepatic gluthathione depletion by 1 h after dosing (Mitchell et al, 1974). Nicardipine was given i.p. at 1,2,3, or 4 h post-paracetamol (PP). Plasma samples were obtained 8 h PP, and GPT concentrations were determined.

A single bolus of 50 mg/Kg nicardipine at 1 h PP reduced GPT concentrations, 1590±903 U/l (n=8) compared with 4185±1297 U/l for controls (n=6) (2 tailed t test, $p < 0.1$). This dose of nicardipine was toxic when given 2 h PP. At a dose of 25 mg/Kg at 2 h PP, nicardipine was not toxic but it had no effect on GPT concentrations. A reduction was observed when nicardipine was given as a priming dose of 25 mg/Kg at 2 h PP followed by a booster dose of 5 mg/Kg two hours later; 2503±664 U/l (n=7) in tests and 5590±642 in controls (n=7) ($p < 0.05$). When the booster dose was increased to 15 mg/Kg, nicardipine was effective in reducing plasma concentrations even when the priming dose was given 4 h PP; 1777±607 U/l (n=9) in tests and 3910±1123 in controls (n=9) ($p < 0.1$). There was an overall trend towards reduction in mortality which was only significant after the 50mg/Kg dose of nicardipine given at 1 h (Fishers exact test, p=0.014).

The results suggest that the administration of calcium channel blocker nicardipine may be an effective late treatment of paracetamol overdose in man.

MITCHELL, J.R. et al. (1974) Clin. Pharmac. Ther., 16, 676.

FARBER, J.L. (1984) Falk Symposium, 38, 111.

EFFECTS OF THE NEW β_2-AGONIST BROXATEROL ON β RECEPTOR-MEDIATED LIPOLYSIS AND PULMONARY SURFACTANT RELEASE IN ISOLATED CELL SYSTEMS

A. Carenzi, D. Della Bella, A. Reggiani and R. Sala *
Zambon Farmaceutici Research Laboratories, Via Lillo Del Duca, 10 - 20091 Bresso (Milan) - Italy

Broxaterol (2-tert-butylamino-1-(3-bromo-isoxazol-5-yl)-ethanol-hydrochloride) is a new β_2 sympathomimetic drug which has been discovered and developed by our Research Laboratories. Its greater selectivity for bronchial than for cardiac β adrenoreceptors was previously shown by receptor binding studies and "in vitro" pharmacological experiments on isolated guinea pig atrium and tracheal chain.

To further investigate the β adrenoreceptor selectivity of Broxaterol, we tested its effects on β receptor-mediated lipolysis in rat isolated adipocytes and on β-mediated surfactant release using cultured A 549 cells.

Lipolysis in rat epididymal isolated adipocytes was stimulated by the agonist isoproterenol (IPNA) $>$ noradrenaline (NE) $\simeq$ adrenaline (EPI). Salbutamol was a thousand times less potent than isoproterenol, but it was able to induce a full lipolytic response. In contrast, Broxaterol was in the same range of potency of salbutamol, but displaied a lower intrinsic activity.

It has been suggested that pulmonary surfactant release from type II alveolar cells depends upon stimulation of β_2 receptors '(Hollingworth & Gilfillan, 1984)'. In the recent years a tumoral cell line, called A 549, derived from human alveolar cells, has been characterized as an "in vitro" model to study surfactant release '(Nardole & Andrews, 1979)'. Here we show that the order of potency of IPNA, EPI and NE in stimulating surfactant release fit the hypothesis of a β_2 receptor mediated event. Salbutamol and Broxaterol were equally effective in causing such a release. Furthermore, both compounds showed a full agonist action as IPNA and EPI did, whereas NE was about 50% as effective.

The results obtained in cultured A 549 cells coupled to those obtained in previous pharmacological studies, indicate that Broxaterol is a very potent and selective β_2 agonist.

Moreover, if β receptor-stimulated lipolysis in rat adipocytes is actually mediated by β_1 receptors, as suggested by many authors '(Bojanic & Nahorski, 1983; Cubero & Malbon, 1984)' the pattern of Broxaterol activity on this experimental model indicates that this compound is very weakly effective in stimulating β_1 adrenergic receptors.

Binding studies in rat heart membrane preparations have shown that Broxaterol has a good affinity for β_1 receptors. The results reported here are in agreement with those obtained on isolated guinea pig atrial preparations and indicate that this compound has very low β_1 intrinsic activity.

Bojanic, D., Nahorski, S.R., (1983) Eur. J. Pharmac. 93, pp 235-243.
Cubero, A., Malbon, C.C, (1984) J. Biol. Chem. 259 (2), pp 1344-1350.
Hollingworth, M., Gilfillan, A.M., (1984) Pharmacol. Rev. 36 (2), pp 69-90.
Nardole, L.L., Andrews S.B., (1979) Biochim. Biophys. Acta 573, pp 276-295.

