IN VITRO CHARACTERISATION OF THE β_2-ADRENOCEPTOR AGONIST, SALMETEROL

D.I. Ball, R.A. Coleman*, L.H. Denyer, A.T. Nials and K.E. Sheldrick, Department of Respiratory Pharmacology & Biochemistry, Glaxo Group Research Ltd., Ware, Herts.

Salmeterol (4-hydroxy-α[[[6-[4-(phenylbutyl)oxy]-hexyl]amino]methyl]-1,3-benzene dimethanol) has been shown to be a potent β_2-adrenoceptor agonist with a long duration of action in the guinea-pig isolated trachea (Bradshaw et al., 1987). In the present study, we have evaluated further the in vitro pharmacology of this compound.

Salmeterol was compared with isoprenaline, salbutamol and clenbuterol for potency and duration of action on the superfused, electrically field-stimulated, guinea-pig isolated trachea preparation (ESGPT, Coleman & Nials, 1986). Infusion of all four agonists caused concentration-related relaxation of ESGPT. The rank order of potency (mean EC_{50} (95% confidence limits)) was: salmeterol (4.2 (1.6-10.9)nM, n=14) > clenbuterol (9.6 (3.7-24.8)nM, n=6) > isoprenaline (20(13-29)nM, n=4) > salbutamol (25 (11-55)nM, n=8). On stopping agonist infusions, the time to 50% recovery from an EC_{50} concentration (Rt_{50}) was measured. Thus, the Rt_{50} values were: isoprenaline (range) = 1-3min (n=4), salbutamol (mean (95% confidence limits)) = 11 (8-15) min (n=8) and clenbuterol = 45 (29-68) min (n=6). However, despite continuous superfusion, little or no recovery of field-stimulated contractile responses was observed with salmeterol over the times examined (7-10h), resulting in Rt_{50} values of >420 min (n=14). These data are similar to those previously obtained in preparations contracted with prostaglandin (PG)$F_{2\alpha}$ although the long recovery times could not be quantified (Bradshaw et al. 1987).

The sustained activity of salmeterol on ESGPT was rapidly and fully reversed by propranolol (0.1μM). Furthermore, in organ bath experiments, the pA_2 value determined for propranolol against salmeterol on $PGF_{2\alpha}$-contracted GPT by the method of Arunlakshana & Schild (1959) was 8.97(8.12-9.81) with a slope of 1.02 (0.57-1.47) (n=11). On β_1-adrenoceptor-containing tissues, such as the rat driven left atrium preparation, salmeterol was >4000-fold weaker than isoprenaline as a positive inotropic agent (n=4). The highest concentration tested (30μM), which resulted in only 18% of the isoprenaline maximum response, caused a <4-fold rightward shift of subsequent curves to isoprenaline. Thus, salmeterol has very low affinity for β_1-adrenoceptors.

In summary, salmeterol appears to exhibit potent, selective, β_2-adrenoceptor agonist activity, with a remarkably long duration of action in vitro. Further data regarding the profile of agonist activity of salmeterol in vitro and in vivo are reported elsewhere (Butchers et al., 1987; Ball et al., 1987; Maconochie et al., 1987).

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INTERACTION BETWEEN ADENOSINE RECEPTORS AND β-ADRENOCEPTORS IN AIRWAYS

I. Kioumis*, B. Grandordy, P.J. Barnes, Department of Clinical Pharmacology, Cardiothoracic Institute, Brompton Hospital, London SW3 6HP.

The effects of adenosine on airway smooth muscle responses are variable and little is known about the role of adenosine receptors in regulating airway smooth muscle responses. Since the effects of adenosine are mediated by stimulation or inhibition of adenylate cyclase, we have studied the action of adenosine on cyclic AMP generation in guinea-pig trachea. Adenosine is also reported to have an anti-adrenergic effect in some tissues (Hosey et al, 1984), so we have also investigated the relationship between beta-adrenoceptors and adenosine receptors in guinea-pig airway and lung.

Isoprenaline caused a dose-dependent increase in cyclic AMP content of guinea-pig trachea (61.8 ± 28.% increase at 10-5M), whereas adenosine (in the presence of indomethacin 10-5M) caused a dose-dependent decrease (42.4 ± 16.2% decrease at 10-4M). Adenosine 10-4M (in the presence of dipyridamole 10-5M) inhibited the rise in cAMP due to isoprenaline 10-5M by 85.45 ± 31%. The effect of adenosine was blocked by 8-phenyltheophylline (10-4M), suggesting that it was mediated by adenosine receptors. (-)-N6-phenylisopropyl-adenosine (PIA) was more potent than 5'N-ethylcarboxamide adenosine (NECA), indicating an A1-receptor. The effect of adenosine on pulmonary beta-adrenoceptors was also investigated. Incubation of lung cell suspensions with adenosine (10-4M) had no affect on density or affinity of beta-receptors, as determined by [125I]iodocyanopindolol binding to lung membranes. Prior incubation with isoprenaline (10-5M for 60 min) caused a 22.0 ± 6.8% (n = 12) reduction in maximum binding (Bmax). Incubation with isoprenaline and adenosine (10-4M) gave a significantly greater reduction in Bmax (30.9 ± 4.8%; $p < 0.01$, n=12), and this effect of adenosine was blocked by theophylline (10-3M). PIA was more potent than NECA, indicating that this effect was also mediated via an A1-receptor.

These results suggest that adenosine inhibits adenylate cyclase in guinea-pig trachea via an A1-receptor and thereby counteracts the effect of beta-agonists. A1-receptor activation also enhances the down-regulation of beta-adrenoceptors in guinea-pig lung, which is consistent with functional studies showing that adenosine increases beta-receptor tachyphylaxis in guinea-pig trachea in vitro (Matran et al, 1987). These studies may be relevant to human asthma, since endogenous adenosine generated by airway inflammation and hypoxia may modulate airway beta-receptor function.

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NEUROPEPTIDE Y MODULATES CHOLINERGIC NERVES IN THE GUINEA-PIG TRACHEA

C.D. STRETTON* & P.J. BARNES, Department of Clinical Pharmacology, Cardiothoracic Institute, Brompton Hospital, London, SW3 6HP.

Neuropeptide Y (NPY), is a 36 amino acid peptide localised to blood vessels and airway smooth muscle within the guinea-pig trachea (Sheppard, et al., 1984). The distribution of NPY-like immunoreactivity appears to be similar to that of dopamine beta-hydroxylase, suggesting localisation to adrenergic nerve fibres. We have studied the effect of NPY on guinea-pig tracheal smooth muscle, and, since adrenergic nerves may modulate cholinergic neurotransmission in trachea, we have also determined the effect of NPY on bronchoconstriction due to electrical field stimulation (EFS) of cholinergic nerves and to exogenous acetylcholine (ACh). Since NPY also interacts with adrenergic mechanisms (Lundberg and Stjarke, 1984), we have also studied the effect of adrenergic blockade on NPY responses.

Guinea-pig tracheas were dissected into transverse strips and mounted in 10ml organ baths containing Krebs-Henseleit solution at 37°C, bubbled with 95% O2/5% CO2, pH 7.4. Concentration-response relationships to ACh (10^{-8}-10^{-1}M) (n=7) and responses to EFS (40V, 0.5ms, 0.5-64 Hz) (n=7) were obtained both in the absence and in the presence of NPY (10^{-10}-3×10^{-7}M). The effects of propranolol and/or yohimbine (2×10^{-6}M) (n=7), 6-hydroxydopamine (10^{-3}M, incubation for 1h to deplete adrenergic nerves; n=8) and bretylium tosylate (5×10^{-5}M, incubation for 40min to prevent the release of noradrenaline; n=9) on responses to EFS in the absence and in the presence of 3×10^{-7}M NPY were also investigated.

NPY had no direct effect on airway smooth muscle contraction, and did not significantly alter the response to ACh. NPY produced both a concentration- and a frequency-dependent inhibition of the cholinergic component to EFS (IC50 = 1.03 ± 0.114 x 10^{-8}M at 0.5Hz, n=7). The presence of either propranolol, yohimbine or 6-OH-DA failed to significantly alter the responses to EFS in the presence of 3×10^{-7}M NPY. Bretylium, however, produced a significant enhancement of the inhibitory effect of NPY on EFS at high frequencies. In the absence of NPY, suitable time controls performed in the presence of 6-OH-DA (n=6) and bretylium (n=5) were not significantly different from controls obtained in the absence of any drug (n=8). By contrast, noradrenaline inhibition of cholinergic nerve induced contraction was significantly blocked by yohimbine, as previously reported (Grundstrom and Andersson, 1981).

In conclusion, NPY appears to reduce the cholinergic component of EFS via a prejunctional mechanism, since there is no effect on comparable contractions due to exogenous ACh. Neither depletion of noradrenaline by 6-OH-DA, adrenergic neuronal blockade by bretylium nor alpha- and beta-adrenoceptor blockade by yohimbine and propranolol, respectively, prevented this effect of NPY, suggesting that NPY acts directly on receptors on cholinergic nerve terminals, rather than by an interaction with adrenergic mechanisms. NPY released by adrenergic nerves may therefore modulate cholinergic neurotransmission in guinea-pig airways.

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THE EFFECTS OF PHI AND NPY ON MUCUS SECRETION FROM THE FERRET IN-VITRO TRACHEA

S.E. Webber*, M. Breen & J.G. Widdicombe, Department of Physiology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.

Peptide histidine isoleucine (PHI) has a relaxant action on airway smooth muscle resembling that of vasoactive intestinal peptide (VIP), and may co-exist with VIP and acetylcholine (ACh) in cholinergic nerves to the airways (Lundberg et al., 1984). Neuropeptide tyrosine (NPY) is present in the mammalian respiratory tract and has a distribution similar to that of sympathetic nerves (Sheppard et al., 1984). Tracheal submucosal glands in the ferret are innervated by both cholinergic and adrenergic nerves; however the effects of PHI and NPY on mucus secretion from these glands have not been examined. We have used the ferret in-vitro whole tracheal preparation (Kyle et al., 1986) to investigate the effects of PHI and NPY on the mucus secretion and lysozyme output (as an index of serous cell secretion) produced by methacholine and phenylephrine.

Methacholine (20 μM) and phenylephrine (100 μM) produced maintained secretions of 0.48 ± 0.04 ($n = 24$) and 0.53 ± 0.07 $\mu g.\mu l^{-1}$ ($n = 28$) respectively. PHI (1-100 nM) produced a dose-dependent inhibition of the maintained methacholine-induced secretion (-20 ro -43%) but had no significant effect on that due to phenylephrine, NPY (1-100 nM) produced a dose-dependent increase in the maintained secretions due to methacholine (8 to 25%) and phenylephrine (6 to 16%).

The rates of output of lysozyme during maintained methacholine- and phenylephrine-induced secretions were 170 ± 23 ($n = 24$) and 169 ± 10 $ng.min^{-1}$ ($n = 28$) respectively, and the concentrations of lysozyme in the mucus were 0.44 ± 0.04 and 0.34 ± 0.02 $\mu g.\mu l^{-1}$ respectively. PHI (1-100 nM) had no significant ($p > 0.05$) effect on the output of lysozyme during maintained methacholine-induced secretion, but dose-dependently increased the lysozyme concentration (12 to 64%). The output and concentration of lysozyme in mucus from maintained phenylephrine-induced secretion was not significantly altered ($p > 0.05$) by PHI (1-100 nM). NPY (1-100 nM) produced a dose-dependent inhibition of the lysozyme output and concentration obtained during methacholine (-7 to -21% and -8 to -21%) and phenylephrine (-9 to -62% and -12 to -57%)-induced secretions.

