CARDIOVASCULAR EFFECTS OF A FISH CAUDAL NEUROPEPTIDE, UROTENSIN II, IN THE PITHED RAT

H. A. Bern[1], A. Gibson, & P. Wallace, Dept. of Pharmacology, Kings College,London, UK and [1]Dept. of Zoology, University of California, Berkeley, USA.

The fish urophysis produces a group of neuropeptides, the urotensins, which were originally classified according to their biological activities (Lederis, 1984). Urotensin II (UII) possessed smooth muscle contracting and osmoregulatory activity in fishes and birds, but was considered to be devoid of activity in mammals. Recently, however, synthetic Gillichthys UII (GUII ; Pearson et al., 1980) has been shown to produce slow relaxations of the mouse anococcygeus muscle (Gibson et al., 1984) and this observation prompted us to examine the pharmacology of GUII on other mammalian systems. In the present study we demonstrate that GUII modifies cardiovascular responses of the pithed rat.

Male Wistar rats (200 - 300 g) were anaesthetised with ether and pithed using the moveable electrode assembly of Gillespie et al. (1970). One jugular vein was cannulated for administration of drugs, and blood pressure and heart rate were monitored via a cannula in a carotid artery. Each animal received gallamine triethiodide (10 mg/kg) to reduce skeletal muscle twitching during spinal stimulation. The thoracic (T_7 - T_{13}) sympathetic outflow was stimulated via the exposed inner wire of the pithing rod using square wave pulses (25V ; 1 ms ; 10 Hz ; 10 s train), leaving an interval of 10 min between trains of stimulation.

GUII (8 - 200 ug/kg) had no effect on basal heart rate of the pithed rat, nor did it affect the rise in heart rate associated with stimulation of the lower thoracic sympathetic outflow. However, GUII did cause an increase in the pulse of the blood pressure trace ; there was both an increase in systolic and a decrease in diastolic pressure, such that there was no overall change in mean arterial pressure. The peak of this effect was reached 10 - 15 min after GUII administration, normal pulse pressure returning after 50 min. In addition, GUII reduced the rise in blood pressure produced by lower thoracic stimulation, the time course of this effect being similar to the action on pulse pressure. GUII (200 ug/kg) also reduced (by 50 - 60 %) the rise in blood pressure produced by administration of noradrenaline bitartrate (160 ng/kg) or Arg-8-vasopressin (8 mU/kg).

GUII shows partial sequence homology with somatostatin. However, somatostatin (200 ug/kg) produced a different pattern of effects on the pithed rat. It had no effect on pulse pressure or on the rise in blood pressure due to sympathetic stimulation. However, basal heart rate and the rise in heart rate following sympathetic stimulation were both reduced.

Thus, GUII produces significant effects on cardiovascular responses of the pithed rat ; it causes an increase in basal pulse pressure and reduces the pressor responses to nerve stimulation and drugs. These results suggest that GUII has vasodilator actions which are probably restricted to a limited number of vascular beds. These actions appear not to be somatostatin-like.

Gibson, A., Bern, H.A., Ginsburg, M., & Botting, J.H. (1984) Proc. Natl. Acad. Sci. USA, 81, 625 - 629.
Gillespie, J.S., MacLaren, A. & Pollock, D. (1970) Br. J. Pharmac. 40, 257 - 267.
Lederis, K. (1984) Frontiers in Neuroendocrinology 8, 247 - 263.
Pearson, D., Shively, J.E., Clark, B.R., Geschwind, I.I., Barkley, M., Nishioka, R.S., & Bern, H.A. (1980) Proc. Natl. Acad. Sci. USA, 77, 5021 - 5024.

THE VASCULAR RELAXANT EFFECTS OF AN ATRIAL PEPTIDE AND NITROPRUSSIDE ARE PARTIALLY ADDITIVE

P.G. Dolamore and J.B. Louttit, Mammalian Physiology Unit,
Glaxo Group Research, Greenford, Middlesex, UB6 OHE.

It has been suggested that atrial natriuretic factors (ANF) and sodium nitroprusside (SNP) share a common mechanism of vasorelaxant action *in vitro* (Winquist *et al*, 1984). Both ANF (Ohlstein & Berkowitz, 1985) and SNP (Schultz *et al*, 1977) have been shown to increase cGMP in vascular smooth muscle (VSM) coincident with their relaxant actions. If ANF and SNP share a common mechanism of action mediated via cGMP then, in contracted VSM in which one treatment has exerted its maximum effect, addition of the other treatment should cause no further relaxation. The present study was designed to test this hypothesis.

Isolated helical strips of rat aorta, bathed in Krebs' solution at 37°C under a resting tension of 1g, were precontracted with KCl (60 mmol l^{-1}). After a maximal relaxation had been obtained in response to either ANF(6-33) or SNP, the maximally effective concentration of the other treatment was applied. If the tissue showed any residual tone after the addition of both ANF(6-33) and SNP, then verapamil (VER) was added to demonstrate that the tissue was capable of further relaxation. The results from these experiments are shown below.

ANF (6-33) = SerLeuArgArgSerSerCysPheGlyGlyArgIleAspArgIleGlyAlaGlnSerGlyLeuGlyCysAsnSerPheArgTyr

Treatment Sequence	1st Treatment	Both Treatments	+Verapamil
1st ANF(6-33), 2nd SNP	26±3.8	42±6.2	92±3.8
1st SNP, 2nd ANF(6-33)	65±6.2	67±6.2	96±1.9

Values are mean percentage inhibitions induced by each treatment (± s.e. n=7). ANF, SNP and VER all added at 1 μmol l^{-1}.

When ANF(6-33) was applied first, the subsequent addition of SNP produced a significant further relaxation (paired t-test, $p<0.05$). However, when SNP was applied first, no further relaxation occurred when ANF(6-33) was added. Furthermore, the combined effect of ANF and SNP was greater when SNP was added first. VER produced an almost complete reduction of residual tone irrespective of the order in which ANF(6-33) and SNP were added.

The addition of acetylcholine (ACh, 1 μmol l^{-1}), which relaxes VSM by stimulating cGMP via endothelium-derived relaxant factor (Rapoport & Murad, 1983), produced a 23±4.1% (n=7) reduction in control KCl-induced contractures. When ACh was added after a maximally effective concentration of ANF(6-33) no additional relaxation was noted. As in the previous experiments, VER produced a large additional relaxation.

These results suggest that, while ANF shares a common mode of action with SNP, part of the relaxation produced by SNP may be due to an additional mechanism.

Ohlstein, E.H. & Berkowitz, B.A. (1985) Hypertension, 7, 306-310
Rapoport, R.M. & Murad, F. (1983) Circ.Res., 52, 352-357
Schultz, K.D. *et al* (1977) Nature, 265, 750-751
Winquist, R.J. *et al* (1984) Eur.J.Pharmac., 102, 169-173

A CONFORMATIONAL STUDY OF MET- AND LEU-ENKEPHALINS

E.C. Griffiths, B. Robson[1] and D. Ward[1], Departments of Physiology and [1]Biochemistry, University of Manchester, Manchester M13 9PT.

Met- and Leu-enkephalins are pentapeptide members of the endogenous opioid neuropeptides (Kosterlitz 1985). Empirical potential energy calculations have been applied to the solution behaviour of these two peptides and the lowest energy conformers calculated have been compared with previous theoretical and experimental studies (Isogai et al.1977; Smith & Griffin 1978; Behman and Deber 1984). From a large number of starting conformations (70 of each), novel gradient minimization techniques incorporating the solvent effects of the Onsager reaction field have been used to identify low energy conformers of Leu- and Met-enkephalins (Finn et al.1984). NMR coupling constants for Met and Phe, and Leu and Phe respectively in the pentapeptides have been calculated for dihedral angles from statistically-weighted stable and metastable conformers.

Both pentapeptides were calculated to share a preferred conformation, which may be described as a β-turn stabilized by 2 hydrogen bonds (1 ← 4 and 2 ← 4) in each case. J(H-NH) coupling constants calculated were closely comparable with those of Behman & Deber (1984).

Table 1 J(H-NH) coupling constants for Met and Phe/Leu and Phe in the enkephalins

Peptide	Amino-acid residue	J(Present Study)	J(Behman & Deber 1984)
Met-enkephalin	Met	7.07 ± 0.5	7.6
Met-enkephalin	Phe	7.31 ± 0.5	7.4
Leu-enkephalin	Leu	7.82 ± 0.5	7.8
Leu-enkephalin	Phe	7.47 ± 0.5	8.0

The β-turn conformation calculated here is in agreement with crystal structure analysis (Cambridge Crystal Data Bank), though the specific hydrogen bonds differ. The 1 ← 4 hydrogen bond of Tyr-OH to C = O of Phe found in the present study agrees with the theoretical calculation of Isogai et al,(1977), which also found that a β-turn structure was preferred. The NMR data from aqueous media (see Table 1) favouring a folded structure are also in agreement. The predicted conformation is also consistent with that produced by replacement of Gly^2 of the pentapeptides by D-Ala. The compact structures of Met- and Leu-enkephalins as calculated in the present study will permit comparison with both rigid opiate structures and more flexible opioid peptide analogues, enabling the structural investigation of specific opioid receptor ligands.

Behman, B.A. & Deber, C.M. (1984) J.Biol.Chem. 259, 14935.
Finn, P.W. et al (1984) Int.J.Pept.Prot.Res. 24, 407.
Isogai, Y. et al (1977) Proc.Nat.Acad.Sci. USA 74, 414.
Kosterlitz, H.W. (1985) Proc.R.Soc.Lond. B225, 27.
Smith, C.D. & Griffin, J.F. (1978) Science 199, 1214.

EFFECTS OF TRH, HISTAMINE AND AN H_2-RECEPTOR ANTAGONIST ON TSH RELEASE FROM THE SUPERFUSED RAT PITUITARY GLAND

C.A. Jones, C.G. Brown, C.K. Atterwill, Dept. of Toxicology, Smith Kline & French Research Ltd., The Frythe, Welwyn, Herts AL6 9AR.

There is evidence that in rats systemic administration of histamine increases basal thyroid stimulating hormone (TSH) release, but reduces the response to thyrotropin releasing hormone (TRH), and these effects may be reversed by histamine H_1- and H_2-receptor antagonists (Maeda & Frohman, 1978; Tuominen et al, 1983). In vitro, at a high concentration (10 mM), histamine has been shown to release TSH from pituitary explants (Bowers et al, 1975) but ranitidine did not alter release from isolated pituitary cells (Yeo et al, 1980). We have investigated direct effects of TRH, histamine and an H_2-receptor antagonist SK&F 93479, and the effect of pre-treatment of rats with SK&F 93479, on TSH release from pituitary glands maintained in vitro.

Freshly isolated whole pituitary glands from male SK&F rats, ≃250 g, were placed in individual superfusion chambers (350 µl volume) and superfused for 5 h by pumping oxygenated Krebs bicarbonate buffer at 37 °C through the chamber at a rate of 200 µl min^{-1}, following the method of Linton et al, 1980. After a 90 min equilibration period, fractions were collected every 10 min. Drugs were added to the superfusion medium either throughout the superfusion period (histamine or SK&F 93479) or during 90-110 mins of the experimental period (TRH or histamine). Whenever histamine was added to the superfusion medium ascorbic acid 20 µM was also included.

Basal TSH release from the pituitary glands resulted in a concentration of 2.2 ± 0.2 ng ml^{-1} (n=33) in the superfusate, as measured by specific radioimmunoassay for rat TSH using reagents from NIAMMD. Stimulation by TRH 5 ng ml^{-1} increased this to 15.1 ± 1.4 ng ml^{-1} (n=18). The response was not significantly different when TRH 1 or 10 ng ml^{-1} was used (15.9 ± 4.9 and 13.0 ± 0.5 ng ml^{-1} respectively, n=3 and 4), suggesting this dose of TRH may cause a maximal response. Histamine (5 µM) had no effect on TSH release when present for a period of 20 min (n=4) nor did it affect basal or TRH-stimulated release when present throughout the superfusion period (n=4).

Basal or TRH-stimulated TSH release from pituitary glands of rats which had been pre-treated with SK&F 93479 (1000 mg kg^{-1} day^{-1} p.o.) for 2 or 4 days (n=8 and 5 respectively) did not differ from control. Similarly addition of SK&F 93479 (10 µM) in vitro to pituitaries from untreated rats did not affect basal or TRH-stimulated TSH release (n=3).

From these studies it appears that, at low concentrations, histamine does not alter the release of TSH, thus confirming the results of Bowers et al (1975) who found no effect at a histamine concentration of 1 mM. The lack of effect of SK&F 93479 agrees with the result of Yeo et al (1980) on the effects of ranitidine and suggests that histamine H_2-receptor antagonists do not alter TSH release.

Bowers, C.Y. et al (1975) In: Stumpf, W.E. & Grant, L.D. (eds) Anatomical Neuroendocrinology, pp333-342, Karger, Basel.
Linton, E.A. et al (1981) J.Endocr. 90, 315-322.
Maeda, K. & Frohman, L.A. (1978) Endocrinology 103, 1903-1909.
Tuominen, R.K. et al (1983) Acta Endocrinologica 103, 88-94.
Yeo, T. et al (1980) Br.J.clin.Pharmac. 10, 171-173.

EFFECT OF BOMBESIN ON RAT PROLACTIN SPECIFIC MRNA LEVELS IN GH_3 CELLS

F. Martin and J. Whelan, Department of Pharmacology, University College Dublin, Dublin 4, Ireland.

GH_3 cells are a clonal rat pituitary cell line which synthesise and secrete prolactin and growth hormone. When thyrotropin releasing hormone (TRH) or bombesin is added to the culture medium, prolactin secretion by GH cells is stimulated. However, TRH also increases the levels of prolactin specific mRNA ($mRNA_{Prl}$) by a transcriptional effect in these cells. It was of interest, therefore, to investigate the effect of bombesin on the $mRNA_{Prl}$ levels in GH_3 cells.

GH_3 cells were maintained in Dulbecco's Minimal Essential Medium supplemented with 15.0% horse serum and 2.5% foetal calf serum in a humidified atmosphere containing 9.0% CO_2. For exposure to hormones cells were place in fresh medium and kept in suspension culture in bacteriological petri dishes for 6-48 hour periods.

$mRNA_{Prl}$ was measured by the cytoplasmic dot hybridization (cyto-dot) technique of White and Bancroft (1982): and also by northern analysis. For cytoplasmic dot hybridization cells were lysed in 5% NP-40 and cytoplasm isolated by centrifugation. The samples of cytoplasm were treated with formaldehyde, serially diluted and applied as dots to a nitrocellulose sheet using a 96-well filter apparatus. After baking and prehybridization the filters were hybridized to ^{32}P-labelled pPrl-1 (a plasmid containing cDNA to rat prolactin mRNA; ^{32}P-labelled by nick-translation, specific activity 5×10^7 dpm. μg DNA^{-1}). After hybridization the filters were washed to high stringency. Specific hybridization was visualised by autoradiography and quantitated by liquid scintillation counting.

Linear increases in $mRNA_{Prl}$ were detected in cytoplasm samples from increasing numbers of GH_3 cells by the cyto-dot technique. No hybridization of the rat prolactin specific cDNA probe to non-pituitary cell cytoplasm was detected. Both bombesin (10 nM) and TRH (27 nM) increased the level of $mRNA_{Prl}$ 24h after their addition to GH_3 cells suspensions, as compared to controls (for bombesin the fold- increase over control was: $1.4 \pm 0.083(5)$ (mean ± SEM (n)), $p<0.05$; for TRH the fold-increase over control was $1.7 \pm 0.187(6)$, $p<0.05$. With TRH, this increase was maintained after 48h (fold-increase over control $1.7 \pm 0.122(6)$, $p<0.05$) but this was not the case with bombesin.

Bombesin has been reported to have potent mitogenic effects on Swiss 3T3 cells. However, we could find no such effect on GH_3 cells either by cell number determinations or by [^3H]-thymidine incorporation measurements.

In further studies we will attempt to characterise further this bombesin induced increase in $mRNA_{Prl}$.

Funded by the Medical Research Council of Ireland. We thank Dr. R. Maurer, University of Iowa for providing pPrl-1.

White B.A. and Bancroft F.C. (1982). J. Biol. Chem. 257, 8569-8572.

PLATELET ACTIVATION INDUCED BY CORONARY ARTERY LIGATION IN THE RABBIT

Deirdre Robertson and G. M. Smith, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen, Scotland.

Many studies have been performed in animals made acutely ischaemic by occlusion of a major coronary artery, and several workers have suggested that platelet activation occurs (1,2). Vik-Mo et al. (3) reported an increased number of circulating platelet aggregates in the coronary sinus during acute coronary occlusion in dogs, and Ohlendorf et al. (4) demonstrated a 30% drop in platelet count within 20 minutes of ligating the left anterior descending (LAD) coronary artery in cats. Elevated plasma levels of TXB_2 have been detected from blood draining from the site of occlusion (5). The aim of this study was to investigate the effects of coronary artery ligation on the platelet count in the rabbit using the technique of continuous *in vivo* platelet counting (6).

Neuroleptanalgesia was induced in male NZW rabbits using diazepam ($5mgkg^{-1}$, i.v.) and fentanyl-fluanisone (Hypnorm, $0.4mlkg^{-1}$, i.m.). BP and heart rate were measured from a cannula in a femoral artery. The platelet count was continuously recorded from a double cannula inserted into the right atria via the right jugular vein. After left thoracotomy, a 6-0 silk suture was placed round either the LAD or circumflex branch of the left coronary artery. Blood samples were obtained from a cannula in a carotid artery for determination of plasma TXB_2 levels by radioimmunoassay. Lead III ECG was recorded. Ligation or sham-ligation was performed after a 30 min equilibration period, and the platelet count continuously monitored for the following 120 mins. The fall in platelet count was taken as a % of that count obtained at the time of ligation (0 min).

In sham-operated animals there was a gradual fall in platelet count with time, which reached a plateau of about 20% at 80 mins and remained at this for the remaining 40 mins. The fall in platelet count upon LAD ligation was significantly different from sham-ligated controls by 20 mins, and reached a maximum of about 40% by 70 mins. Ligation of the circumflex artery resulted in a maximal fall in platelet count of about 30% at 80 mins onwards. Myocardial ischaemia as evidenced by ST-segment deviation was not observed in sham-operated controls but ST-segment depression was seen in 1/4 LAD occlusions and ST-elevation in 2/4 circumflex-ligated animals. TXB_2 was not detected in plasma samples either before or after ligation.

In conclusion, myocardial ischaemia induced by coronary artery ligation in the rabbit results in a fall in the circulating platelet count, suggesting that platelet activation is occurring as a consequence of myocardial ischaemia.

1. Vik-Mo et al., (1974), Scand. J. Haematol., 19, 68.
2. Handin et al., (1978), J. Lab. Clin. Med., 91, 340.
3. Vik-Mo et al., (1978), J. Haematol., 21, 225.
4. Ohlendorf et al., (1980) Thromb. Res., 19, 447.
5. Smith et al., (1980), Can. J. Physiol. Pharmacol., 58, 294.
6. Smith and Freuler (1973), Bibl. Anat., 12, 229.

SK&F 94120 AND MILRINONE, UNLIKE NON-SELECTIVE METHYLXANTHINE PDE INHIBITORS, DO NOT INHIBIT MYOCARDIAL A_1-ADENOSINE RESPONSES

Gillian Bath, R.W. Gristwood, D.A.A. Owen and K.A. Sampford, Smith Kline and French Research Limited, The Frythe, Welwyn, Hertfordshire, U.K.

The methylxanthines theophylline (T) and IBMX, as well as being non-selective phosphodiesterase (PDE) inhibitors, are potent adenosine receptor antagonists. SK&F 94120 and milrinone (M) are selective PDE III inhibitors (Gristwood et al, 1985; Alousi et al, 1983), and we considered it to be of interest to see whether SK&F 94120 and M, like IBMX and T, are adenosine receptor antagonists.

Left atria were obtained from Dunkin-Hartley guinea-pigs. These were incubated in Krebs solution at 37°C, placed under 1g tension and electrically driven (at 1Hz, and threshold + 50% voltage) whilst isometric tension was recorded.

Cumulative concentration-response curves (CRC) were obtained to 2-chloro-adenosine (2Cl.Ado), an adenosine receptor agonist known to exert a negative inotropic effect on the atrial myocardium via an extracellular P_1 purinoceptor displaying characteristics of the A_1 subtype (Collis, 1983). CRC were also obtained to carbachol (Carb), a muscarinic receptor agonist which causes a negative inotropic effect independent of adenosine receptors. For both agonists, repeated CRC were similar to initial CRC.

