THE METABOLISM OF [^{14}C] D-PENICILLAMINE IN NORMAL AND ADJUVANT ARTHRITIC RATS

Sheelagh A. Aird and Lidia J. Notarianni, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY

D-Penicillamine (PSH) is a second line antirheumatic agent shown to be of value in the treatment of rheumatoid arthritis. Its mode of action is unclear although it has been proposed that the mixed disulphide, penicillamine-cysteine (P-C) may be the active species (Nakaike et al, 1983). PSH is not effective in modifying the disease process in the rat model of adjuvant arthritis. This may be due to species variation in the metabolism of PSH. We have investigated the metabolism of PSH in normal and adjuvant arthritic (AA) rats.

Female Sprague Dawley rats weighing 120-160 g were used. Arthritis was induced by a single injection of Mycobacterium Tuberculin (0.05 ml of a 5 mg/ml paraffin solution) into the right hind paw. Fifteen days later the rats were dosed with ^{14}C-PSH (10 uCi;200 mg/kg in saline) via a cannula in the right jugular vein. Serial blood samples (0.3 ml) were collected via a cannula in the left carotid artery in Eppendorf tubes containing EDTA. Blood volume was maintained with saline. Radioactivity was measured in plasma, and protein-free ultrafiltrate by liquid scintillation counting, and individual metabolites by radio-TLC.

Radioactive counts were significantly lower in AA rats during, but not after, the 4-5 minute equilibriation period. Plasma radioactive decay was biexponential - a short rapid half-life ($t1/2_\alpha$) which was longer in the AA rats, followed by a slower decline, $t1/2_\beta$ (Table 1). The major plasma metabolite in both control and AA rats was penicillamine disulphide with a small proportion of free PSH. P-C concentration in plasma was negligible. Plasma protein (P-P) binding rapidly reached an equilibrium of around 55% in both groups although initial binding was significantly higher in the AA rat.

Table 1. Disposition of ^{14}C-PSH in normal and AA rats

	Normal Rats	AA Rats
$t1/2_\alpha$ (initial)	3.14 min	4.52 min
$t1/2_\beta$ (final)	2.13 h	2.33 h
^{14}C P-P binding at 60 sec	44.3±4.1(n=4)	61.5±13.5(n=3)*
^{14}C P-P binding at 30 min	54.5±6.2(n=2)	56.9± 7.4(n=4)

(Figures represent mean ± S.D.: $p<0.05$)

One of the proposed mechanisms of action of PSH is believed to be its ability to 'unblock' sulphydryl (SH) groups in antigenic macroglobulins. The SH reactivity of AA rats is decreased but is returned to normal limits after PSH treatment (Butler et al, 1969). The metabolism of PSH is unaffected in the AA model although its initial disposition is altered, possibly due to the disturbance in SH reactivity PSH metabolism in rats is qualitatively different to that in man. If P-C is indeed the active species this could account for the apparent lack of efficacy of PSH in rat models of arthritis.

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EXPRESSION OF KININ B1 RECEPTORS IN THE RABBIT AORTA - ROLE OF THE ENDOTHELIUM

I.A.Akinosi, E.T.Whalley and H.Whalley, Department of Pharmacology, The Medical School, University of Manchester, Manchester M13 9PT.

Kinins appear to bring about their various actions by acting on at least two different receptor types designated B1 and B2. The B1 receptor is generated de novo in vitro or in vivo in tissues which have been exposed to noxious stimuli (Regoli and Barabe, 1980; Regoli et al., 1981), while the B2 receptor , which is found under normal conditions, is widespread in various species and tissues where it mediates most of the effects of kinins. The sensitivity of tissues containing B1 receptors such as rabbit aorta to the selective B1 receptor agonist des-Arg^9-BK increases progressively during the incubation of the tissue in vitro reaching a maximum in 5-8hrs (Regoli and Barabe, 1980). The response produced by a given agonist on a variety of tissue preparations can be significantly affected by the presence or absence of encothelial cells (Cherry et al, 1982). This study investigates the role of the endothelium in the selective expression of B1 receptors in the in vitro rabbit aorta.

Male New Zealand White rabbits (2-3kg) were used. The aorta was removed and carefully prepared as spiral strips. The endothelium was removed by pulling the strip (endothelium surface down) on Krebs-wetted filter paper. Intact(I) and de-endothelialised(D) tissues were set up (2gT) in 4 ml tissue baths containing Krebs-Henseleit solution at 37^{o}C bubbled with 95%O_2/5%CO_2. The effectiveness of removal of endothelial cells was tested using the ACh functional test (Cherry et al.,1982). After 1h equilibration a concentration-effect curve was constructed to either des-Arg^9-BK, Lys-BK, 5-HT or NAd on I and D tissues. This was repeated at 4h and 7h.

