ACTION OF PHENOXYBENZAMINE AT THE μ-OPIOID BINDING SITE

T.G. Franklin and J.R. Traynor, Department of Chemistry, Loughborough University, Leics. LEII 3TU, UK.

The alpha-adrenergic antagonist phenoxybenzamine causes an irreversible inhibition of opioid binding (Spieler et al,1978). Pre-incubation with opioids protects against this effect, suggesting alkylation occurs at or near to the binding site. Using the sulphdryl reagent N-ethylmaleimide(NEM) it has been shown that two thiol groups are important, one associated with the ligand binding site, and the other associated with the control of agonist affinity (Mullikin-Kilpatrick et al, 1983). In this communication we suggest that alkylation of the mu-receptor by phenoxybenzamine differs from that caused by NEM.

Binding of the mu-ligand (^{3}H)(D-Ala^2,MePhe^4,Gly-ol^5)enkephalin (GLYOL) to homogenates prepared from rat brain was carried out as described by Kosterlitz et al (1981). Alkylation experiments were performed at 37^{0}C for 20 min in the presence or absence of competing opioid ligand added 10 min prior to alkylation, and terminated by extensive washing (Robson and Kosterlitz, 1979). Kinetic experiments were rapidly quenched by addition of a 10-fold excess of dithiothreitol (for NEM) or by lowering the pH to 4.5 (for phenoxybenzamine). Neither process altered control binding.

Phenoxybenzamine (IC_{50}= 2.8±0.3μM) was a more potent inhibitor of (^{3}H)GLYOL(1nM) binding than NEM (IC_{50}= 33.9±7.3μM). Both compounds showed similar pseudo-first order kinetics ($t_{\frac{1}{2}}$ = 7min) for the reduction of (^{3}H)GLYOL binding to 20% of its initial value. The remaining binding was stable to phenoxybenzamine but not to NEM. The alkylating agents reduced the affinity (K_D) of the mu-ligand for its binding sites (control = 3.15±0.75nM, phenoxybenzamine (5μM) treated = 29.68 ± 4.90nM, NEM (150μM) treated = 18.53±3.86nM), but only NEM reduced the Bmax value (control = 92.36±10.35fmol.mg^{-1}, phenoxybenzamine treated = 108.46±15.36fmol.mg^{-1}, NEM treated = 53.80±2.45fmol.mg^{-1}). Alkylation of the binding-site by phenoxybenzamine (5μM) was prevented by prior incubation with morphine (IC_{50} = 38.1± 7.1nM), levorphanol (IC_{50}= 4.8±0.1nM) and naloxone (IC_{50}= 61.0±7.2nM). In contrast protection against NEM (150μM) required higher concentrations of agonists (morphine IC_{50}= 334±54nM, levorphanol IC_{50}= 328±21nM), whilst naloxone afforded maximally only 20.4±2.8% protection. The inhibitory effect of GTP on mu-agonist binding was reduced by both alkylating agents. NEM, but not phenoxybenzamine, enhanced the sensitivity to Na^+ ions.

The results show that both phenoxybenzamine and NEM inhibit the mu-binding site. The alkylation caused by phenoxybenzamine is different from that caused by NEM and may suggest that some of the -SH functionality involved in mu-agonist binding is not accessible to phenoxybenzamine.

TGF is an SERC student.

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EFFECT OF SALAZOPYRIN, 5-AMINOSALICYLIC ACID AND PREDNISOLONE ON AN IMMUNE COMPLEX-MEDIATED COLITIS IN MICE

L.P. Walsh and I.J. Zeitlin. Department of Physiology and Pharmacology, University of Strathclyde, Glasgow.

We have previously described a model of acute colitis based on an early study by Hodgson et al (1978). This model exhibited many features of human clinical colitis and responded to the actions of prednisolone, unlike other similar models (Blackham et al, 1986). In the present study we confirm the findings of Blackham et al, and now report the actions of salazopyrin and 5-amino-salicylic acid (5-ASA) on this model.

Colitis was induced in male BKA mice (20-30g) fed on normal laboratory diet (Oxoid Ltd.) and water ad libitum. Pre-formed human serum albumin (HSA)-antiHSA immune complex was injected i.v., following intra-rectal formalin (1%) (Walsh et al, 1987). Drug (prednisolone i.p.; 5-ASA i.p.; salazopyrin p.o.) or vehicle alone was administered daily for three days prior to induction of colitis and then for a further five days. The animals were killed and samples of distal colon were taken for histological assessment and measurement of tissue water content. Symptomatic colitis was graded according to an arbitrary scoring system.

The increase in tissue water content, seen in the oedematous tissue following the induction of colitis, was reduced in a dose-related fashion in drug-treated mice, reaching statistical significance ($P<0.05$) at 4, 30, 100 and 550mg/kg prednisolone, 670mg, 1.7 and 5g/kg salazopyrin and 30 and 340mg/kg 5-ASA. Prednisolone at all dose levels tested, salazopyrin at 340, 670mg, 1.7 and 5g/kg, and 5-ASA at 30 and 340 mg/kg gave a significant reduction ($P<0.05$) in the colitis score. There was significant correlation ($P<0.05$) between the reduction in symptomatic score and the suppression of the increase in tissue water in the inflamed colons, induced by all three drugs. On a molar basis, the drug potencies were ranked as salazopyrin < 5-ASA < prednisolone.

This animal model of acute colitis has been shown to possess both symptomatic and histological features of clinical colitis (Walsh et al, 1987). We now report that the induced colitis responds in a consistent dose-related manner to both steroidal and non-steroidal anti-colitic drug treatment.

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THE ACTION OF STAUROSPORINE, AN INHIBITOR OF PROTEIN KINASE C, ON THE ACTIVATION OF HUMAN PLATELETS BY THROMBIN

S.P. Watson, L.J. Shipman, J. McNally & P.P. Godfrey[1], Department of Pharmacology, South parks Road, Oxford OX1 3QT and [1]Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE

Studies aimed at investigating the role of protein kinase C (PKC) in human platelets have concentrated on the use of membrane-permeable diacylglycerols or phorbol esters to activate the enzyme. Thus, evidence has emerged implicating a direct role for PKC in secretion and aggregation. In addition, though, this same approach has provided evidence that PKC is also able to exert a negative feedback influence over platelet activation by agonists such as thrombin. For example, the pretreatment of platelets with phorbol esters inhibits thrombin-stimulated hydrolysis of inositol phospholipids and mobilisation of Ca^{2+}, and increases the metabolism of inositol triphosphate. The precise role played by PKC in platelet activation is therefore uncertain, and hence the present study was undertaken, using a newly described inhibitor of protein kinase C, to investigate further the role of this kinase in platelet activation.

The activation of PKC in platelets by thrombin (1 Unit/ml) leads to a 337 ± 47% increase over basal in the [^{32}P]-phosphorylation of a 40,000 dalton protein of uncertain function. The phosphorylation of this protein by thrombin was decreased to -10.2 ± 2.9% (n=3) below basal in the presence of 1 μM staurosporine, the newly described inhibitor of PKC (Tamaoki et al, 1986). In addition, however, staurosporine also inhibited completely the phosphorylation of a 20,000 dalton protein and the secretion of [^{3}H]5-hydroxytryptamine by thrombin. Since the 20,000 dalton protein is phosphorylated predominantly by myosin light chain kinase (MLCK), these data indicate that staurosporine is a non-selective inhibitor of protein kinases in intact platelets. Little can be concluded therefore concerning the role of PKC in the secretion of 5-hydroxytryptamine since it is possible that MLCK also contributes to this response.

Interestingly, however, staurosporine (1 μM) only slowed the rate of thrombin-induced aggregation (not shown) and had no effect on the formation of [^{3}H]inositol phosphates by thrombin (Table 1). Since this concentration of staurosporine inhibits completely the activation of PKC (as judged by the phosphorylation of the 40,000 dalton protein) these data indicate that aggregation can proceed independently of PKC activation, and that the formation of [^{3}H]inositol phosphate is not under regulatory control from PKC. A role for either (or both) PKC and MLCK in the onset of aggregation, however, is indicated by the slowing of the aggregation response observed in the presence of staurosporine.

Thus, in conclusion, the present study has shown that staurosporine is a potent but non-selective inhibitor of kinases in platelets. The complete inhibition of PKC by staurosporine has no apparent effect on the formation of inositol phosphates by thrombin, indicating that their metabolism is not under regulatory control from this kinase.

Table 1 Platelets were incubated with thrombin (1 Unit/ml) for 60 s in the absence or presence of staurosporine (1 μM). Results are shown as mean ± s.e. mean of basal levels (= 100%) from four experiments. IP, inositol monophosphate; IP2, inositol biphosphate; IP3, inositol triphosphate.

	IP	IP2	IP3
Thrombin	204 ± 13.4	277 ± 22.4	531 ± 103
Thrombin & staurosporine	205 ± 16.3	240 ± 16.8	465 ± 62.5

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EFFECT OF SODIUM INTAKE AND CAPTOPRIL ON [^{3}H]-ANGIOTENSIN II BINDING TO RAT RENAL BASOLATERAL MEMBRANES

N.P. Lewis & D.R. Ferguson, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD.

Angiotensin 11 (All) receptor numbers appear to be regulated at several tissue sites by sodium intake and by the circulating concentration of the hormone. (Aguilera & Catt, 1981; Bellucci & Wilkes, 1984; Sernia et al., 1985; Aguilera et al., 1978; Douglas & Brown, 1982). We have investigated the effect of dietary sodium intake and inhibition of converting enzyme with captopril on All receptors on basolateral membranes prepared from the proximal renal tubule of the rat.

Basolateral membranes were prepared, binding was measured and the results obtained were corrected for peptide degradation by TLC as previously described (Lewis & Ferguson, 1986). Estimates of K_D and B_{max} were obtained from this data using the Harwell Library non-linear regression programme VB01A. Binding was measured in membranes prepared from groups of rats maintained on: 1. High sodium intake 2. Normal Sodium intake 3. Low Sodium intake. All dietary regimes were maintained for 14 days. Binding was also measured in rats maintained on low or high sodium intake who received captopril 500mg L^{-1} in their drinking water for 5 days prior to experiments.

Sodium intakes were confirmed by measurement of bladder urinary sodium concentrations showing: 1) Low Sodium = 3.8±1.2mM 2) Normal Sodium = 23±4.3mM 3) High Sodium = 98±17mM (n=5).

Table 1 shows the effect of sodium intake and captopril on B_{max} and K_D. A trend of increasing receptor density with reduced sodium intake, which does not quite reach statistical significance, was found. Inhibition of All production by captopril in high and low soidum groups was associated with a significant reduction in receptor density. These results suggest that All receptor density on the basolateral surface of the proximal renal tubule may be modulated by altered activity of the renin angiotensin system.

Table 1

Diet	B_{max} fmol.mg^{-1}	K_D nM
Low Na (n=5)	505.6±51.6	0.27±0.03
Normal Na (n=5)	465.8±51.5	0.23±0.03
High Na (n=5)	435.9±39.8	0.21±0.05
Low Na + Captopril (n+5)	262.6±19.9**	0.22±0.02
High Na + Captopril (n=5)	294.9±17.5**	0.23±0.02

** $p<0.001$ (analysis of variance)

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REVERSAL OF AGONIST-INDUCED BRONCHOCONSTRICTION IN THE GUINEA-PIG BY THE POTASSIUM CHANNEL ACTIVATOR BRL 34915

D.R. Buckle*, J. Bumstead, G.D. Clarke, J.F. Taylor and S.G. Taylor, (Introduced by B.A. Spicer), Beecham Pharmaceuticals Biosciences Research Centre, Great Burgh, Epsom, Surrey KT18 5XQ.

BRL 34915, a benzopyranol currently undergoing clinical evaluation as a novel antihypertensive agent, has been reported to produce relaxation of bronchial and other smooth muscle preparations in vitro by opening Rb^+-permeable K^+-channels (Allen et al, 1986; Hamilton et al, 1986). In this communication the in vitro effect of BRL 34915 on isolated guinea-pig tracheal spirals is confirmed and preliminary studies in vivo in anaesthetised and conscious guinea-pig models of bronchoconstriction are reported.

Using guinea pig isolated tracheal spirals, prepared for isometric recording under a tension of 2g, BRL 34915 (10^{-7} to 2 x 10^{-5}M) inhibited spontaneous tone in a concentration-dependent manner with an IC_{50} value relative to the maximum relaxation achieved by isoprenaline (10^{-3}M) of 1.1 (0.6 - 1.9) x 10^{-6}M (geometric mean with 95% confidence limits, n=7). Intrinsic activity relative to isoprenaline was 0.89 ± 0.02 (arithmetic mean ± s.e., n=7). Under similar conditions the calcium entry blocker nifedipine had only a partial relaxant effect.

In the anaesthetised guinea-pig (Konzett & Rössler, 1940) BRL 34915, administered intravenously (10 - 400µg kg^{-1}), evoked a dose-related inhibition of the bronchoconstrictor effect of an approximately ED_{50} dose of 5-hydroxytryptamine (5-HT, 3.3 - 5.2µg free base kg^{-1} i.v. given as creatinine salt). The maximal effect of BRL 34915 occurred within 1 to 6 min of administration and the dose producing a 50% inhibition of the 5-HT response at 1 min post dosing was 32 (20-49, n=6) µgkg^{-1}. In contrast, nifedipine (10 - 300µgkg^{-1} i.v.) elicited no inhibition of the bronchoconstrictor response to 5-HT, despite causing large falls in blood pressure at dose levels of 30µgkg^{-1} and above.

In the conscious guinea-pig BRL 34915 (2.5 and 5.0mgkg^{-1}), administered orally prior to a 20 sec exposure to an aerosol of 5 x 10^{-3}M histamine (Herxheimer, 1952) prolonged the time before asphyxic collapse. The protective effect of 2.5mgkg^{-1} p.o. BRL 34915 was maximal 60 min after dosing, at which time approximately half of the animals were protected from collapse during the 4 min observation period. Nifedipine, on the other hand, was inactive in this model at doses up to 5mgkg^{-1} p.o. when it was given 30 min before the histamine challenge.

These results demonstrate that BRL 34915, a drug acting via a novel mechanism, has potential as a bronchodilator for the treatment of asthma. It has a long duration of action in the guinea-pig and a long plasma half life (24 hours) in man (Davies et al, 1987), suggesting that it may have particular value in the treatment of nocturnal asthma, for which current therapy is inadequate. Preliminary studies in man have shown that oral BRL 34915 protects against histamine-induced bronchoconstriction (Baird et al, 1987).

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SALMETEROL: A POTENT AND LONG-ACTING INHIBITOR OF THE RELEASE OF INFLAMMATORY AND SPASMOGENIC MEDIATORS FROM HUMAN LUNG

P.R. Butchers, S.A. Cousins and C.J. Vardey.

Department of Respiratory Pharmacology and Biochemistry, Glaxo Group Research, Ware, Herts.

The release of inflammatory and spasmogenic mediators from human lung contributes to airway obstruction in allergic asthma. β_2-adrenoceptor agonists such as salbutamol have previously been shown to inhibit both histamine and leukotriene (LT) C_4/D_4 release (Butchers et al, 1980, Hughes et al, 1983). We now report the effects of salmeterol, a potent and long-acting β_2-agonist bronchodilator (Bradshaw et al, 1987), as an inhibitor of mediator release from antigen-challenged human lung in vitro.

Human lung fragments were passively sensitised overnight and then challenged with specific antigen as previously described (Butchers, et al 1979). Histamine was assayed on the isolated guinea-pig ileum preparation and LTC_4/D_4 and prostaglandin (PG)D_2 by radioimmunoassay. Agonists were incubated with lung fragments for 30 min prior to antigen challenge. In experiments with propranolol, the antagonist was pre-incubated with lung fragments for 20 min and then the agonist added 10 min before challenge. Duration of action was estimated by incubating lung fragments with agonist for 20 min at 37°C, followed by washing and incubation in Tyrode's solution at 37°C. Fragments of lung were removed and challenged at intervals. The time taken for maximal inhibition to be reduced by 50% (Rt_{50}) was calculated.

Salmeterol was a potent inhibitor of histamine, LTC_4/D_4 and PGD_2 release, being approximately 5-20 times more potent than salbutamol (Table 1). Propranolol antagonised the inhibitory effect of salmeterol in a competitive manner: histamine (pA_2 8.4±0.1, slope 1.0±0.02; n=3) and LTC_4/D_4 (pA_2 8.4±0.04, slope 1.0±0.03; n=3). The inhibition by salbutamol (200nM) of histamine and LTC_4/D_4 release was rapidly reversed by washing (Rt_{50} <1h). In contrast, the inhibitory effect of salmeterol at an equipotent concentration (40nM) was much longer lasting, Rt_{50} values being >8h and significant inhibition still being observed after 20h.

Table 1 Inhibition of lung mediator release:

	EC_{50} (±SEM) nM		
	Histamine	LTC_4/LTD_4	PGD_2
Salmeterol	12.2 ± 6.8 (n=6)	1.8 ± 0.6 (n=6)	1.8 ± 0.8 (n=3)
Salbutamol	56.0 ± 18.4 (n=17)	11.3 ± 1.7 (n=17)	41.3 ± 5.9 (n=3)

Thus, salmeterol is a potent and long-acting inhibitor of antigen-induced mediator release from human lung. The profile of activity of salmeterol should have significant benefit in the treatment of bronchial asthma.

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BRONCHODILATOR ACTIVITY OF SALMETEROL, A LONG-ACTING β_2-ADRENOCEPTOR AGONIST

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

Salmeterol is a potent, selective β_2-adrenoceptor agonist with a long duration of action in vitro (Bradshaw et al., 1987; Ball et al., 1987a). We have now investigated the bronchodilator activity of the compound when given by inhalation and orally in the anaesthetised cat and conscious guinea-pig.

Cats of either sex (1.5-3.0kg) were fasted overnight and anaesthetised with urethane:chloralose (800:80mgkg^{-1} i.p.) or sagatal (48mgkg^{-1} i.p.) and chloralose (40mgkg i.p.), and were prepared for artificial ventilation, intravenous (i.v.) administration of drugs and the recording of tracheal pressure (TP) and soleus muscle twitch tension (SMT) as described by Apperley et al (1976). Continuous i.v. infusion of 5-hydroxytryptamine (28-85nmolkg^{-1}min^{-1}) was used to elevate resting TP by ~50%. In some cats, a three-way tap was introduced into the duodenal wall for dosing by the intraduodenal (i.d.) route. For aerosol administration, an expansion chamber was connected to the in-port of the respiration pump when required, aerosols being generated from solutions of various concentrations of agonists using a Devilbiss 645 nebulizer. Conscious guinea-pigs were challenged with aerosolised histamine (9mM) before and at intervals after ß-adrenoceptor agonists, administered either by inhalation or orally (p.o.) as described by Ball et al., (1987b).