CRC to 2Cl.Ado and Carb were repeated after a 10 minute pre-incubation with SK&F 94120, M, IBMX, T and the P_1 purinoceptor antagonist 8-phenyltheophylline (8PT). Concentrations of these and their effect on resting force of contraction (Fc) are shown in the Table. Dose-ratios (with 95% Fiducial limits) were calculated by analysis of variance (n=4).

Against 2Cl.Ado, at the concentrations used, 8PT, T, and IBMX significantly displaced CRC to the right indicating antagonism; whereas SK&F 94120 and M displaced CRC to the left indicating potentiation. For all drugs with the exception of T, displacements were parallel and calculated dose-ratios for these are shown in the Table. Due to the non-parallel displacement by T, a dose-ratio was not determined. However, in order to quantify its effects, concentrations of 2Cl.Ado giving a 40% reduction in Fc before and after incubation with T were calculated; these were 0.32 µM and 2.0 µM respectively.

Against Carb, all drugs significantly displaced CRC to the left, indicating potentiation (see Table).

Drug	Dose (µM)	% Increase in resting Fc	Dose-ratios	
			2Cl.Ado	Carbachol
8PT	10	13 ± 8	5.89 (4.4 - 7.92)	0.81 (0.68 - 0.95)
T	140	49 ± 9	Displacement Non-parallel	0.66 (0.51 - 0.86)
IBMX	32	105 ± 33	2.59 (1.76 - 4.1)	0.4 (0.35 - 0.63)
M	32	35 ± 14	0.17 (0.13 - 0.21)	0.47 (0.31 - 0.68)
SK&F 94120	10	12 ± 3	0.39 (0.2 - 0.64)	0.39 (0.29 - 0.51)

The above data indicate that 8PT, T and IBMX inhibited responses to 2Cl.Ado and not to Carb. This is consistent with these acting as adenosine receptor antagonists. In contrast, SK&F 94120 and M did not inhibit responses to 2Cl.Ado (or Carb) thus, indicating that these do not antagonise A1-adenosine receptors.

Alousi, A.A. et al (1983). J. Cardiovasc. Pharmac., 5, 792.
Collis, M.G. (1983). Br. J. Pharmac., 78, 207.
Gristwood, R.W. et al (1985). Br. J. Pharmac., 85, 224P.

INTRAVENOUS ADENOSINE STIMULATES RESPIRATION IN CONSCIOUS ADULT RABBITS

D.C. Buss, P.A. Routledge, A.H. Watt. Department of Pharmacology and Therapeutics, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN.

Adenosine is an endogenous nucleoside whose production appears to be related to the balance between oxygen supply and demand within a tissue. Newby (1984) summarised evidence indicating that adenosine may exert feedback effects tending to restore balance in such supply and demand. Studies of adenosine analogues in adult (Wessberg et al., 1985) or neonatal animals (Hedner et al., 1984) have consistently shown respiratory depression, results which are at variance with Newby's postulated role for adenosine in modulating oxygen delivery. We recently demonstrated that adenosine stimulates respiration in a dose-dependent manner when given by serial intravenous boluses to healthy adult human volunteers (Watt and Routledge, 1985), and proposed that adenosine release, possibly in the carotid body, may play some part in the ventilatory response to hypoxia. In order to further characterise the respiratory stimulant effect of adenosine, an animal model appeared desirable.

We administered adenosine by serial rapid intravenous boluses via an indwelling cannula in a marginal ear vein to 11 conscious adult New Zealand White rabbits weighing 2.14-3.93 kg. Respiration was measured by a semi-quantitative method using a Lectromed respiration transducer secured around the rabbits' thorax. Adenosine was given in an initial dose of 40 ug/kg, increasing in steps of 40 ug/kg, to a maximum of 400 ug/kg. Boluses of adenosine or placebo were administered at intervals of about 1 minute, and the respiratory trace was recorded on an Ormed MX216 recorder. Respiratory rate, respiratory depth and ventilation index were measured. Comparisons were made between stable basal respiration prior to an adenosine injection and the peak respiratory effect which occurred about 2-4 seconds after each injection.

Adenosine produced a significant increase in respiration in the dose range 120 ug/kg to 400 ug/kg. There was an increase in respiratory depth over the dose range 40-400 ug/kg. Respiratory rate changed little but showed a slight decrease at 40 ug/kg and 240-320 ug/kg. There did not appear to be a significant relationship between the logarithm of adenosine dose and the increase in ventilation ($r = 0.050$, $n = 110$, $p = NS$). Placebo injections produced no significant change in respiratory variables.

Adenosine, when administered intravenously to conscious adult rabbits, increases respiration by increasing respiratory depth, as occurs in man (Watt and Routledge, 1985). In contrast to our findings in man the respiratory stimulant effect of intravenous adenosine did not appear to be dose-related; this may be attributable to limitations on injection rate into a vein of the size of the rabbit marginal ear vein in conscious animals. Despite this reservation further study of the respiratory effects of adenosine in rabbits may help clarify mechanisms in man.

The mechanism(s) underlying adenosine's respiratory stimulant effect in the rabbit is unclear, but may at least in part involve carotid body stimulation, as has been demonstrated in the anaesthetised cat (McQueen and Ribeiro, 1981).

Hedner T. et al. (1984). Eur. J. Resp. Dis. 65, 153-156.
McQueen D.S. and Ribeiro J.A. (1981). Br. J. Pharmac. 74, 129-136.
Newby A.C. (1984). Trends Biochem. Sci. 9, 42-44.
Watt A.H. and Routledge P.A. (1985). Br. J. clin. Pharmac. (In press).
Wessberg P. et al. (1985). Eur. J. Pharmac. 106, 59-67.

VASODILATING EFFECTS OF L-PENBUTOLOL. MECHANISM OF ACTION

G. Hamon and M. Worcel. Pharmacologie Cardiovasculaire, Centre de Recherches Roussel-Uclaf, 111 Route de Noisy, 93230 ROMAINVILLE, France.

L-penbutolol is a non-selective beta-blocking drug (Kaiser et al., 1980 ; Kaiser, 1980) recently introduced in clinical practice. Preliminary experiments have shown that this compound relaxes vascular smooth muscle. Therefore, the mechanism of the direct vascular effects of L-penbutolol was explored in vivo on the pithed rat and in vitro on the isolated rat tail artery.

In the pentobarbital anaesthetized (50 mg/kg, i.p.) pithed rat, a perfusion of 0.5 µg/kg/min of angiotensin II (At II) in a jugular vein increased blood pressure by 70 to 80 mmHg. After a 10 to 15 min stabilization period, intravenous injections of L-penbutolol produced a dose-dependent decrease in mean blood pressure (MBP) ; the threshold dose being 100 µg/kg and a 20% decrease being obtained at (mean + S.E.M.) 991 + 87 µg/kg (n=8). This effect on MBP was unaffected by 1 mg/kg atropine, 100 µg/kg methysergide, 200 µg/kg cyproheptadine, 300 µg/kg sulpiride, 100 µg/kg mepyramine, 200 µg/kg ketanserine, 1 mg/kg propranolol or 5 mg/kg indomethacine. Dose-response curves to intravenous injections of At II in the pithed rat model were dose-dependently displaced to the right by a previous 10 min i.v. perfusion of L-penbutolol, the threshold dose being 20 µg/kg/min (n=7 to 8). 100 µg/kg/ min DL-propranolol had no effect.

Helical strips of rat tail artery incubated at 37°C in a normal bicarbonate-Krebs solution without calcium were made to contract isotonically every 15 min with a 100 mM KCl depolarizing solution containing 2.5 mM $CaCl_2$. When reproducible contractile control responses were obtained, L-penbutolol was added in increasing amounts 15 min before each induced contraction. Under these conditions, L-penbutolol inhibited the contractions of the artery strips with an IC_{50} + S.E.M. of (4.35 + 0.27) 10^{-7}M (n=8). In comparison, 10^{-4}M DL-propranolol produced an inhibitory effect of only 15.8% (n=6). Contractile responses of helical strips of rat tail artery were also induced by cummulatively increasing calcium concentration up to 10 mM every 3 min in a bicarbonate-Krebs solution containing 100 mM KCl. Under these conditions, a 15 min preincubation period in the presence of L-penbutolol displaced the calcium-dependent contractile curve to the right :the threshold concentration being 3 $x10^{-7}$M and the pA2 5.95 (n=7). $3x10^{-6}$M DL-propranolol was without effect on this curve (n=6).

Therefore, L-penbutolol appears to have a direct vasodilating effect which could result from a calcium antagonist activity. It must be emphazised that this activity is observed at concentrations therapeutically relevant to plasma levels of L-penbutolol.

We are indebted to Ms B. Depouez and M. D. Auberval for their skillfull technical assistance.

Kaiser, Von J. et al. (1980) Arzneim. Forsch. 30, 420-427.
Kaiser; Von J. et al. (1980) Arzneim. Forsch. 30, 427-432.

INFLUENCE OF ADENOSINE ON HEART RESPONSES TO VAGAL NERVE STIMULATION

E.C. Monteiro & J.A. Ribeiro, Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781 - Oeiras, Portugal.

In vitro studies have demonstrated that purines, in particular adenosine, inhibit responses evoked by nerve stimulation (e.g. Ribeiro, 1978; Stone, 1981). This inhibition in preparations innervated by cholinergic nerve endings is associated with decrease in the output of acetylcholine (Ginsborg & Hirst, 1972). The present work was undertaken to investigate whether adenosine could modify the bradycardic responses induced by vagal stimulation in both cats and rats.

Experiments were performed on pentobarbitone-anaesthetized cats (30 mg/kg into the right femoral vein, cannulated under ether anaesthesia) and rats (60 mg/kg i.p.), supplemented every 1-1.5 h (cats) and 0.5-1 h (rats). The animals were tracheotomized and spontaneously ventilated with room air. A cardiotachometer was used to obtain heart rate (HR) from the electrocardiogram or arterial blood pressure. The animals were bilaterally vagotomized and the vagui stimulated during 10-15 s with intervals of 5 min between stimuli at frequencies of 0.8-12.8 Hz. Drug infusions were made through a catheter introduced via the right jugular vein into the right atrium of the heart. The correct position of the catheter was confirmed by post-mortem examination. Atropine (1 mg/kg i.v.) administered at the end of the experiments abolished the responses to vagal stimulation.

Adenosine (100 µg/0.5ml/min was continuously infused for 1.5 min in the cats and 50 µg/0.5ml/min during 1.5 min in the rats) decreased the bradycardic effect induced by vagal stimulation in both animals. A typical effect of adenosine on the bradycardic effect produced by vagal stimulation in a rat is illustrated in Figure 1. The effect was more evident in the rats and antagonized by theophylline (50 µg/0.5ml/min).

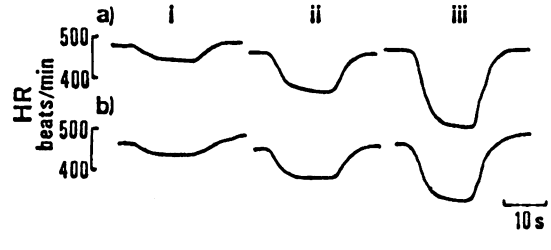


Figure 1 Effect of adenosine on the bradycardic responses induced by vagal stimulation in a rat: (i) 3.2 Hz; (ii) 6.4 Hz; (iii) 12.8 Hz. a) In the absence of adenosine. HR absolute values before stimuli: (i)475; (ii)460; (iii)470 beats/min. b) During adenosine infusion (50µg/0.5ml/min). HR absolute values before stimuli:(i)465; (ii)450; (iii)450 beats/min.

These results suggest that adenosine is able to modulate the bradycardic responses induced by vagal stimulation, an effect probably related to the capacity of adenosine to regulate the output of acetylcholine from nerve endings.

Ginsborg, B.L. & Hirst, G.D.S. (1972) J. Physiol.,Lond. 224, 629-645
Ribeiro, J.A. (1978) Life Sciences 22, 1373-1380
Stone, T.W. (1981) Neuroscience 6, 523-555

ENDOGENOUS ADENOSINE MODULATES TRANSMISSION AT THE NEUROMUSCULAR JUNCTION

J.A. Ribeiro & A.M. Sebastião, Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781 Oeiras, Portugal.

Exogenously applied adenosine decreases the release of transmitter from motor nerve terminals (Ginsborg & Hirst, 1972; Ribeiro & Walker, 1975) and a theophylline-sensitive adenosine receptor at the frog neuromuscular junction has recently been characterized (Ribeiro & Sebastião, 1985). The present work was undertaken to investigate the role of endogenous adenosine on transmission at the neuromuscular junction.

The experiments were carried out at room temperature (22-25°C) on the isolated nerve-sartorius muscle preparation of the frog. The motor nerves were stimulated supramaximally with rectangular pulses of 10 μs duration applied once every 2 s. Intracellular techniques for recording end-plate potentials (e.p.ps) were conventional (see e.g. Ribeiro & Sebastião, 1985). The normal bathing solution contained (mM): NaCl 117; KCl 2.5; Na_2HPO_4 1; NaH_2PO_4 1; $CaCl_2$ 1.8; $MgCl_2$ 1.2 (pH=7.0). Muscle action potentials and twitches were prevented by increasing the Mg^{++} concentration (10-11 mM) in the bath or by adding tubocurarine (0.8-1 μM).

Adenosine deaminase (2.5 IU/ml) and the adenosine receptor antagonists, theophylline (50-200 μM) and 8-phenyltheophylline (2.5-10 μM), reversibly increased the amplitude and quantum content of e.p.ps recorded in high Mg^{++} solutions as well as increased the amplitude of e.p.ps recorded in the presence of tubocurarine (Figure 1). The effect of adenosine deaminase was abolished by the adenosine deaminase inhibitor erythro-9(2-hydroxy-3-nonyl)adenine (EHNA, 5 μM). EHNA (5 μM) by itself was virtually devoid of effect on neuromuscular transmission. The inhibitory effect of adenosine (25 μM), but not that of 2-chloroadenosine (1 μM), was prevented by adenosine deaminase (2.5 IU/ml). The mean increase caused by adenosine deaminase on e.p.ps amplitude was 25±4.3% (n=6), this value being equivalent to the decrease in e.p.ps amplitude caused by 14±5.9 μM of adenosine in the same experiments. Dipyridamole (1 μM) decreased the amplitude of e.p.ps by 21±4% (n=2) and in a lower concentration (0.1 μM) shifted to the left the log concentration-response curve of adenosine but not that of 2-chloroadenosine. In the presence of dipyridamole (0.1 μM) adenosine was about 20 times more potent than in the absence of dipyridamole (n=4).

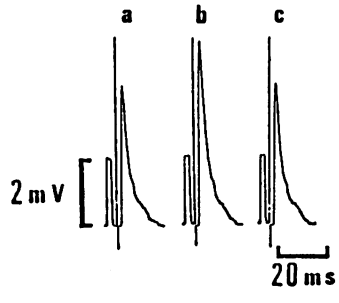


Figure 1. Effect of adenosine deaminase on e.p.ps recorded from a frog sartorius muscle paralysed with tubocurarine (0.8 μM). (a) pre-control; (b) after 20 min in adenosine deaminase(2.5 IU/ml); (c) post-control recorded 25 min after returning to the control situation. Each trace is preceded by a 2 mV,2 ms calibration pulse and is the computed average of 64 successive e.p.ps. Membrane resting potential: 95 mV. The adenosine deaminase-free solution contained equivalent amounts(0.17%v/v) of adenosine deaminase suspension medium.

The results indicate that endogenous adenosine tonically inhibits neuromuscular transmission and suggest that this nucleoside is removed from the synaptic cleft by a dipyridamole-sensitive uptake mechanism.

Ginsborg, B.L. & Hirst, G.D.S. (1972) J.Physiol., 224, 629.
Ribeiro, J.A. & Sebastião, A.M. (1985) Br. J. Pharmac., 84, 911.
Ribeiro, J.A. & Walker, J. (1975) Br. J. Pharmac., 54, 213.

6-NITRO-2-PHENYLISATOGEN SPECIFICALLY ENHANCES THE INHIBITORY EFFECTS OF ADENOSINE ON ELECTRICALLY STIMULATED GUINEA-PIG ILEUM

Diana Wood (introduced by D.F. Weetman), Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP.

Foster et al (1978, 1983) reported that several isatogen derivatives antagonised the relaxant effect of adenosine 5'-triphosphate (ATP) on the isolated taenia of the guinea-pig caecum, a preparation possessing both P_1 and P_2 purinoceptor subtypes (Spedding and Weetman, 1976). Inhibition by adenosine and related nucleotides of the cholinergic twitch response in the transmurally stimulated guinea-pig ileum appears to involve only P_1 purinoceptors (Moody and Burnstock, 1982) and it was therefore of interest to examine the interaction of isatogen derivatives with adenosine in this tissue. From an initial study, 6-nitro-2-phenylisatogen was selected for further experimentation.

Preparations of guinea-pig ileum were arranged for transmural stimulation (0.1Hz 0.2msec pulse width, maximal voltage) in Krebs' solution maintained at 37°C and gassed with 95% O_2, 5% CO_2. Tension changes in the muscle were monitored isometrically. Cumulative concentration response curves for the inhibition of twitch responses by adenosine (0.1-35μM), ATP (0.1-70μM) or noradrenaline (NA, 3nM-3μM) were constructed following exposure of the tissue for 30 min, initially to vehicle (N, N-dimethylformamide, 65mM) and subsequently to increasing concentrations of the isatogen dissolved in the standard concentration of vehicle. For each curve, the EC_{50} value was determined (i.e. the concentration of agonist producing 50% maximum inhibition of twitch). On first exposure to vehicle alone, adenosine, ATP and NA yielded EC_{50} values of 1.45±0.12μM (n=40), 2.11±0.18μM (n=36) and 41.2±6.7nM (n=27) respectively. Reciprocal dose ratios (RDR) were calculated as the ratio: EC_{50} value in the presence of vehicle ÷ EC_{50} value in the presence of isatogen.

6-Nitro-2-phenylisatogen was found to produce significant ($p<0.05$) concentration-related increases in the RDR for adenosine (n=8) and for ATP (n=8) but did not affect the RDR for NA (n=4). The compound at 50μM gave an RDR of 15.78±2.58 (n=8) against adenosine, whilst alone reducing twitch height by 32±5%. The magnitude of the enhancing effect was comparable to that obtained in similar experiments with 0.1μM dipyridamole (RDR 18.78±2.96, n=8), which also reduced twitch height directly, by 19±3%.

Thus, 6-nitro-2-phenylisatogen may be added to the list of drugs which enhance effects of adenosine and ATP and may be of value in the pharmacological investigation of systems involving purinoceptors.

The 6-nitro-2-phenylisatogen used in this study was synthesised by Professor M. Hooper, Department of Pharmaceutical Chemistry, Sunderland Polytechnic.

Foster, H. et al (1978) Br. J. Pharmac. 63, 309-314.
Foster, H et al (1983) Br. J. Pharmac. 79, 273-278.
Moody, C.J. and Burnstock, G. (1982) Eur. J. Pharmac. 77, 1-9.
Spedding, M. and Weetman, D.F. (1976) Br. J. Pharmac. 57, 305-310.

THE PERSISTENCE OF FIELD STIMULATION CAN DICTATE THE ACTION OF NEURONAL 5-HT ANTAGONISTS ON CONTRACTIONS OF GUINEA PIG ILEUM

Amanda J. Bradbury, Brenda Costall, S.J. Gunning, R.J. Naylor & F.D. Tattersall, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP

The neuronal 5-HT antagonists ICS 205-930, MDL72222 and metoclopramide enhance field stimulation (FS)-induced contractions of guinea-pig stomach strips (Buchheit et al, 1985). In the present study, we investigate the actions of these three compounds to modify FS-induced responses of the guinea-pig ileum preparation using a variety of stimulation parameters.

Male Dunkin-Hartley guinea-pigs (450-550g) were killed by cervical dislocation and the gastrointestinal tract removed. Ileal segments (2cm long), obtained 30 cm from the pyloric sphincter, were placed in tissue baths containing oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution at 37°C. 1g tension was applied to the tissues which were allowed to equilibrate for 45 min before being subject to electrical stimulation (platinum wire electrodes placed 6mm apart, 35 volts, 0.2ms pulse width, 0.1-10 Hz) or drug treatments. The significance of differences between treatments was determined using the Mann-Whitney U test ($n = 6-8$).