In conclusion, PHI appears to inhibit secretion from mucous cells produced by methacholine but has no effect on serous cell secretion. NPY inhibits the serous cell secretion produced by methacholine or phenylephrine, but this effect is overshadowed by a stimulatory action on another secretory source, probably mucous cells. Both PHI and NPY may be important as modulators of mucus secretion from the ferret trachea.

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OPIOID CONTROL OF NON-CHOLINERGIC BRONCHOCONSTRICTION IN THE GUINEA-PIG IN VIVO

M.G. Belvisi*, K.F. Chung, D.M. Jackson, P.J. Barnes, Department of Clinical Pharmacology, Cardiothoracic Institute, Brompton Hospital, London. SW3 6HP.

Evidence suggests that opioid receptors may be present on sensory fibres of the vagus nerve (Atweh et al, 1978). Opioids also inhibit neurogenic vasodilatation and plasma extravasation in the rat hind paw, suggesting an inhibition of the release of sensory neuropeptides, such as substance P (SP) (Lembeck and Donnerer, 1985). Non-cholinergic bronchoconstriction caused by vagal stimulation in vivo may be due to the release of neuropeptides such as SP from sensory nerves (Lundberg and Saria, 1983). Opiates inhibit this non-cholinergic (NC) bronchoconstrictor response in guinea-pig bronchi in vitro (Frossard and Barnes, 1987). We have now investigated whether opiates inhibit NC bronchoconstriction in vivo in this species.

Male Dunkin-Hartley guinea-pigs (300-600g) were anaesthetized with urethane (25% solution; 8ml/kg i.p.). The left jugular vein was cannulated for the injection of drugs and the left carotid artery for monitoring of blood pressure. The animal was ventilated via a tracheal cannula and airway pressure was recorded. Both cervical vagus nerves were carefully dissected free and sectioned. The peripheral ends of the cut nerves were placed on platinum electrodes and electrically stimulated (5V pulses of 5ms at 10Hz for 30s). Vagal stimulation produced a consistent bronchoconstrictor response (mean increase in airway pressure=24$\pm$1.9 cmH_2O) which was reduced after atropine (1mg/kg) to 13$\pm$1.4cmH_2O. After atropine, morphine caused a dose-dependent decrease in the NC excitatory component of vagal stimulation; at a dose of 100ug/kg the inhibition was 31$\pm$6.6%(n=5) and at the higher dose of 500ug/kg 76$\pm$6.3% (n=6). Naloxone (1mg/kg) reversed the inhibitory effect of morphine, suggesting it was mediated by a specific opiate receptor. Responses to exogenous acetylcholine (25ug/kg) and SP (25ug/kg) were not inhibited by morphine (500ug/kg). Naloxone alone did not cause any significant increase in the NC constrictor response to vagal stimulation. The inhibition of NC bronchoconstrictor response by morphine (500ug/kg) was still present in animals pretreated with propranolol (1mg/kg) (49$\pm$4.4 % ,n=5).

We conclude that morphine inhibits the NC bronchoconstrictor response to vagal stimulation via an action on opioid receptors which are likely to be localised to sensory nerve endings since morphine has no effect on exogenous SP. Naloxone alone has no effect on the NC response suggesting the endogenous opioids do not inhibit the NC. The inhibition of NC constriction by opioids may have important clinical implications since the release of sensory neuropeptides may be involved in local axon reflexes in the asthmatic airway.

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INHIBITORY EFFECTS OF THE OPIOID PEPTIDE BW443C ON SMALLER DIAMETER SENSORY NERVE ACTIVITY IN THE VAGUS

J.J. Adcock* & T.W. Smith, Department of Pharmacology I, The Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS

The opioid peptide Tyr.D-Arg.Gly.Phe(4-NO_2).Pro.NH_2 (BW443C) was shown recently to inhibit spontaneous activity in vagal sensory Aδ -fibres associated with "irritant" receptors in the respiratory tract (Adcock et al, 1987). Such effects may contribute, at least in part, to the peripherally-mediated antitussive effects of this compound. Besides "irritant" receptors, however, non-myelinated sensory C-fibre endings may also contribute to cough reflexes (Coleridge & Coleridge, 1984). In the present study, therefore, the effects of BW443C have been investigated on histamine-stimulated activity in Aδ -fibres and on spontaneous and capsaicin-induced activity in C-fibres of the vagus arising within the respiratory tract.

Male cats were anaesthetised with chloralose (60-80 mg kg^{-1} iv) following induction with 5% halothane, paralysed with dimethyl tubocurarine and artificially ventilated. Pulmonary mechanics (Buxco, model 4) and cardiovascular parameters were recorded. Thin nerve filaments of the left vagus were dissected clear and discharges in Aδ - and C-fibres were recorded and counted. The fibres were identified by their responses to inflation, deflation, drugs and by their conduction velocities. Drugs were administered intravenously, histamine and BW443C in saline and capsaicin (also given intra-arterially) in an ethanol, Tween 80 and saline mixture.

Histamine (1-100 µg kg^{-1}) produced a dose-related increase in "irritant" receptor discharge. In 10 cats histamine dose-response curves were constructed before and following a 1h infusion (0.1 ml min^{-1}) of saline (n=6) or BW443C (100 µg kg^{-1} min^{-1}, n=4). Impulse activity in the Aδ-fibres were significantly reduced following BW443C.

In 6 cats spontaneous discharges of C-fibre endings were recorded for 20 min prior to 1h infusions of BW443C (100 µg kg^{-1} min^{-1}). During the first 5-10 min of opioid infusion a transient, but not significant ($p>0.05$) stimulation of C-fibre impulse activity was observed followed at 30 min by an inhibition which was then maintained throughout the infusion. In this latter period, the spontaneous activity was reduced significantly from 2.2 ± 0.1 to 1.4 ± 0.4 impulses sec^{-1}. Capsaicin (30 µg kg^{-1} iv or ia) produced reproducible increases in C-fibre ending discharges upon repeated injection. In 12 cats this dose of capsaicin was administered before and following a 1h infusion of saline or BW443C (100 µg kg^{-1} min^{-1}). Capsaicin-induced increases in C-fibre discharges were significantly reduced from 6.4 ± 0.9 to 2.7 ± 0.9 impulses sec^{-1} by BW443C.

In summary, the ability of BW443C to reduce spontaneous and chemically-induced activity in both Aδ - and C-fibres arising from sensory nerve endings in the airways suggests that these effects may contribute to the peripherally-mediated antitussive action of this opioid peptide.

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BIPHASIC RESPONSES OF THE ISOLATED GUINEA-PIG TRACHEAL MUSCLE STRIP TO KALLIDIN AND BRADYKININ

J. Bewley, K.D. Bhoola*, D.M. Crothers & M.I. Cingi, Department of Pharmacology, University of Bristol, Medical School, Bristol BS8 1TD.

Bronchial lavage from asthmatic patients contains high levels of both tissue kallikrein and kinins (Proud, Christiansen & Cochrane, 1987), resurrecting an early view that because kinins are potent bronchoconstrictors (Collier, Holgate, Schachter & Shorley, 1960) they could be implicated in asthma. The aim of the present study was to re-examine the pharmacological response of guinea-pig tracheal muscle strips to kinins.

Adult guinea-pig were killed by cervical dislocation. The trachea was removed, placed in a modified-Krebs buffer solution and divided into two segments, rostral and caudal. Each segment was cut spirally to attain an amount of muscle tissue equivalent to that of six tracheal segments. Each muscle strip was placed in a 10 ml organ bath containing Krebs-buffer at 37^{o}. One muscle strip was mounted isotonically (load, 0.5 gm) and the other isometrically (resting tension, 1.5 gm); responses were monitored on a pen recorder. In a few experiments both strips were mounted isometrically. Each experiment was repeated so that the action of agonists was determined both isometrically and isotonically on the rostral and caudal strips. Control responses were obtained with acetylcholine (10^{-7}M) and adrenaline (10^{-7}M - $5x10^{-7}$M).

Surprisingly, relaxation and a fall in resting tension was observed with low doses (10^{-9}M - 10^{-8}M) of kallidin and bradykinin. However, as the concentration increased mainly biphasic responses were recorded; an initial contraction or rise in tension followed by a prolonged relaxation or fall in tension. In view of this unexpected finding we tested both agonists, simultaneously on the isolated ileum and the tracheal strip of the same guinea-pig. Both kallidin and bradykinin produced contraction of the guinea-pig ileum in a dose-dependent (10^{-9} - 10^{-6}M) manner, in contrast to the observations on the tracheal muscle strips.

Some of the biological actions of kinins are secondary to the release of primary mediators. The bronchoconstrictor action of kinins is inhibited by calcium acetylsalicylate (Bhoola, Collier, Schacter & Shorley, 1962) indicating the possible release of prostanoids. Recent evidence suggests that the loss of tracheo-bronchial epithelium enhances bronchial hyper-reactivity. We therefore examined the question whether the relaxant response could be secondary to mediators released from the epithelium. Alternately, the epithelium of the rostral or the caudal tracheal strip was removed with a nylon brush cleaner. Tracheal muscle strips treated in this way gave responses that resulted in contraction or increased tension, to kallidin and bradykinin. Our results suggest the presence of kinin receptors on epithelial cells that release mediators which modify the contractile response of the guinea-pig tracheal muscle to kallidin and bradykinin. The question whether these actions are mediated by separate receptors may be answered when suitable antagonists are developed.

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SODIUM CROMOGLYCATE INHIBITS THE LATE BRONCHOCONSTRICTOR RESPONSE TO OVALBUMIN IN THE GUINEA-PIG

P.A. Hutson*, M.K. Church & S.T. Holgate, Clinical Pharmacology and Medicine I, Centre Block, Southampton General Hospital, Southampton, SO9 4XY, U.K.

The allergen-induced late asthmatic response in man is inhibited by prior treatment with sodium cromoglycate (SCG) but is less susceptible to beta-adrenoceptor agonists (Hegardt et al, 1981). We have previously reported on a guinea-pig model of multiple late phase reactions and cellular correlates (Church et al, 1987). In this study we have investigated the effects of SCG and salbutamol administered before allergen challenge on these late reactions and cellular correlates.

Male guinea-pigs (500-700g) were sensitized to ovalbumin (1%, inhaled for 3 min weekly for 2 weeks). Groups of 10-19 animals inhaled aerosolized SCG (1%), salbutamol (0.01%) or saline (control) for 2 min, 15 min before challenge with aerosolized ovalbumin (2% for 5 min) under cover of mepyramine (10 mg/kg ip). Specific airways conductance (sGaw) was assessed by whole body plethysmography in conscious animals. Bronchoalveolar lavage (BAL) was performed as previously described (Church et al, 1987).

Ovalbumin challenge of control guinea-pigs produced significant ($p<0.01$) decreases in sGaw of 30.6±4.1%, 40.6±5.1%, 35.6±4.3% and 35.0±3.9% at 5 min, 2, 17 and 72 h respectively. Both SCG and salbutamol inhibited the early reaction to challenge measured at 5 min and 2 h. SCG-pretreatment also inhibited the late reaction at 17 h (0.9±10.9% increase in sGaw from baseline), but not the response at 72 h. Salbutamol pretreatment had no significant inhibitory effect on the decreases in sGaw observed at either 17 or 72 h. Both SCG and salbutamol significantly inhibited ($p<0.05$) the rise in the number of neutrophils present at 17 h after challenge (Table 1). The elevation in eosinophil numbers seen at 17 and 72 h were not inhibited by either drug given as a single dose before challenge.