MODULATION OF GUINEA-PIG TRACHEAL β-ADRENOCEPTOR FUNCTION BY PULMONARY MACROPHAGES

F. Engels*, R.S. Oosting, and F.P. Nijkamp,
Institute for Veterinary Pharmacology, Pharmacy, and Toxicology, State University of Utrecht, P.O. Box 80176, 3508 TD Utrecht, The Netherlands.

Bacterial infections of deeper respiratory airways may be a precipitating factor in exacerbations of chronic asthmatic bronchitis. The gram-negative bacterium Haemophilus influenzae can often be isolated from the deeper respiratory tract of bronchial asthmatics. H. influenzae causes, when administered intraperitoneally to guinea pigs, an imbalance between bronchoconstrictive (cholinergic) and bronchodilatory (beta-adrenergic) receptor systems in the lung (Schreurs et al., 1980, 1983). This effect may be responsible for the symptomatic airway narrowing in bronchial asthmatics. Recently we showed that serum-stimulated pulmonary macrophages (PM) induce a down-regulation of guinea pig tracheal beta-adrenoceptor function (Engels et al., 1985). Here we present evidence that PM, stimulated in various ways, may cause detrimental as well as beneficial effects on tracheal beta-adrenergic receptor function.

In in vitro experiments we evaluated the influence of PM on guinea pig tracheal beta-adrenergic receptor function as follows: PM, isolated through lung lavage in situ, were allowed to adhere to an isolated tracheal spiral from a non-treated animal. PM were stimulated by incubation with serum (2% v/v, 60 min. at 37°C), collected from animals that had received killed, non-capsulated H. influenzae bacteria (10^8 organisms/100 g body weight, i.p.) 4 days beforehand. Where applicable, inhibitors were added 30 min. before serum-stimulation. Tracheal beta-adrenergic receptor function was assessed by an initial precontraction of the tracheal preparation with 10^{-6} M carbachol, after which a cumulative dose response curve to the relaxant isoprenaline was constructed, using an isotonic smooth muscle transducer.

Stimulation of PM from control animals with serum resulted in a significant reduction of tracheal relaxation (-31±5%, mean ± S.E.M., $p<0.05$ vs. non-stimulated PM, ANOVA). Surprisingly, under similar stimulation, PM from H. influenzae-treated animals caused a potentiation of tracheal relaxation. Similar experiments were performed in which PM were stimulated by addition of opsonized H. influenzae bacteria. After induction of phagocytosis with the particulate stimulus, control PM caused a decrease of tracheal beta-adrenoceptor function, as was observed with serum-stimulation. PM from H. influenzae-treated animals also induced a down-regulation of tracheal relaxation this time, as opposed to the potentiation seen after serum-stimulation.
As we have suggested before (Engels et al., 1985), impairment of tracheal beta-adrenoceptor function may be caused by the reactive hydroxyl radical liberated from PM. We do not yet know how up-regulation of beta-adrenoceptors is accomplished. Further research will be required to pinpoint the PM as the cell type responsible for the detrimental effects of bacteria on lung beta-adrenergic receptor function and to clarify the phenomena of potentiation of beta-adrenoceptor function.
(This study was financially supported by the Dutch Asthma Foundation).

Engels, F. et al (1985) Eur.J.Pharmacol. 111, 143
Schreurs, A.J.M. et al (1980) J.Pharmacol.Exp.Ther. 215, 691
Schreurs, A.J.M. et al (1983) Eur.J.Pharmacol. 87, 127

THE EFFECTS OF SEX STEROIDS UPON MUSCARINIC BINDING IN THE RAT BRAIN

Muna I.Al-Dahan & Patrick J.Thomas, Department of Pharmacology, Chelsea College, Manresa Road, London, SW3 6LX.

The number of [^{3}H] scopolamine binding sites in the hypothalamus and amygdala (but not elsewhere in the brain) are modified by hormonal status in adult rats. In females there is a reverse relationship between the numbers of sites and oestrogen and progesterone levels. Binding is high in metoestrous and in ovariectomised animals. It is low in proestrous (when oestrogen levels are high). Hormone replacement therapy in ovariectomised animals lowers the number of binding sites. Oestrogens and progesterone can act both synergistically or independently (Table 1).

In contrast, castration of males reduces the numbers of binding sites and treatment with testosterone increases them to levels observed in the intact (Table 1).

Our results with oestrogens contrast with those of Rainbow et al (1980) who showed that the binding of [^{3}H] QNB was enhanced by oestrogen treatment but are in agreement with, and extend, those of Solovisky et al (1981). Our results with testosterone indicate that it opposes the actions of 'female' sex steroids upon muscarinic binding.

Table 1. Saturation binding capacity (n mols/g prot) ± S.E.M.