Discontinuous FS (0.5-10 Hz) of the ileal tissues for 30s at 5 min intervals caused contraction responses which faded rapidly within each 30s period to stabilise at approximately half the initial contraction height. These responses were repeatable at least 6 times over a 4h period. ICS 205-930 (10^{-10} - 10^{-7}M), MDL72222 (10^{-9} - 10^{-7}M) and metoclopramide (10^{-7} - 10^{-5}M) caused concentration-related enhancements of the initial contraction response (to 150-300% of control values at the highest concentrations, $P<0.001$) and the secondary contraction phase (to 160-210% of control values at the highest concentrations, $P<0.001$). Using continuous FS (0.1 Hz) of the tissues, twitch responses were obtained which remained stable throughout a 4h period. ICS 205-930 (10^{-9} - 10^{-7}M), MDL72222 (10^{-9} - 10^{-6}M) and metoclopramide (10^{-7} - 10^{-5}M) failed to significantly modify the twitch response ($P>0.05$). Higher concentrations of ICS 205-930 could reduce the twitch height (by 57% at 10^{-5}M, $P<0.001$). With the exception of the highest concentration of metoclopramide, the compounds tested failed to modify the spontaneous activity of the tissues or the basal tension. Atropine (10^{-8}M) and tetrodotoxin (10^{-7}M) abolished all FS-induced contraction responses ($P<0.001$).

Low contractions of ICS 205-930, MDL72222 and metoclopramide have previously been shown to enhance FS-induced contractions of stomach muscle (Buchheit et al, 1985), and this ability to enhance contractions is extended in the present studies to the ileum. However, in the ileum the demonstration of this action of the three neuronal 5-HT antagonists is shown to critically depend on the persistence of FS, and ability to enhance contractions is only seen when FS is applied in a discontinuous manner. The failure of ICS 205-930, MDL72222 or metoclopramide to enhance contractions when FS was continuous may reflect a rapid desensitisation of 5-HT receptors, an accumulation of released 5-HT, or a rapid depletion of an endogenous substance relevant to their action.

This work was supported by the Medical Research Council and by Glaxo Group Research (Dr. A.J. Bradbury is a Glaxo Research Fellow).

Buchheit, K.H. et al (1985) J. Pharm. Pharmac. in press

ACUTE AND CHRONIC EFFECTS OF IDAZOXAN ON BLOOD PRESSURE AND PLASMA CATECHOLAMINE CONCENTRATIONS OF RATS

M.J. Brown & D. Harland, Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 OHS

Recent studies have suggested that stimulation and antagonism of prejunctional α_2-adrenoceptors can modulate release of noradrenaline (NA) from peripheral sympathetic nerves of the rabbit in vivo (Majewski et al, 1983a,b). Acute iv administration of the α_2-adrenoceptor antagonist idazoxan to anaesthetised rats results in a large increase in plasma NA concentration which is not accompanied by a significant change in blood pressure and is due mainly to a peripheral action of the drug (Brown & Harland, 1984). In this study we have investigated the acute and chronic effects of idazoxan on blood pressure and plasma catecholamine concentrations of unanaesthetised normotensive rats.

Unanaesthetised male Wistar rats (280-350 g) with indwelling carotid arterial and jugular venous cannulae received a 5 min iv infusion of idazoxan 300 µg/kg or saline 1 ml/kg. Mean arterial pressure (MAP), heart rate (HR) and concentrations of NA and adrenaline (A) in plasma of arterial blood samples (350 µl) were measured 0, 30, 120, 420 min and 24 hours after the end of the infusion. A second group of animals were treated for 7 days with a sc infusion of idazoxan (7.5 mg/kg/day) or saline (1 µl/hr) delivered via an Alzet model 2001 miniosmotic pump. Arterial and venous cannulae were implanted on day 4 of the infusion. MAP, HR and plasma catecholamines were measured in each animal at the same time of day on days 4-7 of the treatment.

Acute iv infusion of idazoxan 300 µg/kg produced an immediate increase in plasma NA to 208 ± 30.4% of the basal value (0.363 ± 0.03 ng/ml, n = 6). Plasma A rose from 0.075 ± 0.02 ng/ml to 0.152 ± 0.02 ng/ml. Plasma catecholamines had returned to a concentration not significantly different from pre-idazoxan levels by 120 min after administration. Saline (1 ml/kg) had no effect on plasma NA and A. In both saline and idazoxan treated animals MAP did not change significantly from pre-infusion levels at any time throughout the study. HR rose by 57 ± 12.7 beats/min ($P < 0.005$, n = 6) during iv infusion of idazoxan but had returned to basal values 30 min after the end of the infusion. In control animals HR fell gradually following iv saline ($P < 0.05$, repeat measures analysis of variance). The MAP and HR of animals treated chronically with idazoxan (7.5 mg/kg/day sc) did not differ significantly from saline treated animals over days 4-7 of the experiment. Plasma concentrations of NA and A were similar in saline and idazoxan treated animals on each day. On day 8 under Inactin anaesthesia (100 mg/kg ip) pressor responses to methoxamine 25 µg/kg iv were 36.6 ± 2.4 (n = 7) and 36.8 ± 3.7 mm Hg (n = 8) in saline and idazoxan treated animals respectively. In control animals, MAP fell by 30.1 ± 3.4 mm Hg 10 min after UK 14-304 10 µg/kg iv and HR by 67.7 ± 7.0 beats/min. Corresponding responses of idazoxan treated animals to UK 14-304 were 4.9 ± 0.8 mm Hg and 18 ± 2.5 beats/min.

These results suggest that blockade of prejunctional α_2-adrenoceptors by idazoxan may increase release of NA from peripheral sympathetic nerves of conscious rats. This effect is short-lived and does not influence blood pressure. Long term α_2-adrenoceptor blockade with idazoxan has no effect on blood pressure of normotensive rats.

DH is an MRC Scholar.

Brown, M.J. & Harland, D. (1984) Br.J.Pharmac. 83, 657
Majewski, H. et al (1983a) J.Cardiovasc.Pharmac. 5, 703
Majewski, M. et al (1983b) Eur.J.Pharmac. 93, 255

BINDING TO α-ADRENOCEPTORS OF THE ANTIHYPERTENSIVE AGENT, ADIMOLOL AND ITS ACTIONS ON THE RAT ANOCOCCYGEUS MUSCLE

B.A. Callingham and A.J. Morton, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD.

Adimolol (MEN 935-CL; 1-[3-[[3-(1-naphthoxy)-2-hydroxypropyl]amino]-3,3-dimethylpropyl]-2-benzamidazolinone hydrochloride) is an antihypertensive agent with both beta- and weaker alpha-adrenoceptor blocking activity (Hoefke & Gaida, 1984; Hoefke et al, 1984), but, unlike similar agents such as labetalol, its actions in vivo are of very long duration. Although evidence in the pithed rat indicated that the antihypertensive action was associated with its effects on alpha-adrenoceptors, pA_2-values against phenylephrine and B-HT 920 in rat and rabbit blood vessels were in the range 4.87 to 6.72 (Palluk et al, 1985).

In this study the ability of adimolol to compete with [^{3}H]dihydroergocryptine (DHE) binding to the alpha-adrenoceptors of rat kidney homogenates and with [^{3}H]prazosin (PRA) and [O-methyl^{3}H]yohimbine (YOH) to the $alpha_1$ and $alpha_2$-adrenoceptors respectively in homogenates of rat cerebral cortex, was first explored. Affinity constants of adimolol for alpha-adrenoceptors were calculated from displacement curves constructed by a weighted non-linear fit to the experimental values (Daum et al, 1982). The Kaff values of adimolol were: against DHE; $2.03 \times 10^5 M^{-1}$, against PRA; $1.25 \times 10^5 M^{-1}$ and against YOH; $2.5 \times 10^5 M^{-1}$. These experiments showed that adimolol bound to both $alpha_1$ and $alpha_2$-adrenoceptors, but with relatively low affinities for both sub-groups.

Anococcygeus muscles of the rat were mounted in modified Krebs-Henseleit solution at 37°C and gassed with 95% O_2 and 5% CO_2 and subjected to a resting tension of 1g. When adimolol was applied at concentrations up to 10^{-4}M for 30sec, no contractile response could be elicited, nor did the drug affect the responsiveness of the tissue to the agonist action of noradrenaline, except at 10^{-4}M when a small but long-lasting reduction ($P<0.05$) in the contraction elicited by 10^{-7}M noradrenaline was seen. Thus in vitro it would appear that adimolol is a poor antagonist at post-synaptic $alpha_1$-adrenoceptors.

Any possible action of adimolol on $alpha_2$-receptors was examined on the anococcygeus muscle stimulated to contract by field stimulation with single supramaximal square wave pulses. In the winter clear evidence that the effect of field stimulation was increased by 10^{-4}M adimolol was obtained, indicating a possible antagonist effect on presynaptic $alpha_2$-receptors overcoming any weak action on postsynaptic $alpha_1$. When these experiments were repeated in the summer the response to field stimulation was reduced, due possibly to a seasonal change in the relative proportions of the two sub-groups of adrenoceptors.

It would appear that the in vitro actions of adimolol on alpha-adrenoceptors are relatively weak and difficult to relate to its long duration of action in vivo. Although the actions on the anococcygeus muscle persisted through several changes of bath fluid no evidence for an irreversible interaction was found.

We thank Boehringer Ingelheim Ltd., Bracknell, U.K. for generous support.

Daum, P.R. et al (1982) Br.J.Pharmac. 77, 347-357
Hoefke, W. & Gaida, W. (1984) IUPHAR 9TH. Internat.Congr.Pharmac.Abstr. 1704P
Hoefke, W. et al (1984) N.S. Arch. Pharmac. 325, R54
Palluk, R. et al (1985) N.S. Arch. Pharmac. 329, R79

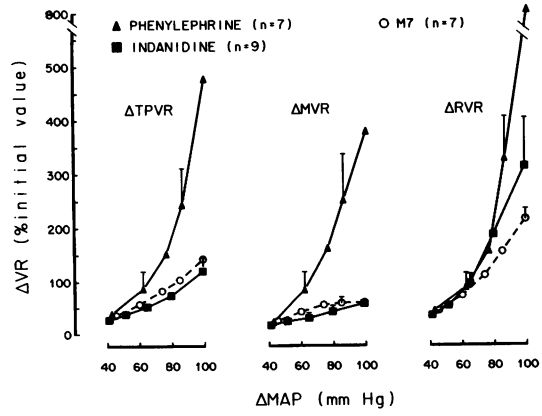
SIMILARITY BETWEEN THE HAEMODYNAMIC PROFILES OF INDANIDINE (Sgd 101/75), M-7 AND UK-14,304, IN PITHED RATS

I. Cavero, P.E. Hicks, J. Lechaire and F. Lefèvre-Borg, Laboratoires d'Etudes et de Recherches Synthélabo, 75013 Paris, France.

In pithed rats, the dose-pressor response curves for M-7 and UK-14,304 (α_2-agonists) are located to the right of and have lower maxima than those for cirazoline and phenylephrine (PHE) (α_1-agonist). These differences were confirmed by a study of the effects of these agonists on the total and regional peripheral vascular resistances (Cavero et al, 1985). In this communication, we compare the hemodynamic effects of indanidine (IND), a partial but selective α_1-adrenoceptor agonist, to M-7, UK-14,304 and PHE, in pithed rats.

Male normotensive Sprague Dawley rats (230-260 g) were anaesthetised with pentobarbitone, pithed, and prepared to measure carotid blood pressure. The abdomen was opened and pulsed Doppler flow probes were placed on the upper abdominal aorta (cardiac output), on the mesenteric and renal arteries and on the terminal aorta (hindquarter blood flow). The vascular resistances (VR) were calculated, by dividing mean carotid blood pressure values by the Doppler blood velocity measurements which were considered to reflect blood flows. Increasing cumulative i.v. doses of IND, M7, UK-14,304 or PHE were injected until the maximal blood pressure response was obtained. The effects on total peripheral (ΔTPVR), mesenteric (ΔMVR), renal(ΔRVR) and hindquarter (ΔHQVR) vascular resistance are expressed as a function of the pressor effects produced by each agonist.

The maximal pressor effects produced by IND, M7, UK-14,304 and PHE were 110 $\pm$ 3 (n=7), 109 $\pm$ 2 (n=7), 104 $\pm$ 4 (n=7) and 128 $\pm$ 5 (n=9) mmHg, respectively. Unexpectly, IND exhibited a similar hemodynamic profile as M7 (see figure) and UK-14,304, but differed from that of PHE.



This hemodynamic finding may explain why pressor effects of IDN, which is a partial but selective α_1-adrenoceptor agonist, are inhibited in a similar manner to those of α_2-adrenoceptor agonists by calcium entry blockers (Timmermans et al 1985).

Cavero et al (1985) Pharmacologist (in press)
Timmermans et al (1985) Naunyn-Schmied Arch Pharmacol 329 404-413

ALFUZOSIN: AN α_1-ADRENOCEPTOR ANTAGONIST WITH PROMISING ANTIDYSRHYTHMIC AND CARDIOPROTECTIVE PROPERTIES

I. Cavero, E. Favennec and A. Grosset*, Laboratoires d'Etudes et de Recherches Synthélabo, 58 rue de la Glacière, 75013 Paris, France.

Antagonists of α-adrenoceptors exert cardioprotective and antidysrhythmic effects (Sheridan et al,1980; Aubry et al, 1985). In this communication we present results indicating that the selective α_1-adrenoceptor antagonist alfuzosin (Cavero et al, 1984) shares these properties.

Cats (2.0-3.5 kg) were anaesthetized with pentobarbitone (40.0 mg/kg i.p. plus 0.1 mg/kg/min i.v.), respired artificially and prepared for heart rate, ECG and blood pressure recordings. Ventricular fibrillation (VF) was provoked by electrical stimulation of the left ventricle with rectangular pulses (1.0 ms , 20.0 Hz) of increasing voltages (0.2 V every 10 s). The VF threshold (VFT) was determined as the minimum current (mA) causing fibrillation. VFT measurements were determined at 40 min intervals three times before and then 10 min after starting an i.v. infusion of saline (0.1 ml/cat/min for 10 min), alfuzosin (0.15 mg/kg/min for 10 min) or an i.v. bolus injection of quinidine sulfate (5.0 mg/kg). In further experiments, the left anterior coronary artery was occluded and 30 min later released. The animals were given i.v. saline (0.5 ml/kg), prazosin (0.5 mg/kg) or alfuzosin (1.0 mg/kg plus 0.03 mg/kg/min throughout the occlusion time) 5 min before the occlusion. Ventricular extrasystoles (VES) were counted and the incidence of VF was noted. Male Wistar rats (350-400 g) were anaesthetized with pentobarbitone (60.0 mg/kg i.p.) and prepared for heart rate, blood pressure and ECG recordings. A 25 min i.v. infusion of saline (0.05 ml/min), alfuzosin (0.66 µg/kg/min) or diltiazem (20.0 µg/kg/min) was started and then 15 min later an i.v. infusion of vasopressin (1 IU/kg/min during 10 min) was installed. The number of VES evoked by vasopressin was counted.

Alfuzosin increased significantly ($p<0.05$, Friedman test) the VFT from 0.30 $\pm$ 0.04 to 0.48 $\pm$ 0.04 mA (n=5). Quinidine enhanced the VFT from 0.22 $\pm$ 0.03 to 0.39 $\pm$ 0.06 mA (n=5). The number of VES during the coronary occlusion was 101 $\pm$ 28 (n=10), 300 $\pm$ 113, (n=10), 27 $\pm$ 17 (n=9, $p<0.05$, Kruskal-Wallis test) in saline, prazosin and alfuzosin-treated cats, respectively. However, during the reperfusion, there was 799 $\pm$ 195, 69 $\pm$ 22 ($p<0.05$) and 273 $\pm$ 109 ($p<0.05$) VES, respectively. The incidence of VF was 6/10, 0/10 ($p<0.05$, Chi^2) and 1/9 ($p<0.05$, Chi^2) in saline, prazosin and alfuzosin-treated cats, respectively. Alfuzosin (16.5 µg/kg total) and diltiazem (0.5 mg/kg total) significantly reduced ($p<0.05$, Kruskal-Wallis test) the number of VES evoked by vasopressin from 539 $\pm$ 92 (n=16, saline group) to 118 $\pm$ 53 (n=6) and 133 $\pm$ 82 (n=6), respectively. None of the compounds studied, modified the hypertensive response or the bradycardia induced by vasopressin.

These results indicate that alfuzosin, like quinidine, can protect the heart against ventricular fibrillation. Furthermore, alfuzosin, like prazosin, reduced dysrhythmias associated with myocardial reperfusion and, like diltiazem, prevented dysrhythmic effects evoked by vasopressin. These antidysrhythmic effects of alfuzosin may be of potential therapeutic value.

Aubry et al (1985) J Cardiovasc Pharmacol 7 Suppl 6 S93-S102
Cavero et al (1984) Br J Pharmacol 81 13P
Sheridan et al (1980) J Clin Invest 65 161-171

AGONIST AND ANTAGONIST EFFECTS OF RX 801080 AT α_1- AND α_2- ADRENOCEPTORS IN RATS

J.C. Doxey, B. Gadie, A.G. Roach, C.F.C. Smith, Diane A. Strachan and I.F. Tulloch, Reckitt and Colman plc, Biology Department, Dansom Lane, Hull, HUB 7DS.

RX801080 ((±)-2-(2,3-dihydro-2-benzofuranyl)-2-imidazoline hydrochloride) is a potent antagonist at prejunctional α2-adrenoceptors in the mouse isolated vas deferens and possesses partial agonist activity at postjunctional α1-adrenoceptors in the rat isolated anococcygeus muscle (Chapleo et al, 1984). Joly et al (1984) described this compound (S9871) as being a more selective α2-adrenoceptor antagonist than idazoxan by virtue of a lower antagonist potency at α1-adrenoceptors; they did not report α1-agonist effects with this compound. We have studied the effects of RX801080 at α1- and α2-adrenoceptors both in vitro and in vivo.

Antagonist activity at α1- and α2-adrenoceptors was assessed against phenylephrine (α1) and p-aminoclonidine (α2) in the rat isolated anococcygeus muscle and rat vas deferens according to the methods described by Doxey et al (1983). In contrast to idazoxan, RX801080 (2-70 μM) contracted some anococcygeus preparations; these tissues were disregarded. In vivo antagonist potencies at peripheral and central α2-adrenoceptors were expressed as AD50 values (μg/kg; cumulative doses producing 50% reversal) against maximal agonist responses to clonidine (100 μg/kg, i.v.) on the stimulation evoked contractions of the vas deferens in pithed rats (Doxey et al, 1983) or guanoxabenz (300 μg/kg, i.v.) induced mydriasis in pentobarbitone-anaesthetised rats (Berridge et al, 1983). Agonist effects of RX801080 were examined on resting diastolic blood pressure (DBP) in pithed rats pretreated with either saline, prazosin (0.1 mg/kg,i.v.) or the selective α2-antagonist RX811059 (0.1 mg/kg,i.v.; Doxey et al, 1984). Values for idazoxan are given for comparison.

RX801080 and idazoxan had pA2 values of 8.23 (8.13 - 8.37) and 8.29 (8.20 - 8.41) at α2-adrenoceptors and 6.17 (6.04 - 6.34) and 6.30 (6.27 - 6.34) at α1-adrenoceptors, respectively. Thus RX 801080 and idazoxan were 115 and 98 times more potent at pre α2- than post α1-adrenoceptors. The AD50 values for RX801080 against clonidine on the vas deferens and the mydriatic response to guanoxabenz were 23±4 and 29±1 μg/kg (n=5/6 per group), respectively. Equivalent AD50 values for idazoxan were 18±2 and 32±3 μg/kg at peripheral and central α2-adrenoceptors. RX801080 did not evoke α2-agonist effects in these test situations. In pithed rats both idazoxan and RX801080 increased basal DBP in a dose-dependent manner. RX801080 was a more potent pressor agent than idazoxan; the ED40 values (cumulative dose producing a 40 mm Hg pressor response) were 7±2 and 270±59 μg/kg and the maximal DBP responses to RX801080 and idazoxan were 81±3 and 62±3 mm Hg, respectively. Idazoxan-induced pressor responses were antagonised by prazosin whereas the effects of RX 801080 were inhibited by both prazosin and RX 811059 indicating that idazoxan is a partial agonist of post α1-adrenoceptors and RX 801080 stimulates both post α1- and α2-adrenoceptors. Due to its marked pressor activity and the fact that these responses were more prolonged than those of idazoxan, the α1-adrenoceptor antagonist potency of RX 801080 was not assessed in vivo.

In accordance with the work of Joly et al (1984), RX 801080 is a potent antagonist of peripheral α2-adrenoceptors. It is also a potent central α2-adrenoceptor antagonist. The pronounced pressor activity of RX 801080 made it impossible to assess α1-antagonist activity and hence α2/α1-selectivity in vivo. Thus, RX 801080 appears to have limitations as a pharmacological tool with which to study α2-mechanisms. In contrast, idazoxan has no detectable agonist activity at α2-adrenoceptors and is a weaker and shorter lasting partial agonist at α1-adrenoceptors. Consequently, idazoxan has a more favourable profile than RX801080 as an antagonist to investigate α2-adrenoceptor mechanisms.