Table 1 Neutrophils ($x10^5$) in BAL

Time	Control (n = 6)	SCG (n = 8)	Salbutamol (n = 6)
17 h	7.2±1.6	2.2±0.3*	2.6±0.4*
72 h	1.6±0.4	0.9±0.1	2.2±0.6

* $p<0.05$ (drug treated vs. control)

These results demonstrate a parallel inhibition by SCG of the 17 h late reaction and neutrophil accumulation in the bronchial lumen. However, the observation that salbutamol inhibited neutrophil accumulation at 17 h without preventing the fall in sGaw at this time suggests that neutrophil accumulation per se is not responsible for the initiation of the late reaction.

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IN VIVO GASTROINTESTINAL PHARMACOLOGY OF FAMOTIDINE IN THE RAT: A COMPARISON WITH RANITIDINE

C. Scarpignato*, R. Tramacere & L. Zappia - Institute of Pharmacology, School of Medicine and Dentistry, University of Parma, Italy.

The successful use of cimetidine and ranitidine in the medical treatment of peptic ulcer disease stimulated the development of new H_2-receptor antagonists with the aim of obtaining more potent and safe drugs. Several hundreds of compounds have been synthesised and only a small number have reached human pharmacology and are now under clinical investigation. One of these is famotidine, a compound containing a thiazole ring like tiotidine. In vivo studies (Takagi et al., 1982; Takeda et al., 1982) have shown that this compound displays strong antisecretory activity (from 40 to more than 100 times that of cimetidine, depending on the experimental conditions). However, a controversy exists as to the duration of its antisecretory action (Buyniski et al., 1984; Humphray et al., 1986; Takagi et al., 1982). The aim of the present investigation was to compare the antisecretory and antiulcer activity of famotidine and ranitidine in rats. Since it was previously shown (Bertaccini & Scarpignato, 1982) that H_2-antagonists can affect gastric motility, the action of both compounds on gastric emptying (GE) was also examined.

Acid Secretion. In the Shay rat preparation (5 h) both famotidine and ranitidine inhibited acid secretion in a dose dependent fashion. In these experimental conditions, the oral ED_{50} values (mg/kg; 95% confidence limits) were 0.80 (0.43-1.28) and 6.84 (5.13-10.90) for famotidine and ranitidine, respectively. However, when equiactive antisecretory doses (i.e. the respective ED_{50} values) were administered orally at different times (1 to 6 h) before pylorus ligation, there was no difference between the duration of action of the two compounds.

Gastric Emptying. After saline administration, the meal leaving the stomach (i.e. GE) was 61.3 $\pm$ 4.7 %. Famotidine was unable to modify gastric emptying, whereas ranitidine induced the well known acceleration in emptying rate (71.5 $\pm$ 1.9 %).

Antiulcer Activity. Gastric and duodenal lesions were induced in the rat by dimaprit (150 mg/kg intravenously) and cysteamine (300 mg/kg subcutaneously), respectively. As far as gastric lesions are concerned, the ED_{50} (i.e. the effective dose which protected 50% of the animals from lesions) was 2.65 (1.36-5.16) mg/kg for famotidine and 5.58 (2.81-11.07) mg/kg for ranitidine. As regards duodenal ulcer, the ED_{50} values were 23.5 (17.5-31.7) mg/kg and 156.9 (105.2-233.9) mg/kg for famotidine and ranitidine respectively.

In conclusion, results of the present investigation, show that - although the antisecretory and antiulcer activity of famotidine are higher than those of ranitidine - the efficacy and the duration of action of both drugs are quite similar.

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600P

INITIAL CHARACTERISATION OF ENZYMES WHICH DESTROY VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN GASTROINTESTINAL TISSUE

S.K. Chakder* & I.J. Zeitlin, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

We recently reported (Chakder & Zeitlin,1987) the presence of VIP-degrading activity (VIP-ase) along the length of the rat gastrointestinal tract. The activity was metal dependent and optimal at pH 7. It was almost completely inhibited by tosyl lysine chloromethyl ketone (TLCK), tosyl phenylalanine chloromethyl ketone (TPCK), inhibitors of trypsin and chymotrypsin respectively and partially by aprotinin, a serine protease inhibitor. We now report further characterization studies of this enzymic activity.

Distilled water homogenates of blood-free gastrointestinal tissue from male Sprague Dawley rats were fractionated using columns of Sephacryl S-300 eluted with Tris buffer (0.1M, pH 8.5, 4^{o}C). VIP-ase and bradykininase activities in the eluates were assayed using the breakdown of [^{125}I]VIP and [^{125}I] bradykinin respectively in phosphate buffer (0.25M,pH 7.4) (Keltz et al,1980). When enzyme inhibitors were used, they were pre-incubated with enzyme for 30min before addition of substrate.

Two major peaks of VIP-ase activity were detected with molecular weights of >400,000 daltons (peak A) and 85,500±4,600 daltons (peak B) respectively. A minor peak (peak C) of molecular weight 23,800±3000 daltons,a molecular weight similar to trypsin (23,800 daltons) and chymotrypsin (25,000 daltons) was also detected. Peak A also appeared to have bradykininase activity. In this peak, the VIP-ase activity was partially inhibited by 40mM EDTA, a chelating agent (69.1±3.5%), by 10mM TLCK (67.0±3.9%), by 10,000 k.i.u.ml^{-1} aprotinin (57.7±4.8%) and by the endopeptidase inhibitor phosphoramidon, 100uM (57.0±4.2%) and not at all by 100uM captopril. The kininase activity was however blocked almost entirely by EDTA (99.2±1.6%) and by captopril (75.6±6.0%) implying the presence of two independent enzymes in this peak. VIP-ase activity in peak B was almost entirely blocked by EDTA (96.4±6.0%) and by aprotinin (106.3±15.3%), partially blocked by TLCK (65.4±10.3%) and phosphoramidon (31.4±12.5%) and not at all by captopril. Too little activity was available from peak C for characterization studies.

Gastrointestinal tissue thus contains at least three different VIP degrading enzymes. Further characterization studies are in progress.

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CANNABINOIDS, THE ACTIVE CONSTITUENTS OF CANNABIS SATIVA L. INHIBIT BOTH HUMAN AND RABBIT PLATELET AGGREGATION

E.A. Formukong*, C.R. Lee, M.F. Roberts, A.T. Evans and F.J. Evans, Dept. of Pharmacognosy, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX

The social use of cannabis has overshadowed the clinical applications of the plant to human disease. Nevertheless, cannabis has been used for the treatment of a number of conditions including asthma. The observation that a reduction in platelet aggregation occurred in human blood following marijuana smoking (Schaeffer et al, 1979) and that Δ'-tetrahydrocannabinol (THC) would antagonise ADP induced aggregation of washed platelets (Levy et al, 1976) prompted our investigations of the abilities of the cannabinoids to antagonise PAF, ADP and TPA (tetradecanoyl-phorbolacetate) induced aggregation.

Platelet rich and platelet poor plasma were obtained by centrifugation. Human blood was used for ADP (6×10^{-6}M) and TPA (1.6×10^{-5}M) induced aggregation, whilst PAF (2.7×10^{-7}M) induced aggregations were performed on rabbit blood. Drugs were administered in a maximum 1% of ethanol, the vehicle having no effect on aggregation or its inhibition, five minutes before the addition of agonist. Aggregations were monitored for a further five minutes (Table 1). ^{14}C-5HT release was determined as previously described (Williamson et al, 1981).

Table 1 Effect of the cannabinoids on PAF and ADP-induced aggregation. Significant ($p<0.05$) using Student t-test* Each value is the s.e. mean of at least 4 experiments.

Drug	PAF-Induced Aggregation IC_{50} (M)	ADP-Induced Aggregation IC_{50} (M)	Maximum effect % inhibition of PAF-induced aggregation s.e.m.	Maximum effect % inhibition of ADP-induced aggregation s.e.m.
Cannabidiol	2.8×10^{-4}	5.8×10^{-4}	77.1 ± 3.6*	79.2 ± 4.0*
Δ'-THC	8.0×10^{-4}	1.1×10^{-3}	60.0 ± 2.0*	66.2 ± 3.7*
Cannabinol	5.6×10^{-4}	marginal activity	76.3 ± 4.3*	33.4 ± 1.4*
Cannabigerol	5.2×10^{-4}	2.7×10^{-4}	100*	90.0 ± 4.3*
Olivetol	9.4×10^{-4}	9.8×10^{-4}	86 ± 2.2*	70.5 ± 2.4*
L652731	3.3×10^{-6}	-ve	not done	-ve
BN52021	6.0×10^{-6}	-ve	not done	-ve

The cannabinoids inhibited PAF induced aggregation and 5-HT release but were considerably less potent than the standard PAF antagonists L652731 and BN52021. These standard drugs had no effect upon ADP induced aggregation whilst the cannabinoids were effective antagonists of that compound. Neither the cannabinoids nor the standard PAF antagonists had an effect on TPA induced aggregation suggesting that in this instance the cannabinoids were not active via antagonism of protein kinase C activation. The cannabinoids are known to affect the activity of phospholipase A_2 (Evans et al, 1987) and their effects on platelets may be mediated by means of membrane associated enzymes. The fact that several cannabinoids other than THC, which are devoid of central activity, exert peripheral effects may be of significance in the understanding of the traditional use of cannabis in the treatment of human disease.

Acknowledgements: we are grateful to the MRC and the Government of Cameroon for financial support.

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THE IMMUNOGENICITY AND DISPOSITION OF BENZYLPENICILLIN AND ITS DEGRADATION PRODUCTS IN THE RAT

G. Christie*, J.W. Coleman, A.M. Breckenridge and B.K. Park, Department of Pharmacology & Therapeutics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX.

The conjugation of penicillin to proteins is thought to be the initial step in the sequence of events which lead to an allergic reaction to the drug. It has been reported that stored solutions of penicillin, in which degradation had occurred, gave rise to a greater antibody response in patients compared to freshly prepared solutions (Neftel et al., 1982). Thus, the aim of this study was to investigate the immunogenicity of benzylpenicillin (BP) and benzylpenicillenic acid (BPE), a degradation product of BP and to correlate this with the degree of protein conjugation in vitro and in vivo, using the male Wistar rat.

The reaction between BP (93 mg, 5 μCi), immediately dissolved or left to stand for 24 hours in 0.5% glucose (stored), and 75% rat plasma proteins (1.0ml) after 3hr at 37°C in vitro was measured by equilibrium dialysis (Kitteringham et al., 1987). The immunogenicity of stored and fresh BP (930 mg ml^{-1}; 1 ml kg^{-1}) in 0.5% glucose was assessed after chronic administration (i.p. and i.m., 4 days) at monthly intervals to rats (n = 8). BPE (9, 0.9 and 0.09 mg kg^{-1}, i.m.) was administered to rats (n = 8) using the same dosing schedule. Serum obtained 7 and 14 days after each series of injections was tested for IgG anti-benzylpenicilloyl (BPO) activity by enzyme-linked immunosorbent assay (ELISA; Kitteringham et al., 1987) (incorporating BPO-human serum albumin coated onto the solid phase). Antibody activity was expressed as the difference in optical density between anti-drug protein conjugate and anti-protein activities at a serum dilution of 1/30 minus the difference in the absence of serum ($\Delta OD_{1/30} - \Delta OD_0$). Circulating antigen was also measured in these samples and in serum samples obtained after each injection of stored and fresh BP i.p. in the first series of injections. The ELISA for circulating antigen was performed as above except that the serum (diluted 1/1000) was coated onto the solid phase, followed by a high titre anti-BPO antisera (diluted 1/5000) raised by immunisation of a New Zealand White rabbit with BPO-keyhole limpet haemocyanin.