All agonists contracted I tissues with significant increases in responsiveness seen at t=4h. At t=7h further significant increases in responsiveness were seen to each kinin but not to 5-HT or NAd. Qualitatively similar effects were seen with all agonists on D tissues at each time interval. In addition, responsiveness of D tissues to each agonist was reduced compared to I tissues, this being greater for the kinins compared to 5-HT and NAd. The progressive increase in responsiveness with time of I and D tissues to all agonists was unaffected by indomethacin (2.8×10^{-6}M) and that seen with the kinins but not 5-HT or NAd was blocked by cycloheximide (7.2×10^{-5}M).

In conclusion we have demonstrated that there is a progressive increase in responsiveness of I and D rabbit aortic strips to selected kinins over a 7h period. Since the increases responsiveness to the kinins is selectively blocked by cycloheximide, de novo generation of B1 receptors seems likely. Finally, the greater reduction in responsiveness of D compared to I tissues to the kinins compared to 5-HT and NAd may indicate that the endothelium plays a role in this phenomenon.

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EICOSANOID SYNTHESIS BY NORMAL AND ULCERATED HUMAN DUODENAL MUCOSA.

K.J. Buller, K. Hillier & C.L. Smith*, Clinical Pharmacology Group and Medicine II*, Faculty of Medicine, University of Southampton, Southampton SO9 3TU.

It is widely accepted that exogenous prostaglandins and their analogues can protect gastric and intestinal mucosa against damage induced by non-steroidal anti-inflammatory drugs (NSAID) and other agents (Hawkey & Rampton, 1985). However, the mucosal damaging or protecting role of endogenous eicosanoids in peptic ulcer disease (UD) remains unclear, particularly in man. Attempts to clarify this by measuring the eicosanoid synthesising capacity or content of gastric and duodenal mucosa have yielded variable results (Hillier et al, 1985).

We have measured PGE_2, TxA_2 (as TxB_2), $PGF_{2\alpha}$ and PGI_2 (as 6-oxo-$PGF_{1\alpha}$) synthesis in short-term incubates of biopsies taken from the ulcer edge in the duodenal cap and from the non-ulcerated second part of the duodenum (2D) (at least 5cm from the ulcer). These were compared with the eicosanoids produced by biopsies from similar sites in patients who were macroscopically normal. Biopsies were incubated for 15 minutes in Krebs solution, and then transferred to fresh Krebs for a further hour (37°C). The eicosanoids synthesised were measured by radioimmunoassay following chromatographic extraction from the supernatant (modified from Hillier et al, 1985). No patients were taking NSAID or ulcer-healing drugs other than antacids.

Patient:	UD	Normal	UD	Normal
Region:	ulcer edge	cap	2D	2D
PGE_2	48.4 ± 7.8	17.4 ± 1.3	16.6 ± 2.2	23.6 ± 2.5
TxB_2	52.6 ± 7.3	25.5 ± 1.8	23.0 ± 3.0	27.5 ± 2.0
$PGF_{2\alpha}$	23.2 ± 2.8	19.5 ± 1.3	17.6 ± 2.4	24.0 ± 1.5
6-oxo-$PGF_{1\alpha}$	35.8 ± 5.9	29.2 ± 2.7	31.1 ± 2.9	41.0 ± 6.0
N	14	34	10	15

Values are ng synthesised/mg protein ± SEM in the combined 75 minute (15 + 60 min) incubation period.

In normal patients, PGE_2 and 6-oxo-$PGF_{1\alpha}$ synthesis was lower in the duodenal cap than in the 2D region ($P<.05$). In UD patients however, PGE_2 and TxB_2 production was higher in mucosa from the ulcer edge (duodenal cap) than from 2D ($P<.01$). The synthesis of PGE_2 and TxB_2 was also much greater in ulcer edge mucosa than in duodenal cap mucosa from normal patients ($P<.001$); this was not apparent when comparing the 2D regions of UD and normal patients, where only $PGF_{2\alpha}$ production was statistically different ($P<.05$).