Berridge, T.L. et al (1983). Br. J. Pharmac., 78, 507-515
Chapleo, C.B. et al (1984). J. Med. Chem., 27, 570-576
Doxey, J.C. et al (1983). Br. J. Pharmac., 78, 489-505
Doxey, J.C. et al (1984). Br. J. Pharmac., 81, Proc. Suppl. 181P
Joly, G. et al (1984). Arch. Int. Pharmacodyn., 269, 277-286

UK14304 PRODUCES BOTH CONTRACTION AND RELAXATION IN ISOLATED ARTERIAL PREPARATIONS

M.T. Hall, A.D. McHarg and R.M. Wadsworth, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, and Department of Pharmacology, Monash University, Clayton 3168, Victoria, Australia.

UK14304 causes vasoconstriction *in vivo* by an action primarily on α_2-adrenoreceptors (van Meel et al, 1981). Experiments were carried out to examine its effects on three isolated arterial preparations.

Ring preparations of the rabbit thoracic aorta, rabbit ileal artery and dog coronary artery were set up in physiological salt solution containing propranolol (1μM), cocaine (10μM), and ascorbic acid (20μM) at 37°C and isometric tension was recorded. The α_1-adrenoceptor agonist methoxamine (0.5-200μM) produced contractile effects in all three tissues. UK14304 (0.5-200μM) produced very slowly developing contractions in the rabbit aorta. In both the rabbit ileal artery and dog coronary artery UK14304 (0.5-200μM) on its own had no contractile effect, whether the endothelium was present or not. However, exposure of these two tissues to KCl (20mM) or to the thromboxane mimetic U46619 (0.1nM) often revealed a contractile effect to UK14304. Thus in these tissues a contractile effect of UK14304 can be demonstrated when the tissues are primed by spasmogens.

In contrast to these contractile effects, a relaxation to UK14304 was revealed in the rabbit ileal artery and dog coronary artery if the tissues were precontracted by noradrenaline (5μM), adrenaline (5μM), methoxamine (10μM) or KCl (20mM). In the rabbit aorta, UK14304 in concentrations that had previously caused a contraction (0.5-100μM) caused a relaxation in tissues precontracted by methoxamine (10μM), KCl (20μM) or angiotensin (0.1μM). In none of the three tissues were these relaxations influenced by removal of the endothelium or by administration of flurbiprofen (6μM).

All of the effects of UK14304 observed, both contractile and relaxant, were blocked by prazosin (10nM) but not by idazoxan (0.1μM) indicating that they were mediated by α_1-adrenoreceptors.

Thus UK14304, considered to be a selective α_2-adrenoreceptor agonist *in vivo* appears to cause contractions *in vitro* via α_1-adrenoreceptors, an effect which occurs more readily in the rabbit aorta than in the rabbit ileal artery or dog coronary artery. A relaxant effect of UK14304 has been demonstrated in all three tissues, and this does not seem to be mediated either by EDRF or by a product of the cyclo-oxygenase pathway.

A.D.M. Is supported by a CASE studentship.

van Meel, J.C.A. et al (1981) J. Pharmac.exp.Ther. 219, 760.

AN AUTORADIOGRAPHIC STUDY OF THE α-ADRENOCEPTORS IN RAT AORTA IN THE PRESENCE AND ABSENCE OF ENDOTHELIUM

M. Jacobs and M. Dashwood[1], Department of Pharmacology and [1]Department of Physiology, Royal Free Hospital School of Medicine, London, NW3 2PF. (Introduced by Professor J.R. Hodges)

The contractile responses of rat aorta to α-adrenoceptor agonists, particularly those selective for the α_2-adrenoceptor subtype, are reduced by the presence of the endothelium (Godfraind et al., 1985). The nature of the receptors which mediate aortic smooth muscle contraction and the putative release of the endothelium derived relaxing factor (EDRF), however, are still disputed. Suggestions for the α-adrenoceptors in the aorta have ranged from the α_1-subtype (Digges and Summers, 1983), a mixture of α_1- and α_2-subtypes (Scarborough and Carrier, 1984) to an, as yet, uncharacterised subtype (Beckeringh et al., 1984). Recently, we have found, using ^{3}H prazosin and ^{3}H rauwolscine binding combined with autoradiography, only a single population of α_1-adrenoceptors although the α_2-subtype were readily detected in the rat tail artery (Dashwood and Jacobs, 1985). Nevertheless, it is possible that the variable preservation of the aortic endothelium may account for the present disagreement between the above studies. We have, therefore, examined the identity of the α-adrenoceptors in preparations of rat aorta containing endothelium and denuded of endothelium by autoradiography. The rat tail artery was again used as a control in which α_2-adrenoceptors can be demonstrated (Dashwood and Jacobs, 1985).

Thoracic aortas and tail arteries dissected from 350 g Sprague Dawley rats were cut into 0.5 mm rings. The endothelial layer was removed from half of these rings by gentle rotation on the tip of forceps. The extent of endothelium removal was assessed by ACh relaxation of the noradrenaline contracted ring and by histological staining (Furchgott and Zawadzki, 1980). The aortic rings and untreated rings of tail artery were then frozen in bundles and transverse sections cut and mounted on subbed microscope slides. Paired sections were incubated in 5 nM ^{3}H prazosin (33 Ci/mmol) or 2 nM ^{3}H rauwolscine (80 Ci/mmol) with or without phentolamine. Sections were then exposed to Ultrofilm (LKB, Stockholm) for 4 weeks (Dashwood and Jacobs, 1985).

Autoradiographs of sections of rat aorta containing endothelium or denuded of endothelium showed only specific ^{3}H prazosin binding whereas the sections of tail artery bound both ^{3}H prazosin and rauwolscine specifically. We conclude that α_2-adrenoceptors, as defined by ^{3}H rauwolscine binding, are absent from both the aortic smooth muscle and its endothelium. In particular, the contractile response and putative release of EDRF by selective α_2-adrenoceptor agonists (Miller et al., 1984; Godfraind et al., 1985) are mediated by α_1-adrenoceptors or, as yet uncharacterised, α-subtypes.

M.D. thanks the British Heart Foundation for financial support.

Beckeringh, J.J. et al., (1984) J. Pharmacol. Exp. Ther. 229, 515.
Digges, K.V. & Summers, R.J. (1983) European J. Pharmacol. 96, 85.
Dashwood, M. & Jacobs, M. (1985) European J. Pharmacol. (in press).
Furchgott, R.F. & Zawadzki, J.V. (1980) Nature 288, 373.
Godfraind et al., (1985) Clinical Science 68 (Suppl 10), 655.
Scarborough, N.L. & Carrier, G.O. (1984) J. Pharmacol. Exp. Ther. 231, 597.

[^{3}H]-ADRENALINE RELEASE FROM RABBIT ISOLATED AORTA EVOKED BY FIELD STIMULATION

J. Abrahamsen & O.A. Nedergaard, Department of Pharmacology, School of Medicine, Odense University, J.B. Winsløws Vej 19, DK-5000 Odense C, Denmark

The aim of the present work was to study the release of adrenaline from sympathetic neurones in a blood vessel.

Rabbit isolated thoracic aorta was used. The method described previously (Nedergaard, 1980) was used. Rings of aorta were preloaded with either (-)-[^{3}H]-adrenaline ([^{3}H]-A; 10^{-6}M) or (-)-[^{3}H]-noradrenaline ([^{3}H]-NA; 10^{-6}M). The incubation period was 45 min. After wash-out of the rings with physiological salt solution, these were subjected to electrical-field stimulation (S_1: 1000 pulses; S_2-S_7: 300 pulses; 3 Hz; 0.5 msec; 225 mA).

In the case of [^{3}H]-NA, the initial stimulation-evoked [^{3}H]-overflow (S_1 and S_2; 300 pulses) is atypical representing mainly [^{3}H]-NA of extraneuronal origin (Schrold & Nedergaard, 1977). This was also the case with [^{3}H]-A, since the [^{3}H]-overflows evoked by S_1 and S_2 (300 pulses) were larger than the subsequent ones (S_3-S_5). Hence S_3 was used as the initial "control" response.

The time course of [^{3}H]-overflows evoked by stimulation (S_3-S_7) of aorta preloaded with either [^{3}H]-A or [^{3}H]-NA was compared. In the case of [^{3}H]-A, the [^{3}H]-overflow increased (up to 125% of S_3) with time, while for [^{3}H]-NA the [^{3}H]-overflow remained unchanged. At 10 Hz, no alteration in [^{3}H]-overflows was seen using either [^{3}H]-amine.

The effect of inhibition of uptake mechanisms and of β-adrenoceptors on the time course of stimulation-evoked [^{3}H]-overflows from aorta preloaded with [^{3}H]-A was examined. Cocaine (3×10^{-5}M) + corticosterone (4×10^{-5}M) + (-)-propranolol (10^{-7}M) had no effect on the time course as compared to untreated tissue.

The relationship between number of pulses (30-1000) and stimulation-evoked [^{3}H]-overflow from rings preloaded with either [^{3}H]-A or [^{3}H]-NA was studied. For both [^{3}H]-amines, the [^{3}H]-overflow increased concomitantly to the same degree with the number of pulses.

The stimulation-evoked [^{3}H]-overflow from rings preloaded with either [^{3}H]-amine was independent of frequency (1-10 Hz); except at 30 Hz where the [^{3}H]-overflow derived from [^{3}H]-A was higher than that using [^{3}H]-NA.

Quantitative aspects of individual stimulation-evoked [^{3}H]-overflows from tissues preloaded with either [^{3}H]-A or [^{3}H]-NA were analyzed. The fractional profile of [^{3}H]-overflow did not differ using either [^{3}H]-amine. In both cases, 96% of the [^{3}H]-overflow evoked by stimulation was contained in the initial four 2-min fractions.

The ratio between stimulation-evoked [^{3}H]-overflow and passive [^{3}H]-outflow was compared for [^{3}H]-NA and [^{3}H]-A. At 10 Hz, the ratio was higher for [^{3}H]-NA, while at 3 Hz it was the same.

Rauwolscine (10^{-6}M), an α_2-adrenoceptor antagonist, enhanced the [^{3}H]-overflow 6-fold, while (-)-propranolol (10^{-7}M) had no effect.

It is concluded that [^{3}H]-A is released by electrical-field stimulation from vascular sympathetic neurones in much the same manner as [^{3}H]-NA. Furthermore, rabbit isolated aorta is a useful preparation for the study of the release of transmitters including the modulation of the release mediated via presynaptic adrenoceptors.

Nedergaard, O.A. (1980) J. Cardiovasc. Pharmac. 2, 629-643
Schrold, J. & Nedergaard, O.A. (1977) Acta Physiol. Scand. 89, 129-143

PROPIONATE POTENTIATES TRYPTAMINE INDUCED CONTRACTION OF THE RAT FUNDIC STRIP

R.P. Allaker, J. Greenman & R.H. Osborne, Department of Science, Bristol Polytechnic, Coldharbour Lane, Frenchay, Bristol BS16 1QY

Gilmour et al (1975) showed that millimolar levels of propionate and butyrate were found in periodontal plaque while Singer et al (1980) reported that gingiva exposed to similar concentrations of these acid salts showed a significant inflammatory response. Allaker et al (1985) reported that bacteria implicated in periodontal disease, such as Bacteriodes gingivalis and Fusobacterium polymorphum produce carboxylic acids, including propionate and butyrate, as well as histamine and tryptamine. All of these substances were shown to cause contraction of isolated gut smooth muscle preparations which were being used to characterize inflammatory effects,caused by bacterial extracts, in terms of specific receptor activation. The aim of this study was to characterize the sodium propionate (pH 7.4) induced contraction of the rat fundus (Vane, 1957) as well as investigating an interaction between this acid salt and tryptamine.

Fundic strips were incubated at 35°C in Ringer-Locke solution and dose response curves constructed for propionate, tryptamine and tryptamine in the presence of a sub-threshold dose (2.5mM) of the acid salt. Exposure of the tissue to NaCl (100 mM), sucrose (100mM), the dicarboxylic acid salt Na succinate (1 - 100mM) and the tricarboxylic acid salt Na citrate (1 - 100mM) did not cause contraction thereby suggesting that the contractile effects of propionate were not caused by osmotic and ionic changes. An attempt was made to characterize the nature of the propionate induced contraction by use of the receptor antagonists atropine (1μM), hexamethonium (1μM), mepyramine (1μM), propranolol (1μM), phentolamine (1μM) and N,N-dimethyltryptamine (NN-DMT, 1μM) and the cyclo-oxygenase inhibitor indomethacin (1 - 10μM). All these antagonists were used separately and in the cases of NN-DMT and indomethacin, the tissue was allowed to equilibrate with the antagonist for 30 min prior to retesting the effects of propionate.

Propionate (3 - 100mM) caused dose dependent contractions of the fundus (ED_{50} = 13.5 ± 3.0mM; n = 6) which were neither reduced nor enhanced by prior administration of any of the antagonists listed above. Tryptamine (1 - 75μM) also caused dose dependent contraction of the tissue with an ED_{50} value of 12.6 ± 2.8μM (n = 6). In the presence of a sub-threshold dose (2.5mM) of propionate the tryptamine dose response curve was shifted to the left along the dose axis with the ED_{50} value being reduced to 4.1 ± 2.5μM (n = 6; significantly different with $P < 0.025$) and the maximum response being increased by 48%. NN-DMT (1μM) reduced all tryptamine responses by 50 - 75%.

The cytotoxic (Greenman et al, 1983), inflammatory (Singer et al, 1980) and smooth muscle activating effects of propionate and related monocarboxylic acids suggests that these substances may play an important role in the aetiology of periodontal and related bacterial inflammatory conditions. Thus, propionate may have a direct inflammatory effect as well as potentiating the inflammatory actions of vasoactive amines (produced by host and bacteria) such as tryptamine and histamine.

Allaker, R.P. et al (1985) J. Dent. Res. 64, 147 Abs
Gilmour, M.N. et al (1975) J. Dent. Res. 54, 299
Greenman, J. et al (1983) J. Dent. Res. 62, 110 Abs
Singer, R.E. et al (1980) J. Dent. Res. 59, 670 Abs
Vane, J.R. (1957) Br. J. Pharmac. 12, 344

REPEATED L-DOPA TREATMENT OF CIRCLING RATS INCREASES ROTATION WITHOUT ALTERING DOPAMINE RECEPTOR NUMBERS

S. Boyce, P. Jenner, C.D. Marsden & C. Reavill, MRC Movement Disorders Research Group, University Department of Neurology and Parkinson's Disease Society Research Centre, Institute of Psychiatry and King's College Hospital Medical School, Denmark Hill, London SE5, U.K.

Chronic therapy in Parkinson's disease may result in a loss of therapeutic benefit (Marsden & Parkes, 1976). One reason may be the development of brain dopamine receptor subsensitivity due to continued agonist bombardment. We investigate the effects of repeated L-DOPA administration on both apomorphine-induced circling behaviour and dopamine receptor numbers in rats with a unilateral 6-OHDA lesion of the medial forebrain bundle (MFB).

Male wistar rats (200-260g; Bantin & Kingman) received unilateral 6-OHDA (8ug) lesions of MFB and 3-5 weeks later were tested for their response to administration of apomorphine hydrochloride (0.5 mg/kg sc; 15 min previously). Animals showing more than 20 contraversive rotations per 4 min were used for subsequent drug treatment. Animals received either carbidopa (18.3-24.7 mg/kg/day) or L-DOPA (149-208 mg/kg/day) plus carbidopa (18.7-26.0 mg/kg/day) in daily drinking water for a 28 day period followed by a 4 day washout period prior to testing. Subsequent determination of striatal dopamine content showed a greater than 90% loss in the lesioned forebrain compared to the intact side.

Spontaneous contraversive circling was observed in some rats during L-DOPA plus carbidopa treatment, but disappeared following withdrawal. Carbidopa treated animals showed no spontaneous rotations. Following drug withdrawal administration of apomorphine hydrochloride (0.06-1.0 mg/kg sc) produced greater circling response in rats treated with L-DOPA plus carbidopa compared to animals receiving carbidopa alone (ANOVA: treatment effect $F_{1,103} = 4.01$ $P < 0.05$).

Specific ^{3}H-spiperone binding (Bmax) (0.03-1.0 nM; defined by 10^{-5}M ($\pm$) sulpiride) in striatal membranes was greater in tissue from the lesioned hemisphere (135%) compared to the intact side in animals receiving carbidopa alone (Table 1). Similarly, in animals receiving L-DOPA plus carbidopa, Bmax for ^{3}H-spiperone was also greater in the lesioned side (127%) compared to intact hemisphere. No difference in Bmax or K_D values for ^{3}H-spiperone binding was observed between treatment groups. Specific ^{3}H-piflutixol (0.15-2.0 nM; defined by 10^{-6}M flupenthixol in presence of 10^{-5}M ($\pm$) sulpiride) binding to striatal membrane was unaltered by either prior 6-OHDA lesion or drug treatment (Table 1).

Table 1 Specific ^{3}H-spiperone and ^{3}H-piflutixol binding in L-DOPA treated rats

Treatment	^{3}H-spiperone binding (Bmax pmol/g) lesioned	intact	^{3}H-piflutixol binding (Bmax pmol/g) lesioned	intact
Carbidopa	26.5$\pm$1.6*	19.6$\pm$1.7	84.9$\pm$11.3	87.1$\pm$10.4
L-DOPA	26.4$\pm$0.6*	20.8$\pm$1.1	102.5$\pm$10.4	84.9$\pm$11.3

Values expressed as mean $\pm$ 1SEM; n = 3-5 unless otherwise stated.
* $P < 0.05$ compared to control sides, Student's t test.

Repeated L-DOPA administration to rats with a prior unilateral 6-OHDA lesion of MFB caused enhanced contraversive rotation without altering the number of affinity of dopamine receptors in the intact or denervated forebrain.

Marsden, C.D. & Parkes, J.D. (1976) Lancet 1, 292.

198P

HUMAN PULMONARY VASODILATATION: INFLUENCE OF ENDOTHELIAL CELLS

P.J. Barnes, B. Greenberg and K.J. Rhoden*, Department of Medicine,
Royal Postgraduate Medical School, London W12 OHS.

The release of a relaxant factor from endothelial cells in response to certain vasodilators, such as acetylcholine (ACh) and adenosine triphosphate (ATP), has been described in systemic vessels (Furchgott, 1984) and in pulmonary vessels (Chand and Altura, 1981) of several species, but not in man. Human pulmonary artery has been shown immunocytochemically to contain vasoactive intestinal peptide (VIP) and the structurally related peptide histidine isoleucine (PHI). In animals these peptides are potent vasodilators (Hamasaki et al, 1983) but their effects in human pulmonary vessels have not been studied. We have examined the effects and endothelial dependence of ACh, ATP, VIP and PHI on human pulmonary artery in vitro.

Pulmonary artery ring segments of diameter 2-4mm were obtained from patients undergoing lung resection. Removal of endothelial cells from half the segments by gentle abrasion was confirmed histolgically. Arterial segments were studied in an organ bath containing oxygenated Krebs solution, and were precontrated with 5-hyroxytryptamine or phenylephrine. Relaxtion dose-response curves were obtained for the four agents.

ACh produced a dose-dependent relaxation of pulmonary artery in the presence of endothelial cells (EC_{50} = 13.0 nM, maximal relaxation = 76%) but failed to relax in the absence of endothelial cells. Relaxation was not inhibited by 1 μM propranolol or 10 μM indomethacin, but was completely blocked by 30 μM quininacrine and by 100 μM nordihydroguiaretic acid. ATP was more potent in relaxing intact segments (EC_{50} = 4.7 μM, maximal relaxation = 89%) and the response was also endothelial-dependent. VIP relaxation was similar in the presence (EC_{50} = 2.5 nM, maximal relaxation = 78%) and absence (EC_{50} = 4.5 nM, maximal relaxation = 71%) of endothelial cells. Similarly PHI relaxation was not endothelial-dependent and although maximal relaxation was not obtained PHI appeared to be less potent than VIP.

Endothelial-dependent relaxation has been demonstrated in human pulmonary artery. As lipoxygenase inhibitors prevent this effect, this suggests a lipoxygenase product might be involved. By contrast VIP and PHI potently relax human pulmonary artery independently of endothelial cells and therefore act directly on smooth muscle receptors.

Supported by the Medical Research Council.

Chand, N. and Altura, B.M. (1981) Science 213: 1376-1379.
Furchgott, R.F. (1984) Ann Rev Pharmacol Toxicol 24: 175-197.
Hamasaki, Y, Mojarad, M. and Said, S.I. (1983) J Appl Physiol 54: 1607-1611.