	Male			Female		
	State	Hyp	Amyg	State	Hyp	Amyg
Low hormone	CAST	0.32±0.04	0.4±0.4	Met	0.83±0.03	1.43±0.04
		-	-	ovx	1.25±0.02	1.51±0.01
High hormone	CAST+T	0.52±0.12	0.81±0.01	PRo	0.46±0.07	0.75±0.05
		-	-	ovx E_2	0.77±0.09	0.95±0.04
		-	-	ovx E_2P	0.81±0.15	0.52±0.07
		-	-	ovx P	-	0.6 ±0.02

CAST = Castrated males, ovx = ovariectomised female
Met = metoestrus, PRo = proestrus, E_2 = 17β
P = progesterone T = testosterone Hyp = hypothalamus

Values refer to binding in the P_2 fraction expressed as a function of protein in the initial homogenate. Binding was measured by the method of Birdsall et al (1978) with minor modifications.

Birdsall, N.M., Burgen, A.S.V. and Hulne, E.C. (1978) Molec.Pharmacol. 14, 723-726.
Rainbow, C.T., Degroff, V., Luine, V.N. and McEwen, B.S. (1980) Brain Research 198, 239-243.
Solovisky, M., Egozi, Y. and Avissars (1981) Proc.Natl.Acad.Sci., 78, 5554-5558.

606P

CHRONIC CAFFEINE TREATMENT REDUCES CAFFEINE BUT NOT ADENOSINE EFFECTS ON CORTICAL ACETYLCHOLINE RELEASE

L.BARTOLINI, R.CORRADETTI, F.PEDATA, G.PEPEU & M.G.VANNUCCHI Department of Pharmacology, University of Florence, Viale Morgagni 65, 50134 Florence, Italy.

Caffeine at concentration of 50 uM increases acetylcholine (ACh) release from electrically stimulated cortical slices and depresses ACh release at concentration of 500 uM. Both effects are antagonized by adenosine derivatives (Pedata, Pepeu & Spignoli, 1984) Chronic treatment with methylxanthines has been shown to up-regulate cortical adenosine receptors (Murray, 1982) and induce some degree of tolerance (Robertson, Wade, Workman, Woosley & Oates,1981). We investigated if chronic treatment with caffeine modifies its bifasic effects on ACh release.

Male Wistar rats, 180 g initial body weight, received 100 mg/Kg caffeine in their drinking water for 30 days. No difference in body weight and mortality was found at the end of treatment. An increase in spontaneous motility was seen on day 3 but was not present on day 30. N^6-cyclohexyl ^3H adenosine (New England Nuclear, spac.act. 13.5 Ci/mmol) specific binding to cortical membranes was measured (Murray,1982). In the rats receiving caffeine a 33% increase in Bmax of the high affinity binding sites was detected. Kd of the high affinity and Kd and Bmax of the low affinity binding sites were not affected.

Cortical slices were prepared, electrically stimulated and superfused with caffeine and adenosine (Pedata et al.,1984). The basal release was 6.3 ± 0.2 $ng.g^{-1}.min^{-1}$ at rest, 10.2 ± 1.5 at 0.2, 37 ± 4.4 at 1 and 85.0 8 10.7 at 5 Hz stimulation frequency. The % changes in ACh release are shown below.

Table 1 Per cent changes in acetylcholine release induced by caffeine and adenosine in rats chronically treated with caffeine.

Controls	caffeine	50 uM	+42 ± 4.8*	+124 ± 27*	+43 ± 4.3*	+31 ± 3*
	caffeine	500 uM	+ 9 ± 0.6	- 47 ± 7*	-35 ± 6.0*	-32 ± 3*
	adenosine	30 uM	-36 ± 4*	- 37 ± 4*	-61 ± 8.4*	-66 ± 3*
Caffeine	caffeine	50 uM	- 3 ± 0.3	+ 3 ± 0.5	-13 ± 0.9	-10 ± 1
30 days	caffeine	500 uM	- 7 ± 0.5	+ 13 ± 2	+ 7 ± 0.6	+17 ± 1
	adenosine	30 uM	-28 + 1.7*	- 38 ± 1*	-45 ± 0.1*	-47 ± 9*

* Statistically significant difference from no drug, P<0.01 (Student's two tailed t test); each point is the mean ± s.e. of 4 - 8 rats.

As shown in table 1, the stimulatory and inhibitory effects of caffeine were abolished in chronically treated rats while adenosine effect was still present.

Investigation supported by a grant from the Ministero P.I.

Murray,T.F. European J. Pharmac. (1982) 82, 113 - 114.
Pedata,F., Pepeu,G. & Spignoli,G. Br. J. Pharmac. (1984) 83, 69 - 73
Robertson,D., Wade,D., Workman,R., Woosley,R.L. & Oates,J.A. J. Clin. Invest. (1981) 67, 1111 - 1117.