Following i.d. administration (0.24-240nmolkg^{-1}) to anaesthetised cats, salmeterol (salm.), like isoprenaline (iso.), salbutamol (salb.) and clenbuterol (clen.), caused dose-related inhibition of TP, the rank order of potency being clen. (1) > salb. (7) ~ salm. (8) > iso. (38). Bronchodilator doses of salm. and salb. also inhibited SMT. The time to 50% recovery (Rt_{50}) for approximate ED_{50} doses of iso., salb. and clen. on TP were similar (57-76 min, n=3-8), whereas those to salm. exceeded 110 min (n=4). On inhalation, all four agonists (10µM-10mM) caused concentration-related inhibition of TP with little or no effect on SMT, the rank order of potency being iso. (1) > salb. (3) ~ salm. (4) > clen. (>30). The Rt_{50} values for iso., salb. and clen. were 3-12 min (n=5-22), but those to salm. were > 20 min (n=5).

In conscious guinea-pigs, salb. (4.2-42µmolkg^{-1}) and salm. (0.24-24µmol kg^{-1}) p.o. gave dose-related protection against histamine-induced bronchoconstriction, salm. being approximately 10-fold more potent than salb. Both compounds had a duration of action of approximately 12h. Salm. (0.12-12mM), like salb. and clen. (0.2-2.0mM) (n=5-8), also inhibited histamine-induced bronchoconstriction when given by inhalation. Inhibition was concentration-related with salb. and clen., and Rt_{50} values for submaximally-effective doses were 60-70 min. In contrast, salm.-induced protection was virtually identical (85-97%) at all the doses tested and the duration of action was 3-6h.

Salmeterol is therefore a potent, long-acting bronchodilator agent in vivo, and appears particularly suitable for aerosol administration.

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AN EXAMINATION OF WHETHER β_2-ADRENOCEPTORS OF ISOLATED LUNG ARE DESENSITIZED BY ISOPRENALINE AND DOPEXAMINE HYDROCHLORIDE

M.L.Herepath and K.J. Broadley, Division of Pharmacology, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff CF13XF

Cardiac β_1-adrenoceptors have been shown by ourselves (Broadley & Herepath, 1987) and others (Kaumann & Birnbaumer, 1976) to undergo desensitization in atria after prolonged *in vitro* exposure to isoprenaline. Lung β_2-adrenoceptors appear to undergo similar desensitization (Van den Berg *et al.*, 1982); however, it is not certain whether they exhibit the same degree of desensitization. The present study examines whether desensitization of β_2-adrenoceptor-mediated relaxation of lung strips can be induced by isoprenaline and dopexamine, a selective β_2-agonist with peripheral dopaminergic activity (Brown *et al.*, 1985).

Guinea-pig paced left atria (2 Hz, threshold voltage + 50%, 5 ms), spontaneous right atria and lung parenchymal strips (n >4) were set up in Krebs-bicarbonate solution containing ascorbic acid (1 mM) at 37.5°C gassed with O_2:CO_2 (95:5). Left atrial and lung strip isometric tension and right atrial rate were recorded. Relaxation responses were obtained in lung strips with intrinsic tone or pre-contracted with carbachol. Cumulative concentration-response curves to isoprenaline or dopexamine were constructed. The maximum effective concentration of agonist remained in contact with the tissue for 4h; washout for 1h was followed by a second curve. Pre-incubation curves were corrected from time-matched controls.

Desensitization of atrial β_1-receptors was exhibited as significant shifts ($p < 0.05$) to the right and depression of the maxima to 54.7±9.1% (tension) and 58.3±10.2% (rate) after incubation with isoprenaline (10^{-5}M). In carbachol-contracted lungs, incubation with isoprenaline (10^{-5}M) caused a significant reduction of the isoprenaline maximum to 76.2±8.6%. The sensitivity to isoprenaline was greater in intrinsic tone than carbachol-contracted lungs. A maximum relaxation could therefore be achieved with 10^{-6}M isoprenaline. This was left in contact for 4h and after washout the second curve was also significantly ($p < 0.05$) reduced to 60.2±11.5%. When this concentration was used in carbachol-contracted lungs, the isoprenaline curves were virtually superimposed indicating no desensitization. In our previous study, this dose of isoprenaline desensitized atrial β_1-adrenoceptors. Dopexamine produced relaxation of the intrinsic tone lung and after 4h incubation with 2 x 10^{-4}M, the second curve was superimposed on the first, confirming the lack of desensitization.

The apparent reduction of sensitivity induced by 10^{-5}M isoprenaline in carbachol contracted lung, and by 10^{-6}M in intrinsic tone preparations required explanation. To examine whether this was due to failure to recover from the prolonged relaxation of isoprenaline, lungs were relaxed with lanthanum (1.4×10^{-5}M) for 4h after constructing a curve for isoprenaline. During this time the isoprenaline was washed out. After washout of the lanthanum for 1h, the maxima of isoprenaline were non-significantly reduced to 74.8±15.1 and 80.3±10.0%, respectively in intrinsic tone and carbachol-contracted lungs. Thus, reduction of the maxima after exposure to isoprenaline could be explained by a failure to restore tone after prolonged relaxation.

Therefore, lung β_2-adrenoceptors are resistant to desensitization by dopexamine and in contrast to atrial β_1-adrenoceptors are less liable to desensitization by isoprenaline.

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A NOVEL IN VIVO METHOD FOR CONTINUOUS MEASUREMENT OF CIRCULATING LEUKOCYTE LEVELS

Jannine F Marr and G M Smith, School of Pharmacy, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen AB2 1FR

Previous in vivo studies on the involvement of leukocytes in various disease states including inflammation, asthma and cardio-vascular disorders have relied upon leukocyte counts obtained from discrete blood sampling, at intervals, from experimental animals. The technique described here has been designed to continuously record circulating leukocyte levels in vivo.

The continuous platelet recording technique (Smith and Freuler, 1973) has been modified to count leukocytes. Blood is continuously withdrawn from an anaesthetised animal, anticoagulated, then diluted with saline and white cell diluting fluid (WCDF). WCDF lyses erythrocytes and platelets and the leukocytes are then counted using a Technicon Autocounter and levels continuously recorded on pre-calibrated chart paper.

This method has been used to count leukocytes both in vitro and in vivo. In vitro a 10ml blood sample was taken from anaesthetised rats and guinea-pigs and mean leukocyte levels of 5738 ± 678 and 4121 ± 585 x 10^6 cells/l respectively recorded. In vivo continuous leukocyte levels of 4947 ± 388 x 10^6 cells/l were recorded in anaesthetised guinea-pigs. To verify the leukocyte counts measured using this technique samples of diluted blood were taken during these experiments and counted using a Coulter Counter. There was no significant difference ($P<0.05$) between leukocyte counts measured using both methods and the results showed a good correlation as shown in Table 1.

	Mean Leukocyte counts* (10^6 cells/l)		Correlation
	Autocounter	Coulter Counter	Coefficient
Rat blood in vitro	5738 ± 678	5502 ± 674	0.991
Guinea pig blood in vitro	4121 ± 585	3971 ± 581	0.975
Guinea pig in vivo	4947 ± 388	4821 ± 388	0.948

Table 1

* mean ± s.e.
n = 10 for each example

Preliminary experiments in anaesthetised male Dunkin-Hartley guinea-pigs measured the fall in circulating leukocyte levels after bolus i.v. administration of Platelet Activating Factor (PAF) and N-Formyl-L-Methionyl-L-Leucyl-L-Phenylalanine (FMLP). PAF (25-200 ng/ug) and FMLP (1-50 ng/kg) produced a dose-dependent fall in leukocyte levels. In sensitised animals allergic challenge produced a fall of 77.5 ± 7.01% in the number of circulating leukocytes.

This technique will allow continuous measurement of leukocyte levels simultaneously with other parameters in in vivo models of various pathological disorders.

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SEMICARBAZIDE-SENSITIVE AMINE OXIDASES OF SHEEP BLOOD VESSELS AND BLOOD PLASMA

J. Elliott, B.A. Callingham and D.F. Sharman, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD.

Amine oxidases, sensitive to semicarbazide and resistant to clorgyline, have been reported in several different species since such an enzyme was found in the cardiovascular tissues of the rat (Coquil et al, 1973). Ruminants possess a semicarbazide-sensitive amine oxidase (SSAO) in the blood plasma capable of metabolising spermine and spermidine as well as many monoamines ("spermine oxidase"; Hirsch, 1953). At present however, there is considerable uncertainty concerning the possible functions of these enzymes.

Five adult, mixed breed sheep (two female and three male), were killed by sodium pentobarbitone and the femoral arteries removed. Tissues were homogenised in 1mM K phosphate buffer, pH 7.8 and the enzyme activity measured with ^{14}C-benzylamine (2.5 - 60 μM). Enzyme activity in sheep plasma was also determined with benzylamine (1.0 - 1000μM). Some amines that might be substrates for SSAO were examined for their ability to interfere with labelled benzylamine deamination by both artery homogenates and sheep plasma. In addition, both fluorimetric and colorimetric methods were used to study the deamination of unlabelled substrates.

Oxidative deamination of benzylamine (10μM) by sheep femoral artery was resistant to inhibition by clorgyline (1mM) and sensitive to inhibition by semicarbazide (0.1mM). The Km for benzylamine was 17.1 ± 1.6μM and the Vmax was 149 ± 10 nmol.h.$^{-1}$ mg^{-1} protein (n = 5). The following amines were found to inhibit benzylamine deamination in this tissue, with approximate Ki values (μM ± s.e.m.): Kynuramine (17 ± 1), spermine (57 ± 10), tyramine (165 ± 8), phenethylamine (350 ± 79), isoamylamine (459 ± 58) and dopamine (648 ± 186), (n = 3-4). Spermidine, histamine and cadaverine were virtually without effect at 250μM. Preliminary findings with a fluorimetric analytical method show that spermine is a very poor substrate for the SSAO in sheep artery. The deamination of benzylamine by sheep plasma was resistant to inhibition by clorgyline (1mM) and sensitive to semicarbazide (10μM). Kinetic analysis (Spears et al, 1971) demonstrated the presence of two activities metabolising benzylamine. One had an approximate Km of 2 - 5μM and the other a Km of 950μM. Spermine and spermidine are substrates for the high Km activity. Spermine interacts with the low Km activity but spermidine appears to have little effect. Dopamine (500μM) interacts effectively with both plasma enzymes.

In conclusion, the sheep femoral artery contains an enzyme activity, capable of deaminating benzylamine, with properties comparable to SSAO of other species. Sheep blood plasma contains two enzymes that will deaminate benzylamine; one similar to the benzylamine oxidase of other species (Blaschko et al, 1959) and the other (spermine oxidase) differs in several respects. The origin of the two plasma enzymes remains to be determined but it is probably not the arterial wall unless the arterial wall enzyme changes its properties on release.

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AN ELECTROPHYSIOLOGICAL INVESTIGATION OF THE MECHANISM OF ACTION OF BRL34915 ON THE GUINEA-PIG BLADDER

K. Fujii, University Department of Pharmacology, South Parks Road, Oxford, OX1 3QT.
(Introduced by A.F. Brading)

BRL34915 produces smooth muscle relaxation which is considered to result from opening of K^+-channels (Allen et al.,1986; Hamilton et al.,1986). I have used electrophysiological techniques to investigate the actions of BRL34915 on the smooth muscle of the guinea-pig bladder, in relation to the K^+-channels present in this tissue.

The resting membrane potential, obtained with microelectrodes, was -60.6 ± 3.2mV (mean ± S.D., n=20, 10 animals) and most cells were spontaneously active. BRL34915 ($5x10^{-7}M$) reduced the spike frequency and, in higher concentrations (10^{-6}-$10^{-5}M$), abolished the spontaneous spikes and produced dose-dependent hyperpolarization (over -25mV at $10^{-5}M$). When the double sucrose gap method was used, BRL34915 (10^{-6}-$10^{-5}M$) hyperpolarized the membrane, increased the membrane conductance and reduced the size of contractions evoked by depolarizing currents. Excitatory junction potentials were only slightly affected, the small reduction in their size probably being caused by the changes in membrane properties. An increase in the extracellular K^+ concentration to 20mM (in which the potassium equilibrium potential is slightly less negative than the resting membrane potential), antagonized the inhibitory action of BRL34915 ($5x10^{-6}M$). These results strongly suggest that BRL34915 may open K^+-channels.

The type of K^+-channel which BRL34915 opens was studied using three K^+-channel blockers, tetraethylammonium (TEA), apamin and procaine.TEA (10mM) and apamin ($10^{-7}M$) caused little, if any, detectable depolarization but increased the spike frequency. TEA slightly prolonged the spike duration and apamin abolished the after-hyperpolarization, but pretreatment with these two agents failed to prevent the effects of BRL34915 ($5x10^{-6}$ M). In contrast, Procaine (5mM) depolarized the membrane with a marked increase in spike frequency. It not only abolished the after-hyperpolarization but also produced a large increase in spike duration, and almost abolished the effects of BRL34915 ($5x10^{-6}M$).

There may be, therefore, at least two K^+-channels in bladder smooth muscle : one responsible for the after-hyperpolarization which is blocked by apamin and procaine, and is possibly Ca^{2+}-activated , and another responsible for the falling phase of the spike which is resistant to apamin, sensitive to procaine and partially sensitive to TEA. Since only procaine effectively antagonized the action of BRL34915, the drug may be opening the latter type of K^+-channel.

I should like to express my thanks to Dr A.F.Brading, University Department of Pharmacology, Oxford, for her help and encouragement. I am grateful to Beecham Pharmaceuticals for providing BRL34915.

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THE EFFECT OF POTASSIUM CHANNEL ANTAGONISTS ON THE BRL 34915 ACTIVATED POTASSIUM CHANNEL IN GUINEA-PIG BLADDER

C.D.Foster and A.F.Brading, University Department of Pharmacology, South Parks Road, Oxford. OX1 3QT.

In smooth muscle, BRL 34915 is thought to act via an increase in the permeability of the membrane to potassium ions. Using guinea-pig bladder detrusor smooth muscle, we have investigated the effects of this compound on both contractile responses and transmembrane fluxes using radio-tracer ion flux analysis. Efflux from tissues loaded with ^{86}Rb or ^{43}K was followed using continuous superfusion. Uptake was measured after a 10 min exposure to the isotope followed by a 5 min wash in ice cold Krebs to remove extracellular tracer. No increase in either flux could be detected with ^{86}Rb. Further experiments with ^{43}K demonstrated a significant increase in both transmembrane fluxes of potassium ions in this smooth muscle. The potassium channels opened by BRL 34915 show a selectivity for ^{43}K over ^{86}Rb.

In order to investigate the nature of the channel, the potassium channel antagonists T.E.A. (10mM), apamin ($10^{-7}M$) and procaine (5mM) were used. Various voltage and calcium activated potassium channels have been described (e.g. Bolton et al., 1985) which may differ in their sensitivity to potassium channel antagonists (e.g. Yamanaka et al., 1985). Apamin is thought to block calcium activated potassium channels. It abolishes the after-hyperpolarisation of the spike in guinea-pig bladder (Fujii, 1987). T.E.A. is thought to be most effective on voltage sensitive potassium channels, and procaine rather non-specifiic in it's action.

The effect of these three antagonists on the efflux of potassium ions was examined. In the presence of apamin ($10^{-7}M$) there was still an increase in potassium ion efflux when BRL 34915 ($10^{-5}M$) was added. Both procaine (5mM) and T.E.A. (10mM) caused an increase in efflux of potassium ions and BRL 34915 caused no further increase. Because BRL 34915 and the potassium channel antagonists may affect the membrane potential and pattern of spontaneous activity of the tissue, the efflux data are difficult to interpret unequivocally. Additional information can also be obtained from uptake experiments. Procaine was found to inhibit the uptake of potassium ions stimulated by the presence of BRL 34915.

The results support the conclusion that BRL 34915 acts by opening procaine sensitive potassium channels in bladder smooth muscle. Similar channels have been characterised in other smooth muscles, (e.g. Weir & Weston 1986), although the channel in the bladder is unusual in being imperment to Rb ions.

BRL 34915 was kindly supplied by Beecham Pharmaceuticals.

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CARDIOVASCULAR EFFECTS OF BRL 34915 IN ANAESTHETISED AND PITHED RATS

E. Donato Di Paola, P.E. Hicks[1] and Janine Lorrain, Laboratoire d'Etudes et Recherches Synthélabo (L.E.R.S.), 58 rue de la Glacière, 75013 Paris, France.

The antihypertensive agent BRL 34915 (BRL) (Buckingham et al., 1986) inhibits phasic activity and reduces vascular responses to noradrenaline and K^+ in the rat portal vein (Hamilton et al., 1986). The compound has been suggested to activate potassium channels in vascular cell membranes (Weir & Weston, 1986). This communication reports the effects of BRL on blood pressure (MAP) and heart rate (HR) in anaesthetised rats, and on vasoconstrictor responses to various stimuli in pithed rats.

Male normotensive rats (220-250 g, n=5-7/group) were anaesthetised with pentobarbitone (55 mg/kg i.p.), respired artificially and prepared for MAP and HR recordings. BRL (0.03, 0.1, 0.3, 1 mg/kg infused i.v. over 5 min; femoral vein) was studied in intact rats. The dose of 0.1 mg/kg was further evaluated in intact, chlorisondamine-vasopressin (CH/VAS) supported and in nephrectomized (NP) rats pretreated with either saline or enalapril (E: 0.3 mg/kg i.v., 10 min previously). Dose-response curves to i.v. cirazoline and UK-14,304 (quantified as $ED_{50\ mmHg}$) or the effect of a single dose of BAY K 8644 (BAY: 0.5 mg/kg i.v.) were determined in bivagotomized pithed rats after 5 min i.v. infusions of saline (0.3 ml/kg) or BRL (0.03 and 0.1 mg/kg).

At the end of infusion, BRL (0.03, 0.1, 0.3 and 1 mg/kg) evoked maximal dose-related decreases (Δ) in MAP (-13 ± 3, -39 ± 3, -59 ± 2, -64 ± 3 mmHg, respectively), with no significant change in HR. Nephrectomy, pretreatment with CH/VAS or E increased the fall of blood pressure (ΔMAP: NP: -66 ± 5; CH/VAS: -57 ± 2; E: -62 ± 2 mmHg) produced by BRL (0.1 mg/kg). However, the administration of E to NP or CH/VAS rats did not further modify the hypotensive activity of BRL. In pithed rats, BRL (0.03 and 0.1 mg/kg) caused small dose-related decreases in MAP (-5 ± 1 and -11 ± 1 mmHg, respectively). BRL (0.03 mg/kg) did not change the vasoconstrictor effect of UK-14,304 but shifted to the right the control dose-pressor response to cirazoline (dose-ratio; DR 2.6) and inhibited the effects of the studied dose of BAY (-64%). BRL (0.1 mg/kg) was, however, equieffective against cirazoline (DR 6.2) or UK-14,304 (DR 6.0). It should be noted that the maximum of the dose-effect curve to the latter agonist was decreased by 32%.