In conclusion, duodenal ulceration appears to be associated with increased PGE_2 and TxB_2 synthesis close to the ulcer site, but this does not extend to the second part of the duodenum. These findings are partly supported by the results of Ahlquist et al (1983), but other workers have failed to detect raised PGE_2 and TxB_2 production at sites close to the duodenal ulcer (Sharon et al, 1983; Hillier et al, 1985).

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CYTOCHROME P-450 DEPENDENT MONO-OXYGENASE ACTIVITY AND ENDOTHELIUM-DEPENDENT RELAXATION OF VASCULAR TISSUE

M.J.Finnen, R.J.Flower, A. Lashenko & K.L Williams.
Pharmacology Group. University of Bath. U.K.

Inhibitors of cytochrome P-450 dependent mono-oxygenase activity have been shown to attenuate endothelial-dependent relaxation of vascular tissue by acetylcholine (ACh), A23187 and arachidonic acid, suggesting that a cytochrome P-450 dependent metabolite of arachidonic acid is responsible for the vasodilator action of these agonists (Peach et al. 1985). Renal cytochrome P-450 has recently been reported to metabolise arachidonic acid to a mixture of epoxy arachidonic acid (EAA) metabolites (Schwartzman et al. 1985). We have investigated the effects of cytochrome P-450 inhibitors on endothelium-dependent relxation, the ability of aortic cytochrome P-450 to metabolise exogenous and endogenous substrates and the effects of chemically synthesised EAA derivatives on vascular tone.

Rat aortic strips were pre-contracted with phenylephrine and a cumulative dose response curve for ACh induced relaxation obtained (10^{-8} - 10^{-3}M). In the presence of the P-450 inhibitors SKF 525A (1uM) or metyrapone (10 uM) the potency of ACh was reduced ten fold and four fold respectively. Mono-oxygenase reactions studied were aldrin epoxidation (AE); ethoxycoumarin dealkylation (EOD); benzpyrene hydroxylation (BP) and ethoxyresorufin dealkylation (ERD). Microsomal fractions from rat aorta were prepared according to standard procedures and used as enzyme source (Finnen et al. 1983). AE, EOD and BP activities were present in aortic microsomes (4.7 ± 1.2:11.4 ± 3.2:3.1 ± 1.2 pmoles/min/mg protein; n=4), whereas ERD was not detectable. Activity was absolutely dependent on NADPH, inhibited by 1uM SKF 525A (70%); 5uM metyrapone (56%) and carbon monoxide (85%), and inducible by phenobarbitone (4 fold). Incubation of rat aorta homogenates with ^{14}C arachidonic acid in the presence of NADPH resulted in the formation of one major and two minor bands of metabolites of arachidonic acid (Rf 1. 0.82 2. 0.63 3. 0.42) as assessed by tlc (ethyl acetate/iso-octane/acetic acid/water 110:50:20:100) and autoradiography. The formation of oxygenated metabolites was dependent on NADPH and inhibited 60 % by SKF 525A (5uM), 56% by metyrapone (10 uM) and 86% by carbon monoxide. Indomethacin (5uM) had no effect on the formation of NADPH dependent metabolites. The major metabolite 1 co-chromatographed with authentic 5,6 EAA whereas meabolites 2 and 3 were more polar derivatives.

EAA derivatives were synthesised by reacting arachidonic acid with 1.05 equivalents of m-peroxy chlorobenzoic acid in dichloromethane for 16 hours. 5,6 EAA; 8,9 EAA; 11,12 EAA and 14,15 EAA were separated and purified by tlc (ethyl acetate/hexane/acetic acid 65:35:0.1). Of the four epoxides 5,6 EAA was effective (1.7×10^{-7} - 3.4×10^{-6}M) in relaxing rat aortic strips pre-contracted with phenylephrine (10^{-7}M).

The present results demonstrate that cytochrome P-450 of aorta is capable of metabolising both exogenous and endogenous substrates and that an arachidonic acid metabolite of aortic cytochrome P-450 is a potent vasodilator.

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AIRWAY EPITHELIUM INFLUENCES RESPONSIVENESS OF GUINEA PIG TRACHEAL STRIPS

Finnen, M.J., Flower, R.J., Lashenko, A. & Williams, K.I. Pharmacology Group, University of Bath, U.K.

Removal of respiratory epithelium from canine bronchi or bovine tracheae potentiates responses to a variety of bronchoconstrictors (Flavahan et al., 1985; Barnes et al., 1985). As bronchial hyperreactivity is a primary asthmatic symptom, experiments were performed to determine whether the airway epithelium of guinea-pig trachea also influences responsiveness, since this tissue resembles human airway smooth muscle in its response to drugs (Foster, 1974).