The degree of irreversible binding of tritiated material to rat plasma proteins in vitro with the stored BP (3.81 ± 0.27%) was significantly greater ($p < 0.05$) than fresh BP (2.55 ± 0.25%). Circulating BPO antigen was significantly different ($p < 0.001$) 2 days after i.p. administration of aged BP (1.109 ± 0.049) compared to fresh BP (0.726 ± 0.040). No BPO antigen was detected after BPE, whereas it was detectable at day 7 after each series of injections of both stored and fresh BP by both routes.

On day 7 after the third series of injections the greatest IgG anti-BPO antibody activity occured after administration of 9 mg kg^{-1} BPE ($\Delta OD_{1/30} - \Delta OD_0$; 1.017 ± 0.266). There was a significant ($p < 0.05$) difference between stored and fresh BP given i.p. ($\Delta OD_{1/30} - \Delta OD_0$: 0.393 ± 0.147 and 0.063 ± 0.027 respectively) but not i.m. ($\Delta OD_{1/30} - \Delta OD_0$: 0.454 ± 0.099 and 0.294 ± 0.044 respectively).

A small amount of BPE was detectable in stored but not fresh BP using a sensitive spectrophotometric method (Levine, 1961). Thus, the increased immunogenicity of stored BP may be due to the formation of BPE in vitro, which can then form immunogenic drug-protein conjugates in vivo.

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THE ESTIMATION OF HEPATIC BLOOD FLOW IN THE RAT USING INDOCYANINE GREEN

E. Burns*, C.E. Ball, G.D. Broadhead, G.T. Tucker and N.D.S.Bax. University Department of Pharmacology and Therapeutics, Royal Hallamshire Hospital, Sheffield S10 2JF.

Indocyanine green (ICG) has been widely used as a marker of hepatic blood flow (HBF). Its high hepatic extraction ratio (ER) in man has facilitated its use for this purpose with estimates of HBF being derived both indirectly from ICG clearance and directly by use of the Fick principle. Recently it has been suggested that fitting a two compartment model to the plasma elimination data of ICG following a bolus and sampling only from a peripheral vein gives valid estimates of HBF in normal subjects and patients with cirrhosis (Grainger et al 1983, Navasa et al 1986). We have examined whether HBF in healthy rats might be estimated by this latter method.

Male Wistar rats (n=33; B.W. 0.28-0.37 kg) each received a single bolus dose of ICG via the external jugular vein under sodium thiobutobarbitone anaesthesia (200 mg/kg). The mean doses are shown in the table. Carotid arterial samples were taken for up to 120 min and plasma ICG assayed by HPLC with a lower limit of detection of 30 ng/ml (C.V. 6%) (Christie et al 1986). A clearance value was estimated from Dose/AUC and ER by the method of Grainger et al (1983).

ICG elimination was biexponential and the estimated ER was high at all doses (Table 1). Clearance appeared to be inversely related to the ICG dose.

Table 1

Mean Dose (mg/kg)	n	Estimated Mean Blood Clearance (ml/min/100g BW)	ER	Estimated Mean HBF (ml/min/100 g BW)
0.50 ± 0.09	7	5.22 ± 1.30	0.86	6.70
1.02 ± 0.16	8	4.45 ± 1.16	0.85	5.23
4.9 ± 0.30	9	3.39 ± 0.72	0.89	3.80
10.21 ± 1.15	9	1.89 ± 0.55	0.87	2.16

To validate these estimates of ER its value was measured directly by transhepatic sampling using a hepatic venous cannula in addition to carotid arterial sampling.

Male Wistar rats (n=14; BW 0.29-0.36 kg) were infused with ICG for one hour via the external jugular vein at various rates (µg/min ± s.d.) (0.53±0.06, n=4; 1.11, n=2; 2.61±0.13, n=4; 5.06±0.12, n=4). Steady state was achieved after approximately 30 min with all doses and mean (± s.d.) ER was 0.43±0.06, 0.59, 0.48±0.09, and 0.51±0.12 respectively.

The ER of ICG was also measured directly in an isolated perfused rat liver preparation (IPRL) during ICG infusions of 0.5 µg/min (n=6) and 2.5 µg/min (n=6). Mean ER (± s.d.) values were 0.51±0.05 and 0.47±0.14 respectively. The ER of ICG suggests that its clearance is not primarily dependent on HBF. The reasons for this discrepancy between the ER values obtained from kinetic modelling and those obtained by direct methods both in vivo and with the IPRL are not known but might include an underestimate of the AUC after bolus dose ICG because of too short a sampling period. Thus the two compartment model analysis did not permit accurate estimation of ICG ER in the rat and the derived HBF values are invalid.

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PHARMACOKINETICS OF PHENYLBUTAZONE IN ADULT CATTLE FOLLOWING INTRAVENOUS INTRAMUSCULAR AND ORAL ADMINISTRATION

P.Lees*, T.Ayliffe, J.B.O.Taylor and T.E.Maitho, R.V.C. Inflammation Unit, The Royal Veterinary College, Hawkshead Campus, North Mymms,Hatfield, Herts.,AL9 7TA.

Phenylbutazone is used widely as an analgesic and anti-inflammatory agent in veterinary medicine, and differences between species in plasma elimination half life, ranging from 4-6 h in the dog, horse and rat (Lees and Higgins,1985) to 72 h in man (Burns et al.,1953), have been reported. In cattle, elimination half-life was found to be 36-65 h in three studies in which i.v. dose rates ranged from 3.0 to 7.5 mg/kg (Debacker et al.,1980; Eberhardson et al.,1979; Toutain et al.,1980).

This study was undertaken to establish more detailed pharmacokinetic parameters and to obtain preliminary data on metabolism and urinary excretion of a clinical dose rate of phenylbutazone (4.4 mg/kg) administered i.v., i.m. (Intrazone, Arnolds Veterinary Products) and orally (Equipalazone Paste, Arnolds Veterinary Products). Six adult non-lactating cows were used in a three-part cross-over study. Hay and water were provided ad libitum.

Following i.v. administration disposition was described by a three compartment open model. Pharmacokinetic parameters for all three routes are reported in Table 1. The somewhat longer $t_{\frac{1}{\beta}}$ values for oral and i.m. routes in comparison with i.v. administration may have resulted, respectively, from sequestration within the gastrointestinal tract and continual uptake from i.m. sites following precipitation as a depot. Absorption was more complete after i.m. than after oral dosing. Double peaks in plasma concentration occurred after oral dosing in some animals, possibly reflecting drug adsorption onto and desorption from hay. There was no evidence for opening of the oesophageal groove and direct passage of drug into the abomasum in any animal. Plasma protein binding exceeded 98%.

Table 1. Pharmacokinetic parameters for phenylbutazone following i.v., i.m. and oral administration to adult cattle (mean ± s.e.m., n=6)

Parameter	Intravenous	Intramuscular	Oral
C_p^o (µg/ml)	88.2 ± 7.0	-	-
Cmax (µg/ml)	-	42.3 ± 5.0	23.9 ± 1.2
tmax (h)	-	7.5 ± 1.0	10.5 ± 1.9
$t_{\frac{1}{\beta}}$ (h)	35.9 ± 3.8	51.2 ± 6.8	44.3 ± 6.0
AUC_o^∞ (µg/ml.h)	3001 ± 597	2987 ± 463	1629 ± 221

Two hydroxylated metabolites of phenylbutazone, oxyphenbutazone and γ-hydroxyphenylbutazone, were present in trace amounts in plasma for 72 h and in much higher concentrations in urine for 168 h. That tubular secretion may have occurred is indicated by approximate urine:plasma (U/P) concentration ratios which sometimes exceeded U/P ratios for creatinine.

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THE EFFECTS OF NICARDIPINE ON PENTYLENETETRAZOLE-INDUCED SEIZURE ACTIVITY IN THE MOUSE

M.C. Allely & B.J. Alps*, Syntex Research Centre, Research Park, Riccarton, EDINBURGH EH14 4AS.

The administration of pentylenetetrazole (PTZ) is a well-documented tool for the production of epileptiform seizure activity (Krall et al, 1978). Since it has been indicated that hippocampal CA_1 and CA_3 neuronal mitochondrial [Ca^{2+}] increases following status epilepticus which may produce Ca^{2+} overload leading to cytotoxicity (Meldrum et al, 1985) the effects of two Ca^{2+} entry-blockers nicardipine HCl (NIC) and nimodipine HCl (NIM) were examined on PTZ-induced seizure activity in the mouse.

NIC and NIM were administered at 500 $\mu g.kg^{-1}$ i.p. to male CD1 mice at three pretreatment times (A - 15 min; B - 60 min; C - 3 days bid + 15 min) before 100 $mg.kg^{-1}$ PTZ s.c. and the results are shown in Table 1. Neither drug affected clonic seizure activity. NIC reduced the incidence of tonic seizures (significantly so at C), the incidence with NIC being significantly lower than with NIM at both B and C. NIC reduced the lethality of PTZ at all three pretreatments, only C attaining significance, but both A and C being significantly lower than the corresponding NIM group.

Drug	Treatment	N	% Clonic	% Tonic	% Death
Saline	---	40	92.5	80	70
NIC	A	20	70	90	40 ++
NIC	B	20	85	60+	50
NIC	C	20	90	*45+	**25+++
NIM	A	20	75	95	90
NIM	B	20	90	95	60
NIM	C	20	80	85	85

Table 1. Effects of NIC and NIM on PTZ-induced seizures and mortality in the mouse. *P<0.02, **P<0.01 (vs controls) +P<0.05, ++P<0.01, +++P<0.001, (vs corresponding NIM group, Chi squared test).

It would therefore appear that NIC but not NIM offers protection against the incidence of tonic seizures and death produced by PTZ in the mouse and that the effects of NIC are improved by "pre-loading". The most marked effects of NIC were following treatment C and it is interesting that in this group the time to onset of clonic or tonic seizure or death was significantly prolonged (P<0.01, P<0.01 and P<0.001 respectively, Student's t-test) whilst latency in the NIM group C was unaltered.

PTZ appears to affect GABA-ergic inhibition of neuronal firing in cultured mouse spinal cord and hippocampus (Macdonald, 1984). If, in vivo, this involves the hippocampal CA_3 neurones (which possess high Ca^{2+} conductance), it may be that NIC, but not NIM, can antagonise the effects of this neuronal Ca^{2+} modulator in provoking seizures.

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LACK OF TOLERANCE TO ETHANOL AFTER CONCURRENT ADMINISTRATION OF NITRENDIPINE

H. J. Little* & S. J. Dolin†. Department of Pharmacology, University Walk, Bristol and †Division of Anaesthesia, Clinical Research Centre, Watford Road, Harrow.