The vasodilator effect of BRL in anaesthetised rats does not require the presence of an operational sympathetic nervous system. BRL appears to reflexly activate the renin-angiotensin system since its hypotensive effects were enhanced by nephrectomy, E or CH/VAS pretreatments. The inhibition by BRL against the pressor responses to cirazoline, BAY as well as UK-14,304 discriminates this compound from calcium-entry blockers.

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[1]present address: Syntex Recherche France, 91310 Montlhéry, France.

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DERIVATIVES OF URAPIDIL WITH HYPOTENSIVE PROPERTIES AND HIGH AFFINITY FOR 5-HT_{1A} RECEPTORS

G.Gross*, G.Hanft and N.Kolassa[1] (*introduced by O.-E. Brodde), Department of Pharmacology, University of Essen, D-4300 Essen and [1]Byk Gulden Pharmazeutika, D-7750 Konstanz, Fed. Rep. of Germany

The antihypertensive agent urapidil is known to produce a vasodilator response due to peripheral $alpha_1$-adrenoceptor antagonism (Schoetensack et al.,1977). However, urapidil also seems to influence the central regulation of blood pressure. Increasing evidence indicates that 5-hydroxytryptamine (5-HT) is involved in the central control of cardiovascular functions. 8-Hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), a selective agonist at 5-HT_{1A} receptors, lowers blood pressure by a central nervous mechanism (Fozard et al., 1987a). Recently, Fozard et al. (1987b) found a rather high affinity of urapidil to these receptors and suggested that this property might be relevant to its antihypertensive effect. Some derivatives of urapidil (5-methyl-,5-formyl-, and 5-acetyl-urapidil) have been reported to lower blood pressure in cats more effectively after injection into the vertebral artery as compared to injection into the femoral vein (Kolassa et al., 1986). In the present study the affinities of these compounds to 5-HT receptors and $alpha_1$-adrenoceptors were studied by radioligand binding techniques. ^{3}H-8-OH-DPAT, ^{3}H-ketanserin and ^{3}H-prazosin were used to label 5-HT_{1A}, 5-HT_2 and $alpha_1$-adrenoceptor binding sites, respectively, in membranes of the rat cerebral cortex.

Table 1. Inhibition of ^{3}H-8-OH-DPAT and ^{3}H-prazosin binding by urapidil and some of its derivatives

	p IC_{50} (-log mol/l)	
Compound	^{3}H-8-OH-DPAT	^{3}H-prazosin
Urapidil	6.4 ± 0.1 (8)	6.1 ± 0.1 (7)
5-Methyl-urapidil	8.4 ± 0.1 (8)	7.3 ± 0.1 (7)
5-Formyl-urapidil	7.7 ± 0.1 (10)	6.7 ± 0.1 (7)
5-Acetyl-urapidil	7.6 ± 0.1 (10)	7.2 ± 0.2 (5)
8-OH-DPAT	8.4 ± 0.2 (4)	

Means ± s.e.mean (n)

Urapidil and especially the 3 derivatives tested possess high affinities for 5-HT_{1A} binding sites (see Table 1; order of potency: urapidil < acetyl- ≤ formyl- < methyl-derivative) as well as for $alpha_1$-adrenoceptor binding sites (order of potency: urapidil < formyl- < acetyl- ≤ methyl-derivative) but lack remarkable affinity for 5-HT_2 binding sites. The potency of 5-methyl-urapidil to inhibit ^{3}H-8-OH-DPAT binding is similar to that of unlabelled 8-OH-DPAT. It is concluded that an action on central 5-HT_{1A} receptors as well as a blockade of peripheral $alpha_1$-adrenoceptors may be involved in the hypotensive action of urapidil as well as its derivatives.

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SK&F 94836 A NOVEL INODILATOR RETAINS ITS ACTIVITY IN THE PRESENCE OF PHARMACOLOGICAL DOSES OF DIGOXIN AND CAPTOPRIL

M.B. Comer, R.W. Gristwood and L.M. Wood, Smith Kline & French Research Limited, The Frythe, Welwyn, Hertfordshire, U.K.

SK&F 94836 (2-cyano-1-methyl-3-[4-(4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl]-guanidine, mw 284, is a novel agent possessing mixed inotropic and vasodilator activities *in vivo* (Gristwood et al 1987). It is currently being developed for the treatment of congestive heart failure. The present investigation reports on the effects of SK&F 94836 when administered in combination with agents currently used to treat congestive heart failure, digoxin an inotrope, and captopril a hypotensive agent (for review see Guyatt 1986).

Six cats anaesthetised with sodium pentobarbitone (60 $mg.kg^{-1}$) were prepared for the measurement of arterial blood pressure, heart rate, and from a cannula placed in the left ventricle via the left common carotid artery, the 1st differential of left ventricular pressure (LVdP/dtmax) as an index of inotropic state of the heart. After 1 hour, an inotropic dose of digoxin, 25 $\mu g.kg^{-1}$, significantly increased LVdP/dtmax (in the absence of any arrhythmias) from 4638 ± 468 to 6082 ± 780 $mmHg.s^{-1}$, with no significant change in mean blood pressure, 106 ± 10 to 114 ± 11 mmHg or heart rate, 172 ± 8 to 168 ± 11 $beats.min^{-1}$. SK&F 94836, 10 $\mu g.kg^{-1}$ i.v., was then given and after 30 minutes, LVdP/dtmax further significantly increased to 7836 ± 1052 $mmHg.s^{-1}$, with no significant change in mean blood pressure, 119 ± 10 mmHg or heart rate 173 ± 11 $beats.min^{-1}$. Thirty minutes later there was no further significant change in any of these parameters. Thus SK&F 94836 retains inotropic activity in the presence of digoxin, comparable with its effects when used alone.

Four dogs were anaesthetised with sodium pentobarbitone (30 $mg.kg^{-1}$) and prepared for the measurement of arterial blood pressure and heart rate. LVdP/dtmax was measured from a cannula placed in the left ventricle via the left atrial appendage. Five minutes after the administration of captopril 3$mg.kg^{-1}$ i.v., there was a significant fall in mean arterial blood pressure, 113 ± 2 to 91 ± 7 mmHg, but no significant change in LVdP/dt, 4000 ± 204 to 4125 ± 239 $mmHg.s^{-1}$ or heart rate, 152 ± 8 to 151 ± 8 $beats.min^{-1}$. SK&F 94836, 50 $\mu g.kg^{-1}$, was then given and after 30 minutes there was a further significant fall in mean blood pressure to 81 ± 6 mmHg accompanied by a significant increase in LVdP/dt to 6000 ± 456 $mmHg.s^{-1}$, and small but significant increase in heart rate to 163 ± 9 beats min^{-1}. Thirty minutes later there was no further significant change in any of these parameters. Thus the activity of SK&F 94836 is retained in the presence of captopril.

These results indicate that SK&F 94836 retains its activity when administered concurrently with digoxin or captopril and is unlikely to result in any adverse effects on the cardiovascular system.

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POSITIVE INOTROPIC ACTIONS OF SK&F 94120 OCCUR VIA A CYCLIC AMP-DEPENDENT MECHANISM: ACTIVATION OF CAMP-PROTEIN KINASE

K.J. Murray, P.J. England and M.L. Reeves (introduced by R.W. Gristwood), Smith Kline and French Research Ltd., The Frythe, Welwyn, Herts. AL6 9AR, U.K.

SK&F 94120 is a potent positive inotropic agent which also possesses vasodilator activity (Gristwood et al., 1986). Like a number of other inotropes eg milrinone and fenoximone it has been shown that SK&F 94120 is an inhibitor of the so called 'low K_m' cyclic nucleotide phosphodiesterase (PDE III) (Reeves et al., 1987a). As the only known target for cAMP is the cAMP-dependent protein kinase (cA-PrK), it would be expected that SK&F 94120, and other similar agents, would cause an activation of cA-PrK in the intact tissue. To date, however, there are no reports indicating that a PDE III inhibitor is a able to achieve this in heart. We have investigated the effects of SK&F 94120 on cAMP levels and the activity of cA-PrK in the perfused guinea-pig heart.

Hearts were perfused for a total of 20 min by the Langendorff technique and the tension recorded as described previously (England, 1976). SK&F 94120 (100 µM) was perfused for 0, 40s, 70s, 120s and 300s. Hearts were then freeze clamped and assayed for cAMP and the % of cA-Prk in the active form (Reeves et al., 1987b).

Summary of the effects of SK&F 94120 on perfused guinea-pig hearts.

Perfusion Time with SK&F 94120	cyclic AMP (n=6) pmol/mg protein	Soluble cA-PrK % active (n=6)	Developed Tension (n=4-12)
0	9.7 +/- 0.9	23.5 +/- 1.7	100
40s	12.7 +/- 1.4	31.7 +/- 2.4*	114 +/- 1.9**
70s	17.1 +/- 1.2**	35.0 +/- 1.0**	120 +/- 4.2**
120s	12.8 +/- 1.2	33.0 +/- 0.4**	126 +/- 6.5**
300s	18.0 +/- 2.7*	36.2 +/- 1.6**	127 +/- 6.7*

(values are means +/- s.e.m.) *p<0.05 **p<0.01

The results in the Table show that SK&F 94120 caused a significant increase in the developed tension and the activity of cA-PrK at all time points studied. In addition, cAMP levels were also significantly increased at 70s and 300s, although increases were also seen at other time points. These data demonstrate clearly, for the first time, that a PDE III inhibitor is able to increase the activity of cA-PrK in intact heart, and provides further evidence that SK&F 94120 acts as an inotrope via a cAMP-dependent mechanism. The relatively small increase in cAMP observed suggests that PDE III inhibitors may affect a specific compartment of cAMP and/or cA-PrK in the intact tissue.

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SELECTIVE VASOCONSTRICTOR ACTION OF GR 43175 ON ARTERIOVENOUS ANASTOMOSES (AVAS) IN THE ANAESTHETISED CAT

W. Feniuk*, P.P.A. Humphrey, M.J. Perren, Glaxo Group Research Ltd., Ware, Herts. SG12 0DJ

The efficacy of ergotamine in migraine may rely on its vasoconstrictor action on carotid AVAs, (Heyck, 1969; Johnston and Saxena, 1978). Since the selective 5-HT_1-like receptor agonist, GR 43175 appears efficacious in the treatment of migraine (Humphrey et al., 1987; Doenicke et al., 1987) we have compared the effect of ergotamine and GR 43175 on the distribution of cardiac output in cats (1.5-3 kg) anaesthetised with chloralose (60 mg/kg i.p.) and urethane (700 mg/kg i.p.).

Blood pressure was recorded from a cannulated femoral artery and drugs administered into a femoral vein. Ascending aortic and common carotid arterial flows were measured with electromagnetic flow probes. The left atrium was cannulated for the administration of radioactive microspheres of 15 μM diameter (Ce^{141}, Ru^{103}, Nb^{95}, Sn^{113}) for measurement of regional changes in vascular resistance as described by Johnston and Saxena (1978).

GR 43175 (30-1000 μg/kg) and ergotamine (0.3-30 μg/kg) both caused increases in carotid arterial vascular resistance (maximum change, 102 ± 29% and 207 ± 34% respectively, all values mean ± s.e. mean). Ergotamine (30 μg/kg) increased diastolic blood pressure (from 73 ± 7 mmHg to 150 ± 10 mmHg) and total peripheral resistance (136 ± 22% increase) whilst GR 43175 (1000 μg/kg) caused little change in diastolic pressure (from 69 ± 5 mmHg to 82 ± 12 mmHg) and total peripheral resistance (14 ± 8% increase). GR 43175 (30-1000 μg/kg) and ergotamine (0.3-30 μg/kg) both caused marked, dose-dependent constriction of AVAs, as judged by the decrease in the fraction of radioactive microspheres trapped in the lung. The highest dose of ergotamine examined (30 μg/kg) also caused increases in resistance in the other vascular beds (Table 1).

Table 1: Regional changes in vascular resistance produced by intravenous ergotamine and GR 43175. Values are mean ± s.e. mean from at least 5 experiments.

	% CHANGE IN VASCULAR RESISTANCE					
	ERGOTAMINE (μg/kg i.v.)			GR 43175 (μg/kg i.v.)		
	0.3	3	30	30	100	1000
AVAs	18±13	299±141	2305±616	57±28	217±98	1240±664
L. KIDNEY	5±15	16±12	88±45	-4±10	-13±11	-13±11
L. ADRENAL	11±13	21±13	173±54	20±17	5±11	23±16
SMALL INTESTINE	12±18	9±12	80±45	11±11	17±13	28±24
LIVER	-3±17	13±21	101±48	-11±10	-15±15	-18±14
R. VENTRICLE	11±13	35±16	83±40	21±14	14±14	6±17
L. VENTRICLE	13±19	24±18	75±40	14±12	16±12	8±16
SEPTUM	10±14	13±9	67±40	25±18	17±13	16±23
R. CEREB. HEMIS	5±13	9±14	51±26	17±14	3±11	5±19
BRAINSTEM	5±12	10±12	68±38	24±19	9±17	14±27
CEREBELLUM	2±13	16±18	78±30	17±16	3±12	8±24
R. EYE	12±13	39±23	180±55	34±16	33±15	130±38

GR 43175 appears to have a selective vasoconstrictor action on AVAs which may explain its efficacy in the treatment of migraine.

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CHARACTERISATION OF 5-HT RECEPTORS MEDIATING CONTRACTION IN DOG AND PRIMATE ISOLATED BASILAR ARTERY

H.E. Connor*, W. Feniuk and P.P.A. Humphrey, Pharmacology Division, Glaxo Group Research Ltd., Ware, Herts. SG12 ODJ

The aim of this study was to characterise the 5-HT receptor mediating contraction of dog and primate basilar artery using selective 5-HT agonists and antagonists. Basilar arteries were obtained from beagle dogs or cynomolgus monkeys after sacrifice with pentobarbitone sodium (100 $mgkg^{-1}$ i.v.). Arteries were cut into ring segments (3-4 mm length) and suspended in modified Krebs-Hensleit solution (Apperley et al., 1976) at a final resting tension of 1g. Cumulative concentration-effect curves for agonists were constructed. When antagonists were used, 30 min contact time was allowed prior to the second agonist concentration-effect curve. One preparation remained as a control to assess spontaneous changes in tissue sensitivity. Both the dog and primate basilar arteries were contracted by 5-hydroxytryptamine (5-HT) with EC_{50} values of 45 nM (31-65 nM, n=17) and 67 nM (37-123 nM, n=12) respectively (mean, 95% limits). 5-Carboxamidotryptamine (5-CT) and GR 43175 (Humphrey et al., 1987) also caused contraction in these preparations; relative potencies and maximum responses, relative to 5-HT, are shown below.

Agonist	Dog basilar artery		Primate basilar artery	
	EPCR	% 5-HT max	EPCR	% 5-HT max
5-HT	1	100	1	100
5-CT	0.9 (0.7-1.1)	22.7±7.2*	1.4 (0.4-3.8)	74.3±14.8
GR 43175	8.8 (4.9-10.9)	56.3±14.4	6.4 (4.0-8.4)	78.3±1.9

Values are geometric means (range) or mean ± s.e.m. from between 3-5 experiments. Equipotent concentration ratio (EPCR) calculated at the level of 50% 5-HT maximum unless test agonist maximum < 50% 5-HT maximum (*) when calculated at the level of 50% test agonist maximum.

The contractile effects of GR 43175 in the dog basilar artery were unaffected by ketanserin (1 μM) and MDL 72222 (1 μM). However methiothepin (0.1 μM) antagonised the effects of both 5-HT (in the presence of ketanserin 1 μM) and GR 43175 producing concentration ratios of 35±9 and 35±11 respectively (mean ± s.e.m., n=3 and 4). This concentration of methiothepin did not antagonise the contractile effects of the thromboxane A_2-mimetic, U-46619.

We suggest that both the dog and primate basilar artery contain a 5-HT receptor which is not of the $5\text{-}HT_2$ or $5\text{-}HT_3$ type. This receptor can be characterised by the high agonistic potencies of 5-CT and GR 43175 and is similar to the $5\text{-}HT_1$-like receptor mediating contraction in the dog saphenous vein (Feniuk et al., 1985; Humphrey et al., 1987).

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EFFECTS OF THREE ACYL CARNITINES ON THE ISOLATED CORONARY AND MESENTERIC VASCULAR BEDS OF THE RAT

D.N. Criddle, A.J. Higgins[1] and B. Woodward, Pharmacology Group, University of Bath, Claverton Down, Bath BA2 7AY and [1]Pfizer Central Research, Sandwich, Kent LT13 9NJ

We have shown that palmitoyl carnitine has vasoconstrictor and vasodilator actions on the coronary circulation of the isolated rat heart (Criddle et al, 1987). As acyl carnitines accumulate in the ischaemic myocardium (Neely and McDonough, 1984) we were interested to see if other acyl carnitines had a similar profile of action to palmitoyl carnitine. The effects of myristoyl (C_{14}), palmitoyl (C_{16}) and stearoyl (C_{18}) carnitines have therefore been studied on the coronary circulation of the rat. In addition their actions on the perfused mesenteric vascular bed of the rat have been examined.

Hearts and mesenteric beds from Wistar rats (University of Bath strain) were perfused at constant flows of 10 and 4 ml min^{-1} respectively at 37°±0.5° using modified Krebs Henseleit solution containing 3.2mM or 5.9mM K^+ respectively. Perfusion pressure changes were used as a measure of vasoconstriction and dilation. In some mesenteric preparations vascular tone was increased by perfusing with Krebs-Henseleit solution containing 65mM K^+(Na^+ reduced to maintain osmolarity), or 10^{-5}M phenylephrine. Acyl carnitines were made up in perfusate and administered as bolus injections into the perfusion stream.

In isolated hearts all three acyl carnitines (1-10nmoles) produced vasoconstriction with no effect on heart rate or developed tension. At higher doses (10-80 nmoles), myristoyl and stearoyl carnitines (n=6) produced a dose related vasoconstriction; occasionally this was followed by a slowly developing but small vasodilator component. In contrast, palmitoyl carnitine (10-80nmoles) produced a large, long lasting vasodilation (n=12) the magnitude of which was dependent on the initial perfusion pressure, this attained values of 50-60 mmHg in many cases; at the two highest doses used (40 and 80 nmoles) palmitoyl carnitine produced a pronounced initial vasoconstrictor action. At these high doses all three acyl carnitines depressed myocardial contractility with no effect on heart rate.