Male guinea-pigs (Dunkin Hartley and strain 2, 300-450 g) were killed by a blow on the head. The trachea was excised and tracheal strips prepared. These were placed under 1g resting tension in 20 ml organ baths containing Krebs' solution maintained at 37^{o}C and gassed with 5% CO_2 in O_2. Bath fluid was changed at 15 min intervals and a 60 min equilibration period allowed. Agonist drugs were added cumulatively and inhibitors added 15 min prior to agonist addition. Epithelium was removed mechanically with a fine probe.

In intact tracheal preparations acetylcholine (ACh), 10^{-8} to 10^{-3}M, and 5-hydroxytryptamine (5-HT), 10^{-9} to 10^{-5}M, both produced full dose-response curves. The EC_{50} values for ACh and 5-HT were 1.1 x 10^{-5}M $\pm$ 0.5 (n=5) and 2.7 x 10^{-7}M $\pm$ 0.3 (n=3) respectively. Following epithelial cell removal the EC_{50} values were 1.2 x 10^{-6}M $\pm$ 0.4 for ACh and 1.1 x 10^{-7}M $\pm$ 0.3 for 5-HT. These values were markedly lower than those obtained with intact tracheal strips. In addition, tracheal strips without epithelium showed an increase in maximal response to ACh over controls (126% $\pm$ 12). When tracheal strips were prepared from guinea-pigs sensitised to ovalbumen they developed less tension to added ACh than normal strips. Furthermore de-epithelialisation did not reduce the EC_{50} value for ACh (n=3), a finding which contrasted with normal preparations.

In 5 experiments using normal intact tracheal strips dose-response curves were obtained to ACh before and after exposure to SKF 525 -A (5 x 10^{-6}M), an inhibitor of cytochrome p450 mono-oxygenase. This treatment increased the maximal response to 126% $\pm$ 6 relative to controls. Similarly the maximal response to 5-HT was increased by 125% $\pm$ 16 (n=3) over controls.

Epoxides of arachidonic acid were prepared as described by Oliw et al (1982) and separated by thin-layer chromatography. Each epoxide was then tested for relaxant activity in pre-contracted tracheal strips. The 5,6-epoxide of arachidonic acid was found to be the most potent in inducing dose-dependent relaxations (1.4 x 10^{-7}M to 1.4 x 10^{-6}M).

These results suggest that in guinea-pig trachea the airway epithelium exerts an inhibitory influence upon the underlying smooth muscle cells. Any impairment of this mechanism e.g. in respiratory disease could lead to bronchial hyperreactivity.

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A NOVEL AND VERSATILE MULTIPLE SUPERFUSION SYSTEM: A REPLACEMENT FOR THE ORGAN BATH?

R.A. Coleman, A.T. Nials, K.E. Sheldrick and R.L.G. Sheldrick, Department of Respiratory Pharmacology & Biochemistry, Glaxo Group Research, Ware, Herts.

We have developed a novel eight-chamber superfusion system which can be used for many applications involving different smooth muscle preparations, including the study of both contraction and relaxation and the effect of agents which interfere with these actions. It allows measurement of not only potency, but also of rate of onset and offset of drug action. Preparations may be stimulated electrically to induce neuronally-mediated responses and agents which interfere with neurotransmission may then be evaluated.

The system consists of eight individual perspex chambers in parallel, each comprising two halves, a fixed dome-shaped upper part and a funnel-shaped lower part, hinged to allow access. Preparations are tied to a stainless steel hook inside each chamber, a thread attached to the other end of each preparation passes out through a hole in the upper part for attachment to a strain gauge. Preparations are superfused at 2ml min^{-1} with oxygenated (5% CO_2 in O_2) physiological salt solution, maintained at $37^{o}C$. The superfusion fluid is applied with a Watson Marlow 502S peristaltic pump, with a 502AA pumphead, via silicon rubber tubing to stainless steel tubes which pass through the perspex roof of each chamber (at approximately 30^{o} to vertical) and thence down the thread and over the tissue within each chamber. The fluid then drips through a hole in the lower part of the chamber to waste. Bipolar platinum electrodes may also be introduced through the roof of the upper chamber, shaped so that they are parallel with and in close proximity to the superfused tissue. The surface tension of the superfusion fluid ensures electrical contact between electrode and tissue. Electrical stimuli are then delivered by a square wave stimulator with suitable amplification, to all eight preparations. Drugs may be delivered to the tissue either by addition to the superfusion fluid reservoir or by infusion at a slow rate (0.02ml min^{-1}) into the superfusion fluid immediately before entry into the chamber. Preparations examined to date in this system include guinea-pig trachea, dog saphenous vein, human bronchus, dog iris and guinea-pig vas deferens.