There is now considerable evidence that neuronal calcium channels are functionally involved in ethanol dependence. The dihydropyridine calcium channel antagonists, nitrendipine and nimodipine, were very effective in preventing the ethanol withdrawal syndrome (Little et al, 1986). They have little action on neurones in normal circumstances, but their effects, and those of the calcium channel agonist, BAY K 8644, on neurotransmitter release and on inositol phospholipid turnover were significantly increased after chronic ethanol treatment (Hudspith & Littleton, 1986; Dolin et al, 1987; Littleton et al, 1987). Chronic ethanol administration increased the number of dihydropyridine binding sites in rat cortex (Dolin et al, 1987). Nitrendipine and nimodipine increased the acute effects of ethanol (Dolin & Little, 1986). We now investigate the effects of nitrendipine on ethanol tolerance.

Groups of male Sprague-Dawley rats (150 - 170g), n = 7 or 8, were injected, i.p., once daily for ten days (days 1-10) with the following drugs: (i) ethanol 1.5 mg kg^{-1}, (ii) ethanol 1.5 mg kg^{-1} + nitrendipine 50 mg kg^{-1}, (iii) nitrendipine 50 mg kg^{-1}, or (iv) vehicles (saline + Tween 80, 0.5%). On day 11 they were tested on a rotorod, every 30 min for 3h, after all groups had been given ethanol alone, 2 mg kg^{-1}. The results are expressed as median (and interquartile range) for the time (sec) spent on the rotorod, with a cut-off time of 3 min. The parameters of the test were such that untreated animals stayed on the rod for approximately 3 min.

Chronic treatment/ Test time:	30 min		60 min		'U' test
Vehicles	14	(10.5-18.5)	13	(8-24)	
Ethanol alone	65 *	(24-180)	180 *	(140-180)	a
Ethanol + nitrendipine	8.5 *	(8-16)	34.5 *	(23.5-149)	b
Vehicles	6.5	(4.5-12.5)	12.5	(7.5-67.5)	
Nitrendipine alone	3	(2-3)	11	(5-16)	

* $P<0.01$, a: compared with vehicle treated group, b: compared with ethanol alone

Ethanol treatment for 10 days caused tolerance to its effects, but significant tolerance did not develop when nitrendipine was given with each dose of ethanol. Treatment with nitrendipine alone did not alter the effects of ethanol. The same pattern was seen when the experiment was repeated, using a higher dose of ethanol. The dose of nitrendipine used slightly decreased the blood pressure. Administration of a dose of hexamethonium (equieffective with the nitrendipine with respect to blood pressure), with each dose of ethanol, did not affect tolerance development. Nitrendipine did not alter brain ethanol concentrations. The results suggest that nitrendipine decreases the development of tolerance to ethanol. This may be related to the decrease in central dihydropyridine binding site numbers reported after chronic nitrendipine treatment (Panza et al, 1985), in contrast to the increased number we found after chronic ethanol treatment (Dolin et al, 1987)

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We thank the Wellcome Trust and the Brewers' Society for financial support.

BEHAVIOURAL EFFECTS OF CALCIUM CHANNEL BLOCKERS SUGGESTING A CENTRAL SEROTONINERGIC MECHANISM

D. J. Boullin * & D. G. Grahame-Smith, MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford OX2 6HE.

Recent work suggests that calcium channel blockers have actions on serotoninergic mechanisms: Uhr et al (1986) report reduced platelet 5-HT in normal and schizophrenic subjects given verapamil. Calcium channel blockers have been reported to inhibit platelet 5-HT uptake and imipramine binding in brain and platelets, (Carmi, et al, 1987); to have synergistic effects when given in combination with lithium (Valdiserri, 1985) and carbamazepine (Brodie & Macphee, 1986). Diltiazem may be effective in tardive dyskinesia (Ross et al 1987).

We have studied the actions of calcium channel blockers upon central serotoninergic function using the behavioural syndrome provoked in rats following administration of tranylcypromine and (-)-tryptophan, and believed to be mediated by 5-HT (Grahame-Smith, 1971).

Motor activity of Sprague-Dawley male rats (150-300g) was monitored using activity meters coupled to digital counters recording 5 minute and cumulated counts. Individual behaviours were scored by the method of Deakin & Green (1978). All drugs were injected intraperitoneally in saline unless stated. The following compounds were used: tranylcypromine; (-)-tryptophan; diltiazem; 5-methoxy, N, N-dimethyl-tryptamine (5MeODMT); parachlorophenylalanine methyl ester (PCPA); all from Sigma, Poole. (-)-Propranolol, (ICI Pharmaceuticals); 8-hydroxy-2-(di-propyl-amino) tetralin (8-OHDPAT, Research Biochemicals, Wayland, MA, USA); quipazine (Miles Labs, Elkhart IND, USA); verapamil hydrochloride (Knoll, Sussex); nifedipine (in DMSO:saline: 1:20 v/v, Bayer); clomipramine hydrochloride (Geigy).

In rats pretreated 45 min earlier with 20 mg/kg tranylcypromine, 10-100 mg/kg diltiazem, 5 mg/kg nifedipine, 10-25 mg/kg verapamil all provoked the classical
5-HT-mediated behavioural syndrome, without the need to give (-)-tryptophan. 25-50 mg/kg diltiazem also enhanced the behavioural syndrome produced by 20 mg/kg tranylcypromine followed by 50 mg/kg (-)-tryptophan.

The tranylcypromine/diltiazem-evoked syndrome is blocked by 20 mg/kg (-)-propranolol and by pretreatment with PCPA (300 mg/kg daily for 3 days prior to behavioural testing). 25 to 100 mg/kg Diltiazem did not enhance behaviours induced by 10 mg/kg quipazine; 1-5 mg/kg 5MeODMT or 1 mg/kg 8-OHDPAT. The 5-HT uptake blocker clomipramine also dramatically enhanced the motor activity and individual behaviours.

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A NEW ASSAY FOR MEASURING BASAL ACETYLCHOLINE LEVELS IN RAT BRAIN DIALYSATE

W.John Albery[1], Martyn Boutelle[2], Anthony E.G. Cass[3], Sally L.T. Durrant[*1], Marianne Fillenz[2] and Tyra Zetterström[2]. [1]Department of Chemistry, [3]Centre for Biotechnology Imperial College of Science and Technology, London SW7 2AY. [2]University Laboratory of Physiology, Parks Road, Oxford OX1 3PT.

The measurement of acetylcholine (ACh) release in rat brain has proved difficult. We have developed a novel HPLC assay which uses a packed enzyme bed (PEB) wall jet electrode. The enzymes acetylcholinesterase (AChE)(Ec 3.1.1.7), choline oxidase (ChOx)(EC 1.1.1.17) and horseradish peroxidase (HRP) (EC 1.11.1.7) are immobilised onto solid supports by covalent linkage. The analytes react through the sequence shown in Fig.1 where ferrocene monocarboxylic acid (Fc) acts as an efficient redox mediator. The analytes ACh and choline (Ch) are separated using a reverse phase HPLC column. This technique has a detection limit of better than 100 attomoles (10^{-18}M) per 50µl sample.

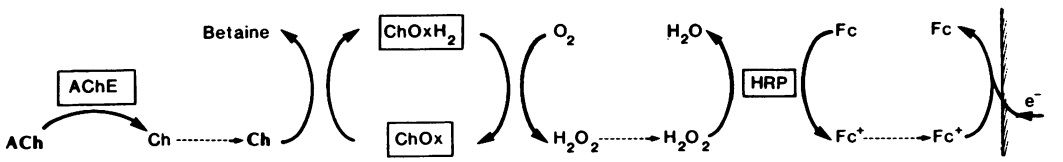

Fig.1

We have measured concentrations of ACh and Ch in dialysis samples from striata of unanaesthetised rats: in the absence of AChE inhibitors the basal values were 12 fmoles for ACh and 80 fmoles for Ch in a 50µl sample. On addition of 10^{-5}M eserine to the dialysis medium the ACh content of the dialysate increased to 35 fmole per 50µl sample. The estimated extracellular concentration for ACh in the striatum is 1.1 nmolar (allowing for the recovery via the dialysis loop).

The PEB wall jet electrode has a number of advantageous characteristics. The operating potential is very low (-27mV), which avoids interference from other electroactive species in the dialysate. The use of Fc avoids the poisoning of the electrode inherent in H_2O_2 oxidation. The immobilisation of the enzymes onto a solid support confers increased activity and stability of the enzymatic assay. As a consequence, the assay is stable for a period of 2 months. These characteristics distinguish the present assay from those which use direct detection of H_2O_2 (Damsa et al., 1987).

We wish to thank the SERC, MRC and the Swedish Medical Research Council for financial support.

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BRAIN REGIONAL [^{3}H]-D-ASPARTATE AND [^{3}H]TCP BINDING IN ALZHEIMER'S DISEASE AND DOWN'S SYNDROME

M.D.C. Simpson*, M.C. Royston, A.J. Cross, P. Slater, D.M.A. Mann, J.F.W. Deakin & G.P. Reynolds[1], Department of Physiology, University of Manchester, Manchester M13 9PT and [1]Department of Pathology, Queen's Medical Centre, Nottingham NG7 2UH.

Previously described transmitter deficits in Alzheimer-type dementia (ATD) have involved neurones projecting to cerebral cortex. Recent reports of altered glutamate (Glu) function may correlate with the cortical pathology in ATD. Both the binding of [^{3}H]D-aspartate ([^{3}H]Asp) to glutamate uptake sites, and the uptake of Glu were reduced in ATD cortex (Cross et al., 1987; Hardy et al., 1987). We have measured [^{3}H]Asp binding in several areas of ATD brains, together with the binding of [^{3}H]TCP to the Glu (NMDA) receptor complex (Loo et al., 1986). Similar measurements have been made on brains from Down's syndrome (DS) subjects with ATD pathology.

Brains were matched for age and p.m. delay. Alzheimer's disease was confirmed neuropathologically in all ATD and DS brains. [^{3}H]Asp (100 nM) and [^{3}H]TCP (10 nM) binding to crude membrane preparations were performed as described previously (Cross et al., 1986; Loo et al., 1986).

[^{3}H]Asp binding was reduced in temporal cortex and caudate nucleus in ATD. [^{3}H]TCP binding (5-100 nM) to human frontal cortex was saturable (Bmax 618 $\pm$ 52 fmol/mg protein; Kd16 nM) and was displaceable by compounds (phencyclidine (PCP) >> ketamine = dextromethorphan > pentazocine = haloperidol) which showed preference for PCP-sigma sites. [^{3}H]TCP binding was reduced in frontal cortex and caudate in ATD brains. [^{3}H]Asp binding in DS was greatly below normal in all areas studied, whereas [^{3}H]TCP binding was not altered.

Table 1 [^{3}H]Asp and [^{3}H]TCP binding in ATD, DS and control (C) brains

		Temporal cortex	Frontal cortex	Hippocampus	Caudate	Putamen	Globus pallidus
[^{3}H]Asp	C	808(107)	2013(314)	3606(528)	3700(617)	1268(274)	1567(224)
	ATD	452(58)*	1410(150)	2221(350)	1990(450)*	940(87)	1235(260)
[^{3}H]TCP	C	143(11)	143(13)	117(11)	108(12)	-	-
	ATD	132(13)	93(17)*	136(20)	82(10)*	-	-
[^{3}H]Asp	C	470(69)	1306(86)	1097(90)	1135(138)	-	-
	DS	152(27)*	462(76)*	363(54)*	218(29)*	-	-
[^{3}H]TCP	C	269(29)	199(9)	303(15)	242(28)	-	-
	DS	194(40)	209(44)	251(44)	251(34)	-	-

Values are mean fmol/mg($\pm$ S.E.M.) (n=5-9 in ATD, 8-10 in DS). *P<0.05.