In the mesenteric vascular bed all three acyl carnitines (10-640 moles) produced vasoconstriction with no vasodilator component. The vasoconstrictor action was much less than that seen in the isolated heart. In preparations in which tone had been increased by high K^+ or phenylephrine, palmitoyl carnitine only produced vasoconstriction; this was potentiated by phenylephrine but not by high K^+. Palmitoyl carnitine did not produce vasodilation in these precontracted mesenteric preparations.

These results show that the large coronary dilator action seen with palmitoyl carnitine is not common to all acyl carnitines, also the vasodilator action of palmitoyl carnitine shows organ specificity as it does not dilate mesenteric vessels in the rat. The vasoconstrictor action of palmitoyl carnitine in the mesenteric vascular bed is potentiated by the α agonist phenylephrine.

D.N. Criddle is an SERC CASE student.

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QUANTITATIVE CHEMILUMINESCENCE MEASUREMENTS OF XANTHINE OXIDASE AND DEHYDROGENASE ACTIVITY IN FOUR TYPES OF CARDIOVASCULAR CELL

I.A. Bailey[1], C.P. Blackwell and B. Woodward, Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY.
[1]Bioscience II, ICI Pharmaceuticals plc, Alderley Park, Macclesfield, Cheshire SK10 4TG.

The activities of xanthine oxidase (XO) and xanthine dehydrogenase (XD) were quantitatively measured using luminol-enhanced chemiluminescence. Cell monolayers of neonatal rat myocytes, rat vascular smooth muscle (vsm) cells, rat cardiac fibroblasts and human umbilical endothelia, and a cell suspension of adult rat myocytes were used. Cells were introduced into the light-tight sample compartment of a phototube in appropriate culture medium containing 0.4 mM xanthine and 40 µl (2 mg/ml) luminol. Emitted photons were measured for 10 consecutive periods of 10 s initially and after each agent addition. Cells were lysed with 40 µl 1% Triton-X100 to release any XO present. 40 µl of 5mM DTNB was then added to oxidise any XD present to the XO form. Finally 40 µl (5 m.i.u.) commercial XO was added as a positive control.

Table 1: XO and XD activities of five cell preparations. Values are the mean + s.e.mean of 3 experiments throughout.

CELL TYPE	ENZYME ACTIVITY (m.i.u./mg total cell protein) XO	XD
Endothelial cells	27.4 ± 7.2	16.5 ± 5.8
Adult myocytes	0.4 ± 0.3	0.5 ± 0.2
A10 vsm cells	15.4 + 2.8	4.4 + 2.4
Cardiac fibroblasts	2.2 ± 0.5	1.8 ± 0.2
Neonatal myocytes	4.7 ± 0.3	8.0 ± 4.5

Each medium had similar and negligable anti-oxidant properties (data not shown). Table 1 shows that the greatest XO activity is located in vascular cell types, predominantly the endothelia. Chemiluminescence in endothelial cells was greatly reduced in the presence of 0.001 mM oxypurinol (data not shown), indicating that photo emissions were the result of XO activity. These results are in agreement with earlier qualitative data (Bruder et al, 1982; Jarasch et al, 1981; Werns et al, 1986), although Gerlach et al (1985) published data to the contrary. The method for quantitative XO/XD activity measurements was both rapid and reproducible. The vascular location of XO, and its role in producing free radicals, may be important in the pathology of myocardial infarction, microvascular abnormalities and in arrhythmogenesis.

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INCREASED CARDIAC β-ADRENOCEPTOR SENSITIVITY IN THE DIABETIC RAT

C.E. Austin, A.M. Breckenridge & R. Chess-Williams, Department of Pharmacology & Therapeutics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX.

Several studies have reported a desensitization or "down-regulation" of cardiac β-adrenoceptors in diabetes of several months duration (Williams et al., 1983; Latifpour & McNeil, 1984). In the present study the effect of diabetes of 14 days duration has been examined on cardiac adrenoceptors.

Diabetes was induced in rats (200g) by a single intravenous injection of streptozotocin (50 mg Kg^{-1}). Age matched controls were injected with the citrate buffer vehicle. 14 days later animals were killed and left atria (LA) and papillary muscles (PM) set up in an aerated krebs solution at 37°C. Tissues were paced at 1Hz and isometric developed tension recorded. Cumulative concentration-response curves to isoprenaline, phenylephrine and calcium chloride were constructed in the presence of desipramine (1μM) and metanephrine (10μM). The remaining ventricular tissue was assayed for [^{3}H] dihydroalprenolol (DHA) binding using 200μM isoprenaline to determine non-specific binding.

In diabetic animals, cardiac sensitivity to isoprenaline was increased, the EC_{50} being reduced from 38.4 (21.9-67.3)nM to 5.1 (2.7-9.7)nM for LA ($P < 0.001$) and from 57.6 (27.6-119)nM to 10.6 (3.5-32.6)nM for PM ($P < 0.05$). The maximum developed tension to isoprenaline was similar in control (LA = 0.41 ± 0.05: PM = 0.69 ± 0.10g) and diabetic tissues (LA = 0.45 ± 0.07: PM = 0.49 ± 0.05g). Phenylephrine responses were not significantly altered in diabetic rats, the EC_{50} values and maximum responses as a percentage of the isoprenaline maxima in control LA (6.2 (4.3-9.0)μM; 44.3 ± 12.2%) and control PM (2.0 (0.9-4.9)μM; 40.8 ± 8.5%) were not significantly different to those of LA (2.4 (0.5-12.6)μM; 65.9 ± 13.2%) and PM (0.6 (0.01-27.2)μM; 53.6 ± 16.1%) from diabetic animals. EC_{50} values and maximum responses to calcium were also similar in control (LA = 4.2 (1.6-11.0)mM; 0.40 ± 0.09g: PM = 6.1 (1.2-32.0)mM; 0.31 ± 0.09g) and diabetic tissues (LA = 4.9 (2.4-9.8)mM; 0.36g ± 0.10 : PM = 3.9 (2.5-6.1)mM; 0.46g ± 0.07).

The dissociation constant for [^{3}H] DHA binding was not altered in diabetic animals (1.3 ± 0.4nM compared with 1.5 ± 0.4nM in controls). However the number of [^{3}H] DHA binding sites was increased from 40.6 ± 3.9 to 65.1 ± 14.2 fmoles mg^{-1} protein in diabetics although this increase was not statistically significant.

These results suggest that in diabetes of short duration in the rat, cardiac β-adrenoceptor mediated responses are enhanced. This may possibly be due to an increase in β-adrenoceptor number.

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DESENSITIZATION OF CARDIAC AND AORTIC ADRENERGIC RESPONSES BY ADRENALINE

R. Chess-Williams, Department of Pharmacology & Therapeutics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX.

It has recently been demonstrated that incubating tissues with noradrenaline (50μM) results in a desensitization of aortic but not cardiac α-adrenoceptor mediated responses (Chess-Williams, 1987). The present study investigates whether adrenaline also exhibits this differential desensitizing action at cardiac and aortic α_1-adrenoceptors.

Rat left atria (LA) papillary muscles (PM) and thoracic aorta were set up in aerated Krebs solution at 32°C. Aorta were cut spirally into a strip, the endothelium removed, and set up under 1g tension. Following 120 min incubation with or without 50μM adrenaline (AD), tissues were washed and cumulative concentration-response curves to phenylephrine or potassium chloride constructed. Cardiac tissues were paced at 1 Hz and isometric developed tension recorded. After 30min equilibration, the stimulator was turned off and the tissues incubated with or without adrenaline (50μM) for 120 min. After washout tissues were again paced at 1 Hz and cumulative concentration-response curves to isoprenaline calcium or phenylephrine were constructed in the presence of desipramine (1μM) and metanephrine (10μM).

Incubation of cardiac tissues with AD resulted in a desensitization of responses to isoprenaline with an increase in EC_{50} value from 1.7 (1.0-3.0)nM to 17.3 (5.9-50.4)nM for LA ($P < 0.001$) and from 17.5 (11.0-27.9)nM to 72.9 (52.7-100.8)nM for PM ($P < 0.001$). The maximum developed tension to isoprenaline was not significantly altered by adrenaline incubation.

Cardiac responses to phenylephrine were also desensitized following AD incubation, with a significant reduction in the maximum response from 0.67 ± 0.08g to 0.35 ± 0.09g for LA ($P < 0.01$). Phenylephrine EC_{50} values for control (6.8(5.0-9.3)μM) and desensitized LA (10.3(2.8-34.4μM) were not significantly different. Responses of PM to phenylephrine were almost abolished after incubation with AD. Responses to calcium were unaffected by incubation with AD, EC_{50} values in control (LA = 4.4 (2.9-6.6; PM = 3.5 (2.8-4.4)mM) and desensitized tissues (LA = 5.4 (4.1-7.2); PM = 4.8 (2.7-8.3)mM) were similar, as were the maximum responses to calcium.

Incubation of aorta with adrenaline also resulted in a selective desensitization of α-mediated responses. The EC_{50} of phenylephrine was increased ($P < 0.001$) from 25.0 (15.8-39.4)nM to 824 (95.1-7149)nM, whereas EC_{50} values for potassium chloride were similar in control 57.7 (26.9-123.6mM) and desensitized aorta (48.1(33.0-69.8)mM).

These results suggest that adrenaline desensitizes vascular α- and myocardial β-adrenoceptor mediated responses, but unlike noradrenaline, also desensitizes myocardial α_1-mediated responses.

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A COMPARISON OF THE EFFECTS OF NORADRENALINE ON SHEEP BASILAR AND MIDDLE CEREBRAL ARTERIES

A.J. Gaw, P.P.A. Humphrey[1] & R.M. Wadsworth, Department of Physiology and Pharmacology, Strathclyde University, Glasgow G1 1XW. [1]Glaxo Group Research Ltd, Ware, Herts SG12 ODJ.

It has been reported that postjunctional α_2-adrenoceptors occur in the cerebrovascular bed [Skarby et al, 1983]. We have studied several arteries from the sheep cerebrovascular bed, comparing contractile responsiveness to noradrenaline (NA) and investigating the adrenoceptor types involved.

Ring segments (3-4mm length) were prepared from sheep basilar or middle cerebral arteries (MCA). Some preparations were rubbed to remove the endothelium (confirmed by abolition of relaxation caused by acetylcholine). The rings were suspended on parallel wires under 1g resting tension in Krebs solution. Cumulative concentration-response curves to NA were obtained. Cocaine (10^{-5}M) or prazosin ($3x10^{-9}$M) was then added to the bath and allowed to equilibrate for 30 minutes before carrying out a second concentration response curve to NA.

Taking the maximal 5-hydroxytrypamine (5HT) contraction as standard, a much larger contraction could be produced by NA in the MCA than in the basilar artery ($P<0.01$). The potency of NA was similar in the two arteries, whether rubbed or unrubbed (Table 1). Cocaine significantly potentiated NA in the MCA (unrubbed, $P<0.01$; rubbed, $P<0.05$) but not in the basilar artery. Cocaine had no effect on the maximum NA contraction. NA contractions in the MCA were antagonised by prazosin with a dose ratio of 5.8±1.0 (n=6) which is equivalent to a pA_2 of 9.2 and suggestive of α_1-adrenoceptor blockade. The selective α_2-adrenoceptor agonist, UK14304 (up to 10^{-4}M), had no effect in either artery.

Table 1. Noradrenaline EC_{50} values and maximal contractions (expressed as a percentage of the 5HT maximum) (mean±s.e.m. n=4).

ARTERY		NO COCAINE		COCAINE(10^{-5}M)
		EC_{50}	MAX.CONTRACTION	EC_{50}
BASILAR	(+E)	$8.7\pm3.9x10^{-6}$M	23.6±3.8%	$3.8\pm1.7x10^{-6}$M
	(-E)	$6.8\pm3.9x10^{-6}$M	20.0±3.2%	$1.6\pm1.2x10^{-5}$M
MCA	(+E)	$3.5\pm0.5x10^{-6}$M	78.1±3.6%	$6.3\pm2.3x10^{-7}$M
	(-E)	$3.8\pm0.8x10^{-6}$M	77.1±4.3%	$8.0\pm2.4x10^{-7}$M

In the sheep NA has a much stronger contractile effect on the MCA than the basilar artery. This is not attributable either to a greater neuronal uptake of NA or to a greater release of EDRF by NA in the basilar artery. The NA contraction is mediated predominantly by α_1-adrenoceptors in the MCA.

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THE EFFECTS OF ISOSORBIDE DINITRATE AND DILTIAZEM ON THE RAT TAIL ARTERY AND PORTAL VEIN

E.K.I. Omogbai and G.M. Smith, School of Pharmacy, Robert Gordon's Institute of Technology, Aberdeen AB9 1FR.

Changes in the cytosolic calcium levels mediate vascular smooth muscle tone. Increases in calcium may occur through agonist-induced influx through voltage - or receptor - operated calcium channels in the cell membranes or through release from intracellular sites (Van Breeman et al, 1981; Meisheri et al, 1981). Noradrenaline (NA) produces part of its contractile response in various arteries by releasing calcium from intracellular stores (Saida and Van Breeman, 1983) while the spontaneous myogenic activity of the portal vein depends on the influx of extracellular calcium (Rahwan et al, 1979).

This presentation will examine the degree of dependence of the rat tail artery and portal vein on the mobilisation of intracellular calcium and the effects of isosorbide dinitrate (ID) and diltiazem (DZ) on intracellular mobilisation. Male Sprague-Dawley rats (400-450gm) were used. The portal vein was opened longitudinally and the tail artery spiralled over a fine stainless steel wire. Both tissues were mounted in the same 50ml organ bath at 37°C. They were aerated with 95% O_2 and 5% CO_2 and contractions measured isometrically. Two different modified Krebs-Hensleit solutions were used depending on the level of calcium required for loading the tissues. Solution A and B contained 1.25mM and 3.5mM Ca^{++} respectively. Each solution also contained propranolol (10^{-6}M) and imipramine ($3x\ 10^{-6}$M. In the zero-calcium procedures, $CaCl_2$ was omitted and 2mM EGTA included. Tissue responses to a submaximally effective dose of NA ($5x10^{-6}$M) in normal and zero-calcium solutions were recorded. In the lower Ca^{++} containing Krebs solution, portal vein responses to NA were observed to start 7.5 ± 1.4 sec. after the tail artery had started to contract. When KCl was the contracting agent the artery was slower than the vein to respond (8.9 ± 1.2 sec. n=10). Differences in the time of response in high-calcium solutions were difficult to quantify because of the marked spontaneous activity of the portal vein. The portal vein did not respond to NA stimulation from 1 min. after exposure to zero-calcium (in normal K^+). The tail artery responses to NA in zero-calcium were phasic and declined from 69.1 ± 2.5% in 1 min. to 2.5 ± 1.5% in 10 min. ID inhibition of NA stimulation of the tail artery in normal calcium was significantly greater than of the portal vein (IC_{50} $2.4x10^{-7}$ and $5.3x10^{-5}$M respectively; $P<0.05$). ID (10^{-7}M) significantly reduced the response of the tail artery in zero-calcium to NA in 5 min. from 40.2 ± 3.1% of control to 21 ± 3.4% whereas DZ ($5x10^{-6}$M) had no significant effect. ID had no effect on the spontaneous activity of the portal vein, which is known to be due to extracellular calcium influx even at 10^{-5}M.

The rat tail artery therefore seems to depend more on intracellular Ca^{++} mobilisation for contraction than the portal vein. ID inhibits intracellular Ca^{++} mobilisation in the tail artery and this may partly account for the greater susceptibility of the artery than the vein to ID inhibition following NA stimulation.

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REPERFUSION-INDUCED ARRHYTHMIAS: EFFECT OF ORAL ADMINISTRATION OF TWO XANTHINE OXIDASE INHIBITORS IN ANAESTHETISED RATS

N. Lad & A.S. Manning, Department of Biology, Roche Products Ltd., Welwyn Garden City, Hertfordshire AL7 3AY

Recently it has been shown that oral administration of the xanthine oxidase inhibitor, allopurinol, to rats 24 hrs prior to experimentation and then again intravenously 15 minutes prior to coronary artery occlusion (CAO) greatly reduced the incidence of ventricular arrhythmias and mortality upon reperfusion (Manning et al, 1984). It was suggested this reduction may be due to inhibiting the formation of free radicals by xanthine oxidase (XO) during the early reperfusion phase. In the present study we have investigated whether allopurinol is effective when given solely as an oral dose 1 hour prior to CAO and also whether another potent XO inhibitor, amflutizole, is equally beneficial. For this study we have used an anaesthetised rat preparation with transient (7 min) CAO followed by reperfusion as described previously (Manning et al, 1984). The XO inhibitors studied, allopurinol and amflutizole, were dissolved in PEG 400–Carboxymethylcellulose vehicle (1:1 v/v).

Table 1. Effect of xanthine oxidase inhibitors on reperfusion-induced arrhythmias in the anaesthetised rat

	Incidence (%)			Arrhythmia score
	VF	VT	Mortality	(mean ± s.e.m.)
Control	76	100	46	5.2 ± 0.4
Allopurinol				
50 mg/kg	38	100	31	4.0 ± 0.5
100 mg/kg	54	100	15	3.8 ± 0.4*
Amflutizole				
10 mg/kg	54	100	23	4.1 ± 0.5
50 mg/kg	46	85	15	3.8 ± 0.5*
100 mg/kg	31*	92	8	2.9 ± 0.4**

* $p<0.05$; ** $p<0.001$ (n = 13 in each group)

Allopurinol reduced the overall arrhythmia score (based on criteria described by Johnson et al, 1985) from 5.2 ± 0.4 to 3.8 ± 0.4 ($p<0.05$) with 100 mg/kg. Amflutizole reduced the incidence of ventricular fibrillation (VF) from 76% to 31% ($p<0.05$) with 100 mg/kg and arrhythmia score from 5.2 ± 0.4 to 3.8 ± 0.5 ($p<0.05$) with 50 mg/kg and to 2.9 ± 0.4 ($p<0.001$) with 100 mg/kg. Thus this study shows that both XO inhibitors investigated reduced the incidence of reperfusion-induced arrhythmias even when given orally 1 hour prior to the onset of ischaemia and that at equal doses amflutizole may be more effective than allopurinol.

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ACUTE EFFECTS OF CILAZAPRIL AND OTHER ANTIHYPERTENSIVE AGENTS, ON THE BAROREFLEX OF CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS

I.S. Cowlrick, (introduced by J.F. Waterfall), Biology Department, Roche Products Ltd., Welwyn Garden City, Hertfordshire AL7 3AY

Clinical studies with essential hypertensive patients show that angiotensin converting enzyme inhibitors (ACEI) reduce blood pressure through vasodilation with no reflex tachycardia. Giudicelli et al (1985) in clinical studies and Takeda et al (1986) in the rat suggested that the absence of reflex tachycardia after ACEI may be due to changes in the baroreflex. However, Ajayi and Reid (1986) and Coleman et al (1981) could show no change. Since the spontaneously hypertensive rat is a widely used animal model of human essential hypertension, it has been used to study the effects of the ACEI cilazapril, and other antihypertensive agents on baroreceptor mediated tachycardia.