The advantages of such a continuous superfusion system over immersion techniques are: (1) drug contact time with the tissue is reduced allowing examination of relatively unstable compounds (2) potentially toxic metabolites will not accumulate, (3) baths do not have to be repeatedly washed throughout the experiment and (4) drug kinetics may be readily measured (see Coleman et al, 1986). The present system may therefore be complementary to, or even replace the classical organ bath.

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Coleman, R.A., Nials, A.T., (1986) This meeting.

THE CHARACTERISATION AND USE OF THE ELECTRICALLY-STIMULATED, SUPERFUSED GUINEA-PIG TRACHEAL STRIP PREPARATION.

R.A. Coleman and A.T. Nials, Department of Respiratory Pharmacology & Biochemistry, Glaxo Group Research, Ware, Herts.

We have previously described an eight chamber superfusion system, which will permit electrical stimulation of a variety of smooth muscle preparations (Coleman et al., 1986). We now report some results obtained on the guinea-pig isolated tracheal strip (GPT) using this technique. We have investigated both the nature of the electrically-induced response and its suitability for the evaluation of potency and kinetics of action of some spasmolytic agents.

GPT was prepared as described by Coleman and Kennedy (1980) and mounted in superfusion chambers. The preparations were superfused with Krebs solution, containing indomethacin (2.8uM) and were stimulated with 10s trains of pulses every 2 min. The frequency of the pulses was 5Hz, pulse width 0.1ms and supramaximal voltage (10-14v). The preparations responded with contractions equivalent to 500-1500mg in amplitude. Once equilibrated, these contractions were highly reproducible for periods of up to 14h. Infusion of tetrodotoxin (30nM) or atropine (1-10nM) into the superfusion fluid caused complete inhibition of the contractile responses, indicating that they were due to stimulation of cholinergic neurones. Administration of propranolol (100nM) enhanced the amplitude of contraction by 10-180% (n=6) in preparations from normal guinea-pigs, but not in those from animals pretreated with 6-hydroxydopamine (6OHDA, 200mg/kg i.p., 24-60h previously; n=5) or after adrenergic neurone blockade with bethanidine (10-1000nM; n=2). Thus it appears that both cholinergic (mediating contraction) and adrenergic (mediating relaxation) nerve fibres are stimulated and propranolol potentiates the contractile response by blocking the adrenergic component. In all subsequent experiments, preparations were taken from animals pretreated with 6-OHDA. We have evaluated the potency (EC_{50}) and time to 50% onset of action (Ot_{50}) and 50% recovery from time of stopping drug infusion (Rt_{50}) for some spasmolytic agents in inhibiting electrically-induced contractile responses (Table 1).

TABLE 1

Agonist	EC_{50}(nM)	Ot_{50} (min)	Rt_{50} (min)	n
Salbutamol	24.7 (11.1 - 54.9)	3.1 ± 0.8	11.4 ± 1.6	8
Clenbuterol	9.6 (3.7 - 24.8)	6.7 ± 2.2	47.9 ± 8.9	6
Theophylline	5924*(127 - 27486)	<2 - 4.5	<2 - 5.0	3
Papaverine	3400 (2300 - 4800)	5.7 ± 1.2	10.8 ± 0.7	3

(*EC_{30} value)

All four compounds inhibited contractions of GPT in a concentration-related fashion, salbutamol and clenbuterol being more potent than theophylline and papaverine. Theophylline was particularly weak, 50% inhibition not always being achieved. While the Ot_{50} values for all four agonists were similar, their Rt_{50} values differed, with theophylline being the shortest-acting whilst clenbuterol was at least four times longer acting than salbutamol or papaverine.

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AIRWAY HYPERREACTIVITY IN RATS TREATED WITH PROTEIN-COATED LATEX PARTICLES.

M.F. Burgess; G.D. Clarke; S.M. Laycock and H. Smith
Beecham Pharmaceuticals, Biosciences Research Division, Great Burgh, Epsom, Surrey, KT18 5XQ

Two characteristic features of patients with asthma are the exquisite sensitivity of their airways to diverse stimuli and the presence of increased numbers of eosinophils in blood and sputum. The exact cause(s) of the bronchial hyperreactivity and the relationship between the eosinophil and asthma is unknown. This study was undertaken to determine whether techniques known to produce blood and lung eosinophilia (Schriber and Zucker-Franklin, 1974; Eady et al, 1978) would influence bronchial reactivity in the rat.