The findings show that the loss of Glu uptake sites in ATD involves not only temporal cortex but also a subcortical area (caudate). The NMDA receptor complex ([^{3}H]TCP binding) in frontal cortex is also affected in ATD. The low levels of [^{3}H]Asp binding recorded in DS may be related to the Alzheimer-type changes in this disorder.

Financial support was given by North West Regional Health Authority.

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BLADDER HYPERREFLEXIA FOLLOWING MPTP TREATMENT OF THE COMMON MARMOSET

A. Albanese[1], P. Jenner[1], C.D. Marsden[1] & J.D. Stephenson[*], Department of Pharmacology, [1]MRC Movement Disorders Research Group, University Department of Neurology & Parkinson's Disease Society Research Centre, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill, London SE5 8AF.

Detrusor hyperreflexia is the most common urological complaint associated with Parkinson's Disease affecting about 90 % of patients (Pavlakis et al., 1983; Fitzmaurice et al., 1985). It manifests itself as urgency and frequency of micturition, symptoms which are exaggerated by therapy with L-DOPA (Fitzmaurice et al., 1985). We now report on the effect of the nigral toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), on the bladder activity of marmosets.

Common marmosets of either sex received MPTP 2-4 mg/kg for up to 5 days to render them parkinsonian. At the time of experiment, approximately 3 months following cessation of MPTP administration, the animals exhibited akinesia, rigidity, and postural tremor. These animals were compared with 3 age-matched controls. The marmosets were anaesthetized with sodium pentobarbitone (36 mg/kg i.m.). After making an abdominal incision, a silicone catheter (0.6 mm i.d.) was introduced into the bladder through its' dome, for recording intravesical pressure and for infusing saline (0.1 ml/min). The urethra was ligated.

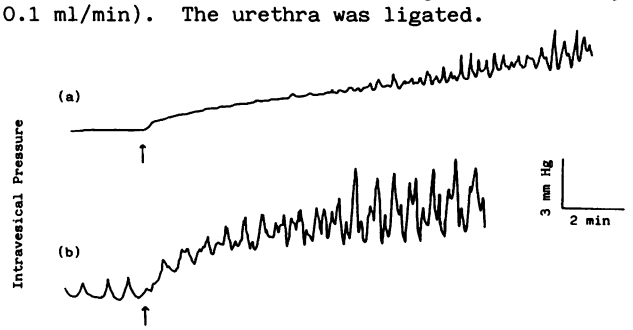

Figure 1 Cystometrograms from a control marmoset (a) and an MPTP-treated marmoset (b). Bladder filling commenced at the arrow.

In control marmosets, the empty bladder was quiescent and remained so on infusing saline, until approximately half of the total volume had been infused when small spontaneous rises in pressure appeared (Figure 1a). In contrast, in 4 of the 5 MPTP-treated marmosets regular and spontaneous rises in intravesical pressure were present when the bladder was empty (Figure 1b) and in the 5th animal these appeared immediately upon commencing the infusion. Thereafter the contractions were continually present and were of greater amplitude than in controls. L-DOPA (200 mg/kg ip) 15 min after carbidopa (100 mg/kg ip), increased activity of the detrusor in all animals but the effect was most pronounced in the control animals because the detrusor was initially less active.

The results demonstrate that MPTP induces bladder hyperreflexia, a common symptom of Parkinson's Disease. The worsening of the effect by L-DOPA suggests that it will be a suitable primate model upon which to devise means of controlling hyperreflexia in Parkinsonian patients receiving L-DOPA treatment.

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PILOT STUDY OF TRANSPLANTATION OF FOETAL NIGRAL CELLS INTO STRIATUM OF MARMOSETS WITH MPTP-INDUCED PARKINSONIAN SYNDROME

W.H. Oertel[1*], A. Fine[2], P.N. Chong[1], S. Hunt[2], M. Nomoto[1], J.A. Temlett[1], S. Dunnett[3], L. Annett[3], P. Jenner[1] & C.D. Marsden[1], [1]University Department of Neurology & Parkinson's Disease Society Research Centre, Institute of Psychiatry & King's College Hospital Medical School, London; [2]MRC Molecular Neurobiology & [3]Department of Experimental Psychology, University of Cambridge, Cambridge, U.K.

Grafting of foetal ventral mesencephalic cells may provide an alternative or adjunct to the drug treatment of Parkinson's disease (Brundin and Bjorklund, 1987). In this pilot study, we investigated the survival of foetal mesencephalic cells bilaterally grafted into the striatum of marmosets with an MPTP-induced parkinsonian syndrome using pharmacological and immunohistochemical methods.

Six common marmosets of either sex received a total of 11-13 mg/kg MPTP i.p. each to induce an initially severe parkinsonian syndrome. Two animals served as 'Normal Controls'. MPTP-treated animals partially recovered motor function after 3-4 months. At this point two MPTP-treated animals received bilaterally multiple injections of a dispersion of foetal ventral mesencephalic cells into the striatum ('S Nigra Transplanted'). For control, two MPTP-treated animals received similar stereotaxic bilateral injections of a dispersion of foetal striatal cells ('Striatum Transplanted'). The remaining two MPTP-treated animals did not receive a graft ('MPTP-Controls'). Embryos with crown rump length of 10 mm and 14 mm were used. Cell dispersions were prepared according to Bjorklund et al. (1983). Animals were subsequently assessed by measurement of locomotor activity without and after treatment with L-DOPA or amphetamine. 6-7 Months after transplantation the animals were anaesthetised and perfused with 0.1 m phosphate buffer (pH 7.4)/4% paraformaldehyde. Brain sections were stained with antiserum to tyrosine hydroxylase (TH).

Immunohistochemical analysis revealed presence of TH-immunoreactive neurons and fibres in striata of 'S Nigra Transplanted' animals. 'MPTP-Controls' showed only mildly impaired motor function at the end of the observation period. 'Striatum Transplanted' animals recovered less than 'MPTP-Controls', whereas 'S Nigra Transplanted' animals could not be definitively distinguished from 'MPTP-Controls' by behavioural observation. Overall spontaneous locomotor activity of 'S Nigra Transplanted' animals was higher than for 'Striatum Transplanted' animals or 'MPTP-Controls'. 'Normal Controls' exhibited a variable degree of spontaneous locomotor activity. Challenge with L-DOPA (12.5 mg/kg)/ benserazide (12.5 mg/kg) i.p. enhanced locomotor activity in all MPTP-treated animals and did not distinguish between 'MPTP-Controls' and 'S Nigra Transplanted' animals. In contrast treatment with amphetamine (0.5 mg/kg i.m.) led to an enhanced locomotor activity only in 'S Nigra Transplanted' animals, whereas 'Striatum Transplanted' animals exhibited an increase in checking behaviour, but not in locomotor activity.

This pilot study shows, that foetal dopaminergic neurons survived for 6 months when grafted into striatum of parkinsonian marmosets. There was a tendency to higher spontaneous locomotor activity in animals with a mesencephalic graft. In pharmacological tests, treatment with amphetamine increased the locomotor activity only in the animals with a mesencephalic graft and therefore distinguished the 'S Nigra Transplanted' animals from the other groups of marmosets.

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W.H.O. is supported by Deutsche Forschungsgemeinschaft Oe 95/3-1

A PHYSIOLOGICAL ROLE FOR MONOAMINE OXIDASE-B IN BRAIN DOPAMINE METABOLISM?

I.S. Fairbrother, S.P. Butcher*, J.S. Kelly and G.W. Arbuthnott. MRC Brain Metabolism Unit, University Department of Pharmacology, University of Edinburgh, U.K. and *Department of Pharmacology, University of Edinburgh, U.K.

The catecholamine degrading enzyme, monoamine oxidase (MAO) exists as two distinct isoenzymes within the brain. These are differentially localised within neurones and glia, with MAO-A being found predominantly within neurones, and MAO-B mainly in glia (see Francis et al, 1985). The major route of dopamine (DA) inactivation in the brain is believed to involve high affinity uptake followed by enzymatic breakdown by MAO-A. However, recent microdialysis data have questioned this assumption (Fairbrother et al, 1987), since DA uptake inhibitors do not influence extracellular levels of the primary DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC). The mode of DA inactivation is therefore unclear, and the present study is designed to examine whether MAO-B plays a physiological role in this process.

Cannula design dialysis probes (Sandberg et al, 1986) were implanted into rat striata (AP ± 0.5, ML ± 2; from Paxinos and Watson, 1982) under halothane anaesthesia. These were perfused with oxygenated Krebs bicarbonate buffer at 1.25µl/min. After a 60 min washout period, 20 min fractions were collected throughout the experiment and DA and related metabolites were detected by high performance liquid chromatography with electrochemical detection. Selegiline (10mg/kg), an inhibitor of MAO-B (Housley et al, 1976) was administered as an intraperitoneal injection 60 min later. Amphetamine (4mg/kg; i.p. injection), veratrine (100µg/ml; via dialysis probe) and ouabain (100µM; via dialysis probe) were administered 60 min after selegiline injection.

Basal dialysate concentrations of DA and related metabolites were: DA, 7nM; DOPAC, 0.8µM; homovanillic acid (HVA), 0.4µM; 3-methoxytyramine (3-MT), 2nM. Selegiline did not influence basal efflux of DA, HVA or 3-MT. However, basal DOPAC efflux was consistently decreased by selegiline (80-85% of control). Amphetamine induced an identical increase in DA efflux (700-800% of control) and decline in DOPAC (20-30% of control) and HVA (35-40% of control) in the presence and absence of selegiline. 3-MT efflux was consistently increased by amphetamine (150% of control) only in the presence of selegiline. Veratrine and ouabain also evoked similar patterns of DA overflow in the presence and absence of selegiline. 3-MT efflux induced by veratrine and ouabain was slightly greater in selegiline treated animals (250-300% of control) than in control animals (200-250% of control).

These data suggest that MAO-B plays a minor role in dopamine degradation both in unstimulated and chemically stimulated striata. The reduction in basal DOPAC efflux induced by selegiline may reflect a MAO-B mediated breakdown of extracellular DA to DOPAC in glia. However, a partial inhibition of MAO-A could also be responsible for these effects. The chemically induced stimulation of 3-MT efflux noted in the presence of selegiline most probably results from a MAO-B mediated degradation of released dopamine in glia. However, this relatively small response suggests that MAO-B does not play a major role in the degradation of extracellular DA.

Selegiline was a gift from Britannia Pharmaceuticals, Ltd.

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CONTRIBUTION OF α-ADRENOCEPTOR BLOCKADE TO THE HYPOTENSIVE ACTION OF RITANSERIN IN ANAESTHETISED RATS

S.J. Coker* and A.G. Barlow, Department of Pharmacology & Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX.

The 5-HT_2 antagonist ritanserin has been reported to be a very weak adrenergic antagonist (Janssen, 1985) with limited hypotensive action (Conolan et al., 1986). Ketanserin, however, has appreciable α_1-adrenoceptor blocking activity which is thought to account for its ability to reduce blood pressure (Fozard, 1982). In recent experiments we observed that both ketanserin and ritanserin reduced blood pressure to a similar extent (Coker & Ellis, 1987). The present study was designed to investigate this further by assessing the contribution of α-adrenoceptor blockade to the effects of each drug.