Groups of 5–6 male rats (275–350 g) were anaesthetised with brietal (50 $mgkg^{-1}$ ip). The left femoral vein and artery were cannulated and exteriorised at the back of the neck. The rats were allowed a minimum of 4 hours to recover from anaesthesia. They were placed in perspex tubes 30 min before experiment to reduce restraint related stress. Blood pressure was recorded from the femoral artery and heart rate (HR) was derived from the pulse pressure signal using a tachograph. Drugs were administered via the left femoral vein.

The effects of cilazapril (1 $mgkg^{-1}$; an effective angiotensin converting enzyme inhibiting dose, Natoff et al (1985)), lisinopril (1 $mgkg^{-1}$), enalapril (1 $mgkg^{-1}$), prazosin (0.003 $mgkg^{-1}$),and saline vehicle (1 $mlkg^{-1}$) on the baroreflex were assessed. Increasing bolus doses of sodium nitroprusside (1–100 μgkg^{-1}) evoked increasing falls in mean arterial pressure (MAP) which were accompanied by increasing reflex tachycardias. Linear regression analysis was performed on at least three data pairs (maximum change in HR to maximum change in MAP). Pretreatment values of the slope of the regression line, the resting MAP, and the resting HR were each compared with their respective values 15 min after treatment using the paired 't'-test.

Prazosin (195.7 $\pm$ 13.4 to 184.3 $\pm$ 11.7 mmHg) and enalapril (196.0 $\pm$ 9.3 to 184.0 $\pm$ 11.3 mmHg) significantly ($p<0.05$) reduced MAP, and none of the drugs affected HR. Baroreflex sensitivity as measured by the slope of the HR/MAP relationship was unchanged by cilazapril (1.3 $\pm$ 0.3 to 1.1 $\pm$ 0.2), lisinopril (1.6 $\pm$ 0.2 to 1.3 $\pm$ 0.3), enalapril (1.3 $\pm$ 0.2 to 1.2 $\pm$ 0.1), and saline vehicle (1.2 $\pm$ 0.2 to 1.6 $\pm$ 0.3) but was significantly reduced by prazosin (1.3 $\pm$ 0.1 to 0.5 $\pm$ 0.1, $p<0.01$). Pierce and Shepperson (1984) reported a similar inhibition of baroreflex sensitivity by prazosin in conscious normotensive rats.

These results are consistent with the view that ACEI do not alter baroreflex sensitivity and that the absence of reflex tachycardia may be due to other cardiovascular mechanisms.

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ENDOTHELIUM-DEPENDENT VASODILATOR RESPONSES TO ACETYLCHOLINE IN HYPERTENSION AND DIABETES

A. Templeton*, G.M. Drew+ and J.C. McGrath*. Institute of Physiology, University of Glasgow, Glasgow, G12 8QQ* and Department of Cardiovascular Pharmacolgy, Glaxo Group Research, Ware, SG12 ODJ+.

Acetylcholine (ACH)-induced relaxation of isolated blood vessels is endothelium dependent and is mediated through the release of an endothelium derived relaxing factor (EDRF). EDRF stimulates soluble guanylate cyclase activity in the vascular smooth muscle and increases intracellular cGMP. Thus, EDRF depresses responses to vasoconstrictor compounds (Furchgott, 1984). Increased reactivity of vascular smooth muscle to vasoconstrictor agents in hypertension is well documented and hypertension is a common complication of diabetes. It is possible that a decline in the release of EDRF contributes to increased vascular reactivity in these conditions. Accordingly we have evaluated the influence of spontaneously released EDRF on the vasoconstrictor activity of noradrenaline (NA) in isolated rings of aorta from untreated, diabetic (streptozotocin, 80 mg/kg i.v.) and hypertensive (2 kidney-1 clip; DOCA/salt) rats (SDG strain). The ability of ACH to relax NA-induced tone in these vessels was also established and compared with that of sodium nitroprusside (SNP) which also stimulates soluble guanylate cyclase activity, but is endothelium-independent (Ignarro and Kadowitz, 1985). Thus, responses to SNP reflect the capacity of vascular smooth muscle to respond to stimulation of soluble guanylate cyclase. Differences in the relationship between sensitivities to SNP and ACH should, therefore, reflect changes in endothelial function.

Two adjacent aortic rings (3-4 mm) were obtained from each rat, and the endothelium on one was deliberately disrupted by rubbing. Both rings were suspended under 1.5 g resting tension in Krebs-bicarbonate solution at 37°C, bubbled with 95% O_2/5% CO_2. An hour later a concentration effect curve to NA ($1x10^{-10}$-$1x10^{-6}$ M) was constructed in each ring. After washout, the ability of ACH ($1x10^{-8}$-$3x10^{-5}$ M) to relax the sustained contraction to NA ($3x10^{-8}$ M) was then determined. This procedure was repeated using SNP ($1x10^{-10}$-$3x10^{-8}$ M) in place of acetylcholine.

Noradrenaline concentration-effect curves in endothelium-disrupted aortic rings from the different treatment groups were almost superimposable. The presence of the endothelium depressed the sensitivity (at IC_{50}) to, but not the maximum tension produced by, NA in untreated and renal hypertensive rats. The sensitivity and the maximum response to NA were reduced in endothelium-intact rings from diabetic rats ($P<0.05$). In contrast, the presence of the endothelium in rings from DOCA hypertensive rats had no significant effect on the responses to NA.

ACH reduced NA-induced tone in endothelium-intact, but not disrupted, rings from all groups. There was no significant difference in the ACH IC_{50} in any treatment group, compared with the untreated group. SNP also reduced NA-induced tone, in endothelium-intact and -disrupted rings. SNP was 78 and 58 times more potent than ACH in endothelium-intact rings from untreated and diabetic rats, respectively. In contrast, it was only 8 to 9 times more potent than ACH in rings from hypertensive animals. These results suggest that ACH-induced release of EDRF may be enhanced in hypertensive animals to compensate for a decreased responsiveness to guanylate cyclase stimulation.

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EFFECTS OF BRL 34915, NICORANDIL AND SODIUM NITROPRUSSIDE ON Ca^{2+} EFFLUX AND CYCLIC GMP ACCUMULATION IN RABBIT VASCULAR TISSUE

D.R. Howlett
Beecham Pharmaceuticals Research Division, Coldharbour Road, Harlow, Essex, U.K.

The vascular smooth muscle relaxant effects of BRL 34915 are associated with a hyperpolarisation of the smooth muscle cell membrane and an increase in potassium efflux (Hamilton et al 1985). However, the causal relationship between relaxation and potassium efflux remains enigmatic. The antianginal drug nicorandil has also been shown to produce hyperpolarisation at 10 times greater concentrations than BRL 34915. However the vasodilator effects of nicorandil may arise by virtue of increasing cyclic GMP levels (Holzman 1983). It has recently been argued that the relaxant properties of nitrovasodilators are attributable to a dephosphorylation of myosin-light-chain kinase activity, mediated by increases in cyclic GMP and decreases in intracellular calcium (Collins et al 1986). The present communication compares and contrasts the effects of BRL 34915 and nicorandil on intracellular Ca release and cyclic GMP accumulation.

Calcium efflux was measured by the method of Aaronson et al (1979) as developed by Collins et al (1986) for rabbit aorta. Briefly, tissue was loaded with calcium-45 (250 µCi/100 ml) for 3 h at 37°C in oxygenated HEPES-physiological salt solution (HEPES-PSS; composition (mM), NaCl, 140; KCl, 4.6; $MgCl_2$, 1; $CaCl_2$, 1.5; HEPES, 5; glucose, 10; pH 7.3 at 37°C). Pre-loaded tissue was transferred to ice-cold buffer containing $CaCl_2$ (6.5 mM) (to prevent membrane dysfunction) and EGTA (5 mM) (to remove extracellular calcium) for 45 min. The efflux of calcium-45 from the tissue into HEPES buffer at 37°C was determined at 5 min intervals over the subsequent 50 min. Under these conditions, the efflux of calcium-45 is thought to reflect the release of intracellular calcium (Aaronson et al 1979).

Cyclic GMP accumulation was determined in rabbit isolated mesenteric artery. Following exposure to drugs for 90 sec, tissue was clamped with tongs cooled in liquid nitrogen and cyclic GMP levels were determined by RIA.

Noradrenaline (NA) (present from 30-45 min of the efflux period) produced a concentration related (1-10 µM) increase in calcium-45 efflux; basal efflux 2.0±0.1 (% released/min, n>5 for all groups ± sem); NA (10 µM) 3.8±0.3. The response to NA was totally inhibited by sodium nitroprusside (100 µM) and also by nicorandil (100 µM). BRL 34915 (100 µM) was devoid of any activity either on basal efflux or against NA stimulated efflux. Sodium nitroprusside (100 µM) and nicorandil (100 µM) stimulated cyclic GMP levels to 158±32 and 39±9 pmol/mg protein, respectively ($p<0.01$ for both compared to basal levels). BRL 34915 (10 µM) had no effect on cyclic GMP accumulation (basal 20±4 pmol/mg protein, BRL 34915 16±3).

These data suggest that the vasorelaxant properties of nicorandil, but not BRL 34915, may result, at least in part, from a decrease in the cytosolic calcium available for contraction. Although nicorandil has been shown to produce hyperpolarisation, the lack of effect of BRL 34915 on cyclic GMP accumulation and calcium-45 efflux suggests that the smooth muscle relaxant effects of BRL 34915 are not attributable to a decrease in intracellular calcium availability.

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BRL 34915 INDUCED K^+ CHANNEL ACTIVATION: TEMPORAL RELATIONSHIP BETWEEN DRUG EXPOSURE AND OPENING/CLOSING OF THE CHANNEL

M.C. Coldwell (introduced by C. Wilson)
Beecham Pharmaceuticals Research Division, Coldharbour Road, Harlow CM19 5AD

We have shown that in a calcium free medium, the potassium channel activated by BRL 34915 in rabbit isolated mesenteric artery (RIMA) remains open following removal of the drug (Howlett & Coldwell, 1987). This study has examined further the temporal relationship between exposure to drug and the opening/closing of the channel.

Segments of RIMA were loaded with 86-rubidium (Rb) as described previously (Howlett & Coldwell, 1987). Efflux of Rb was determined at 3 min intervals over the next 60 or 120 min in either (i) a HEPES physiological salt solution or (ii) calcium free HEPES containing 2 mM EGTA and 10 mM $MgCl_2$ (CAFH). Tissues were exposed to drug for varying times after minute 30 of the efflux period. Data were calculated as % of remaining counts released during each 3 min efflux period. Basal efflux rate was defined as the average efflux over minutes 21-30 of the efflux period.

In HEPES a 30 sec exposure to BRL 34915 (10 μM) caused a 56% increase in Rb efflux rate of RIMA which was maximal after 6 min but gradually returned towards basal levels. A similar efflux profile was observed after 1, 3 or 18 min exposure. With 15 sec exposure, the maximum increase in Rb efflux rate was halved, and with a 1-2 sec exposure the response was abolished. Experiments in which ^{3}H-BRL 34915 was present showed that the carry over of drug was such that the concentration fell 200 fold in the first transfer. In CAFH, the maximal increases in efflux rate following exposure to BRL 34915 for 18 or 3 min, 30, 15 or 1-2 sec were 106, 102, 77, 55 and 23% respectively. These experiments demonstrate that in the presence or absence of calcium ions a short exposure of RIMA to BRL 34915 stimulates an increase in Rb efflux which continues to develop after the removal of the drug.

Between 30 and 120 min, control basal efflux rates decreased only slightly in HEPES, and were unchanged in CAFH. The efflux rate in tissues exposed to BRL 34915 for 18 min in HEPES returned to basal levels (1.9% per 3 min) over 40 min after first exposure. However in similar experiments in CAFH, the efflux rate was still above basal levels (2.5 as opposed to 2.1% per 3 min) 90 min after the first exposure. In tissues exposed to the drug for only 3 min in CAFH, the efflux rate returned to basal levels by 90 min. Thus, once developed, the response only slowly returns to basal values.

We have shown previously large increases (up to 100%) in Rb efflux caused by noradrenaline (NA) (30 μM) and K^+ (30 mM) in RIMA following 18 min exposure in HEPES (Coldwell & Howlett, 1986). A 1 min exposure to either of these stimulants, however, produced only very small (17 and 15% for NA and K^+ respectively) increases, of short (3 min) duration. We were unable to demonstrate any significant increase in Rb efflux caused by NA or K^+ in CAFH following 1 or 18 min exposure. Thus the increase in efflux rate caused by NA or K^+ is dependent both on the continued presence of the stimulant and of extracellular calcium.

In conclusion, the ability of BRL 34915 to develop and maintain an increase in Rb efflux for considerable periods of time after the removal of the drug, implies either that bound BRL 34915 dissociates very slowly from the potassium channel or that BRL 34915 need not be bound to this channel for it to remain open.

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CAN DIFFERENTIAL CALCIUM CHANNEL BLOCKER POTENCY IN CARDIAC MUSCLE BE EXPLAINED BY THE MODULATED RECEPTOR HYPOTHESIS?

P Lumley and MJ Robertson, Department of Cardiovascular Pharmacology, Glaxo Group Research Ltd, Ware, Herts, SG12 ODJ, United Kingdom.

The potency of calcium channel blocking drugs, to produce negative inotropic effects or antagonise the positive inotropic effect of Ca^{++} in cardiac muscle can be influenced by experimental conditions used in vitro (Nabata, 1977; Lumley and Robertson, 1986a) and by ischaemia in vivo (Smith et al., 1976). Thus, the frequency of stimulation, the level of membrane depolarisation and the presence or absence of catecholamines can all influence the potency of these drugs. However, although the frequency-dependence of verapamil is well established, that of nifedipine is equivocal (see Woods and West, 1985).

We have determined the ability of verapamil and nifedipine to antagonise the positive inotropic effect of Ca^{++} in guinea-pig, isolated, electrically stimulated (5 ms pulse width, threshold voltage) papillary muscles maintained at 32°C in physiological salt solution bubbled with 95% O_2: 5% CO_2. Potency was expressed as a CR_2 value (concentration of drug (μM) required to produce a two-fold rightward displacement of the Ca^{++} concentration-effect curve). Increasing the frequency of stimulation from 0.5 to 3 Hz decreased the CR_2 for verapamil from 13 to 0.5 μM and for nifedipine from 0.2 to 0.03 μM respectively. In preparations stimulated at 0.5 Hz, raising the K^+ concentration in the physiological salt solution to 27 mM and inclusion of isoprenaline (0.2 μM) also enhanced the potency of verapamil and nifedipine (CR_2 values of 0.5 and 0.04 μM respectively). Thus these interventions enhanced the potency of the two drugs to a similar extent to that produced by the increase in frequency alone. In contrast, exposure of preparations stimulated at 3 Hz, to conditions in vitro which mimic the consequences of ischaemia in vivo (physiological salt solution pH 6.9, K^+ 9.2 mM and bubbled with 55% O_2: 40% N_2: 5% CO_2) (see Lumley and Robertson, 1986b) failed to alter the potency of nifedipine compared with control (3 Hz) conditions (CR_2 = 0.03 μM $P>0.05$) but resulted in a further enhancement of verapamil's potency (CR_2 = 0.05 μM $P<0.001$). Thus the potency of both verapamil and nifedipine can be enhanced by certain changes in experimental conditions such as increase in stimulation frequency and depolarisation plus catecholamine. However a combination of conditions which occur during ischaemia (decreased pH, elevated extracellular K^+ and hypoxia) lead to a selective increase in the potency of verapamil.

The calcium channel is thought to exist in three interconvertible states; rested, open and inactivated (modulated receptor hypothesis, see Hondeghem and Katzung, 1984). Rapid repeated channel stimulation, or depolarisation plus catecholamines, increases the probability of the calcium channel being in the open and then inactivated state. The increase in potency of verapamil and nifedipine under these conditions could be explained if both drugs bound preferentially to the inactivated state of the calcium channel so reducing the number of functioning channels. However an alternative explanation for the selective increase in the potency of verapamil in 'ischaemic' conditions may be necessary.

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Ca^{2+} MODULATORS AND ATROPINE AS ANTIDOTES TO ACETYLCHOLINE LETHAL TOXICITY IN RAT

J.F.Démus, G.Nahas, M.Sitbon, C.Latour & R.Trouvé, Laboratoire de Pharmacologie et de Toxicologie Cellulaires, INSERM, Hôpital F.Widal, 75010 PARIS (France).

The antagonistic and antidote effects of flunarizine, a Ca^{2+} channel entry modulator, on the functional and morphological toxicity of imipramine have been reported. It was then suggested that the toxicity of imipramine was related to its properties of releasing and inhibiting reuptake of major neuro-excitatory transmitters : dopamine, norepinephrine and serotonin. The toxic effects of the latter could be antagonised by flunarizine. In the present study, a combination of atropine and flunarizine was tested in the course of acetylcholine continuous administration until lethal outcome. Presently, no treatment has been suggested for such an occurence except with atropine like substances which only neutralize in part the muscarinic action of acetylcholine, but do not antagonize the others effects (nicotinic) of this neurotransmitter and do not prevent lethal outcome.

Twenty fasting Sprague Dawley rats weighing 300 ± 40 g are fitted, under ether anesthesia, with a caudal artery catheter connected to a recorder for on-line recording of arterial blood pressure which is analysed by a microcomputer. Measurements of heart rate and pulse pressure are displayed every 30 seconds. When the animal has awakened, it is placed in a restraining grid.

In the first series, 5 rats are administered intraarterially 10 mg/kg/min of acetylcholine, survival time is 25'22"± 3'10". In the second and third series, 5 minutes after the start of acetylcholine administration, animals are given atropine intramuscularly (total dose: 0.5 mg/kg) or flunarizine intraarterially (total dose : 0.4 mg/kg). In these series survival time is not significantly different from that of the controls. In a fourth series, 5 minutes following the start of acetylcholine infusion, fractionated amounts of atropine intramuscularly (total dose: 1 mg/kg) and of flunarizine intraarterially (total dose : 0.9 mg/kg) are administered. In this last series, a total amount of 350 mg/kg of acetylcholine was administered (instead of 252 ± 31 mg/kg in the control series). All animals treated with the combination of atropine and flunarizine were alive and active 48 hours later.

Table 1.

Number of rats	Acetylcholine intraarterially	Flunarizine intraarterially(b)	Atropine intramuscularly(b)	Survival time
5	10 mg/kg/min	none	none	25'22" ± 3'10"
5	10 mg/kg/min	none	0.5 mg/kg	71'47" ± 8'25"
5	10 mg/kg/min	0.4 mg/kg	none	38'14" ± 7'10"
5	10 mg/kg/min(a)	0.9 mg/kg	1 mg/kg	> 48 h (c)

(a) : up to a total dose of 350 mg/kg
(b) : in fractionated amount during the period of treatment
(c) : animals conscious and active

Nimodipine, a Ca^{2+} modulator with effects on the central nervous system, given in association with atropine displays a protective action against acetylcholine poisonning.