SR 24870, A NOVEL AGENT WITH POSITIVE INOTROPIC ACTIVITY

Barthelemy G., Boutin M.M., Broussy R., Doumaux P. (introduced by Keane P.E.)
SANOFI RECHERCHE, 195 route d'Espagne, 31035 TOULOUSE Cedex France.

SR 24870, ethyl-1 methyl-3 (pyridil-3)-7 triazolo (4,3-a) pyrimidinone-5, is a novel structure which has been shown to exert a potent positive inotropic activity in vivo and ex vivo models. The present study reports the results in anaesthetized dogs of haemodynamic comparison with amrinone and vardax. Dogs were anaesthetized by pentobarbital (5 mg/kg) and chloralose (80 mg/kg). A Millar Mikro Tip catheter pressure transducer was introduced into the left ventricule for the measurement of heart contractility by dp/dt x 1/p, and the preload. Afterload, systemic and pulmonary arterial pressures were measured by traditionnal blood pressure transducers connected to catheters positioned just above the sigmoïd valves, and in the carotid and pulmonary arteries respectively. Cardiac output was determined using a thermodilution system, and femoral and carotid outflow using an electromagnetic flowmeter. Three drugs were injected by intravenous route at equimolar doses : SR 24870 10 mg/kg, amrinone 7,3 mg/kg, vardax 11,4 mg/kg. Results are established with five animals for each drug.

The three molecules had about the same positive inotropic action (+60%) : the effects of SR 24870 and vardax lasted for more than three hours, and two hours for amrinone. Amrinone had no lusitropic effect, but relaxation velocity increased with SR 24870 (+70%) and vardax (+50%) for more than three hours. SR 24870, but not vardax and amrinone, increased cardiac output (+50%) and reduced the total peripheral resistances for more than three hours. Heart rate was not modified by amrinone, but tachycardia of about forty per cent was induced by SR 24870 and vardax. The stroke volume was unchanged by SR 24870 and reduced by about 20% by amrinone and vardax. SR 24870 did not change the diastolic arterial pressure, so heart work was correlated to cardiac output and rose by 50%. Vardax and amrinone did not modify this parameter. Diastolic arterial pulmonary pressure was slightly increased (+20%) by SR 24870, but decreased by amrinone and vardax (-20%). Preload was reduced for more than three hours by -40% with SR 24870, -70% with amrinone and -160% with vardax. Afterload was unchanged by SR 24870 and vardax, but amrinone produced a slight reduction (-15%). Femoral and carotid outflow were not modified by SR 24870 and vardax. No electrocardiographic anomaly appeared with the three molecules.

We conclude that, in our experimental conditions, SR 24870, amrinone and vardax have a similar haemodynamic profile characterized by a potent and long-lasting positive inotropic activity. However, only SR 24870 induced an important increase in cardiac output. Similar cardiovascular effects were also seen after intraduodenal treatment by the same dose of 10 mg/kg SR 24870.
The mecanism of the action of SR 24870 is not well established, but it is different from that of catecholamines and digitalics : preliminary biochemical studies reveal an inhibitory effect on dog heart CAMP phosphodiesterases, a good affinity for A1 adenosine receptors, and an interaction with calcium-dependent process.

200P

EFFECTS OF ISOPRENALINE ON CONTRACTILE FORCE AND ON THE CYCLIC AMP LEVEL IN THE ISOLATED HUMAN RIGHT ATRIUM

O.-E.Brodde, K.Ikezono*, M.C.Michel, X.L.Wang and H.-R.Zerkowski
Med.Klinik&Poliklinik,Div.Renal&Hypertensive Diseases and Div.Thoracic and Cardiovascular Surgery, University of Essen,D-4300 Essen,FRG

By radioligand binding studies it has been demonstrated that in human right atrium β_1- and β_2-adrenoceptors(R) coexist (Brodde et al., 1983;Stiles et al.,1983). Both β-R subtypes seem to be coupled to the adenylate cyclase (Brodde et al.,1984), although this has been recently challenged (Waelbroeck et al.,1983). We have determined on the isolated electrically driven muscle strip of human right atrium the time course of the effects of isoprenaline(IPN) on contractile force and on the cAMP content to find out whether also in human heart positive inotropic effects induced by β-R agonists are mediated by the cAMP system.

Human right atrial appendages were obtained from patients undergoing elective coronary bypass grafting. The atria were set up in Krebs-Henseleit solution at 37°C and gassed with 5% CO_2 in O_2. Tension responses of the electrically driven atria (1 Hz, 5 msec, 20% above threshold) were recorded via a strain gauge on a Hellige recorder. The preparations were equilibrated for 2 h with 5 µM phenoxybenzamine; after washout of phenoxybenzamine 0.3 µM IPN was administered and the developed tension was recorded. At certain time intervals after the administration of IPN the muscles were removed from the organ bath and quickly frozen in liquid nitrogen. The muscles were homogenized in 5% trichloroacetic acid and the cAMP content was assessed by the protein binding method of Gilman (1970) as previously described (Brodde et al.,1978).

The basal cAMP content of human right atrium was 1.37±0.23 pmol/mg w. w. (N=16). 0.3 µM IPN caused a rapid increase in cAMP content, which was maximal after 60 sec (2.4fold increase), while contractile force reached its maximum after 120-240 sec. Preincubation of the muscles with papaverine exaggerated the effects of IPN: in the presence of 10 µM papaverine basal cAMP level was increased to 2.48±0.31pmol/mg w.w. (N=8) and 0.3 µM IPN produced significant greater increases in cAMP and contractile force. On the other hand, the β-R antagonist propranolol (0.1 µM) completely abolished IPN induced increase in the cAMP content.

According to these results important criteria required for a mediator role of cAMP (Sutherland et al.,1968) are fulfilled: IPN induced cAMP increases preceded increases in contractile force and contractile as well as cAMP responses to IPN were potentiated by inhibition of phosphodiesterase. It is concluded, therefore, that in human heart increases in contractile force induced by β-R stimulation are mediated by cAMP.

Brodde,O.-E. et al. (1978) J.Mol.Cell.Cardiol. 10,207-219
Brodde,O.-E. et al. (1983) Circ.Res. 53, 752-758
Brodde,O.-E. et al. (1984) J.Cardiovasc.Pharmacol. 6, 1184-1191
Gilman,A.G. (1970) Proc.Natl.Acad.Sci. USA 67, 305-312
Stiles,G.L. et al. (1983) Life Sci. 33, 467-473
Sutherland,E.W. et al. (1968) Circulation 37, 279-306
Waelbroeck,M. et al. (1983) Molec.Pharmacol. 24, 174-182

A FLEXIBLE MICROCOMPUTER CONTROLLED SYSTEM FOR IN VITRO SUPERFUSION EXPERIMENTS

M. Cooper, J.R. Coughlan, S.E. Holt and J.H. Wyllie, Department of Surgery, The Rayne Institute, University College London, London WC1.

We have designed a semi-automated system for controlling electrical stimulation parameters and addition of drugs to an isolated superfused tissue using a cheap computer - the Sinclair Spectrum. The system uses microsolenoid valves (Lee Products) as a means of introducing drugs to the superfusate. The computer controls the valve opening sequence and stimulation parameters via an interface to a modular valve driver and pulse generator (Square One Instruments). This interface interprets signals from the computer as 4-bit patterns. There are 16 such permutations, each controlling an individual operation, e.g. valve 1 on, stimulation on, etc.

Valve control is easily achieved using Sinclair BASIC; stimulation parameters are controlled by calling a machine code routine from the BASIC programme. This gives greater speed and accuracy to pulse width and frequency. The flexibility of this system allows pulse width, pulse number and frequency of stimulation to be changed automatically during the course of the experiment. Only the voltage has to be changed manually.

The system has been used to investigate the action of 5-HT in the mouse bladder, a robust tissue which gives reproducible responses in experiments lasting 15 hours or more, (Holt et al, 1985). The bladder is stimulated with trains of 16 2ms pulses at 10 Hz and 8V every ten minutes throughout the experiment. Various concentrations of 5-HT are added to the superfusate via the valves for ten minutes duration to obtain a concentration-response curve.

Supported by the M.R.C. S.E.H. is a S.E.R.C. scholar.

Holt, S.E., Cooper, M. & Wyllie, J.H. (1985), Eur. J. Pharmac. (in press).

DIETARY LIPIDS AND THE SUSCEPTIBILITY TO DYSRHYTHMIA IN THE RAT HEART

W.F. Dryden and N.H. Tawfik*, Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7

Dietary fatty acids are not only a source of energy, but are also incorporated into the phospholipids of cell membranes. From this location, it is supposed that they can influence cellular activity either by biophysical or pharmacological means (Gudbjarnason & Hallgrimsson, 1979; Stubbs & Smith, 1984). There is, however, conflicting evidence on the effects of polyunsaturated fatty acids on cardiac response to catecholamines in vitro (Logan et al., 1977; Crandall et al., 1982). The present work was undertaken to investigate both in vitro and in vivo the effects of common dietary triglyceride lipids of differing composition on the rhythmicity of the rat heart.

Four groups of male Sprague-Dawley rats weighing 363±53 gm were fed for 10-12 weeks on diets of rat pellets supplemented with 14-18% w/w lipid. Erucic acid deficient rapeseed oil (canola variety - CAN), linseed oil (LIN), sunflower seed oil (SSO) or sheep perirenal fat (SKF) were used. Left atria and papillary muscles from the left ventricle were dissected at sacrifice and stimulated at 1 Hz in an organ bath containing oxygenated Bretag's synthetic interstitial fluid (Bretag, 1969). Threshold concentrations at which spontaneous twitches developed during cumulative concentration-effect studies with either calcium or isoprenaline were noted.

The incidence of dysrhythmia was least in the CAN group and greatest in the SKF group when challenged with calcium. In the presence of increasing concentration of isoprenaline CAN atria had a lower incidence of dysrhythmia, while CAN papillaries required a much higher concentration before developing dysrhythmia, in comparison to other groups. In vivo experiments were performed on similar groups of animals (n=10) anaesthetized with pentobarbitone (60 mg/kg i.p.). The E.C.G. and arterial blood pressure were monitored before and after ligation of the left coronary artery near its origin (Selye et al. 1960). Mortality was greatest in the SSO group (100%; 3 fibrillation, 7 hypotension and bradycardia) while it was least in the CAN group (40%; 2 fibrillation, 2 hypotension and bradycardia). SKF and LIN groups were intermediate (80% and 70% respectively).

While no correlation has been established between the membrane fatty acid composition and the susceptibility of these tissues to dysrhythmia, the results indicate a clear link between the quality of dietary lipid, and myocardial response to inotropic and dysrhythmogenic agents or stimuli.

Supported by Alberta Heart Foundation. NHT is an AHFMR post-doctoral fellow.

Bretag, A.H. (1969). Life Sciences 8, 319-329.
Crandall et al. (1982). Toxicol. Appl. Pharmacol. 62, 152-157.
Gudbjarnason, S. & Hallgrimsson, J. (1979) in Ischaemic Myocardium and Antianginal Drugs. edit. M.M. Winburg and Y. Abiko, Raven Press, New York.
Logan, R.L. et al. (1977). Atherosclerosis 27, 265-269.
Selye, H. et al. (1960). Angiology 11, 398-407.
Stubbs, C.D. & Smith, A.D. (1984). Biochem. Biophys. Acta 779, 89-137.

EFFECTS OF DOPAMINE ON HUMAN VENTRICLE IN VITRO: COMPARISON WITH EFFECTS OF ISOPRENALINE, EPININE AND IBOPAMINE

T.A.H. English[+], R.W. Gristwood, D.A.A. Owen, K.A. Sampford and J. Wallwork[+], Smith Kline and French Research Ltd., The Frythe, Welwyn, Hertfordshire and Papworth Hospital, Cambridgeshire[+], U.K.

Dopamine, a positive inotropic agent which is used clinically in the treatment of congestive heart failure (CHF), increases ventricular contractility in animal species largely via release of endogenous catecholamines (eg Gristwood & Owen, 1983). There is evidence that endogenous cardiac catecholamines are depleted in patients with CHF (Chidsey et al, 1965) and we considered that it would be useful to investigate the effects of dopamine (DA) on ventricular tissue obtained from patients with CHF. In addition, the effects of isoprenaline (ISO), epinine (n-methyl dopamine, EP) and an epinine pro-drug, ibopamine (a di-isobutyric ester of epinine, IB) were investigated for comparison.

Samples of left ventricular papillary muscle were obtained from cardiac transplant recipients (n=5) and from patients with mitral valve disease (n=7).

On electrically driven (at 1 Hz) preparations (incubated at 37°C in Krebs solution) cumulative concentration-response curves were obtained to ISO and then, following washout and recovery, also to DA (n=8). All tissues were very responsive to ISO which, with a threshold concentration of 1×10^{-8}M, caused concentration dependent increases in force of contraction (Fc). The mean maximum increase was 255 ± 65% at 1×10^{-5}M and the geometric mean EC_{50} value was 1.5×10^{-7}M (8.7×10^{-8}M - 2.5×10^{-7}M 95% confidence limits). In contrast all tissues were poorly responsive to DA, increases in Fc being; 0% at 1×10^{-6}M, 13 ± 8% at 1×10^{-5}M, 24 ± 7% at 1×10^{-4}M and 42 ± 9% at 1×10^{-3}M.

The effects of DA, EP and IB were studied in separate preparations obtained from each of 4 patients. EP with a threshold concentration of 1×10^{-6}M caused concentration related increases in Fc. The maximum observed increase at 1×10^{-3}M was 294 ± 81%. The paired comparison showed that EP was significantly more potent than DA, concentrations causing a mean 50% increase in Fc were 9.5×10^{-6}M and 1.1×10^{-4}M respectively. IB up to 1×10^{-4}M caused concentration related, slowly developing, increases in Fc in 3 of 4 tissues. In 2 tissues responses to IB exceeded those of DA at similar concentrations and in 1 tissue IB and EP were equipotent.

The above data indicate that despite being very responsive to ISO, ventricular tissue from CHF patients is only poorly responsive to DA. When tested, tissues were found to be also unresponsive to tyramine (n=5), suggesting that there was not sufficient catecholamines present to elicit a response. Since DA acts largely indirectly this may explain the poor responses. Tissues were, however, found to respond well to EP, which in ventricles from animal species has been found to be equipotent with DA (Gristwood & Owen, 1983). The much greater potency of EP in the present study is likely due to the finding that EP has a larger direct component than DA (Gristwood & Owen, 1983).

The slowly developing responses and evidence in tissue from one patient of an equal potency with EP is consistent with responses to IB caused by hydrolysis to EP.

Chidsey, C.A. et al (1965). Am. J. Med. 39, 442-451.
Gristwood, R.W. and Owen, D.A.A. (1983). Br. J. Pharmac. 78, 30P.

OESTROGEN SECRETION BY PROGESTOGEN-TREATED RAT OVARIES IN VITRO

Jacqueline I. Fox and M.A. Shaw (Introduced by I.E. Hughes), Department of Pharmacology, University of Leeds, Leeds LS2 9JT.

There is evidence that progesterone can act directly on ovarian tissue in vitro to inhibit oestradiol secretion (Schreiber et al, 1980), but little is known about the in vitro effects of synthetic progestogens on oestradiol secretion. This study reports the effects of several progestogens on oestrogen secretion by the rat ovary in vitro.

Pro-oestrus female rats were killed with ether and the ovaries dissected out and incubated as described by Uilenbroek et al (1983) in Medium 199 containing 10 I.U. Pregnant Mare Serum Gonadotrophin. Progesterone, dydrogesterone or norethisterone were added in 20µl ethanol to give a final concentration of 1, 10 or 100µM; one ovary from each rat was used as control. After 4h, the incubation medium was decanted and oestrogen concentrations measured by radioimmunoassay; the oestradiol antiserum used showed 7.5% cross-reaction with oestrone, and thus is not completely specific. Intra- and inter-assay coefficients of variation were 6.8 and 7.8% respectively.

Progesterone tended to inhibit oestrogen secretion at all concentrations. At concentrations of 10 and 100µM, norethisterone also tended to inhibit oestrogen secretion, while dydrogesterone tended to increase it (Table 1). However, due to wide variations, statistical significance only occurred with a concentration of 10µM progesterone.

Table 1 Oestrogen secretion (% control ovary); mean ± s.e.mean, n = 4; *P < 0.05

	Progesterone	Norethisterone	Dydrogesterone
1µM	73.6 ± 23.0	126.5 ± 56.2	121.3 ± 14.1
10µM	57.2 ± 3.9*	76.4 ± 7.4	115.6 ± 26.7
100µM	69.6 ± 27.8	62.4 ± 24.6	189.6 ± 51.3

Although these results did not reach statistical significance, they do suggest that progestogens differ in their effects on oestrogen secretion, and it is interesting that, unlike progesterone or norethisterone, dydrogesterone does not suppress follicular development in vivo (Lenton, 1984). These differences presumably reflect differences in the steroid nucleus: dydrogesterone is a retro-progesterone, and norethisterone a 19-nortestosterone. Further studies are necessary to clarify the relationship between chemical structure and effects on oestrogen secretion.

Dydrogesterone was kindly supplied by Duphar Laboratories Ltd.

Lenton, E.A. (1984) Clin. Endocrinol. 20, 129
Schreiber, J.R. et al (1980) Mol. Cell. Endocrinol. 19, 165.
Uilenbroek, J.T.J. et al (1983) J. Endocrinol. 99, 469.

EFFECT OF LOCAL ANAESTHETICS ON TONE AND MECHANICAL RESPONSES OF HUMAN SAPHENOUS VEIN AND BOVINE CORONARY ARTERY IN VITRO

E.Greenidge and F.A.Wali. Department of Physiology and Anaesthetics Unit, The London Hospital Medical College,Whitechapel,London El.

Local anaesthetics can either relax or contract the vascular smooth muscle,the effects being dependent on the concentration and the type of anaesthetic drugs used.In the present investigation,the effects of 5 local anaesthetics,lignocaine, prilocaine,etidocaine,mepivacaine and bupivacaine,on tone and contractility of human saphenous vein and bovine coronary artery were studied and compared in vitro.

Human saphenous vein was obtained as leftovers from coronary by-pass graft of male patients aged 48-64 years.Coronary artery was obtained from bovine hearts within 20 min of slaughter.Tissues were cut spirally into strips approximately 3 mm wide and 20-30 mm long (Docherty & Hyland,1985;Campbell,Marshall & Winlow,1983;Cheung, 1985).The preparations were set up,under initial tension of 1 g, in separate organ baths containing 20 ml of Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% CO_2 in O_2 .The mechanical responses of vascular smooth muscle were recorded isometrically.

Lignocaine (0.035-5.6 mM) and etidocaine (0.7-4.8 mM) produced relaxation of the human saphenous vein and bovine coronary artery in a dose-dependent manner. Prilocaine (0.1-3.9 mM),mepivacaine (0.04-5.25 mM) and bupivacaine (0.07-2.0 mM) had a dual action at the vascular smooth muscle.At low concentrations,they produced contraction whereas at high concentrations they relaxed the vascular smooth muscle.Table 1 shows summary of the results.

Table 1.Effects of local anaesthetics on tone and mechanical activity of human saphenous vein and bovine coronary artery in vitro.

	Human saphenous vein		Bovine coronary artery		
	(g) Max. Response mean±s.e.	EC50 (mM) * mean±s.e.	Max.Response mean±s.e.	EC50 (mM) mean±s.e.	n
Lignocaine^{r}	0.4±0.1	2.8±0.3	1.2±0.3	1.5±0.2	6
Prilocaine^{c}	0.3±0.1	0.8±0.2	0.4±0.1	0.6±0.1	6
Etidocaine^{r}	0.3±0.1	1.5±0.1	0.3±0.1	1.4±0.1	6
Mepivacaine^{c}	0.4±0.1	1.4±0.1	0.4±0.1	0.9±0.2	6
Bupivacaine^{c}	0.5±0.1	1.2±0.3	0.5±0.1	0.9±0.1	6

c: contraction, r: relaxation, *: EC50 value (concentration to produce 50% of maximum response,relaxation or contraction).Note that lignocaine produced a marked relaxation of coronary artery,compared to its action on human saphenous vein. Etidocaine produced the smallest relaxations in the blood vessels.Among the drugs producing contraction of vascular smooth muscle,bupivacaine was slightly more effective than mepivacaine and prilocaine.

The dual effects of some local anaesthetics have been reported in rat portal vein (Aberg & Wahlstrom,1972) and in human volenteers (Aps & Reynolds,1976).The relaxation/contraction pattern produced by local anaesthetics of vascular smooth muscle has been attributed to stabilization/mobilization of tissue bound calcium(Aberg, 1972).