Male Wistar rats (250-300g) were anaesthetised with sodium pentobarbitone 60 mg kg^{-1} i.p. A carotid artery and a femoral vein were cannulated to permit blood pressure recording and drug administration respectively. Ketanserin did not alter pressor responses to noradrenaline whereas ritanserin caused significant reductions e.g. the increase in systolic blood pressure caused by 1μg kg^{-1} noradrenaline was reduced from 74 ± 9 to 56 ± 13 mm Hg by 3 mg kg^{-1} ritanserin (n=5). A similar reduction in the pressor response to noradrenaline was achieved with 0.01 mg kg^{-1} prazosin (59 ± 6 to 41 ± 3 mm Hg, n=3). Ketanserin did, however, reduce pressor responses to phenylephrine e.g. the increase in systolic blood pressure of 50 ± 9 mm Hg induced by 3μg kg^{-1} phenylephrine was reduced to 23 ± 2 mm Hg after 3 mg kg^{-1} ketanserin (n=3). In contrast, ritanserin did not alter pressor responses when phenylephrine was the agonist.

Both ketanserin and ritanserin caused similar dose-dependent reductions in heart rate and blood pressure. For example, heart rate was reduced from 392 ± 16 beats min^{-1} to 340 ± 17, 319 ± 17, 303 ± 24 then 281 ± 15 beats min^{-1} by 0.1, 0.3, 1.0, then 3.0 mg kg^{-1} ritanserin (n=8). Correspondingly blood pressure was reduced from 157 ± 7/116 ± 7 mm Hg to 150 ± 10/108 ± 8, 141 ± 9/ 100 ± 9, 137 ± 9/96 ± 7 then 121 ± 5/80 ± 5 mm Hg respectively. In rats which had received prazosin 0.1 mg kg^{-1}, prior to either ketanserin or ritanserin the 5-HT antagonists failed to cause any further reduction in blood pressure but significant decreases in heart rate were still observed. For example, prazosin did not significantly alter heart rate, 419 ± 14 to 415 ± 13 beats min^{-1} but the four doses of ritanserin reduced heart rate to 372 ± 13, 319 ± 9, 311 ± 16 then 326 ± 20 beats min^{-1} respectively. Prazosin reduced blood pressure from 168 ± 9/123 ± 7 to 124 ± 5/88 ± 5 mm Hg but after this increasing doses of ritanserin failed to cause any further reduction, the values being 117 ± 4/85 ± 5, 113 ± 2/81 ± 5, 115 ± 3/78 ± 5 and 123 ± 6/83 ± 8 mm Hg (n=5).

These results suggest that blockade of α-adrenoceptors contributes to the hypotensive action of ritanserin to a similar extent as it does with ketanserin but does not affect the bradycardia induced by either drug. The observation that ketanserin appeared to block responses to the α_1-selective agonist phenylephrine whereas ritanserin seemed to be more effective against noradrenaline warrants further investigation.

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DEMONSTRATION OF VASCULAR DOPAMINE RECEPTORS ON HUMAN RESISTANCE VASCULATURE

*A. Hughes, P. Sever. Clinical Pharmacology, St. Mary's Hospital Medical School, Norfolk Place, London, W.2.

The existence of vascular dopamine (DA_1) receptors in the renal and mesenteric vasculature of several species including man is generally accepted (Goldberg, 1972) and DA_1 receptors have been demonstrated on human basilar arteries in vitro (Forster et al., 1983). Recent studies in man have demonstrated potent vasodilatory effects of dopamine and the selective DA_1 receptor agonist fenoldopam in vivo, implying that such receptors are present on human resistance vasculature. However, their location has not been demonstrated directly.

Human resistance arteries (internal diameter 100-600 μm) were obtained from subcutaneous fat or omentum removed from 21 patients (13 male, age range 27-80 yrs) undergoing surgery. Arterioles were isolated using a dissection microscope and mounted as intact segments between two 40 μm wires in a myograph chamber (Aalkjaer & Mulvany, 1981) containing 10 ml of Krebs buffer oxygenated with 95% O_2 5% CO_2 and maintained at 37°C to allow direct isometric measurement of tension. All vessels were set to an optimal resting tension corresponding to 0.9 L100, where L100 = diameter of vessel under a 100 mm Hg distending pressure calculated from the passive length tension relationship. Following 1 hr equilibration vessels were preconstricted by a depolarising (K^+ 125 mM) Krebs solution. Agonists were added cumulatively once a stable contraction was obtained. Concentration response data to dopamine were obtained in the presence of propranolol 4×10^{-6}M, cocaine 10^{-5}M and 17βoestradiol 10^{-5}M. Responses were calculated as % relaxation of precontracted tension. Dopamine (10^{-9} - 10^{-5}M) either alone or in the presence of phentolamine (10^{-5}M), the selective DA_1 receptor agonists fenoldopam (10^{-7} - 10^{-4}M) and SKF 38393 (10^{-6} - 10^{-3}M) all relaxed preconstricted arterioles in a concentration dependent fashion. Agonist potency was dopamine > fenoldopam > SKF 38393. Relaxation in response to dopamine or fenoldopam was competitively antagonised by the DA_1 receptor antagonists SCH 23390 (3.10^{-8} - 3.10^{-7}M) and (+) butaclamol (10^{-7} - 10^{-6}M) but not by (-) butaclamol 10^{-6}M, indicating that the responses were mediated by an action on DA_1 receptors.

In conclusion, these data provide direct evidence for the existence of vascular dopamine (DA_1) receptors on human arterioles.

This work was supported by a grant from the British Heart Foundation. A.H. is a Squibb Cardiovascular Fellow.

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BLOOD PRESSURE EFFECTS OF THE FLUORO ANALOG OF FENOLDOPAM, SK&F 87516, AN AGONIST AT DA_1, β_2, α_2 AND $5\text{-}HT_2$ RECEPTORS IN RATS

Anne-Charlotte Le Monnier de Gouville*, Kim Lawson and Icilio Cavero, Rhône-Poulenc Santé, Centre de Recherche de Vitry, 13 quai Jules Guesde, B.P. 14, 94403 Vitry-sur-Seine Cedex, France.

SK&F 87516 is the R-enantiomer analog of the relatively selective DA_1 dopamine receptor agonist, fenoldopam, in which the chlorine in the 6 position of the benzazepine has been replaced by fluorine. This compound has been reported to stimulate DA_1 dopamine receptors and α_2-adrenoceptors (Weinstock et al., 1985). The aim of this study was to investigate the mechanisms of the blood pressure effects of SK&F 87516 in anaesthetized rats.

Male normotensive Sprague-Dawley rats (230-260 g) were anaesthetised with pentobarbitone (55 mg/kg i.p.) and placed under artificial respiration ; for certain studies, the rats were pithed. Mean blood pressure was measured from the cannulated left carotid artery and injections were given via the femoral veins. SK&F 87516 was always administered as a single 15 min i.v. infusion. Initially, a dose (1.25 - 80 µg/kg/min)-response study to SK&F 87516 was performed in intact rats. In subsequent studies, the effects of 20 µg/kg/min were investigated in intact and pithed rats pretreated with i.v. saline (0.25 ml/kg), atenolol (1 mg/kg), enalapril (0.3 mg/kg), ICI 118551 (1 mg/kg) idazoxan (0.3 mg/kg), propranolol (1.5 mg/kg), SCH 23390 (25 µg/kg), methysergide (0.1 mg/kg) or a combination of these antagonists. The responses to SK&F 87516 were also determined in intact rats pretreated with chlorisondamine (0.5 mg/kg), methylatropine (0.3 mg/kg) or S-sulpiride (0.3 mg/kg). Finally, the hypotensive effects of an i.v. bolus injection of fenoldopam (20 µg/kg) were studied in intact rats 10 min after pretreatment with an infusion of saline (0.25 ml/kg/min) or SK&F 87516 (5 - 40 µg/kg/min).

Infusions of SK&F 87516 (1.25, 5, 20, 80 µg/kg/min over 15 min i.v.) produced dose-related maximal decreases (14 ± 1, 32 ± 4, 39 ± 2, 47 ± 3 mm Hg), in mean carotid blood pressure. These effects occurred within the initial 2-5 min of the infusion, then waned by 50-80 % during the subsequent 10 min. This "tolerance" phenomenon was more marked with the higher doses of SK&F 87516 and was not modified by enalapril or idazoxan. The maximal hypotensive effect of SK&F 87516 (20 µg/kg/min i.v.) was inhibited (85%) by SCH 23390 and (25 %) ICI 118551, but not changed by atenolol, idazoxan or S-sulpiride ; however, after the removal of the autonomic tone with chlorisondamine or pithing, SK&F 87516 evoked an increase in blood pressure. In the pithed rat, this effect was biphasic : an initial response reaching a maximum (15 ± 1 mm Hg, n = 10) after 3 min of infusion and then a second further 16 ± 2 mm Hg rise in pressure which appeared during the last 3 min of the infusion. Idazoxan reversed the first phase to a hypotension (-5 ± 1.6 mm Hg, n = 7), and inhibited slightly (25 %) the secondary hypertensive response, whereas methysergide abolished only the latter effect. In contrast SCH 23390, enhanced by 200 % the pressor effects of SK&F 87516. The combination of idazoxan and SCH 23390 abolished, both the hypotensive phase of SK&F 87516 seen after idazoxan, and the enhancement of the pressor response observed after SCH 23390. Finally, the hypotensive response (-52.3 ± 2.3, n = 14) evoked by fenoldopam in intact rats was inhibited (50%) by SK&F 87516 (10 µg/kg/min).

In conclusion, SK&F 87516 produced a decrease in mean carotid blood pressure in intact pentobarbitone anaesthetised rats which was mediated by a concomitant stimulation of DA_1 and β_2-adrenoceptors. In pithed rats, the pressor effects of this compound results mainly from the activation of vascular α_2-adrenoceptors and $5\text{-}HT_2$ receptors. The development of the "tolerance" to the hypotensive effects to SK&F 87516, in intact rats, is possibly due to the pressor mechanisms as well as by the property of SK&F 87516 to antagonize functionally the hypotensive activity of the DA_1 agonist, fenoldopam.

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GR 43175 - A SELECTIVE AGONIST FOR FUNCTIONAL 5-HT_1-LIKE RECEPTORS IN DOG SAPHENOUS VEIN

P.P.A. Humphrey*, W. Feniuk, M.J. Perren, A.W. Oxford, I.H. Coates, D. Butina, R.T. Brittain and D. Jack, Glaxo Group Research Ltd., Ware, Herts. SG12 0DJ

It has been suggested that 5-HT_1-like receptors are heterogeneous (Bradley et al., 1986) but their definitive classification awaits the identification of selective compounds in functional studies. We now describe the actions of a novel, selective agonist, GR 43175 (3-[2-dimethylamino]ethyl-N-methyl-1H-indole-5 methane sulphonamide), in a range of isolated tissues, containing different 5-HT receptors (see Table 1), which were prepared as described previously (Feniuk et al., 1979, 1983, 1985; Ireland & Tyers, 1987).

In the dog isolated saphenous vein, GR 43175 was a potent agonist, being only 4-7 times weaker than 5-HT at causing contraction and at inhibiting the contractile response to electrical field stimulation. In contrast, GR 43175 was devoid of both agonistic and antagonistic activity (vs 5-HT) in the cat isolated saphenous vein, rabbit isolated aorta and rat vagus nerve (Table 1).