Such a combination of atropine with a centrally acting Ca^{2+} modulators might be tested in conditions associated with dysfunction of cholinergic receptors.

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REGIONAL HAEMODYNAMIC EFFECTS OF β-ADRENOCEPTOR ANTAGONISM IN CONSCIOUS, UNRESTRAINED, LONG EVANS RATS

Sheila M. Gardiner & T. Bennett*, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, U.K.

We have used chronically-implanted, miniaturized, pulsed Doppler probes (Haywood et al., 1981) to monitor changes in regional vascular resistance in response to administration of propranolol or the β_2-adrenoceptor antagonist ICI 118551, in conscious, unrestrained, Long Evans rats. Animals (n=15) were anaesthetized with sodium methohexitone (60 mg/kg I.P., supplemented as required) and pulsed Doppler probes were sutured around the renal and superior mesenteric arteries and distal abdominal aorta. The wires were led subcutaneously and soldered to a miniature connector held in a harness worn by the rat. At least 7 days later, when animals were healthy and gaining weight, they were briefly re-anaesthetized (as above) and catheters implanted in the abdominal aorta and jugular vein. The next day, following baseline recordings of mean arterial pressure (MAP) and mean Doppler shifts for at least 1 h, propranolol (1 mg/kg, 0.5 mg kg^{-1} h^{-1}, n=8) or ICI 118551 (0.2 mg/kg, 0.1 mg kg^{-1} h^{-1}, n=7) were administered and measurements made for 1 h. During the baseline recording, variables were steady, so the averages over the 2 min before drug administration were taken as control levels. For analysis, measurements at 1, 5 and 60 min after the onset of drug administration were selected. Changes in regional vascular resistances were calculated from the percentage change in MAP/mean Doppler shift for each vascular bed. The results are shown in the table.

Table 1 Changes in MAP and HR and percentage changes in regional vascular resistances following propranolol (P) or ICI 118551 (I). Values are mean (s.e.m.).

Time after injection	1 min		5 min		60 min	
	P	I	P	I	P	I
MAP (mmHg)	+9 (2)*	+5(2)	+2(2)	+2 (2)	-1(2)	-1 (2)
HR (beats/min)	-43 (5)*	+6(6)+	-45(4)*	+16(12)+	-53(9)*	0 (6)+
Renal resistance (%)	+13 (7)	+6(3)	+4(4)	+2 (4)	11(9)	-2 (7)
Mesenteric resistance (%)	+5 (4)	+1(6)	+11(5)	+4 (9)	-3(6)	-6 (8)
Hindquarters resistance (%)	+30(10)*	+8(5)+	+7(7)*	+23 (5)*+	18(7)*	+12 (6)

*denotes a significant change from baseline ($P<0.05$).
+denotes a significant difference between propranolol and ICI 118551 ($P<0.05$).

The results indicate that the marked vasoconstriction seen in the hindquarters vascular bed immediately following administration of propranolol was not due solely to antagonism of β_2-adrenoceptors.

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RESTING AND PHASIC V_{max} BLOCK INDUCED BY 5-HYDROXYPROPAFENONE IN GUINEA-PIG VENTRICULAR MUSCLE FIBRES

C. Valenzuela, E. Delpón & J. Tamargo. Department of Pharmacology. School of Medicine. Universidad Complutense. 28040 Madrid. Spain.

5-hydroxy-propafenone (5-OH-P) has been identified as the main unconjugated metabolite of propafenone in human plasma (Philipsborn et al., 1984). In isolated cardiac preparations 5-OH-P dose-dependently inhibited the maximum rate of depolarization (Vmax) and thus, it was classified as a class I antiarrhythmic drug (Delgado et al., 1987). In the present experiments the resting and phasic Vmax block induced by 5-OH-P has been analyzed.

The experiments were performed in guinea-pig papillary muscles excised from the right ventricle. Vmax values obtained by electronic differentiation were used as an indirect estimative index of the magnitude of the I_{Na}.

5-OH-P, 10^{-6}M, 5 x 10^{-6}M and 10^{-5}M, produced a 5.8 ± 0.7%, 15.1 ± 1.3% and 31.6 ± 4.0% inhibition, respectively, of the Vmax of the first action potential of a train of impulses (resting block). 5-OH-P also produced a phasic, rate-dependent, Vmax block which increased not only with the concentration of the drug but also by decreasing the inter-stimulus interval from 0.5 to 5 sec. In muscles driven at 1 Hz the time and rate constants for the onset kinetics of phasic Vmax block induced by 10^{-5}M 5-OH-P were 13.0 ± 1.7 sec and 0.07 ± 0.01 AP^{-1} (AP = action potential), respectively (n = 7). In muscles partially depolarized by 10 mM K these values were 10.8 ± 1.1 sec ($P < 0.05$) and 0.09 ± 0.008 AP^{-1} ($P < 0.05$), which indicated that the development of phasic block was faster in depolarized ventricular fibres. Furthermore, 5-OH-P (10^{-5}M) prolonged the time constant of recovery of phasic Vmax block to 200.0 ± 9.2 sec (n=7).

Each type of drug-induced Vmax block exhibited its own individual apparent disociation constants (km) and apparent Hill coefficients (nH). In fibres perfused with Tyrode solution (5.4 mM K) the km and nH values for the resting and phasic block were 3.6 x 10^{-5}M and 0.937, and 5.0 x 10^{-6}M and 1.007, respectively. Similar values for tonic (km = 3.4 x 10^{-5}M, nH = 1.239) and phasic block (km = 5.5 x 10^{-6}M, nH = 0.852) were found in fibres depolarized by 10 mM K (Em = 70.0 ± 1.6 mV. n = 9).

The present experiments confirmed that 5-OH-P exhibits class I antiarrhythmic effects and can be satisfactorily explained in terms of the modulated receptor hypothesis.

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SK&F 94836, A POTENT INOTROPIC AGENT, IS A SELECTIVE INHIBITOR OF TYPE III PHOSPHODIESTERASE ACTIVITY FROM SEVERAL TISSUES

M.L. Reeves, R.W. Gristwood, B.K. Leigh and P.J. England, Smith Kline and French Research Ltd., The Frythe, Welwyn, Hertfordshire, AL6 9AR, U.K.

SK&F 94836 is a novel and potent positive inotropic/vasodilator agent (Gristwood et al., 1987). Other agents of this class (eg Amrinone, Fenoximone and SK&F 94120) have been shown to be specific inhibitors of the so called 'low K_m' PDE III activity. Inhibition of PDE III has been suggested to be the primary mechanism by which such agents act as inotropic agents. Further evidence to support this hypothesis is the observation that SK&F 94120 can cause significant increases in cyclic AMP and activate cyclic AMP-dependent protein kinase (Murray et al., 1987, this meeting), the only known mediator of the effects of cyclic AMP.

SK&F 94836 specifically inhibited cardiac ventricle PDE III activity from several species including guinea-pig, cat and man with IC_{50}'s of 2.9 μM, 2.8 μM and 0.81 μM respectively. SK&F 94836 had no effects on other cardiac ventricle PDE activities, including the recently described PDE IV activity (Reeves et al., 1987), at concentrations up to 100 μM. SK&F 94836 also inhibited PDE III activity from human platelets (IC_{50}=0.8 μM) and pig aorta (IC_{50}=0.7 μM). The specific inhibition of vascular smooth muscle PDE III by SK&F 94836 may explain its significant vasodilator properties.

The rank order of potency of SK&F 94836 compared to other PDE III inhibitors correlated well with its inotropic potency as measured by the dose required to cause a 50% increase in force of contraction of guinea-pig ventricle strips (see Table).

These data suggest that the primary mechanism of action of SK&F 94836 as a positive inotropic agent is via PDE III inhibition.

Comparison of IC_{50} and $EC_{50\%}$ for a range of positive inotropic/PDE III inhibitors.

	IC_{50} PDE III (μM)	$E_{50\%}$ ventricle strip (μM)
SK&F 94836	2.8	1.7
Milrinone	2.2	3.4
CI 914	3.7	4.2
SK&F 94120	10.9	6.0
Fenoximone	8.0	8.8
Amrinone	51.8	15.0

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EVIDENCE AGAINST A ROLE FOR EXCITATORY 'NON-α-ADRENOCEPTORS' IN SYMPATHETIC NEUROTRANSMISSION IN THE RABBIT SAPHENOUS ARTERY IN VITRO

R.J. Allcorn & T.C. Cunnane, University Department of Pharmacology, South Parks Road, Oxford. OX1 3QT

Many of the responses of blood vessels to sympathetic nerve stimulation are resistant to α-adrenoceptor blockade (Holman & Surprenant, 1980). In an attempt to explain this phenomenon, two hypotheses have been proposed. First, that ATP may act as a co-transmitter with noradrenaline (NA) in sympathetic nerves (Sneddon & Burnstock, 1984). Second, it has been suggested that NA may produce some of its postjunctional effects by activation of adrenoceptors that are distinct from α- and β-adrenoceptors, the so-called 'γ-adrenoceptor' (Hirst & Neild, 1984). Recently it has been demonstrated that after treatment of arterial smooth muscle with irreversible α-adrenoceptor antagonists, high concentrations of NA can produce contractile responses, probably via an interaction with sites distinct from α- and β-adrenoceptors (Laher *et al.*, 1986).The aim of this study was to examine contractile responses of the rabbit saphenous artery to sympathetic nerve stimulation *in vitro* to determine the extent to which such responses were resistant to α-adrenoceptor blockade and to compare any resistant responses with those evoked by high concentrations of NA after irreversible blockade of α-adrenoceptors.

Contractile responses of ring segment preparations of rabbit saphenous artery to field stimulation and exogenous agonists were studied. Agonists were added cumulatively (NA, adrenaline and phenylephrine) or non-cumulatively (ATP) to the Krebs solution with which the baths were perfused. Antagonists were added to the Krebs solution at least 30 min before repetition of stimuli. Treatment with benextramine, an irreversible α-adrenoceptor antagonist (Melchiorre *et al.*, 1978) was achieved by perfusing the bath with 10 μM benextramine for 30 min, followed by 30 min wash.

Field stimulation (20 pulses, 0.05 ms, 1-20 Hz) produced contractile responses via activation of sympathetic nerves which were reduced, but not abolished, by prazosin (0.1 μM) and benextramine (10 μM). Neurogenic contractions that were resistant to α-adrenoceptor blockade were not reduced by high dose (10 μM) prazosin, but were abolished by α,β-methyleneATP (α,β-MeATP;1-10 μM). Exogenous NA (0.1 μM-0.1 mM) produced dose-dependent contractions that were antagonised by prazosin and benextramine. After treatment with benextramine, high concentrations of NA (0.1-1 mM) produced large contractile responses that were not antagonised by α,β-MeATP (10 μM) prazosin (0.1 μM) or propranolol (1 μM), but were abolished by a high dose of prazosin (10 μM). Adrenaline, but not phenylephrine, also produced large benextramine-resistant contractions of the saphenous artery, as did exogenous ATP. Responses to 5 mM ATP were not reduced by prazosin (10 μM) or benextramine (10 μM), but were abolished by α,β-MeATP (10 μM).

These findings suggest that excitatory 'adrenoceptors' distinct from α- and β-adrenoceptors may be present in the rabbit saphenous artery, but that they appear to play no part in sympathetic neurotransmission *in vitro*. The results support the proposed role of ATP as a co-transmitter in this tissue (Burnstock & Warland, 1987).

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ISOLATION OF POST-JUNCTIONAL α_2-ADRENOCEPTORS IN THE RABBIT ISOLATED SAPHENOUS VEIN WITH PHENOXYBENZAMINE

C.J. Daly, J.C.McGrath and V.G. Wilson. Autonomic Physiology Unit, Institute of Physiology, University of Glasgow. Glagow G12 8QQ

We have recently suggested that contractions to (-)-noradrenaline ((-)-NA) in the rabbit isolated saphenous vein are mediated by both α_1- and α_2-adrenoceptors (Daly, McGrath and Wilson 1987). However, the reversible selective antagonists employed (eg: prazosin and rauwolscine) failed to uncover a component of the responses to (-)-NA which was prazosin-resistant. The irreversible antagonist phenoxybenzamine possesses selectivity for post-junctional α_1-adrenoceptors (Starke 1981) and has been employed in the dog isolated saphenous vein to "isolate" the post-junctional α_2-adrenoceptor population (Constantine, Lebel and Archer 1982). We have attempted to use phenoxybenzamine to similar effect in the rabbit isolated saphenous vein.

3mm segments of the rabbit isolated saphenous vein were set up for isometric tension recording as previously described (Daly et al., 1987). Following completion of a control cumulative concentration-response curve (CRC), preparations were exposed to either; (a) saline, (b) phenoxybenzamine (0.003μM-0.1μM), or (c) 2.5μM rauwolscine and 0.1μM phenoxybenzamine (rauwolscine added 5min before phenoxybenzamine) for a total of 30min. Each preparation was then washed at least 5 times over a period of 45min and the (-)-NA CRC repeated.
After completion of the 2nd CRC, preparations were exposed to either 0.1μM prazosin or 2.5μM rauwolscine for a further 45min and a 3rd CRC constructed . All responses were expressed as a percent of the maximum response to (-)-NA in the 1st CRC and the agonist concentration-ratios (ACR - Arunlakshana and Schild 1959) were determined at the 50% level of the maximum response in the 2nd CRC.

In preparations exposed to saline the maximum response increased by 5.4±2.7% (n=5) and 0.1μM prazosin produced a 10-fold parallel displacement of the (-)-NA CRC. In contrast, 2.5μM rauwolscine produced a non-parallel displacement of the (-)-NA CRC which was associated with a resistant component (~30% of maximum response) which we have previously shown to be prazosin-sensitive (Daly et al., 1987) . Phenoxybenzamine (0.003μM-0.1μM) effected a concentration-dependent rightward displacement of the (-)-NA CRC and a progressive reduction in the maximum responses .Subsequent exposure to 2.5μM rauwolscine failed to indicate "selective" inhibition of the prazosin-sensitive, rauwolscine-resistant response to (-)-NA (α_1-) or "sparing" of the rauwolscine-sensitive response (α_2) by prior exposure to phenoxybenzamine: both components were inhibited by each concentration of phenoxybenzamine to a similar degree . However, following exposure to the combination of 2.5μM rauwolscine and 0.1μM phenoxybenzamine, the maximum response to (-)-NA was significantly increased ($p<0.05$) from 10.7±9.4% (0.1μM phenoxybenzamine alone, n=6) to 53.3±3.5% (combination, n=8), and the remaining response was judged to be resistant to 0.1μM prazosin (ACR < 1.5) but sensitive to 2.5μM rauwolscine (ACR > 100)) - a property consistent with the characteristics of α_2-adrenoceptors (Starke 1981).

In conclusion, although we have demonstrated that phenoxybenzamine cannot distinguish between post-junctional α_1- and α_2-adrenoceptors on the rabbit isolated saphenous vein, isolation of the α_2-subtype can be achieved by prior protection of this subtype with the selective α_2-adrenoceptor antagonist rauwolscine.

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α-ADRENOCEPTOR-MEDIATED CONTRACTIONS IN THE RABBIT SAPHENOUS VEIN- THE INFLUENCE OF ENDOTHELIAL CELLS

C.J. Daly, J.C. McGrath and V.G. Wilson. Autonomic Physiology Unit, Institute of Physiology, University of Glasgow, Glasgow G12 8QQ.

α-adrenoceptor-mediated contractile responses of the isolated thoracic aorta of the rat and the rabbit and the dog isolated coronary artery (Collins et al., 1986) are, to varying degrees, under the control of a spontaneously released endothelial derived relaxatory factor (EDRF). Much of this evidence has been derived from a comparison of contractions in rubbed and unrubbed preparations. Few isolated venous preparations have been examined in a similar manner. We have, therefore, examined the effect of rubbing the intimal side of isolated preparations of the rabbit saphenous vein on α-adrenoceptor function.

The intimal surface of 3mm segments of the rabbit isolated saphenous vein were either gently rubbed or left intact and then set up for isometric tension recordings as previously described (Daly et al., 1987). The integrity of the endothelium was assessed by determining the relaxation produced by 1μM acetylcholine against 3μM (-)-noradrenaline ((-)-NA) . Cumulative concentration-response curves (CRC) to the α-adrenoceptor agonists (-)-NA, amidephrine, B-HT-920 and xylazine were then constructed in both rubbed and unrubbed preparations (a maximum of 3 agonists in one preparation) and the maximum responses (grams tension) and the pD_2 values (concentration producing 50% of maximum response) compared using a Mann-Whitney U test.

In unrubbed preparations, 1μM acetylcholine caused 90.6±2.8% (n=10) reduction in responses to 3μM (-)-NA, a concentrations that produced approx' 95% of the maximum contractile response. In rubbed preparations, no relaxatory response to acetylcholine was observed. In contrast, sodium nitroprusside was equieffective in relaxing both rubbed and unrubbed preparations exposed to 3μM (-)-NA . At concentrations greater than 10nM, contractile responses to (-)-NA in rubbed preparations were significantly greater than those elicited in unrubbed preparations but, based upon pD_2 values, there was no difference in their sensitivity to NA (Table 1). Similarly, responses to amidephrine were significantly greater in rubbed than in unrubbed preparations and there was no significant difference in the sensitivity of the two preparations. In contrast, neither the magnitude of the responses or the sensitivity of the preparations to either B-HT-920 or xylazine were affected by the removal of the endothelium (Table1).

Table 1: Maximum contractile responses and pD_2 values for various agonist in rubbed (R) and unrubbed (U) preparations of the rabbit isolated saphenous vein (n=6-10). * $p<0.05$

	Max. Response (g)		pD_2	
	(U)	(R)	(U)	(R)
(-)-NA	3.01±0.26	3.98±0.25*	7.34±0.06	7.49±0.07
Amidephrine	1.80±0.23	2.58±0.30*	5.27±0.08	5.34±0.08
B-HT-920	1.84±0.19	2.03±0.21	6.42±0.11	6.41±0.11
Xylazine	1.50±0.22	1.59±0.28	5.74±0.14	5.60±0.14

Removal of the endothelium in the rabbit isolated saphenous vein, as judged by the absence of a relaxatory response to acetylcholine, appears to be associated with an enhancement of contractile responses to both (-)-NA and amidephrine, without a similar effect on responses to either B-HT-920 and xylazine. Since this preparation possesses α_1- and α_2-adrenoceptors (Daly et al., 1987), and both B-HT-920 and xylazine are selective for α_2-adrenoceptors while (-)-NA and amidephrine can stimulate α_1-adrenoceptors (McGrath 1982), a selective effect of spontaneously released EDRF on the function of the α_1-subtype is suspected but by no means proved.