Aberg,G.(1972). Acta Pharmacol et Toxicol 31 , 46 P
Aberg,G.& Wahlstrom,B.(1972). Acta Pharmacol et Toxicol 31 , 255-266
Aps,C.& Reynolds,F.(1976). Br J Anaesth 48, 1171-1174
Campbell,J.K.,Marshall,R.J.& Winlow,E.(1983). Eur J Pharmacol 86, 217-228
Cheung,D.W.(1985). Br J Pharmac 85, 317-319
Docherty,J.R.& Hyland,L.(1985). Br J Pharmac 84, 573-576

DIFFERENT RECEPTOR SYSTEMS IN INTESTINAL SMOOTH MUSCLE VARY IN THEIR ABILITY TO TRANSLOCATE CALCIUM

Judith M. Hall & I.K.M. Morton, Department of Pharmacology, King's College London, Strand WC2R 2LS.

In intestinal smooth muscle it is well known that many different receptor types may elicit contraction and it seems likely that a number may act through opening a common pool of receptor-operated ion channels (ROCs).

In the present experiments, receptor systems activated by carbachol, histamine, bradykinin, substance P and eledoisin were compared in a matched manner in preparations of intestinal smooth muscle, principally the taenia caeci of the guinea-pig. With regard to contractile actions of the agonists in normal Krebs solution there seemed no very marked differences in their maxima at 37°C, but at 23°C it emerged that carbachol gave a greater response than the other agonists.

Such differences might be attributable to a number of factors so, to investigate one of these, experiments were designed to compare the relative abilities of the agonists to open Ca^{2+}-ROCs or release Ca^{2+} from internal stores. In experiments performed with solutions where Na^{+} was largely replaced by K^{+}, the development of tension was dependent on external Ca^{2+}; but for a given $[Ca^{2+}]o$ the maxima of the agonists differed. For instance, in the guinea-pig taenia caeci at 23°C with 2 mM $[Ca^{2+}]o$ the maximum for carbachol was greater than the maxima of the other agonists; about double those of bradykinin and eledoisin and more than double those of histamine and substance P. These differences may reflect relative ability to open Ca^{2+}-ROCs. Similar differences between agonists have been found in intestinal smooth muscle for tracer measurements of K^{+}-ROC opening, and also for conductance measurements (eg, Benham & Bolton, 1983; Bolton et al., 1981).

The reasons for these differences remain to be elucidated. However, it may be speculated that, even if there were the same quantitative mechanism of excitation, a difference in effective functional reserve could occur at any stage between efficacy on binding through to varying efficiency of coupling of receptors to ROCs or $[Ca^{2+}]$ translocation processes. Nevertheless, later stages of signal amplification eg, propagated action potentials, which are attenuated or abolished by low temperature or depolarization, might under more physiological conditions ensure final contractile responses of effectively equal magnitude.

J.M. Hall has an MRC studentship.

Benham, C. & Bolton, T.B. (1983) Br.J.Pharmac. 80, 409-420.
Bolton, T.B., Clark, J.P., Kitamura, K. & Lang, R.J. (1981) J.Physiol. 320, 363-379.

EVIDENCE AGAINST THE INVOLVEMENT OF ADENOSINE IN ENDOTHELIUM MEDIATED RESPONSES OF RAT AORTA

C.J. Long and T.W. Stone, Department of Physiology, St. George's Hospital Medical School, University of London, SW17 ORE, U.K.

Many vascular relaxants are dependent upon the presence of an intact endothelium for the expression of their action. It is thought that in response to the relaxant agent the endothelium secretes a locally active vasodilatory factor which then causes the smooth muscle to relax. We have recently shown that the endothelium derived relaxant factor (EDRF) is not only active in a cascade perfusion system (indicating that it is a chemical moiety) but that its release is calcium dependent (Long and Stone, 1985a,b). The identity of the EDRF has remained elusive due to the short half life of the factor but it has been suggested that adenosine may play a role since it is endogenous, it is known to induce vasodilatation in many vascular systems (Berne et al., 1976), it is released from the endothelium in response to acetylcholine (ACh) stimulation (Deussen et al., 1985) and most importantly, it is known to act independently of the endothelium in vasodilatation responses (DeMey and Vanhoutte, 1981).

Male Wistar rats (250-350g) were killed by cervical dislocation and the thoracic aorta rapidly removed into Krebs solution. The aorta was cut into a helical strip and mounted in an organ bath under 0.8g tension. De-endothelialisation, where used, was achieved by gentle rubbing with moist filter paper. All precontractions were with 1μM NA.

We have confirmed in this tissue that adenosine (0.1-300μM) acts independently of the endothelium and also that ACh (10nM-10μM) has an absolute requirement on the endothelium for relaxant responses. The adenosine uptake inhibitor dipyridamole (10μM) increased the potency of adenosine by a factor of around 10 whilst ACh responses were unchanged. In experiments in which the tissue was incubated with adenosine deaminase (ADA) at 10 units/ml, there was no significant alteration in the potency of ACh. ADA was also used in a perfusion experiment in which an endothelium bearing aortic segment was perfused with Krebs solution, the effluent of which then superfused a de-endothelialised aortic strip. ADA was added to the perfusate at 1 or 5 units/ml and had no effect on the relaxant action of 1μM ACh. Furthermore the time courses of relaxation due to ACh and adenosine were significantly different.

It is concluded that adenosine plays only a minor role, if any, in ACh-induced relaxation in the rat aorta.

We thank the British Heart Foundation for support.

Berne, R.M., Herlihy, J.T., Schrader, J. and Rubio, R. (1976) In Ionic actions on vascular smooth muscle. ed E.Betz.Springer-Verlag, Berlin pp137-140.
DeMey, J.G. and Vanhoutte, P.M. (1981) J. Physiol. 316, 347-355.
Deussen, A., Moser, G. and Schrader, J. (1985) In Purines: Pharmacology and Physiological Roles. ed. Stone, T.W. Macmillan, London,p.259.
Long, C.J. and Stone, T.W. (1985a) Br. J. Pharm. 84, 152P.
Long, C.J. and Stone, T.W. (1985b) Blood Vessels. In Press.

GASTROINTESTINAL ACTION OF BALSALAZIDE, A NOVEL PRO-DRUG FOR INFLAMMATORY BOWEL DISEASE

D.V. Parke*, K.H. Peh, P. Sacra & B.Y.C. Wan, *Department of Biochemistry, University of Surrey, Guildford, Surrey, GU2 5XH and Biorex Laboratories, Canonbury Villas, London N1 2HB, England.

Balsalazide (BS) is an effective pro-drug for the potential treatment of ulcerative colitis (Chan et al., 1983). BS is cleaved in the gastrointestinal tract to release 5-aminosalicylic acid (ASA, the therapeutic moiety) in man and animals similarly to Sulphasalazine (SZ). The present study investigates the pharmacological action of BS on the rat gastrointestinal tract.

Experimental ulcerative colitis was induced in groups of 10 male Biorex Wistar Rats (BWR) by daily administration of degraded carrageenin (3% in drinking water) and prednisolone (5mg.kg^{-1} s.c.) for 8 days, with concurrent oral administration of BS (200mg/kg) to one group. On day 9 the colon and rectum were excised and fixed in 2% formal saline (FS). The erosion index was a function of the erosion number and area of each erosion and erosion severity. All rats in the control group (no BS) had erosions with a mean score of 2.1$\pm$1.3 (sd). Only 5 out of 10 rats treated with BS had erosions, with a mean score of 0.9$\pm$1.3 ($p < 0.05$). BS considerably reduced the incidence and severity of erosion formation in the colon and rectum.

The effect of BS and SZ on ethanol-induced necrosis in rat colon and rectum was studied. BS and SZ were administered by intubation through the anus to the lumen of the rectum and colon 2h prior to the challenge with 30% ethanol (2ml/rat), followed immediately by the injection of 3ml of air to clear the ethanol. All rats were killed 30 min after ethanol challenge. The colon and rectum were excised, fixed in 2% FS, coded and examined blind macroscopically. The erosion index was calculated for each specimen. Local application of BS or SZ produced a significant inhibition and BS was more effective than SZ.

Table 1 Effect of BS and SZ on ethanol-induced necrosis in the colon and rectum

Group	Treatment (mg.kg^{-1})	n	Erosion index mean $\pm$ sd	Inhibition (%)
Control	0	6	16.0 $\pm$ 2.4	-
BS	200	6	7.7 $\pm$ 4.2	52 ($p < 0.01$)
SZ	200	6	11.3 $\pm$ 3.9	29 ($p < 0.05$)

The effects of BS on ethanol-induced gastric necrosis in rats were studied (Wan & Gottfried, 1985). Oral BS (30, 70, or 140mg.kg^{-1}) produced a dose-related inhibition of necrosis (60, 88 and 92% respectively, $p < 0.001$). Pretreatment with indomethacin (10mg.kg^{-1} s.c.) 75 min prior to BS (70mg/kg orally) significantly ($p < 0.05$) reduced the inhibitory action of BS from 89% (BS alone) to 61% (Indomethacin + BS). SZ (70mg.kg^{-1} orally) alone produced only 26% inhibition ($p > 0.05$).

Chan, R. et al (1983) Dig. Dis & Sci, 28, 609-615.
Wan, B.Y.C. & Gottfried, S. (1985). J.Pharm.Pharmacol (In press).

EFFECTS OF CANNABINIODS ON NEUROTRANSMISSION IN HUMAN AND RAT ISOLATED VAS DEFERENS

P.B. Patra* & R.M. Wadsworth, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

Cannabinoids have widespread effects in the body and these probably occur through interaction with neurotransmitters, although the type(s) of receptor(s) involved are not yet known (Kaymakcalan, 1979; Smith, 1980). The synthetic cannabinoid nabilone is used in medicine as an antiemetic and other compounds related to THC may also have therapeutic potential (Razdan & Howes, 1983).

Pieces of human vas deferens were obtained from the vasectomy clinic. Vasa deferentia were removed from rats and bisected. All tissues were suspended in Krebs-Henseleit solution at 37°C and isometric tension recorded. The nerve supply was stimulated using parallel silver wire electrodes.

In the human vas deferens, nabilone (40-80 µg/ml) usually augmented the contractions evoked by trains of stimuli at 30Hz. The effect of nabilone was inhibited by pretreatment with naloxone (10 µg/ml). Nabilone (40 µg/ml) inhibited contractions produced by noradrenaline. No effect was seen with crude marihuana extract (CME), delta-9 tetrahydrocannabinol (THC), cannabinol (CBN) or cannabidiol (CBD).

In the rat vas deferens, twitches elicited by single pulse stimulation were augmented 2.8 fold by CBD (40µg/ml) and slightly by CME (40µg/ml). This augmentation was confined to the late phase of the twitch and was not seen when trains of stimuli were applied. The effect of CBD was not affected by pretreatment with indomethacin (10µg/ml) but was not seen in the presence of idazoxan (0.1µg/ml). However idazoxan itself augmented twitches, and CBD did not prevent UK 14304 from inhibiting twitches. The effect of CBD was inhibited by naloxone (10µg/ml). Nabilone, THC and CBN had no effect on contractions following twitches or trains of stimuli.

Since augmentation was produced specifically by nabilone in the human and by CBD in the rat vas it is possible that the cannabinoids are acting on specific receptors. Our results suggest that this effect is not exerted through α_2-adrenoreceptors nor endogenous prostaglandins but may involve opiate receptors in the vas deferens.

PBP is supported by ORS and David Livingstone bursaries. We thank NIDA for THC, CME, CBD and CBN, Sister Leckie and Mr Dean for human tissue and Eli Lilly for nabilone.

Kaymakcalan, S. (1979) in Marihuana Biological Effects. Ed G.G. Nahas & W.D.M. Paton, 591
Razdan, R.K. & Howes, J.F. (1983) Med. Res. Revs 3, 119
Smith, C.G. (1980) NIDA Research Monographs 31, 120

EVIDENCE FOR A NORADRENALINE-INDEPENDENT DOPAMINE POOL IN THE DOG MESENTERIC ARTERY

P. Soares-da-Silva, Laboratório de Farmacologia, Faculdade de Medicina, 4200 Porto, Portugal

In previous studies[1,2] we have identified a noradrenaline-independent dopamine pool as defined by selective 6-OHDA depletion of noradrenaline (NA) in the proximal branches of the mesenteric artery but not in the main trunk from the same blood vessel.

An obvious question concerns the cellular localization of this dopamine pool (DA), i.e. if it is found in dopaminergic neurones or in noradrenergic neurones, in a structure not affected by 6-OHDA. Taking advantage of two characteristics of dopaminergic neurones, the absence of dopamine B-hydroxylase (DA B-H) and the presence of an uptake system sensitive to benztropine (BZ) and not demethylimipramine (DMI), experiments have been done in order to further characterize this dopamine pool.

Since the rate-limiting step in catecholamine synthesis is tyrosine hydroxylase, the change in DA content in both segments of the mesenteric artery, proximal branches and main trunk, after DA B-H inhibition would give an estimate of the amount of DA which is substrate for the enzyme. The effects of DA B-H inhibition by disulfiram on DA and NA content were investigated at various times, and the rate constant of DA accumulation in the main trunk ($0.114\ h^{-1}$) was significantly higher than that in the proximal branches ($0.079\ h^{-1}$). However, the rate constant of NA decline after DA B-H inhibition was approximately the same in both segments ($0.0520\ h^{-1}$).

The effects of DMI and BZ on the uptake of exogenous DA in the proximal branches of the mesenteric artery were also studied; only DMI blocked the uptake of exogenous DA.

These results suggest the existence of a non-precursor DA pool in noradrenergic neurones supplying the proximal branches of the dog mesenteric artery, and provide evidence against the presence of independent dopaminergic neurones in this vascular area.

[1]Soares-da-Silva, P. & Davidson, R. (1985) Naunyn-Schmiedeberg's Arch Pharmacol. 329, 253-257.

[2]Caramona, M.M. & Soares-da-Silva, P. (1985) Br.J.Pharmac. in press

ANTAGONISM OF THE VASCULAR PERMEABILITY ACTIONS OF BRADYKININ IN THE RABBIT

J.M.Stewart[1], R.J.Vavrek[1] & E.T.Whalley, Department of Pharmacology, Madical School, University of Manchester, Oxford Road, Manchester M13 9PT and [1]Department of Biochemistry, University of Colorado Medical School, Denver, Colorado, U.S.A.

Two different receptor types for kinins have been described, namely B_1 and B_2 (Regoli & Barabé, 1980). The B_1 receptor is characterised by the selective agonist des-Arg^9-BK and the selective competitive antagonist des-Arg^9-Leu^8-BK. Recent evidence suggests that replacement of proline-7 and phenylalanine 5 & 8 in the bradykinin (BK) stucture (I) by certain D-aromatic residues results in compounds which antagonise the action of BK on certain tissues thought to contain B_2 receptors (Stewart & Vavrek, 1984). This study investigates the effect of some such substituted analogues of BK on the vascular permeability action of BK in rabbit skin, a response believed to be mediated via activation of B_2 receptors (Marceau et al, 1981, Whalley, 1985).

Male New Zealand White rabbits (3.5 kg) were injected with captopril, 1 mg kg^{-1} s.c. 30 minutes later the back was shaved and Evans Blue Dye, 10 mg kg^{-1} injected intravenously. BK, 10^{-10} moles or histamine, 10^{-7} moles were then injected alone or in combination with compounds II-VII (all 10^{-8} moles) intradermally in a volume of 0.1 ml. One hour later the animals were killed, the skin removed from the back and the diameter of the vascular leakage site determined on the internal surface.

BK alone produced a vascular leakage site of 11.3±0.4 mm diameter (n=12). The results for the analogues in combination with BK are shown in the table below.

	1	2	3	4	5	6	7	8	9	a)
I		Arg—	Pro—	Pro—	Gly—	Phe—	Ser—	Pro—	Phe—	Arg	—
II		Arg—	Pro—	Pro—	Gly—	Phe—	Ser-D-Phe-	Phe—	Arg.TFA		0
III		Arg—	Pro—	Pro—	Gly—	Thi—	Ser-D-Phe-	Thi—	Arg.HOAC		89.5
IV		Arg—	Pro—	Hyp—	Gly—	Thi—	Ser-D-Phe-	Thi—	Arg.TFA		70.8
V	D-Arg—	Arg—	Pro—	Hyp—	Gly—	Thi—	Ser-D-Phe-	Thi—	Arg.TFA		100
VI	Lys—	Lys—	Arg—	Pro—	Pro—	Gly—	Thi—	Ser-D-Phe-	Thi—	Arg.TFA	100
VII	Lys—	Lys—	Arg—	Pro—	Pro—	Gly—	Thi—	Ser-D-Pal-	Thi—	Arg.TFA	24.8

Thi=beta-(2-thienyl)-L-alanine; Hyp=L-4-hydroxyproline; Pal=beta-(2-pyridyl)-L-alanine; HOAC=acetic acid salt; TFA=trifluoracetic acid salt; a)% inhibition of response to BK (I) alone, n=4.

Replacement of proline-7 with phenylalanine(D) alone (II) resulted in no antagonism of BK. Insertion of Thi in positions 5 & 8 and elongation of the amino terminal (III, V & VI) produced compounds which antagonised BK. Introduction of Hyp in position 3 (IV) or D-Pal in position 7 (VII) reduced antagonistic action (cf III & VI respectively). Compounds II-VII did not antagonise the action of histamine which produced a vascular leakage site of 12.6 ± 0.3 mm diameter (n=12). These results demonstrate that modification of the BK structure by the insertion of certain D-amino acids in positions 5, 7 & 8 in addition to elongation of the amino terminal results in the production of compounds which antagonise the action of BK on rabbit vascular permeability, a response thought to be mediated via direct activation of B_2 receptors.

Marceau, F. et al (1981) Can. J. Physiol. Pharmac., 59, 921.
Spector, W.G. et al (1958) Pharmac. Rev., 10, 475.
Regoli, D. & Barabé, J. (1980) Pharmac. Rev., 32, 1.
Stewart, J. & Vavrek, R.J. (1984) Proc. of Kinin '84 Savannah. Int. Cong. p134.
Whalley, E.T. (1985) Proc. Brit. Pharmac. Soc. Edinburgh.

SOME EFFECTS OF NICORANDIL ON THE AORTA AND PORTAL VEIN OF THE RAT

S.W. Weir* and A.H. Weston. Department of Pharmacology, Medical School, University of Manchester, Manchester M13 9PT.

In a variety of smooth muscles, the inhibitory effects of nicorandil are often associated with hyperpolarisation, an effect attributed to the opening of membrane potassium (K^+) channels (Inoue et al., 1983, 1984; Kajiwara et al., 1984). However, such inhibition is not always accompanied by a hyperpolarisation (Itoh et al., 1981; Karashima et al., 1982) and the presence of a nitro-group within the structure of nicorandil may also play an inhibitory role (Maruyama et al., 1982). In the present study, the inhibitory actions of nicorandil on rat isolated blood vessels have been investigated.

Whole portal veins and segments of aorta were removed from male Wistar rats (250-350 g) and mounted for isometric tension recording in a MOPS-buffered physiological salt solution (PSS), at 37°C. In portal vein, nicorandil ($5\text{-}500\times10^{-6}$M) abolished spontaneous electrical and mechanical activity and produced a significant concentration-dependent reduction in electrical and mechanical responses to noradrenaline ($0.1\text{-}100\times10^{-6}$M). The appearance of these reduced responses was delayed by up to 47 sec. In aorta, nicorandil ($8\text{-}32\times10^{-6}$M) produced a concentration-dependent inhibition of responses to noradrenaline ($0.001\text{-}1\times10^{-6}$M) with no evidence of a time delay.

In portal vein, nicorandil ($5\text{-}500\times10^{-6}$M) produced a significant rightward shift of the K^+ concentration-effect curve ($5\text{-}80\times10^{-3}$M, added to the PSS) with little reduction of the maximum response. In aorta, nicorandil ($8\text{-}32\times10^{-6}$M) reduced the responses to all concentrations of added K^+.

When aortae and portal veins were loaded with ^{86}Rb for 2 h and the ^{86}Rb was allowed to efflux into Rb-free PSS, the ^{86}Rb efflux rate coefficient became constant after approximately 10 min. Portal veins were then challenged with nicorandil (500×10^{-6}M) for 8 min and a significant increase in the ^{86}Rb efflux rate coefficient was observed. However, when aortae were exposed to nicorandil (32×10^{-6}M) for 8 min, no such increase in the ^{86}Rb rate coefficient was detected.

It is concluded that the primary action of nicorandil in rat portal vein is to open potassium channels thereby producing inhibitory effects by creating a low resistance pathway in the membrane. These actions are similar to those recently reported for nicorandil in guinea-pig taenia caeci and are qualitatively similar to those of the benzopyran derivative BRL34915 (Hamilton et al., 1985; Weir and Weston, 1985). In rat aorta the inhibitory effects of nicorandil do not appear to be associated with the opening of potassium channels.