Sprague-Dawley female rats (200-300g) were injected intravenously, in a foot vein, with latex particles, 45µm in diameter, coated with bovine gamma globulin (BGG). The BGG was adsorbed onto the particles by mixing the particles in a 3.0mg/ml solution of antigen for one hour at room temperature. After the initial injection some animals were tested on day 5. Other animals received identical booster injections on day 13, or days 11 and 13, before use on day 15; control rats were given intravenous saline.

The respiratory effects of intravenous 5-HT *in vivo* (0.1 - 25µg/kg) were monitored in anaesthetized, artificially respired rats according to the technique of Konzett and Rössler (1940). The animals were anaesthetized with sodium pentobarbitone (60mg/kg i.p.) and ventilated using a constant volume respiratory pump at 90 strokes/min. The effects of cumulative administration of 5-HT or carbachol on isolated lung strips were measured *in vitro* with the tissues arranged for isometric recording using standard organ bath techniques. Blood eosinophils were counted in treated and control animals in parallel experiments.

Intravenous injection of BGG-coated latex particles produced a blood eosinophilia and an increased responsiveness of the respiratory tissues of rats *in vivo* to the spasmogenic action of 5-HT and *in vitro* to 5-HT and carbachol. Both the degree of hyperreactivity *in vivo* and the blood eosinophilia were related to the extent of the dose schedule. The effects on airway contractility *in vivo* were shown by decreases in threshold, ED_{25} and ED_{50} values for 5-HT; maximum responses were not affected. In isolated parenchymal strip preparations the contractile response to 5-HT, and to a lesser extent to carbachol, was enhanced over the agonist dose range employed compared to responses in control tissues. For example, the mean tension (±s.e.m.) evoked by the maximum dose of 5-HT (2×10^{-4}M bath concentration) was 334 ± 34mg in tissues from animals given injections of BGG/latex particles and 42 ± 5mg in tissues from control animals.

Airway hyperreactivity *in vivo* and lung smooth muscle hyperreactivity *in vitro* have been found in animals subjected to procedures shown to induce blood eosinophilia. The data suggest that increased responsiveness of peripheral lung smooth muscle *per se* may be responsible for the hyperreactivity observed *in vivo*.

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EFFECT OF AMINOPHYLLINE ON RESPIRATORY DEPRESSION PRODUCED BY INTRAVENOUS ADENOSINE IN NEONATAL RABBITS

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

We have found that adenosine when administered intravenously to neonatal rabbits commonly causes respiratory depression. Such a respiratory depressant effect of intravenous adenosine contrasts with the respiratory stimulant effect produced in adult rabbits (Buss et al., 1986) and in man (Watt and Routledge, 1985). The adenosine antagonist aminophylline reduces the number of "spontaneous" episodes of apnoea (possibly precipitated by hypoxia) in human neonates. It therefore appeared of interest to examine the effect of aminophylline on the respiratory effects of intravenous adenosine in neonatal rabbits.

We administered adenosine to 11 neonatal New Zealand White rabbits on days 1 and 2 of life by repeated bolus injections in a dose of 120 ug/kg via a cannula positioned in an external jugular vein. Respiration was measured non-invasively and semi-quantitatively using a Lectromed respiration transducer, to avoid any possible effects of general anaesthetics. Following a series of intravenous boluses of adenosine, aminophylline was administered in a dose of 10 mg/kg over 1 minute as a slow intravenous injection. Serial injections of adenosine in a dose of 120 ug/kg were then repeated. Comparisons were made between stable baseline respiration before each adenosine injection and the peak change in respiration which occurred about 2-4 seconds after adenosine injection.

Adenosine produced a significant reduction of ventilation in 10 of 11 neonatal rabbits. In one animal there was no significant change. In 7 animals the reduction in respiratory depth reached statistical significance, and in 3 the respiratory rate decreased after adenosine injections. After aminophylline adenosine increased ventilation in 7 animals, and produced no significant change in 4. Of the 10 animals in which adenosine initially depressed respiration, it stimulated respiration in 7. In all 7 animals adenosine produced an increase in respiratory depth and in 4 of these an increase in respiratory rate also occurred. In one animal adenosine did not significantly alter respiration before or after aminophylline administration. For the groups adenosine depressed ventilation from 1058 $\pm$ 584 units to 697 $\pm$ 419 units before aminophylline ($p < 0.001$) but adenosine increased ventilation from 1197 $\pm$ 715 units to 1564 $\pm$ 890 units after aminophylline ($p < 0.001$).