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INHIBITION OF UPTAKE 1 IN THE DOG BY DOPEXAMINE HYDROCHLORIDE

G.W. Smith & I. Naya, Department of Pharmacology, Fisons plc - Pharmaceutical Division, Loughborough, Leicestershire, LE11 ORH

Dopexamine hydrochloride, a dopamine receptor and β_2-adrenoceptor agonist under development for the acute treatment of heart failure (Brown et al, 1985), has also been shown to be an inhibitor of Uptake 1 in vitro (Smith et al, 1987). The purpose of this study was to establish if dopexamine infusions, known to be therapeutically active in man, lead to Uptake 1 inhibition in vivo.

Pentobarbitone anaesthetised dogs (n = 8) were used to examine the effect of dopexamine upon the changes in cardiac contractility ($dP/dt.P^{-1}$ and dP/dt, measured by a Gaeltec catheter) and blood pressure (BP) induced by noradrenaline (NA, 1 nmol kg^{-1}, i.v.), tyramine (Tyr, 340 nmol kg^{-1}, i.v.) and stimulation of the left ansa subclavia nerves (NS, $3H_Z$, 0.5 ms, supramax. voltage for 30s).

Dopexamine produced a dose-related fall in BP with rises in $dP/dt.P^{-1}$, dP/dt and heart rate (HR) when given as stepwise i.v. infusions of 3, 10 and 30 nmol kg^{-1} min^{-1} (1.3-13 µg kg^{-1} min^{-1}). The responses produced by Tyr were attenuated both by dopexamine as well as by desmethylimipramine (DMI, 0.5 mg kg^{-1}, i.v.) given after the recovery period as shown in the Table.

Table 1 Effect of dopexamine infusion and DMI on responses to tyramine

Infusion rate (nmol kg^{-1} min^{-1})	BP (mmHg)	$dP/dt.P^{-1}$ (s^{-1})	dP/dt (mm Hg s^{-1})
Control	24 ± 4	39 ± 4	4675 ± 398
Saline	22 ± 5	39 ± 3	4763 ± 466
3)	16 ± 2*	21 ± 2**	2375 ± 256**
10) Dopexamine	14 ± 2**	10 ± 1**	1350 ± 176**
30)	10 ± 2**	5 ± 1**	675 ± 105**
Recovery - 60 min	25 ± 4	25 ± 2**	3263 ± 296**
DMI	12 ± 2**	5 ± 1**	388 ± 332**

Values are absolute increases (mean ± s.e. mean) of 8 dogs. DMI dose was 0.5mg kg^{-1} i.v. * P <0.05, ** P <0.01 compared with control (Student's paired t-test).

The increase in contractility produced by both NA and NS (and the rise in BP caused by the former) were by contrast potentiated by dopexamine eg. the dP/dt rise was increased (P <0.01) from 4775 ± 541 to 7963 ± 1153 mm Hg s^{-1} and 3529 ± 471 to 4900 ± 769 mmHg s^{-1} respectively at the lowest dopexamine infusion rate, qualitatively resembling the action of DMI. The HR responses were inconsistent presumably reflecting the differing integrity of the baroreflex.

We conclude that as a consequence of Uptake 1 inhibition, clinically relevant infusions of dopexamine can potentiate the cardiac stimulant effect of neuronally released and circulating noradrenaline. This may contribute to the inotropic response observed in patients (Dawson et al, 1985).

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EFFECTS OF ANOXIA, ROTENONE AND ALTERED CYCLIC GMP CONCENTRATION ON REACTIVITY OF RAT PORTAL VEIN AND MESENTERY

E.M.A. El Muradi & Janice R. McCurrie (introduced by J.M. Foy), Postgraduate School of Pharmacology, University of Bradford, Bradford, BD7 1DP

Previous studies have shown differences in the dilator responses of two models of resistance vessels, rat mesentery and hepatic portal vein (El Muradi and McCurrie, 1985). The latter resembles the innervation, myogenic activity and calcium-dependence of the resistance vessels more closely than do large arteries (Pegram, 1980). The present work demonstrates further differences in both dilator and constrictor responses of these vessels.

Rat portal vein (PV) and perfused mesentery (MES) were set up as previously described (El Muradi and McCurrie, 1985). Each preparation was maximally constricted by noradrenaline (NA) (5×10^{-6}M in PV and 10^{-5}M in MES). One dilator agent was tested in each tissue; N = 4-8. Dilator responses to Dantrolene, DANT, 17-130μM; nitroprusside, NP, 0.1-4μM and verapamil, VP, 0.01-1μM were unaffected by propranolol (2×10^{-6}M), flurbiprofen (10^{-5}M) or removal of endothelium. The endothelium was removed from both tissues by perfusion with distilled water (Bolton et al, 1984). Removal was confirmed histologically in PV and functionally in MES by acetylcholine (Ach) (10^{-8} to 2×10^{-6}M). Ach caused only constriction in PV.

In MES anoxia (tissues were gassed with 5% CO_2, 95% N_2) and rotenone, 5×10^{-9}M (an inhibitor of mitochondrial electron transport) did not affect responses to NA, NP and VP, enhanced the dilator effect of DANT at most doses but markedly reduced responses to Ach (Table 1). Methylene blue, MB1, 10^{-5}M (which prevents the increased cGMP associated with dilators such as EDRF and those releasing NO in tissues, Kukovetz et al, 1982) did not affect responses to NA, VP, NP or sodium nitrite (5×10^{-5}-4×10^{-3}M), enhanced responses to low doses of DANT and markedly reduced Ach responses.

In PV anoxia and rotenone markedly reduced NA responses so that tension was inadequate for testing dilators. MB1 caused no change in response to NA or VP but relaxant effects of NP were markedly reduced. M&B 22948 (an inhibitor of cGMP phosphodiesterase) enhanced NP-induced relaxation. The relaxant effect of DANT was increased by MB1 and slightly reduced by M&B 22948.

Table 1 - Comparison of dilator activity in portal vein and mesentery

		Maximum reduction in vasoconstriction, %			
		VP	NP	DANT	Ach
PV	Control	86.8±14.3	56.2± 4.8	44.2±3.1	-
	MB1	79.3± 7.4	21.6± 3.0**	72.1±6.6**	-
MES	Control	64.3± 6.5	79.0±12.4	44.6±5.0	65.0±9.8
	Anoxia	65.2± 5.2	81.3±10.0	66.6±6.8**	26.5±4.5**
	Rotenone	67.3±10.5	85.8± 7.1	62.5±8.0*	27.2±4.2**
	MB1	65.9±13.7	78.9± 6.8	59.3±7.8	19.8±2.4**

Figures: mean and S.E.M. calculated from original data. * **Different from reduction in control, $p<0.05$ and 0.01 respectively (Mann-Whitney U test).

The results show that responses of MES, but not PV, are resistant to anoxia and a mitochondrial ATP deficit. The differential effects of MB1 in the two preparations do not support a direct role for cGMP in relaxation. It is difficult to interpret the potentiation of DANT by anoxia, rotenone and MB1 in these tissues.

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PLASMA CREATINE KINASE AS AN INDICATOR OF MYOCARDIAL INFARCT SIZE IN A CANINE CORONARY ARTERY OCCLUSION/REPERFUSION MODEL

B. Hughes, D.J. Longridge, G.O. Evans & G. Allan. Departments of Pharmacology and Drug Safety Evaluation, Wellcome Research Laboratories, Beckenham, Kent

Evaluation of the isoenzymes of plasma creatine kinase (CK) is used clinically to diagnose myocardial infarction (Pateghini et al., 1986) but in experimental canine models of coronary artery occlusion/reperfusion there is conflicting evidence regarding the relationship between serum CK estimates and the histologic extent of infarction (Vatner et al., 1978; Roe et al., 1977). The present study was undertaken to clarify the relationship between CK levels and infarct size in an an anaesthetised dog model and thus assess the potential of CK measurement as a non-invasive indicator of myocardial cell damage.

Anaesthetised beagle dogs of either sex (n=12, wt 10-15kg) instrumented for blood pressure, blood gas monitoring and lead II electrocardiogram were used. A left thoracotomy was performed and the left anterior descending coronary artery cleared of all surrounding connective tissue. An occlusive snare was placed around the vessel distal to the first major diagonal branch and occluded for 90 minutes with subsequent reperfusion (120 min). Blood samples were taken at 30 minute intervals for measurement of blood gas status, lactate and total CK (enzyme kit-method Boehringer Mannheim). The area of myocardium at risk and infarcted was delineated by the simultaneous dual dye perfusion technique (Jolly et al., 1984) and the areas of risk and infarction were measured by computer asssisted planimetry as previously described (Allan et al., 1986).

Correlation coefficients (r) relating extent of infarct to peak CK release are summarized in the table below:

Parameter 1	Parameter 2	n	r	P
% Infarct of Left Ventricle	Peak CK	12	0.805	$p<0.05$
Infarct/Risk ratio	Peak CK	12	0.887	$p<0.001$

The CK isoenzymes, MM, MB and BB were assessed qualitatively using agarose electrophoresis: the MM isoenzyme was the dominant form, the MB isenzyme was only apparent on reperfusion. There were small increases in plasma CK on occlusion (from 314 ± 87 i.u./l to 787 ± 134 i.u./l) but maximal levels were attained between 1-2 hour reperfusion (9320 ± 2017 i.u./l). This elevation of total CK was not due to sustained leakage resulting from operative procedures since marked increases in serum CK did not occur in animals subject to left thoracotomy alone or those animals exhibiting very small infarct (<5%, CK approx. 1000 i.u./l) n=2.

These data show that serial plasma CK measurement correlate well with myocardial infarct size and thus may provide a useful adjunct for assessment of myocardial cell injury in a beagle dog model of coronary artery occlusion/ reperfusion.

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THE INFLUENCE OF ENDOTHELIUM ON HYPOXIC RESPONSES OF RAT ARTERIES

C. M. Waters, K. A. Wilson & O. A. Downing. Pharmaceutical Sciences Institute, Aston University, Birmingham, B4 7ET, U.K.

De Mey and Vanhoutte (1983) observed that contractions of canine femoral artery to NA were augmented by hypoxia and that this hypoxic augmentation was not seen following endothelium removal. In the present study we have investigated the effects of hypoxia, in the presence and absence of endothelium, on NA induced contractions of three arterial preparations from the rat.

Male Wistar rats (180-250g) were killed by stunning followed by cervical dislocation. 5-7mm circular preparations of thoracic aorta (AO), superior mesenteric artery (MA) or femoral artery (FA) were dissected and mounted in organ baths containing Krebs solution ($CaCl_2$=2.5mM) gassed initially with 5% CO_2 in O_2 (pO_2=395mmHg) and maintained at 37°C. The resting tension applied was AO 3g, MA + FA 1.5g. In some rings the endothelium was removed, prior to mounting, by gently rubbing the intimal surface of the artery on stainless steel wire. Tissues were equilibrated for 1hr, after which reproducible responses to NA(1μM) were obtained. Relaxation of NA contracted preparations by ACh (1μM) was used to assess the viability of the endothelium (Furchgott and Zawadzki 1980). ACh (1μM) caused a relaxation of the response to NA (1μM) of 58.2±7.2% (n=10) in AO and 61.9±5.1% (n=17) and 57.6±5.8% (n=12) in MA and FA respectively.

After a contraction to NA (1μM) had been elicited and reached a plateau, hypoxia was imposed by rapidly switching the gassing mixture to 5% CO_2 in N_2 (pO_2=59mmHg). Hypoxia produced a transient reduction of contractile responses to NA of 22.1±3.8% (n=10) and 32.3±3.4% (n=10) in AO and MA respectively. After 4-5 min in hypoxia the tension in these tissues started to increase again and reached levels slightly, but not significantly, higher than normoxic control levels within approximately 6 min in AO and 8 min in MA. Indomethacin (10^{-6}M) did not affect the inhibitory or contraction responses of the AO or MA during hypoxia. Endothelial removal did not affect the size of normoxic contractions to NA. In the absence of the endothelium, tissues still showed a relaxation in response to hypoxia of 33.5±5.3% (n=10) in AO and 19.7±5.7% (n=10) in MA. However, no secondary contraction was observed.

In FA the initial relaxation in response to hypoxia amounted to only a 11.1±2.0% (n=12) decrease of the control normoxic response. A secondary contraction started to occur after approximately 1 min and tension rose 13.3±10.4% higher than control. However, this response was poorly maintained and within 14 min tissues had relaxed by 81.9±5.5% (n=12). Removal of the endothelium resulted in a reduction in the control response of FA to NA of 27%. However, responses to hypoxia in endothelium denuded preparations were unaltered.

These results suggest that hypoxia is able to produce a secondary contraction in all three arteries tested but only in AO and MA is this endothelium dependent.

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COMPARISON OF THE CONTRACTILE EFFECTS OF NEUROPEPTIDE Y AND OTHER AGONISTS ON RABBIT ISOLATED BLOOD VESSELS

Katharine A. Thorpe, T. L. Grant and M. H. Todd. ICI Pharmaceuticals Division, Bioscience Department II, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

Neuropeptide Y (NPY) is co-stored with noradrenaline in several regions of the CNS and in sympathetic nerve fibres surrounding cerebral and peripheral blood vessels. NPY acts directly as a vasoconstrictor in some vessels and has been reported to have both pre-and post-junctional modulatory effects at the sympathetic neuroeffector junction (Wahlestedt et al, 1986). The vasoconstrictor action of NPY appears to be more potent in the cerebral as opposed to peripheral blood vessels (Edvinsson, 1985). The aim of the present study was to compare the contractile effects of NPY, on isolated cerebral and peripheral blood vessels, with the action of angiotensin II (AII), serotonin (5-HT) and, in particular, noradrenaline (NA).

Male NZ white rabbits (2-3kg) were killed by an overdose of Sagatal and exsanguinated. The portal vein plus basilar, carotid, femoral and renal arteries were removed and rings of each vessel set-up in organ baths (20ml), bathed in normal Kreb's solution ($[Ca^{2+}]$ = 2.5mM) at 37°C, and bubbled with 95% O_2 : 5% CO_2. Resting tensions were 0.5g for the basilar artery and 1g for the four peripheral vessels. After 60 min equilibration, peripheral vessels were sensitised with an estimated ED_{50} dose of the agonist; the basilar artery was administered a single dose of KCl (30mM). Non-cumulative dose response curves ($1x10^{-9}$M to $1x10^{-5}$M, with 30 min between each dose) were constructed for each agonist. Contractions were measured isometrically and expressed as a percentage of the response to KCl (80mM).

NPY was of low efficacy in the four peripheral vessels; maximum responses were no more than 10% of the responses to KCl (80mM : n = 4-5). In contrast, NPY produced potent dose-related contractions in the basilar artery (ED_{50} = 1.0 ($\pm$0.6)$x10^{-8}$M, n = 5). At the highest dose of NPY studied ($1x10^{-7}$M) the response was 92% that of KCl (80mM). NA was of low potency and efficacy in the basilar artery (ED_{50} = 1.0($\pm$0.4)$x10^{-6}$M, max = 43% of KCl, n = 4), compared to its effects in several of the peripheral vessels (renal: ED_{50} = 3.9($\pm$0.9)$x10^{-8}$M, max 109% of KCl; carotid: ED_{50} = 1.9($\pm$0.4)$x10^{-7}$M, max = 94% of KCl; femoral : ED_{50} = 6.8($\pm$0.9)$x10^{-7}$M, max = 89% of KCl; portal vein : ED_{50} = 1.0($\pm$3.9)$x10^{-6}$M, max = 95% of KCl; (n =4-6). Unlike NPY and NA, 5-HT and AII did not show selectivity for cerebral or peripheral vessels.

The results show that NPY and NA have contrasting potency and efficacy. NPY was more selective for the cerebral vessel, having low efficacy at the peripheral vessels, whereas NA was relatively selective for the peripheral vessels. Since NPY and NA are co-stored and co-released from sympathetic perivascular nerves this suggests that NPY may play a dominant role in the control of the cerebral circulation.

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POSSIBLE ROLE OF Na^+-K^+-Cl^--COTRANSPORT IN THE PRODUCTION AND/OR RELEASE OF ENDOTHELIUM-DERIVED RELAXING FACTOR

S. Topouzis & R.C. Miller. Laboratoire de Pharmacologie Cellulaire et Moléculaire, Université Louis Pasteur, B.P. 10, F-67048 Strasbourg, France.

Basal and stimulated release of endothelium-derived relaxing factor (EDRF) are dependent on extracellular Ca^{++}, and a Na^+/Ca^{++} exchange mechanism could be implicated in the entry of Ca^{++} into the endothelial cells (Winquist et al., 1985; Schoeffter & Miller, 1986). However, the antagonist compounds used in these studies are not entirely specific for the Na^+/Ca^{++} exchanger and there is a possibility that another ion-exchange system, such as the Na^+-K^+-Cl^- cotransporter, could be implicated. To test this possibility, we have examined the effects of the loop diuretic muzolimine (MZ), a cotransporter antagonist (Knorr et al., 1985) on endothelium-dependent responses in rat isolated thoracic aorta.

Intact and de-endothelialized aortic rings were mounted for isometric tension recordings as previously described (Schoeffter & Miller, 1986). Maximal contractions were elicited by either phenylephrine (PE, 10 µM) or protaglandin F2alpha (PGF2alpha, 30 µM) and relaxant responses to cumulative additions of either acetylcholine (Ach, 3 nM to 10 µM) or sodium nitroprusside (1 nM to 1 µM) were studied. Between consecutive contraction-relaxation cycles there was a 2 h wash period. In some experiments MZ 100 or 300 µM was present during the last hour of the wash period and throughout the contraction-relaxation cycle.

MZ 100 µM reduced relaxations to Ach, expressed in either actual magnitude or as % of previously developed tension (Table 1). 300 µM MZ abolished Ach-induced relaxations in PE-precontracted rings and markedly reduced them in PGF2alpha-precontracted rings. MZ also increased acetylcholine EC50 values. This antagonism of relaxant responses was observed in preparations in which MZ antagonized the initial contractions (Table 1), conditions that might be expected to enhance endothelium-mediated relaxant responses (Furchgott, 1984). Rings without endothelium, maximally precontracted by PE, were completely relaxed by sodium nitroprusside 1 µM and MZ 100 or 300 µM had no significant effect on these responses.

Table 1. Effects of muzolimine on endothelium-dependent acetylcholine-induced relaxations of precontracted rat aortic rings.