S.W.W. is in receipt of an SERC Case Award. We acknowledge the help and support of Dr Jan Poloniecki (May and Baker).

Hamilton, T.C. et al (1985) Br. J. Pharmac. in press
Inoue, T. et al (1983) Br. J. Pharmac. 80, 459
Inoue, T. et al (1984) J. Pharmac. exp. Ther. 229, 793
Itoh, T. et al (1981) J. Pharmac. exp. Ther. 218, 260
Kajiwara, M. et al (1984) J. Pharmac. exp. Ther. 230, 462
Karashima, T. et al (1982) J. Pharmac. exp. Ther. 221, 472
Maruyama, M. et al., 1982) Arch. int. Pharmacodyn. 258, 260
Weir, S.W. and Weston, A.H. (1985) J. Physiol. submitted for publication

THE EFFECT OF PULSE-DURATION ON THE RESPONSES OF RAT VAS DEFERENS TO ELECTRICAL FIELD STIMULATION

M.A. Zar, Department of Pharmacological Sciences, Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH

Rodent vas deferens is extensively used as an isolated preparation for pharmacological investigations. A large number of such investigations have involved electrical field stimulation of the isolated preparations. Despite the widespread use of field-stimulated isolated vas deferens preparation, there has been little systematic study of the effect of pulse-duration on its response to field stimulation (Ambache & Zar, 1971; McGrath, 1978). In the present investigation, the prostatic third of the vas deferens, obtained from freshly-killed albino Wistar rats, 300-350g, were suspended between two platinum electrodes in a 2 ml. organ bath containing Krebs-Henseleit solution, bubbled with 95% O_2 + 5% CO_2 mixture, at 37°C. After allowing equilibration period of 30 min, the preparation was subjected to electrical field stimulation (frequency: 8.3 mHz, current: 690mA, pulse-duration: 0.01-2.0ms) and the changes in tension were recorded isometrically. With step-wise increase in pulse-duration from 0.01 to 0.08ms, the tension developed in response to electrical stimulation displayed a corresponding increase, peaking at 0.03ms and remained constant between 0.03 and 0.08ms pulse duration (Response A). With further step-wise increase in pulse-duration from 0.09ms to 1ms the tension developed by the preparation grew paralleling the rise in pulse-duration up to 0.4ms and remained steady thereafter up to 1ms. This further rise in tension, superimposed on response A has been labelled as Response B. Additional increases in pulse-duration from 1.1ms to 2ms evoked a decline in twitch tension; the decline peaked at 1.6ms and remained constant thereafter up to 2.0ms. This inhibitory component in the response to field stimulation has been termed as response C. The tensions developed by A, B and C were 374 ± 71, 219 ± 49 and 161 ± 40 (mg; mean ± SEM; n= 7), respectively. The responses to electrical stimulation were abolished by tetrodotoxin, 0.6μM. The effects of clonidine, guanethidine, phentolamine, propranolol and cooling (30°C) on A, B and C were studied. Cooling (30°C) greatly augmented A, modestly inhibited B and abolished C (mean % ± s.e.mean of control values for A, B and C respectively 246 ± 18, 86 ± 7, and 0.0; n = 4). Both clonidine 10nM and guanethidine, 10μM virtually abolished A and C but exerted a much lower inhibitory effect on B. Phentolamine, 5μM potentiated A and inhibited B and C (mean % ± s.e.mean of the respective control values 124 ± 6, 76 ± 11 and 89 ± 7, n = 4). Propranolol, 5μM did not have any significant effect on A, B and C.

The results indicate that at least three different populations of nerve terminals are distinguishable in the prostatic third of rat vas deferens, on the basis of their respective neuroeffector sensitivities to physical and chemical stimuli.

Ambache,N. and Zar,M.A. (1971) J.Physiol, 216, 359-389
McGrath,J.C. (1978) J.Physiol., 283, 23-39

A MODEL OF ASYMMETRY AND CIRCLING BEHAVIOUR FOLLOWING ASYMMETRIC LESION OF MOUSE MEDIAL RAPHE NUCLEUS

Amanda J. Bradbury, Brenda Costall, R.J. Naylor & E.S. Onaivi, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

5-HT agonists and antagonists affecting different populations of peripheral 5-HT receptors are being rapidly developed using many different test systems (Humphrey, 1984), but models available for assessing their potential to modify central mechanisms are more limited (see Green, 1984). Here we describe the development of a simple circling model in the mouse based on limbic serotonergic dysfunction following medial raphe nucleus (MRN) lesions.

Male BKW mice, 40-50g, were subject to standard stereotaxic surgery for asymmetric electrolesion of the MRN (0.64 mm diameter electrode, insulated excepting at the tip, angled 30°C posterior for lowering to F-4.7, Vert. 5.4, Lat. 0.1, atlas of Slotnick & Leonard, 0.5 mA for 5s). Circling and asymmetric behaviour, apparent on recovery from anaesthetic, were measured daily for 60 days. Circling was assessed in circular glass cages, 20 cm diameter, as complete rev/min. Asymmetry was scored 1-4 which ranged from weak body bending with movements in wide circles, predominantly in one direction, to a tight posture, nose to tail, with pivotal circling. Some animals were killed on day 6 for determination of levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic and (HVA), noradrenaline (NA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the striatum and limbic tissue using HPLC with electrochemical detection.

An intense contralateral circling response (25-30 rev/min when disturbed) with maximum score asymmetry developed on recovery from anaesthetic and persisted for 8 or 9 days. The response gradually reduced to approximately 15 rev/min, score 1-2 asymmetry, which was maintained for at least 60 days. Biochemical determinations showed significant reductions in 5-HT and 5-HIAA levels in the left limbic system (side of lesion) with reduced DA, DOPAC and HVA in the right striatum.

Table 1. Changes in limbic and striatal biochemistry in the left (L) and right (R) hemispheres following lesion of the left side of the MRN.

Brain region	5-HT pg/mg	5-HIAA pg/mg	DA ng/mg	DOPAC pg/mg	HVA pg/mg
Control	397±64	196±17	11.17±0.8	751±39	872±16
L Striatum	-15%	-10%	+2%	-11%	-4%
R Striatum	-33%*	-18%	-33%**	-29%**	-23%
Control	694±42	173±22	3.32±31	359±29	295±26
L Limbic	-23%**	-39%**	-14%	-28%*	-21%
R Limbic	-15%	-30%	-17	-33%**	-9%

n = 5. S.E.M.s given. Significant reductions in levels indicated as *P<0.05, **P<0.01 (Student's 't' test).

It is concluded that contralateral asymmetry and circling which develops after asymmetric lesion of the MRN may reflect removal of an inhibitory 5-HT input to the limbic system and a decreased DA activity in the opposite striatum. The reliable, marked and persistent response obtained is being used to elucidate 5-HT agonist/antagonist action on 5-HT receptors modulating motor activity.

Green, A.R. (1984) Neuropharmacology 23, 1521-1528
Humphrey, P.P.A. (1984) Neuropharmacology 23, 1503-1510

BEHAVIOURAL RESPONSES TO NICOTINE MEASURED IN RATS WITH SELECTIVE LESIONS OF THE HIPPOCAMPAL 5-HT SYSTEM

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

Previous studies in this laboratory have shown that habituation of the adrenocortical response to an aversive stimulus appears to be associated with a regionally-selective reduction in hippocampal 5-hydroxytryptamine (5-HT) and that nicotine can attenuate this process of adaptation (Benwell and Balfour, 1982). In the present study the effects of nicotine and of selective lesions of the hippocampal 5-HT system on behavioural habituation to an elevated X-maze, incorporating open runways as an aversive stimulus, have been examined.

The 5-HT pathways innervating the hippocampus were lesioned by the stereotaxic administration of 5, 7-dihydroxytryptamine using a procedure described by Quik and Azmitia (1983). Control animals received injections of the vehicle (0.02% ascorbic acid in saline). Starting 14 days after the stereotaxic injections both lesioned and sham-injected rats were given daily subcutaneous injections of nicotine (0.4 mg/kg) or saline for 40 days. Three minutes after each injection the rats were placed at 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) for 20 minutes. Entries into each arm of the maze were recorded on days 1, 5, 10, 20, 30 and 38 - 40. On days 38 - 40 half the rats previously treated with nicotine were given injections of saline. The rats were killed on day 40 and brain samples taken for the measurement of hippocampal, hypothalamic and cerebrocortical 5-hydroxyindole levels (method of Reinhard et al, 1980).

The lesion caused significant reductions ($P < 0.01$; $n = 21$) in hippocampal 5-HT and 5-hydroxyindole acetic acid (5-HIAA) from 0.176 ± 0.031 to 0.059 ± 0.008 µg/g and from 0.448 ± 0.032 to 0.179 ± 0.027 µg/g respectively. Cerebrocortical 5-HIAA was also reduced ($P < 0.05$) from 0.384 ± 0.041 to 0.273 ± 0.029 µg/g. No other significant changes in brain 5-hydroxyindoles were observed. Analysis of the behavioural data for days 1 to 30 showed that nicotine increased entries into both enclosed ($F(2,12) = 8.9$; $P < 0.01$) and open runways ($F(2,12) = 11.2$; $P < 0.01$) and that there was an interaction between these effects and the number of days of treatment (F enclosed $(8,48) = 5.5$; $P < 0.01$; F open $(8,48) = 3.1$; $P < 0.01$). In lesioned rats open and enclosed runway entries were also increased although only significantly so for the enclosed runways ($F(1,6) = 8.43$; $P < 0.05$). There was an interaction ($F(4,24) = 3.0$; $P < 0.05$) between the effects of the lesion and the number of days of treatment, the greatest difference being observed on day 10. The lesion had no effects on the behaviour of the nicotine-treated rats. In sham-injected rats nicotine withdrawal on days 38 to 40 caused significant reductions ($P < 0.05$; $n = 7$ per gp) in both open and enclosed runway entries from 20.4 ± 8.3 to 4.6 ± 2.4 per trial and from 35.0 ± 7.0 to 9.1 ± 2.0 per trial respectively. However, enclosed, but not open, runway entries in the withdrawn rats remained significantly greater ($P < 0.05$) than controls (4.1 ± 0.7 entries per trial). The lesion attenuated ($P < 0.05$) the reduction in enclosed runway entries caused by nicotine withdrawal. No other significant effects on the responses to nicotine withdrawal were apparent in the lesioned rats.

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

Benwell, M.E.M. & Balfour, D.J.K. (1982). Psychopharmacology, 76, 160-162.
Quik, M. & Azmitia, E. (1983). Eur. J. Pharmac., 90, 377-384.
Reinhard, J.F., Moskovitz, M.A., Sved, A.F. & Fernstrom, J.D. (1980). Life Sci, 27, 905-911.

THE EFFECT OF CHRONIC ICV INFUSION OF TRH ANTIBODIES ON THE BEHAVIOURAL RESPONSE TO A TRH ANALOGUE (CG 3509)

G.W. Bennett, Celia Lighton & C A. Marsden, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK.

Chronic antidepressant treatment increases, and repeated electroconvulsive shock decreases, the synthesis and ex vivo release of thyrotrophin releasing hormone (TRH) in rat nucleus accumbens (Lighton et al, 1984; 1985), and these responses are accompanied by reciprocal changes in the behavioural responses to a TRH analogue injected in the same region (Bennett et al, 1985a,b). The present study was undertaken to evaluate whether direct modulation of endogenous TRH released in brain could alter the behavioural response to a TRH analogue.

Rats were chronically infused for 14 days with TRH antibodies or control serum ICV using osmotic mini-pumps and the CG3509-induced reversal of pentobarbitone sleep time, hypothermia and respiratory depression were measured before and after infusion. Male Wistar rats under pentobarbitone anaesthesia (60 mg/kg i.p.) were bilaterally implanted with cannulae ICV and a third cannula into the medial septum. Paired osmotic mini-pumps (Alzet) containing TRH antibodies or control serum were implanted intrascapularly and connected to the ICV cannulae. The TRH antibodies raised in sheep were shown to be specific for TRH by RIA and the TRH and control sera were partially purified by ammonium sulphate precipitation prior to infusion. On day 4 the analeptic response to intra-septal injection of CG 3509 (Bennett et al, 1985a) was measured (acute response). Sleep time was measured as the duration of loss of righting reflex (LRR) following sodium pentobarbitone (35 mg/kg i.p.). Twenty min after LRR intra-septal CG 3509 (5 μg in 1 μl) was injected. The response to central saline was assessed 24h later. Rectal temperature and respiration rate were measured following LRR every 10 min. On days 13, 14 of antiserum infusion the analeptic response to CG 3509 and saline was repeated (chronic response). The effects of the TRH antibody on the K^+ (56 mM) stimulated release of TRH-LI was tested on a range of brain regions in vitro. Brain slices were incubated in the presence of the TRH antibody or control antiserum, at a final dilution of 1:20 for 20 mins as previously described (Lighton et al, 1985) and TRH-LI in the supernatant measured by RIA (Lighton et al, 1984). Addition of purified TRH antibody to the medium during incubation of brain slices significantly reduced ($p<0.05$, $n=6$) the levels of TRH-LI in the supernatant with slices of accumbens (60%), hypothalamus (76%), septum (78%) and ventral spinal cord (52%).

Chronic infusion of TRH serum ICV more than doubled the duration of anaesthesia and potentiated the hypothermia and respiratory depression following pentobarbitone. However, there was an increased CG 3509 reversal of sleep time following chronic infusion of TRH serum (51.6%) compared with the animals infused with control serum (36.0%). The increased arousal in response to CG 3509 was associated with an increased recovery from hypothermia and respiratory depression.

None of these responses was observed following acute infusion of TRH serum. The results indicate that chronic immunological blockade of endogenous extracellular TRH ICV can increase responsiveness to TRH and further suggests the importance of TRH in arousal mechanisms.

We thank the Mental Health Foundation for financial support and Prof L. Flohe, Grunenthal GMBH for CG 3509. CAM is a Wellcome Senior Lecturer.

Bennett, G.W. et al (1985a) Br.J.Pharmac. 85, 244P
Bennett, G.W. et al (1985b) Br.J.Pharmac. (in press)
Lighton, C. et al (1984) Neuropharmacology 23, 963-966
Lighton, C. et al (1985) Neuropharmacology 24, 401-406

THE "PLUS-MAZE TEST OF ANXIETY": VALIDATION IN DIFFERENT RAT STRAINS AND EFFECT OF A WIDE VARIETY OF ANTIDEPRESSANTS

Mike Briley, Philippe Chopin and Murielle Veigner. Biochemical Pharmacology Department, Centre de Recherche Pierre Fabre, 81106 Castres, France.

We have recently described a behavioural screening test for the identification of anxiolytic and anxiogenic drug effects in the Olac Hooded rat (Pellow, Chopin, File & Briley, J. Neurosci. Meths. In Press). The apparatus consists of a plus maze elevated to a height of 50 cm with two open and two (opposite) enclosed 50 cm arms. The number of entries into each type of arms was noted for a 5 min. period. Total exploratory activity was measured as the total number of entries. The percentage of open arm entries provided a measure of fear-induced inhibition of exploratory activity. To provide a measure of spontaneous locomotor activity rats were placed in a Panlab activity monitor for 5 min. before placement on the maze.

In the present investigation, to test the importance of strain differences, four strains of rats other than the Olac Hooded were tested : Wistar (Iffa-Credo : France), Sprague-Dawley (Charles River : France), Long-Evans (Janvier : France) and Fawn-Hooded (Bred in our laboratory). In addition we examined the effect of a wide variety of clinically effective antidepressants given acutely to Sprague-Dawley rats.

In each rat strain the number of entries into the open arms was significantly lower than the number of entries into the closed arms, Clinically effective anxiolytics (diazepam and chlordiazepoxide) significantly elevated, and yohimbine (which is anxiogenic in man) significantly attenuated the percentage of open arm entries in each rat strain.

The following antidepressants, having different mechanisms of action, given intraperitoneally in a single dose (3 to 5 doses within the ranges given below) 30 min. before the test were without significant effect on the fear-induced inhibition of exploratory activity :

(1) Monoamine oxidase (MAO) inhibitors ; pargyline (10-30 mg/kg) which exhibits a somewhat greater inhibitory activity against MAO type B and clorgyline (1-10 mg/kg), a rather selective inhibitor of MAO type A.
(2) Tricyclics antidepressants ; imipramine (3-30 mg/kg) and amitryptyline (3-30 mg/kg), noradrenaline (NA) and serotonin (5-HT) uptake inhibitors.
(3) Amineptine (5-50 mg/kg), a dopamine (DA) uptake inhibitor.
(4) Nomifensine (2-10 mg/kg), inhibiting the uptake of both NA and DA.
(5) Mianserine (5-20 mg/kg), an atypical antidepressant, not inhibiting amine uptake.
(6) Citalopram (0.1-1 mg/kg) selective 5-HT uptake inhibitor.
(7) Midalcipran (1-20 mg/kg) inhibiting the uptake of 5-HT et NA equipotently.

In conclusion, it appears that the present procedure provides a valid and reliable test for the detection of anxiolytic and anxiogenic drug effects in many rat strains and that false positives are not obtained with a wide variety of antidepressants having different pharmacological profiles.

THE EFFECT OF VASOPRESSIN ON LEARNING MOTIVATED BY FOOD REWARD

I.S. Ebenezer (Introduced by M.A. Zar), Department of Anatomy, Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne.

De Wied (1971) has demonstrated that vasopressin can improve memory retention and recall in a shock-avoidance experiment. However, there is little evidence to suggest that vasopressin facilitates memory processes in positively rewarded paradigms (Alliot & Alexinsky 1982; Ettenberg et al 1983). The present study was therefore designed to investigate the effects of vasopressin on a food finding task.

Male Wistar rats (n=60; wts: 280-320g) were used. The apparatus consisted of a T-maze with a 50x15cm stem, 12x15cm choice area and two 50x15cm arms. Twenty four hr prior to the start of the experiment the rats were deprived of food. Rats were fed ad lib. at the end of the procedures on day 1 and day 2 for 30 min.

Day 1: Exploration Trial - The rat was placed in the starting position of the T-maze and given 5 min to explore the maze. All rats entered both arms, and a note was made of the arm which the rat first entered.

Day 2: Learning Trials - (a) Food (rat pellets) was placed in a container in one of the arms of the T-maze, and entry into the opposite arm was blocked. Food was placed in the arm opposite that initially entered by the rat on day 1. The rat was placed in the starting position and allowed to enter the arm containing the food. The rat was allowed 30s to eat the food before it was removed.

(b) The arm of the T-maze without food was now opened while entry into the arm with food was blocked. Five min after the end of procedure (a) the rat was placed in the starting position and allowed to enter the arm with no food. The rat was allowed 30s to explore the arm before it was removed.
(c) Five min after procedure (b), procedure (a) was repeated. Immediately after (c) the rats were injected s.c. with either saline (n=24), arginine-8 vasopressin (AVP) (1.2 IU/kg) (n=24) or lithium chloride (LiCl) (50 mg/kg) (n=12) and removed.

Day 3: Retention Trial - Both arms were opened, and food removed from the containers in each arm. Twenty four hr after the learning trial the rat was placed in the starting position and the arm of entry and latency for entry were noted.

Analysis of the results obtained showed that rats treated with AVP or LiCl (i) displayed a significant ($p < 0.05$, Chi^2 test) preference for the arm of the T-maze that did not contain food during the learning trials, (ii) displayed significantly ($p < 0.05$, Mann Whitney U-test) longer latencies to enter either of the arms.

It is well known that rats will tend to avoid food that has been previously paired with aversive stimuli (Silverman, 1978). It has been demonstrated that both LiCl and AVP produce aversive effects (i.e. make the rat feel ill) when injected s.c. (Ettenberg et al, 1983; Ebenezer, 1985). It is thus possible that rats given LiCl and AVP associate the unpleasant (aversive) effects of these drugs with the food reward in the T-maze, and consequently avoid the arm which contained food during the retention trial. These results therefore suggest that the claim that vasopression facilitates memory processes is complicated by the fact that vasopressin appears to have aversive effects.

Acknowledgements: I wish to thank Professor J.W. Thompson and the Wellcome Trust.

Alliot, J. & Alexinsky, T. (1982) Physiol.Behav. 28, 525-530
De Wied, D. (1971) Nature (Lond.) 232, 58-60
Ebenezer, I.S. (1985) Br.J.Pharmac. 84, 107P
Ettenberg et al (1983) Behav.Brain Res. 7, 331-350
Silverman, P. (1978) Animal Behaviour in the Laboratory. Chapman & Hall, Lond.