The mechanism(s) whereby adenosine stimulates respiration in the adult rabbit but decreases respiration in the neonatal rabbit are, as yet, unclear. Thus it is difficult to analyse the site(s) of action of aminophylline by which it not only antagonises but commonly reverses the respiratory depressant effect of adenosine in rabbit neonates. One possibility is that aminophylline alters some central effect of adenosine in respiratory regulation. It is also possible that the effect of aminophylline described here may have some relevance to the therapeutic effect of the methylxanthines in the treatment of neonatal apnoea. Further studies are required to clarify these uncertainties.

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RESPIRATORY EFFECTS OF INTRAVENOUS ADENOSINE IN NEONATAL RABBITS

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

Neonatal apnoea is not uncommonly observed in human neonates but the pathophysiology is obscure. The methylxanthines theophylline and caffeine have been found to be effective in the treatment of this problem (Kuzemko and Paala, 1973; Bednarek and Roloff, 1976). Both drugs are competitive adenosine antagonists and it appeared possible that adenosine might be involved in neonatal apnoea. However, adenosine analogues depress respiration in both adult (Wessberg et al., 1985) and neonatal (Hedner et al., 1984) animals. In contrast to previous observations we recently identified a respiratory stimulant effect of intravenous adenosine in man (Watt and Routledge, 1985) and in adult rabbits (Buss et al., 1986). We therefore examined respiratory responses of neonatal rabbits to intravenous adenosine. Repeated injections of one dose were given to each animal, as there is considerable interindividual variability in respiratory responses in neonatal animals.

To avoid any possible effects of general anaesthesia on respiration we used a non-invasive semi-quantitative method of measuring respiration using a Lectromed respiration transducer (which we confirmed on a dummy to give a linear response in the format used in this study). The neonatal rabbit was positioned on a foam pad. An intravenous cannula was placed in an external jugular vein and maintained there manually. Adenosine, at a concentration of 0.1 $mg.ml^{-1}$, was administered at intervals of approximately 1 minute by serial rapid intravenous bolus doses of 120 ug/kg to 30 neonatal New Zealand White rabbits. Placebo injections were given to 11 neonatal rabbits. Comparisons were made between stable baseline respiration before injections and peak change in respiration which occurred about 2-4 seconds after injections.

Adenosine produced significant respiratory depression in 13 of 30 animals; in 8 this was attributable solely to a reduction in respiratory depth. In 15 animals adenosine produced no significant change in respiration, and in 2 neonates adenosine produced a significant increase of ventilation (due to an increase in respiratory depth). For the group as a whole adenosine reduced ventilation from 738 $\pm$ 390 units to 674 $\pm$ 418 units ($p < 0.002$, paired t test). Placebo injections produced no significant change in respiratory variables.

Intravenous adenosine commonly depressed respiration in neonatal rabbits in this study. This contrasts with adenosine-induced respiratory stimulation in man (Watt and Routledge, 1985) and adult rabbits (Buss et al., 1986). The mechanisms underlying the different respiratory responses in the neonatal and adult rabbits are unclear. It is possible that altered central processing of peripheral chemoreceptor signals or altered access of adenosine to the brain may be involved. The results of this study suggest that further study of the possible role of adenosine in the pathophysiology of neonatal apnoea is appropriate.

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RESPIRATORY EFFECTS OF INTRAVENOUS ADENOSINE IN ANAESTHETISED RABBITS BEFORE AND AFTER CAROTID NERVE SECTION

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

Intravenous adenosine stimulates respiration in man (Watt and Routledge, 1985) and the conscious rabbit (Buss et al., 1986). The site of action of adenosine is unknown, but as adenosine increases neural discharges in the carotid sinus nerve of the cat (McQueen and Ribeiro, 1981), we proposed that adenosine exerts its respiratory stimulant effect on the carotid body. We examined the respiratory effects of intravenous adenosine in anaesthetised rabbits.