	Muzolimine (µM)		
	0	100	300
PE contraction (%)	100	84.4 ± 2.0	27.8 ± 1.4
Ach relaxation (%*)	86.3 ± 3.2	58.7 ± 7.1	0
PGF2alpha contraction (%)	100	74.0 ± 7.0	17.5 ± 2.6
Ach relaxation (%*)	89.8 ± 3.0	83.6 ± 3.8	31.8 ± 7.5

* Relaxation responses are expressed as % of the contraction just prior to addition of acetylcholine.

The concentrations of MZ used are those that inhibit Na^+-K^+-Cl^--cotransport (Knorr et al., 1985) and the results therefore provide some indirect evidence that this cotransport mechanism could be involved in EDRF production and/or release, perhaps by affecting Na^+/Ca^{++} exchange mechanisms. In this regard it is interesting that bradykinin, a compound that releases EDRF (Furchgott, 1984), stimulates this cotransporter in cultured endothelial cells (Brock, 1986).

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RESPONSES OF THE ISOLATED PERFUSED RAT MESENTERY TO AII, 5-HT, PHE AND ACH: ROLE OF THE ENDOTHELIUM

Helen Whalley and E.T. Whalley, Smooth Muscle Research Group, Department of Physiological Sciences, The Medical School, University of Manchester, Oxford Road, Manchester M13 9PT.

The role of the vascular endothelium on the responses to vasoactive agents has been studied extensively in vitro (Furchgott, 1983), the majority of studies being limited to large capacitance vessels, typically the aortae from rabbit (Furchgott and Zawadzki, 1980) and rat (Martin, et al 1985). The present study investigates the role of the endothelium in the responses of the isolated perfused mesenteric bed of the rat to the spasmogens angiotensin II (AII), 5-Hydroxytryptamine (5-HT) and phenylephrine (PhE) in addition to the well documented endothelium-dependent vasodilator agent acetylcholine (ACh).

Isolated perfused mesenteric beds from Wistar rats (200-300g) were prepared according to a modification of the method described by McGregor (1965). The mesentery was perfused at a rate of 2m l min^{-1} with Kreb's solution at 37°C and oxygenated with 95% O_2/5% CO_2. Perfusion of the mesentery with distilled water for 10 min was used to remove endothelial cells (Criscione, et al 1984). Preparations were also perfused with Kreb's solution containing either indomethacin (IND) at 2.8 μM or oxyhaemoglobin (Hb) at 10 μM. Agonists were administered as a 10 μl bolus injection, proximal to the mesenteric artery, and the change in perfusion pressure recorded via a side arm of the arterial cannula. Where a maintained increase in vascular tone was required, PhE (10-4M) was added to the perfusing Kreb's solution.

The functional integrity of the endothelium was assessed using ACh on intact (I) and de-endothelialised (D) tissues under sustained tone. ACh produced dose-dependent dilatations of I and had no effect on D tissues respectively. IND had no effect on ACh responses on I or D tissues. Hb produced a significant (P>0.05) parallel rightward shift of the dose response curve to ACh on I tissues and had no effect on D tissues. The dose dependent increases in perfusion pressure seen with AII, 5-HT and PhE on I tissues under normal tone were significantly (P>0.05) enhanced on D tissues. IND had no effect on the responses to AII or 5-HT of either I or D tissues, however the responses of I but not D tissues to PhE were significantly (P>0.05) depressed. Hb significantly (P>0.05) enhanced responses of I but not D tissues to PhE and 5-HT but not to AII.

In conclusion, the vasoconstrictor actions of AII, 5-HT and PhE and the vasodilator action of ACh are significantly modified by the presence of endothelial cells in the perfused mesenteric bed of the rat. The inability of Hb to inhibit completely the effect of ACh in the perfused mesenteric bed are in contrast to other studies using isolated arterial strips or vascular rings. This may reflect experimental protocol.

H.W. is an SERC CASE student with ICI plc.

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RESTING PLASMA ANGIOTENSIN CONVERTING ENZYME ACTIVITY IN SEVERAL SPECIES

S W Holmes, Research Division, Roche Products Ltd., Welwyn Garden City, Hertfordshire, AL7 3AY.

Plasma is the only source of angiotensin converting enzyme (ACE) which is readily accessible in living animals and plamsa ACE activity is a commonly used index of activity of ACE inhibitors. However there is only one study in the literature where a range of animals species has been compared for resting serum ACE activity (Horiuchi et al, 1982) and there are no comparative studies using plasma. Therefore it was decided to investigate the levels of plasma ACE in a range of laboratory animal species to provide baseline data for inhibitor studies.

Blood samples were collected into heparinised tubes by venepuncture of conscious animals. Plasma was removed and stored at -20°C. Plasma ACE activity was determined radioenzymatically by measuring the quantity of hippuric acid released upon incubation at 37°C with ^{14}C Hip.His.Leu. Incubation times for the various species were chosen to provide 5 to 20% substrate utilisation.

Table 1 Resting plasma ACE activity

Species	n		Mean ACE activity ($u.l^{-1}.min^{-1}$ ± s.e.mean)		
	Male	Female	Male	Female	Total
Beagle	4	4	2.9 ± 0.1	3.8 ± 0.4*	3.3 ± 0.4
Cat	11	9	6.6 ± 0.9	6.4 ± 0.7	6.5 ± 0.6
Human	5	5	26.2 ± 3.6	22.4 ± 2.0	24.3 ± 2.1
Marmoset	6	6	49.4 ± 4.6	40.6 ± 4.2	45.0 ± 3.3
Cynomolgus monkey	2	2	78.3 ± 2.9	35.7 ± 14.2	57.0 ± 13.7
Baboon	4	4	59.8 ± 5.9	71.6 ± 10.7	65.7 ± 6.1
AP1/R2 rat	8	8	77.6 ± 7.7	48.4 ± 3.1*	63.0 ± 5.5
CD rat	4	4	90.2 ± 2.4	71.9 ± 5.4*	81.0 ± 5.4
BalbC/R mouse	8	8	352 ± 19	184 ± 19*	268 ± 25
MF1/R2 mouse	8	8	378 ± 21	245 ± 6*	312 ± 20

*Significantly different from males, $p<0.05$

None of the species tested had plasma ACE activity close to that of humans but the furthest removed of those commonly used for toxicology studies was the beagle. This suggests that this species would not be a good model for man in testing ACE inhibitors as the amount of drug potentially bound to the enzyme in beagles will be considerably less. These results are consistent with those of Horiuchi et al (1982) and extend their observations to non-human primates and cats.

Horiuchi, M., Fujimura, K., Terashima, T. & Iso, T. (1982) J. Chromatog. 233, 123-130.

EFFECTS OF ATRIAL NATRIURETIC PEPTIDE (ANP) ON HAEMODYNAMICS AND REGIONAL BLOOD FLOW IN EXPERIMENTAL CHRONIC HEART FAILURE

I.S. Anand, J. Gurden[1] and P.A. Poole-Wilson. The Cardiothoracic Institute, 2 Beaumont Street, London, W1N 2DX, and [1]Department of Cardiovascular Pharmacology, Glaxo Group Research, Priory Street, Ware, Herts, SG12 0DJ (Introduced by GM Drew).

Atrial natriuretic peptide (ANP) is stored in granules in mammalian atria, and released in response to increased blood volume and sodium levels (De Bold and Flynn, 1983). A conscious rabbit model of heart failure, produced by the repeated administration of adriamycin, has been developed (Wanless, et al., 1987). Because of its potential role in heart failure, we studied the effect of ANP infusion on haemodynamics and regional blood flow in a group of normal (n=5) and adriamycin-treated (n=5) rabbits. Two days before the study, catheters were implanted into the right atrium, left ventricle and ascending aorta. A thermistor catheter was inserted into the descending aorta via the femoral artery. Studies were carried out on conscious animals at rest. Cardiac output was measured by thermodilution and regional blood flow by use of radiolabelled microspheres injected into the left ventricle. ANP was infused via the right atrium and final measurements taken after stable responses had been achieved to a dose of 3.3 μg/kg/min. The effects of ANP on various parameters in both groups of animals are shown in Table 1.

Table 1: Cardiovascular effects of ANP

Parameter	Untreated		Adriamycin-treated	
	ANP		ANP	
	Before	After	Before	After
Cardiac Output (ml/min/kg)	334 (280-399)	371† (308-447)	234* (204-267)	278† (248-312)
Stroke Vol. (ml/beat/kg)	1.15 (0.99-1.33)	1.28† (1.07-1.52)	0.89* (0.71-1.13)	1.02† (0.86-1.21)
Total Peripheral Resistance (TPR units.kg)	0.233 (0.168-0.323)	0.192† (0.145-0.252)	0.355* (0.327-0.385)	0.277† (0.233-0.330)
Myocardial Blood Flow (ml/min/g heart)	5.66 (3.28-9.77)	7.28† (4.23-12.51)	3.24* (2.28-4.63)	4.02† (2.64-6.11)
Renal Blood Flow (ml/min/g kidney)	3.69 (2.95-4.63)	4.16† (3.11-5.55)	3.40 (2.15-5.37)	3.84 (2.39-6.17)

All data expressed as geometric mean (and 95% confidence interval).
* significantly different ($P<0.05$) from corresponding untreated group - unpaired "t"-test after log transformation. † significant ($P<0.05$) ANP-induced changes - paired "t"-test after log transformation.

Resting blood pressure and heart rate were similar in both groups and were unaffected by ANP infusion. Resting blood flows to spleen and brain were similar in both groups of rabbits, and were significantly ($P<0.05$) decreased by ANP in the adriamycin-treated, but not the untreated, rabbits.

Thus ANP increases cardiac output and stroke volume and reduces total peripheral resistance, but has different effects on regional blood flow in normal rabbits compared with those in heart failure.

De Bold, AJ and Flynn, TG (1983), Life Sci., 33, 297-302.
Wanless, RB et al. (1987), Cardiovasc. Res., 21, 7-13.

THE ROLE OF THE ADRENAL GLANDS IN THE PRESSOR RESPONSE TO CALCITONIN

J.E. Parker, R.F.L. Bates, G.A. Buckley and S.E. Mireylees. Department of Life Sciences, Trent Polytechnic, Nottingham.

The pressor response observed after peripheral administration of salmon calcitonin (sCT) to rats rendered hypotensive by haemorrhage is abolished after acute sympathetic blockade using guanethidine. However, the pressor response to centrally administered sCT in haemorrhaged rats is significantly attenuated, but not abolished, after guanethidine (Bates et al, 1987).

We have investigated the role of the adrenal glands, after acute sympathetic blockade, in the response to intracerebroventricular (i.c.v.) administration of sCT, both in normotensive rats and those subjected to haemorrhagic shock.

Male Sprague-Dawley rats (200-300g) were anaesthetised with urethane (1.6g.Kg^{-1}; 50% i.p. and 50% s.c.) and bilaterally adrenalectomised (ADX), or sham adrenalectomy (SADX) in control animals. Animals were prepared for drug administration as previously reported (Bates et al, 1987). After a 20 min. stabilisation period, guanethidine (10mg.Kg^{-1} i.v.) or vehicle (0.9% NaCl i.v.) was administered. Ten minutes later, sCT (5U.Kg^{-1} i.c.v. in 25μl) or vehicle (50mM tris-buffer containing 0.1% BSA i.c.v. in 25μl) was administered by stereotaxic placement. Mean arterial pressure (M.A.P.) was monitored for 60 min. Statistical analysis was by the Student's 't' test. Results are expressed as mean area under the blood pressure curve (AUC).

Table 1. Mean AUC (mmHg.min.) after 5U.Kg^{-1} sCT (i.c.v.) in normotensive and haemorrhaged rats. (mean ± s.e.m.; n=5; *p<0.05, *p<0.01.

PRETREATMENT	NORMOTENSIVE 60 min.		POST-HAEMORRHAGE 60 min.	
SADX	+1058 ± 304		+2227 ± 290	
ADX	+ 798 ± 103		+1944 ± 438	
SADX + Guanethidine	+ 850 ± 130	**	+ 758 ± 122	*
ADX + Guanethidine	+ 175 ± 146		+ 200 ± 199	
ADX	+ 798 ± 103	**	+1944 ± 438	**
ADX + Guanethidine	+ 175 ± 146		+ 200 ± 199	

In both the normotensive animals and the haemorrhaged animals, ADX alone has no effect on the response to sCT. In the presence of guanethidine, however, ADX leads to a significant further reduction in this pressor response.

In normotensive animals either the peripheral sympathetic nervous system or the adrenal glands can maintain the pressor response to sCT. In haemorrhaged animals the peripheral sympathetic nervous system is more important than the adrenal glands in the pressor response to sCT.

Bates, R.F.L. et al. Br. J. Pharmacol 91 Suppl. 376P

CHRONOTROPIC AND DROMOTROPIC ACTIONS OF ADENOSINE IN THE ANAESTHETISED GUINEA-PIG, RAT AND DOG

P. Bhalla, M.F. Gurden & I. Kennedy, Department of Respiratory Pharmacology & Biochemistry, Glaxo Group Research Ltd., Ware, Herts SG12 0DJ.

It has recently been demonstrated that bolus injections of adenosine in man cause transient bradycardia, frequently accompanied by first and second degree AV block (Watt & Routledge, 1986), whilst intravenous infusions produce only tachycardia (Barnes et al, 1986). Since the early work of Drury & Szent-Gyorgi (1929), a detailed analysis of the cardiac actions of adenosine in intact animals has not been carried out and most studies have utilised isolated cardiac tissue. We have therefore conducted a systematic study to investigate the cardiac actions of adenosine in the anaesthetised guinea-pig, rat and dog.

Male Dunkin-Hartley guinea-pigs (350-450g) were anaesthetised with sodium pentobarbitone (60mg/kg, i.p.), beagle dogs (8-10kg, male or female) with sodium thiopentone (25mg/kg, i.v.) and sodium barbitone (300mg/kg, i.p.), and male AH/A strain rats (250-300g) with sodium thiobutobarbitone (130-150mg/kg, i.p.). The animals were either ventilated with room air (guinea-pigs and dogs) or allowed to breathe spontaneously (rats). Blood pressure (BP) was measured via the left carotid artery (guinea-pig and rat) or the left femoral artery (dog). Drugs were administered via the left femoral vein (rat and dog) or left jugular vein (guinea-pigs). In all experiments, heart rate (HR) was derived electronically from the BP signal. ECG recordings were made using standard limb lead II and displayed on a Cambridge model VS500 single channel ECG recorder. Adenosine was administered either as bolus injections or intravenous infusions. ED_{50} values were calculated as mean ± SEM.

Bolus injection of adenosine ($1x10^{-8}$-$1x10^{-5}$ mol/kg) produced transient dose-dependent falls in BP and HR in all three species (ED_{50} for reduction in BP (mol/kg): 7.4±1.5 x 10^{-7}, guinea-pig; 7.3±0.5 $x10^{-7}$, rat; 5.8±0.5 $x10^{-7}$, dog; ED_{50} for reduction in HR (mol/Kg): 1.3±0.2 $x10^{-6}$, guinea-pig; 3.8±0.3 $x10^{-6}$, rat; 1.8±0.2 $x10^{-6}$, dog). ECG analysis however revealed important differences. In the guinea-pig, second, but not first degree AV block was observed. The fall in HR appeared to be exclusively due to AV block, there being little or no reduction in sinus rate. In the rat, sinus bradycardia was accompanied by first and second degree AV block, whereas in the dog, the major effect was a reduction in sinus rate and there was no evidence of AV block. The effects of intravenous infusions of adenosine ($1x10^{-7}$ mol/kg/min -$1x10^{-4}$ mol/kg/min) were qualitatively similar to those of bolus injections.

The results show species differences with respect to the cardiac actions of adenosine. At one extreme in the guinea-pig, adenosine has a major effect on the AV node, and at the other extreme in the dog, the predominant effect is on the SA node. The rat occupies an intermediate position with both the SA and the AV nodes being affected. Interestingly, these latter effects resemble man most closely (Watt & Routledge, 1986), with bolus injections of adenosine causing bradycardia and AV block. The effects of intravenous infusions of adenosine are different in man compared with the rat, but this could be due to the influence of anaesthesia in the rat.

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IS PD116,948 A SELECTIVE ADENOSINE A_1 RECEPTOR ANTAGONIST?

M.F. Gurden, P. Bhalla and I. Kennedy, Department of Respiratory Pharmacology and Biochemistry, Glaxo Group Research, Ware, Herts, SG12 0DJ.

Adenosine receptors have been classified into two types, A_1 and A_2, on the basis of the relative potencies of agonists (see Collis and Brown, 1983). Recently, 1,3-dipropyl-8-cyclopentylxanthine (PD116,948) has been proposed as a selective adenosine A_1 receptor antagonist (Haleen et al, 1987). We have therefore investigated the action of this compound in three isolated tissue preparations previously reported to contain either A_1 or A_2 receptors; the guinea-pig aorta (A_2, Collis and Brown, 1983), the guinea-pig left atria (A_1, Collis, 1983) and the rat right atria (A_1, Kurahasi and Paton, 1986).

The tissues were set up in a modified Krebs solution at 37°C (aorta) or 32°C (atria), gassed with 5% CO_2 in O_2 and containing indomethacin (5.6μM) for aorta, and ascorbic acid (0.1mM) and corticosterone (40μM) for left atria. In the aorta, the effect of PD116,948 on 5'N-ethylcarboxamide adenosine (NECA)-induced relaxation of phenylephrine (3μM) tone was studied. In right atria (spontaneously beating) and left atria (stimulated at 1ms, 3Hz, 5V and isoprenaline, 3-10nM) preparations, the effect of PD116,948 on NECA-induced negative chronotropism and negative inotropism respectively was investigated. Antagonist equilibration times of upto 45 (atria) and 60 (aorta) min. were used.

PD116,948 produced concentration-related antagonism of the response to NECA on all three preparations (see Table 1). In contrast, PD116,948 (10μM) was without effect on sodium nitroprusside-induced relaxation of the aorta and carbachol-induced negative chronotropic and inotropic responses of atria.

Table 1 **Antagonist Potencies of PD116,948**

	Guinea-pig aorta	Rat right atria	Guinea-pig left atria
pA_2 (95% C.L.)	6.72 (6.0-7.5)	8.74 (8.26-9.24)	7.25 (7.0-7.55)
Slope (95% C.L.)	0.9 (0.56-1.49)	1.16 (0.83-1.6)	1.16 (1.01-1.34)
n	7	4	4

The pA_2 value obtained on the rat right atria is significantly different from both the guinea-pig aorta ($P<0.001$) and the guinea-pig left atria ($p<0.01$). However, the pA_2 values in the latter two tissues are not significantly different from each other ($p>0.05$).

These results are difficult to reconcile with previous conclusions, based on agonist potencies, that both atrial preparations contain A_1 receptors, whilst the aorta contains A_2 receptors. Further work will be necessary to determine the value of PD116,948 as a tool for the classification of adenosine receptors.

PD116,948 was synthesised by Dr F Ellis, Chemistry Dept., Glaxo Group Research.

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