Table 1: Agonist potencies of 5-hydroxytryptamine (5-HT) and GR 43175 in a range of isolated tissues containing different 5-HT receptors

Receptor	Preparation	Response	EC_{50} 5-HT (nM)	EC_{50} GR 43175 (nM)
5-HT_1-like	Dog saph. vein	Contraction*	98 (55-174)	670 (330-1343)
5-HT_1-like	Dog saph. vein	Inhibition**	11 (4.4-27)	40 (19-87)
5-HT_1-like	Cat saph. vein	Relaxation***	44 (21-90)	inactive (> 10,000)
5-HT_2	Rabbit aorta	Contraction*	2440 (1150-5180)	inactive (> 50,000)
5-HT_3	Rat vagus nerve	Depolarisation	2260 (1370-3740)	inactive (> 100,000)

Values are means (95% confidence limits) from at least 4 experiments.
* In presence of (all 1 μM) atropine, phentolamine, mepyramine, ketanserin (not aorta).
** Inhibition of electrically induced contractions in presence of atropine, mepyramine, cyprohetadine (all 1 μM), cocaine (3 μM), indomethacin (2.8 μM).
*** Relaxation of cat saphenous vein contracted with α-methyl 5-HT (10 μM).

The contractile effect of GR 43175 in the dog isolated saphenous vein was resistant to antagonism by atropine, phentolamine, mepyramine, ketanserin, MDL 72222 and cyanopindolol (all at 1 μM). Methiothepin (0.1 μM) antagonised the contractile effect of 5-HT and GR 43175 (but not the thromboxane A_2 mimetic, U-46619) with concentration ratios of 15 ± 6 and 18 ± 8 (mean ± s.e. mean from 4 and 5 experiments respectively).

We suggest that GR 43175 is a selective agonist at the 5-HT_1-like receptors which occur pre- and post-junctionally in the dog saphenous vein and that these receptors are different to the 5-HT_1-like receptors which mediate smooth muscle relaxation in the cat saphenous vein.

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THE EFFECT OF ENDOTHELIAL REMOVAL ON CONTRACTION OF CANINE CORONARY ARTERY RINGS

A.D.McHarg * & R.M.Wadsworth, Department of Physiology and Pharmacology, Strathclyde University, Glasgow G1 1XW.

It has been proposed that endothelial damage of the large coronary arteries may contribute to the intense constriction of the large coronary arteries observed clinically in coronary vasospasm (Lamping et al, 1985). The aim of these experiments was therefore to investigate the effect of endothelial removal on the concentration response curves to 5-HT, adrenaline and U46619, the thromboxane mimetic.

Rings (2-3 mm long) of the left circumflex coronary artery from greyhounds or beagles were suspended on parallel wire hooks under a resting tension of 1.5 g. The rings were placed in a physiological salt solution containing propranolol (1μM), cocaine (10μM) and flurbiprofen (6μM). The endothelium was removed by gently rubbing the lumen with a metal rod and the functional integrity of the endothelium was confirmed in each ring by the presence or absence of relaxation induced by acetylcholine (1μM) during contraction with adrenaline (5μM).

In Ca-HEPES buffer, 5-HT (1-50μM), U46619 (1-10nM) and adrenaline (0.1-10μM) concentration response curves of greyhound coronary arteries were not affected by endothelial removal (Table 1). When expressed as force per cross sectional area, contractile responses to adrenaline and U46619 in endothelium intact arteries were identical in greyhounds and beagles using Ca-HEPES buffer (pD_2 and E_{max} values were compared using the Mann-Whitney test).

Table 1. pD_2 and E_{max} values for adrenaline, U46619 and 5-HT contractions in endothelial intact (+E) and rubbed (-E) greyhound coronary artery rings (mean±s.e.m., n=5-10)

	pD_2		E_{max} (g)	
	+E	-E	+E	-E
adrenaline	5.66±0.03	5.92±0.07	2.36±0.29	2.08±0.30
U46619	8.66±0.10	8.63±0.11	3.00±0.64	2.45±0.33
5-HT	5.56±0.10	5.65±0.07	1.51±0.18	1.69±0.24

The beagle coronary arteries, however, differed from the greyhound in that addition of 5-HT caused a predominantly relaxant response when they were precontracted with 40mM KCl. The contractile component of this response was significantly augmented by endothelial removal (Mann-Whitney U-test, $P<0.05$). The adrenaline concentration-response curve in Krebs-Henseleit lay to the left (2 fold) of that in Ca-HEPES buffer. In Krebs there was also no difference in the pD_2 values for adrenaline in control (5.71±0.09, n=7) and rubbed (5.79±0.05, n=7) arteries nor in E_{max}(Student's t-test).

It is concluded that the endothelium has only a minor modulating influence on contraction of canine coronary artery rings.

A.D.McHarg was supported by an SERC CASE award.

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GR 43175 SELECTIVELY CONSTRICTS THE CANINE CAROTID ARTERIAL BED VIA STIMULATION OF 5-HT_1-LIKE RECEPTORS

R.T. Brittain, D. Butina, I.H. Coates, W. Feniuk*, P.P.A. Humphrey, D. Jack, A.W. Oxford and M.J. Perren, Glaxo Group Research Ltd., Ware, Herts, SG12 0DJ.

The selective and novel 5-HT-receptor agonist, GR 43175 (3-[2-(dimethyl amino)ethyl]-N-methyl-1H-indole 5 methane sulphonamide) appears useful in the treatment of acute migraine (Doenicke et al., 1987). We now describe the haemodynamic effects of GR43175 in anaesthetised (barbitone 300 mg/kg i.p.) beagle dogs (7-12 kg). Blood pressure, heart rate and tracheal inflation pressure were recorded using standard techniques. Ascending aortic, common carotid, circumflex coronary and mesenteric blood flows were recorded (in different studies) with electromagnetic flow probes and changes in vascular resistance calculated.

In preliminary studies GR 43175 (1-1000 µg/kg cumulatively i.v.) produced a long-lasting dose-dependent increase in carotid arterial vascular resistance (maximum change 161 ± 32%; values are mean ± s.e. mean from 4 experiments). The dose producing 50% of the maximum response was 36 ± 3 µg/kg i.v. In contrast GR 43175 had little or no effect on blood pressure, heart rate or tracheal inflation pressure.

In other experiments we have examined the haemodynamic effect of GR 43175 in more detail. GR 43175 produced a selective vasoconstriction in the carotid artery bed (Figure 1) which was resistant to antagonism by pretreatment with MDL 72222 (1 mg/kg i.v.), ketanserin (0.1 mg/kg i.v.) and phentolamine (3 mg/kg i.v.) but was markedly attenuated by pretreatment with methiothepin (1 mg/kg i.v.).

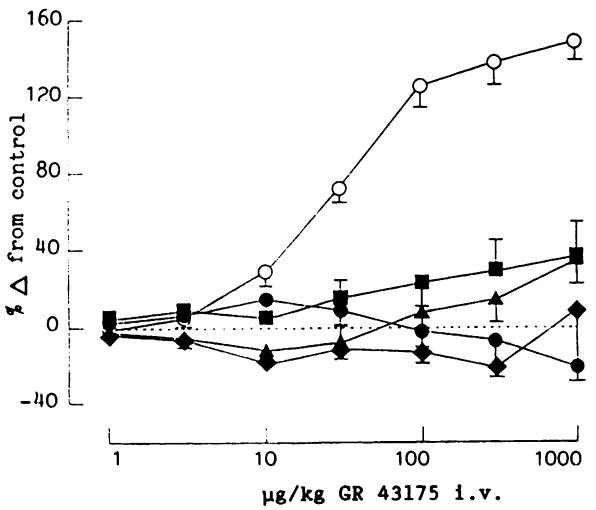

Figure 1: Regional changes in vascular resistance produced by GR 43175

O carotid arterial resistance

● ascending aortic flow

■ mesenteric arterial resistance

▲ total peripheral resistance

◆ coronary arterial resistance

Values are mean ± s.e. mean where n = 3 or more.

We conclude that the carotid arterial vasoconstrictor action of GR 43175 is due to stimulation of 5-HT_1-like receptors similar to those mediating contraction of the dog isolated saphenous vein (Humphrey et al., 1987).

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EXPERIMENTAL CONDITIONS AFFECT THE QUANTITATIVE DEMONSTRATION OF THE EFFECT OF BASAL AND STIMULATED RELEASE OF EDRF

I.A. Dainty*, J.C. McGrath, M.Spedding[1], and A.G.B. Templeton, Autonomic Physiology Unit, Institute of Physiology, Glasgow University, G12 8QQ, U.K., [1]Department of Pharmacology, Syntex Research Centre, Edinburgh, EH14 4AS, U.K.

EDRF has been shown to be the mediator of the action of a number of vasodilators including acetylcholine (see Furchgott 1983). In addition, spontaneous release of EDRF can indirectly modulate the response of vascular smooth muscle to contractile stimuli (Martin et al., 1986). There is often variation between laboratories with respect to the demonstration of both the basal and the stimulated release of EDRF. This suggests that variations in experimental protocol might affect the perceived influence of the endothelium on contractile responses of the smooth muscle. In this study we examine the influence of the initial stretch of the smooth muscle and the degree of agonist-induced tone on the quantitative demonstration of the effects of basal or stimulated release of EDRF.

For length-tension experiments paired aortic ring segments from male Wistar rats were used. When required endothelium was mechanically removed from one of the pair before suspension for isometric tension recordings in Krebs' bicarbonate solution gassed with 5% CO_2 in O_2 at 37°C. The apparatus used allowed accurate measurement of the separation of lower and upper tissue holders and measurement of 'stretch' (length) was estimated from this distance. The tissue holders were separated by an initial distance of 1500μm from which point each tissue was 'stretched' to a final separation of 2700μm. After each alteration of length, the rings were allowed 15 minutes equilibration before administering 1μM phenylephrine (PhE) followed by 1μM acetylcholine (ACh). The resting tension at various degrees of stretch was recorded as was the contractile response to PhE, and any subsequent relaxation to ACh. When examining the effect of induced tone, intact aortic rings were suspended in a similar manner but at a fixed resting tension of 1500mg. After a suitable equilibration period a cumulative concentration response curve to noradrenaline (NA) (0.003-10μM) was obtained. Muscle tone was raised with a fixed amount of NA (0.03, 0.10, or 0.30μM) prior to cumulative addition of ACh (0.03-10μM). This was repeated for each of the three different concentration of NA in each tissue.

With increasing stretch, removal of the endothelium had no effect on the resting tension of the preparations whilst the responses to PhE were markedly increased. Both contractile and relaxant responses of the preparations were dependent on the degree of stretch. Responses to PhE were significantly greater at all resting lengths in the vessel with a disrupted endothelium. The optimum lengths for demonstration of the absolute and proportionate difference in contractile response between the rubbed and unrubbed preparations did not coincide. In rings with an intact endothelium, ACh induced concentration-dependent relaxations. The optimum lengths for demonstration of the absolute and proportionate effect of ACh did not coincide with each other nor (in the case of the latter) with that for demonstration of PhE-induced contractions. The sensitivity of the intact vessels to ACh showed no significant change with time but did vary with the degree of induced tone. When the concentration of NA used was low, the apparent sensitivity of the tissue was high. Increasing the degree of NA-induced tone caused a decrease in sensitivity to ACh. Analysis of correlation showed an inverse correlation which was statistically significant indicating an apparent decrease in tissue sensitivity with increasing initial tone.

These results indicate that the quantitative demonstration of the effects of basal and stimulated release of EDRF can be dramatically altered by the initial stretch and degree of induced tone used in the experimental design. Therefore conditions used to compare vessels from different species, anatomical regions or patho-physiological states, must be carefully chosen to avoid obtaining misleading data and to optimise demonstration of the effects of basal, and stimulated, release of EDRF.

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