CHRONIC LITHIUM TREATMENT IN RATS HAS DIFFERENT EFFECTS ON MOTOR RESPONSES MEDIATED BY 5-HT_1 AND 5-HT_2 RECEPTORS

P.E. Harrison-Read, Academic Department of Psychiatry, St. Charles' Hospital, Exmoor Street, London, W10 6D7.

Rats administered lithium (Li) for a week or more show forepaw treading (FPT) during periods of spontaneous activity (Harrison-Read, 1983a). This behaviour can also be evoked by drugs which stimulate brain 5-HT_{1A} receptors (Tricklebank, 1984). 'Wet dog shakes' (WDS) are evoked by stimulation of 5-HT_2 receptors in rodents (Goodwin & Green, 1985), but Li effects are unclear (Harrison-Read, 1978). I now report the effects of Li and 5-HT antagonists on FPT, WDS, and other 5-HT dependent behaviours evoked by the 5-HT precursor 5-HTP plus zimelidine (ZIM), a selective 5-HT reuptake inhibitor.

Adult male hooded rats (4 per cage) were injected daily with 0.15M NaCl or LiCl (2 mmol/kg i.p.). On day 25, 3h after the last injection, rats were given carbidopa (25 mg/kg i.p.) followed ½h later by either ZIM (10 mg/kg i.p.) or saline (SAL). ZIM enhanced spontaneous FPT in Li rats, but otherwise had no obvious behavioural effects on its own. Rats were next injected i.p. with one of the following: cyproheptidine(CYP, 0.075 mg/kg), (-)-propranolol (PRL, 1.5 mg/kg), haloperidol (HAL, 0.15 mg/kg) or a control solution (CON). 45 min later, all rats were given i.p. DL-5-HTP (50 mg/kg), and then placed in individual containers where they remained undisturbed for 1h. An observer blind to drug pretreatment counted the number of WDS over 5 min and then rated 0,1,2 or 3 the following behaviours: tremor, forepaw treading, hindlimb splay, head-weaving or circling, and reactivity to handling. Treatment effects were assessed by Mann-Whitney U tests (2-tailed).

Table 1 Mean scores for FPT and WDS evoked by 5-HTP in NaCl and LiCl treated rats

		SAL CON	ZIM CON	ZIM CYP	ZIM PRL	ZIM HAL			SAL CON	ZIM CON	ZIM CYP	ZIM PRL	ZIM HAL
	Na	0.00*	0.70	0.20	0.18*	0.20		Na	3.00*	11.50	5.50*	10.36	12.60
FPT		P<.01	<.01	<.01	<.01	<.01	WDS		NS	P<.01	<.05	NS	P<.01
	Li	2.11	2.70	1.70*	2.18	1.70*		Li	1.78*	5.70	3.50	7.09	6.00

*significantly smaller than corresponding ZIM CON score, P<0.05 (n=9-11 per group)

The mean (± s.d.) plasma level of Li measured next day in 24 rats, 4-7 h after injection was 0.7 ± 0.2 mmol/l. ZIM markedly potentiated all rated behaviours in NaCl and LiCl rats given 5-HTP, showing that Li did not prevent raised 5-HT release. Li significantly raised FPT scores, and the 3 antagonists tended to reduce them. Only PRL had a significant effect on FPT in NaCl rats, whereas the increased FPT in LiCl rats was relatively resistant to PRL. Li probably increases 5-HT release onto 5-HT_{1A} receptors, since FPT results directly from activation of these receptors which are blocked by PRL. Although 5-HT_2 and catecholamine receptors have a facilitatory action on FPT (Tricklebank,1984; Goodwin & Green, 1985), probably explaining inhibition of FPT by CYP and HAL, the increase in FPT due to Li is unlikely to be mediated by these receptors. WDS are mediated by 5-HT_2 receptors and inhibited by 5-HT_1 receptors (Goodwin & Green, 1985; Harrison-Read, 1983b), and WDS and most other rated behaviours were reduced by Li (only WDS data shown). Since the inhibition of WDS by Li was increased by ZIM and reduced by PRL, this effect of Li may also be explained by enhanced release of 5-HT at 5-HT_1 receptors.

I thank the Nuffield Foundation for a grant and Astra Ltd for a gift of zimelidine.

Goodwin, G.M. & Green, A.R. (1985) Br.J.Pharmac. 84, 743-753
Harrison-Read, P.E. (1978) In'Lithium in Medical Practice' MTP, Lancaster
Harrison-Read, P.E. (1983a) Br.J.Pharmac. 79, Proc. Suppl., 394P
Harrison-Read, P.E. (1983b) Br.J.Pharmac. 78, Proc. Suppl., 92P
Tricklebank, M.D. et al (1984) Eur.J.Pharmac. 106, 271-282

220P

COMPARISON OF THE FOOD INTAKE AND LOCOMOTOR EFFECTS OF LEVALLORPHAN, LEVORPHANOL AND NALTREXONE

Helen C. Jackson & R.D.E. Sewell, Division of Pharmacology, The Welsh School of Pharmacy, UWIST, Cardiff CF1 3XF, U.K.

Levallorphan has been classified as a mixed agonist-antagonist opioid (Wood, 1982). In the current study we have examined the effects of this agent on day-time and night-time food intake, comparing it with the more traditional opioid agonist levorphanol and the opioid antagonist naltrexone. Spontaneous locomotor activity was also examined where appropriate in order to determine whether opioid-induced changes in food intake were derived from primary changes in motility.

Subjects were individually-housed male Wistar rats (300-350g) fed with powdered standard rat diet and tap water ad libitum and maintained on a 12h light-dark cycle (08.00h-20.00h). Drugs or saline were administered i.p. in a dose volume of 1ml/kg. Experiments were carried out during the day (commencing at 10.00h) and during the nocturnal feeding period (injecting at 20.00h). Feeding jars were weighed at the time of drug administration and after 1, 2 and 3h to enable the calculation of mean cumulative food intakes (g/kg rat weight ± s.e.m.). Locomotor activity was monitored using photobeam activity boxes, cumulative counts being recorded every 15 min for 60 min. Conditions in the activity boxes were closely matched to those during the nocturnal feeding period. Statistical comparisons were made using analysis of variance and Dunnetts t-test.

Levallorphan (1, 10mg/kg i.p.) led to an increase in food intake during the light phase in a way comparable to levorphanol (1mg/kg i.p.) while naltrexone (0.1-10mg /kg i.p.) had no significant effects on food consumption in this period. The hyperphagia induced by both levallorphan and levorphanol was inhibited by naltrexone (1mg/kg i.p.). Levallorphan (10mg/kg i.p.), levorphanol (10mg/kg i.p.) and naltrexone (1, 10mg/kg i.p.) all decreased nocturnal food intake. Locomotor activity, however, was significantly suppressed by levorphanol during the first hour of the dark period but neither levallorphan nor naltrexone had any effect on this parameter.

In conclusion, levallorphan appears to increase day-time feeding in a naltrexone-reversible manner which is analogous to that of the opioid agonist levorphanol. Nocturnal food intake, by comparison, was decreased by both levallorphan and levorphanol and by the opioid antagonist naltrexone. However, the reduced feeding levels observed with levallorphan and naltrexone during this period - unlike those of levorphanol - do not appear to be related to any detectable depression of locomotor activity and as such this suggests that these particular compounds may exert a more specific anorectic effect on night-time feeding behaviour.

The authors gratefully acknowledge gifts of levallorphan and levorphanol (Roche) and naltrexone (Endo). HCJ is supported by SERC.

Wood, P.L. (1982) Neuropharmacology 21: 487-497

THE INFLUENCE OF THYROID HORMONE (T_3) ON THE EFFECTS OF DESIPRAMINE ON α_2- AND β-ADRENOCEPTOR FUNCTION IN RAT BRAIN

C.K. Atterwill, J. Bloomfield[1], L.J. Bristow[1], L.C. Catto, J.M. Elliot[1] & D.J. Heal[1]. Department of Toxicology, Smith Kline & French Research Ltd, The Frythe, Welwyn, Herts AL6 9AR. [1] MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford, OX2 6HE.

Tri-iodothyronine (L-T_3) accelerates the onset of the clinical action of some antidepressants (Wilson et al, 1974) and also affects central monoamine function and receptor activity in rat brain (Atterwill et al, 1984). In view of these findings we have examined the effects of a single injection of T_3 on the desipramine(DMI)-induced changes in α_2-adrenoceptor function and α_2-and β-adrenoceptor binding in rat brain.

Male CD rats (Charles River; 60-70g) were injected twice daily with DMI (5mg/kg, i.p.) plus either T_3 (100μg/kg,s.c.) on day 1 or vehicle. Animals were killed at 18 hours after the final dose and membrane fractions prepared from cerebral cortex (Atterwill et al, 1984). β-Adrenoceptor binding was measured (days 1,3 & 14) with [^{3}H]-dihydroalprenolol (DHA; 0.5-4nM) and α_2-adrenoceptor binding (days 2 & 14) using the selective antagonist [^{3}H]-idazoxan (0.4-4nM) with isoprenaline (200μM) and phentolamine (5μM), respectively, to estimate specific bound ligand. Clonidine-induced sedation responses (days 2,4 & 14) were rated from (0-3) on four behavioural parameters, as described previously (Heal et al, 1981).

After 2 days DMI treatment, animals receiving drug alone showed no observable decreases in clonidine-induced sedation response, whereas those receiving a single T_3 injection on day 1 plus DMI showed a significant decrease. Injection of T_3 alone had no effect on behaviour at this time. However, at 14 days there were no differences in the net 'down-regulated' clonidine sedation responses to DMI alone or in combination with T_3.

In rat cortex, DMI caused an 18% down regulation of α_2-adrenoceptor binding at 14 but not at 2 days. This effect was unaltered by prior treatment with T_3. Similarly, DMI caused 30% reduction in β-adrenoceptor binding at 14 days. However, in contrast to [^{3}H]-idazoxan binding, down-regulation was observed after 2 days treatment. Administration of T_3 did not affect the responses to DMI but did slightly decelerate the rate of β-adrenoreceptor down-regulation.

Thus, although a single injection of T_3 accelerates the onset of the DMI-induced decreases in behavioural responses to clonidine, there are not parallel changes in α_2-adrenoceptor binding. However, whereas the behaviour probably represents presynaptic α_2-adrenoceptor function (Zebrowska-Lupina et al, 1977) ligand receptor binding predominantly reflects postsynaptic sites (U'Prichard et al, 1980). Since DMI produced a rapid decrease in cortical β-adrenoceptor number, this may account for the failure of T_3 to influence the onset of this effect. In conclusion, this study demonstrates that T_3 injection influencies one, but not all, of the possible therapeutic actions of DMI administration.

C.K. Atterwill et al (1984) J. Neural. Transm. 59, 43.
D.J. Heal et al (1981) Eur. J. Pharmacol. 75, 231.
D.C. U'Prichard et al (1980) Brain Res. 180, 143.
I.C. Wilson et al (1974) In: The Thyroid Axis, Drugs & Behaviour (Ed. A.J. Prange) Raven Press p.49.
I. Zebrowska-Lupina et al (1977) Naunyn-Schmiedeb. Arch. Pharmac. 297, 227.

DIFFERENTIAL AGONIST INTERACTIONS WITH β_2-ADRENOCEPTORS ON INTACT HUMAN PLATELETS SUGGESTS RECEPTOR INTERNALISATION

D B Barnett, N Cook & S R Nahorski. Department of Pharmacology & Therapeutics, University of Leicester, Leicester, LE2 7LX.

Previous studies employing ^{125}I(-)pindolol (^{125}IPIN) have demonstrated the presence of a high affinity, low capacity binding site on human platelet membranes with the pharmacological characteristics of the β_2-adrenoceptor (Cook et al, 1985). It has been shown in intact heart cells that ^{125}IPIN labels both surface and intracellular receptors at 37°C and only surface receptors at 4°C (Linden et al, 1984). We have in the present study employed this differential temperature effect to investigate catecholamine interactions and possible regulation of the β_2-adrenoceptor on intact human platelets.

Whole platelets, prepared using modifications of previously described methods (Cook et al, 1985) were finally resuspended in assay buffer (50mM Tris HCl, 100mM NaCl, 5.0mM EDTA, 0.2mM sodium metabisulphite, pH 7.8) at a concentration of 5-9 x 10^8 cells/ml. Platelets were incubated to equilibrium with ^{125}IPIN and appropriate concentrations of drugs in a final volume of 500µl assay buffer, for 40 min at 37°C, or 5 hrs at 4°C. Assays were terminated by the addition of 10ml ice-cold hypotonic assay buffer, left for 30 min on ice followed by rapid vacuum filtration. Non-specific binding assessed in the presence of 1µM (-)propranolol or 1µM (±)CGP 12177 gave equivalent results (20-30%). Maximal binding capacities (B_{max}) and equilibrium dissociation constants (K_d) were identical whether defined in the presence of the lipophilic antagonist (-) propranolol or the hydrophilic antagonist (±) CGP 12177. There was no significant difference in binding parameters at 4°C or 37°C (B_{max} = 1.23 ± 0.06 fmol/mg, 1.21 ± 0.13; K_d = 16.17 ± 1.49pM, 22.61± 0.31 respectively). The β-agonists isoprenaline, adrenaline and noradrenaline displaced ^{125}IPIN with similar overall affinity in whole cells at 4°C and 37°C (Table 1). However, agonist competition curves at 37°C yielded shallow binding isotherms best fitted to a 2 site model for isoprenaline and adrenaline, and a steeper curve for noradrenaline which is only a partial agonist at the β_2-adrenoceptor. At 4°C all agonist displacement curves showed an increased slope factor.

TABLE 1 IC_{50} Values* and Hill Slope Factors (nH) for Agonist Displacement of ^{125}IPIN Binding to Intact Human Platelets.

	IC_{50} [M] (nH) 4°C	IC_{50} [M] (nH) 37°C
(-) isoprenaline	1.73×10^{-7} (0.76)	4.12×10^{-7} (0.61)
(-) adrenaline	6.08×10^{-7} (0.77)	2.31×10^{-6} (0.58)
(-) noradrenaline	1.36×10^{-5} (0.94)	3.93×10^{-5} (0.85)

* Values are the means of at least three separate experiments performed in duplicate.

These results suggest that all β_2-adrenoceptors measured at 37°C in the absence of agonist are on the platelet surface. Also when intact platelets are incubated with specific agonists at 37°C internalisation of a proportion of the cell surface receptors may occur with consequently shallow binding isotherms due to impaired access of the agonist.

Cook N, Nahorski SR, Barnett DB (1985) Eur J Pharm, 113, 247-254
Linden J, Patel A, Spanier AM, Weglicki WB (1984) J Biol Chem, 259, 15115-15122

COMPARISON OF THE EFFECTS OF CHRONIC INFUSION OF XAMOTEROL AND ISOPRENALINE ON RAT VENTRICULAR β-ADRENOCEPTORS

D B Barnett & M Maguire, Department of Pharmacology, University of Leicester, Leicester, LE2 7LX.

Xamoterol is a β_1-adrenoceptor (βAR) partial agonist presently being evaluated for treatment of cardiac failure. Chronic therapy with full βAR agonists in animals leads to down regulation of βAR's and tachyphylaxis to these agents in heart failure where cardiac βAR density is already reduced (Bristow, 1984).

Using subcutaneous osmotic minipumps we administered, isoprenaline (I) (400μg/kg-/hr), Xamoterol (X) (900μg/kg/hr) or saline vehicle to male AS rats (weight 200-300g). After 6 days rats were sacrificed, ventricular membranes prepared using previously described methods (Baker et al, 1980), resuspended (0.5-1.0mg protein-/ml) in assay buffer (50mM Tris HCl pH 7.8 with 0.2mM sodium metabisulphate) and used immediately in the binding assays. Membranes were incubated to equilibrium with the non selective βAR antagonist ligand ^{125}I(-)pindolol (PIN) and appropriate concentrations of drugs in a final volume of 250μl for 40 mins at room temperature. Non specific binding (< 10%) was defined by 200μM isoprenaline. Compared to saline controls maximal binding capacity (B_{max}) was significantly reduced by 43% after I but unaffected by X (Table 1). Equilibrium dissociation constants (K_d) were unchanged by any treatment.

TABLE 1 B_{max} (fmol/mg protein) and K_d (pM) of ^{125}IPIN Binding to Ventricular Membranes After 6 Day Subcutaneous Infusions (mean ± SEM)

	Saline (n = 8)	Isoprenaline (n = 3)	Xamoterol (n = 3)
B_{max}	17.5 ± 1.1	10.1 ± 1.2*	19.9 ± 0.8
K_d	35.0 ± 3.1	35.4 ± 6.2	43.3 ± 3.1

* significantly different to saline ($P < 0.05$)

The effect of the addition of 0.1mM Gpp(NH)p on isoprenaline displacement of ^{125}IPIN binding to ventricular membranes in the presence of 8mM $MgCl_2$ was used to assess the efficiency of coupling of the βAR adenylate cyclase after each treatment. Gpp(NH)p produced an approximately 3-4 fold rightward shift and steepening of the agonist binding isotherm in control membranes which was indistinguishable from that seen after X treatment. After I treatment the remaining βAR's exhibited reduced sensitivity to guanine nucleotide with little or no effect on overall isoprenaline IC_{50} or slope factor.

Plasma catecholamines in blood obtained by cardiac puncture at the end of each treatment period were not significantly different. Plasma X concentrations (161 + 21ng/ml) were similar to those found in clinical studies of effective oral X therapy and equivalent to the in vitro IC_{50} of X in binding studies (0.25μM).

These results suggest that chronic therapy with I but not X causes down regulation and probably uncoupling from cyclase of rat cardiac βAR's.

Baker SP, Boyd HM, Potter LT 1980 Brit J Pharm, 68, 57-63.
Bristow MR 1984 New Eng J Med, 311(13) 850-851

REGIONAL CHANGES IN MET-ENKEPHALIN-LIKE IMMUNOREACTIVITY IN RAT BRAIN AFTER TEMPORARY CEREBRAL ISCHAEMIA

B. Carruthers & I.A. Silver (introduced by K.D. Bhoola), Department of Pathology, Medical School, University of Bristol, BS8 1TD.

The ability of naloxone to improve neurological function both in stroke patients and in animal models of stroke is at present a matter of controversy (reviewed by Faden 1984), but has led to the suggestion that endogenous opioids might be important in the pathophysiology of cerebral ischaemia. The direct effects of cerebral ischaemia on brain opioid peptides have not been systematically investigated. In the present study we have measured regional brain content of met-enkephalin-like immunoreactivity (MELI) immediately after temporary cerebral ischaemia and in the first hour after restoration of circulation, in an animal model of stroke (Erecińska et al., 1984).

Rats were anaesthetised with sodium pentobarbitone (50 mg/kg i.p.). After cannulation of the trachea, ischaemia was induced by means of a hydraulic cuff placed around the neck. Ventilation was maintained artificially via the tracheal cannula. After 30 min the cuff was removed to allow restoration of circulation. Groups of 6-8 animals were killed at times 0, 15, 30 and 60 min after removal of the cuff. In each experiment an equal number of sham-operated rats was included as control. The brain of each animal was removed and dissected on ice into the following regions: cortex, hippocampus, striatum, diencephalon, midbrain, hindbrain, cerebellum. Tissue samples were extracted into acetic acid (1M), and after centrifugation the supernatants were applied to Amberlite XAD-2 columns. Columns were washed with 0.1M HCl followed by distilled water before MELI was eluted with methanol. Samples were then dried under nitrogen and the residues dissolved in 0.05M Tris-HCl buffer (pH 7.4 at 25°C). MELI was measured by radioimmunoassay using an antiserum characterised and kindly donated by Dr. T. Ashwood (Southampton University).

In animals killed immediately after removal of the hydraulic cuff MELI levels in all brain regions were not significantly different from those in sham-operated rats. 15 min after the restoration of circulation a significant reduction in MELI was observed in the hippocampus (88.2% of the control value, $p<0.05$). A further reduction was apparent in the hippocampus at 30 min (84.3% of control; $p<0.05$) and at 60 min (58.3% of control, $p<0.01$). Significant reductions in MELI were also observed in the striatum (67.5% of control, $p<0.01$) and midbrain (78.9% of control, $p<0.05$) 60 min after removal of the cuff. No changes in MELI were detected in cortex, diencephalon, hindbrain or cerebellum at any time.

In summary, loss of MELI appears to take place after the blood flow has been restored to ischaemic brain tissue, and this loss is restricted to the hippocampus, striatum and midbrain.

Erecińska, M., Nelson, D., Wilson, D.F. & Silver, I.A. (1984) Brain Res. 304, 9-22.
Faden, A.I. (1984) Stroke 15, 575-578.