Eight male New Zealand White rabbits weighing 4.26-4.88 kg were anaesthetised using pentobarbitone 30-50 mg/kg. An indwelling cannula was sited in a marginal ear vein. The carotid arteries were exposed and the bifurcation and the glossopharyngeal nerves identified but care was taken not to disrupt the carotid sinus branch of the nerve. Bolus doses of adenosine and saline were injected in duplicate, with at least 90 seconds between injections. The carotid sinus nerves were then divided bilaterally and bolus doses of adenosine and saline were administered again.

Respiratory rate, tidal volume and minute ventilation were measured for 30 seconds before and after injections using a Lectromed respiration transducer calibrated at the end of each experiment. The dose of adenosine chosen was 4000 ug/kg, pilot studies having shown that such high doses were required in the anaesthetised rabbit to consistently produce respiratory stimulation. The volume of 5 mg/ml adenosine solution in saline was approximately 3.2-4.0 ml. To control for volume effects comparisons were made between changes in respiratory variables produced by adenosine and saline, both before and after the carotid sinus nerves were divided. Comparisons were made using log-linear transformed data.

With the carotid sinus nerves intact adenosine produced an increase in minute ventilation of 232 ± 146 ml.min^{-1} which was significantly greater ($p < 0.005$) than the increase of 39 ± 79 ml.min^{-1} produced by saline. This was attributable to both a greater increase in respiratory rate and tidal volume produced by adenosine. Following division of the carotid sinus nerves the increase in minute ventilation produced by adenosine of 14 ± 118 ml.min^{-1} did not differ significantly ($p > 0.20$) from that produced by saline (10 ± 70 ml.min^{-1}). Changes in respiratory rate and tidal volume produced by adenosine and saline also did not differ significantly after division of the nerve.

Our results demonstrate that in the anaesthetised rabbit the respiratory stimulant action of adenosine is not detectable following division of the carotid sinus nerve. Adenosine increases neural discharges in that nerve in the cat without producing changes in blood pressure, suggesting that adenosine may act on the carotid body rather than the carotid sinus (McQueen and Ribeiro, 1981). We previously suggested (Watt and Routledge, 1985) that endogenous adenosine may play a role in the ventilatory response to hypoxia. Exogenous adenosine exerts its respiratory stimulation on the carotid body, the organ initiating the ventilatory response to hypoxia. It remains to be established whether endogenous adenosine exerts such an effect in physiological situations.

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INTERACTIONS OF VERAPAMIL, NIFEDIPINE AND DILTIAZEM WITH TUBOCURARINE AT THE RAT NEUROMUSCULAR JUNCTION

E.Greenidge and F.A.Wali. Department of Physiology and Anaesthetics Unit,The London Hospital Medical College,Whitechapel,London E1.U.K.

Recently,a great interest in the use of calcium entry blockers has been developed in anaesthesia,especially in connection with their use during surgical relaxation. Previous reports have indicated that calcium entry blockers,e.g. verapamil,intensifies neuromuscular blockade produced by muscle relaxants,e.g. gallamine,pancuronium,atracurium and tubocurarine (Bikhazi,Leung & Foldes,1982;1983;Durant,Nguyen & Katz,1984;Wali,1985). Furthermore,the effects and interactions of calcium entry blockers with muscle relaxants could be reversed on washing out the preparation with control Krebs solution and with neostigmine or edrophonium (Carpenter & Mulroy,1983;Wali,1986).

In the present investigation,the effects and interactions of verapamil,nifedipine and diltiazem with tubocurarine were studied at a rat neuromuscular junction to further analyse the potentiation by calcium entry blockers of neuromuscular blockade produced by muscle relaxants in an in-vitro preparation.

The preparation,rat phrenic nerve-hemidiaphragm,was set up in an organ bath containing 80 ml of Krebs-Henseleit solution maintained at 38± 2°C and bubbled with 5% carbon dioxide in oxygen. Mechanical responses (both direct and indirect twitch responses),elicited at 0.2 Hz with 5-10 V (maximal) and 0.2 msec pulse duration, were recorded isometrically,by means of a force-displacement transducer and a Washington pen recorder.

Verapamil (1-100 μM),nifedipine (15-1500 μM) and diltiazem (2-200 μM) reduced the mechanical twitch responses (both directly and indirectly-elicited twitch tension) in a dose-dependent manner.However,the directly-elicited twitch response was only reduced by about 20% of the maximum indirect twitch depression. Tubocurarine (0.127-127 μM) reduced the indirect twitch response but had no effect on the direct twitch response. Verapamil,nifedipine and diltiazem increased the neuromuscular blockade produced by tubocurarine.The mean IC50 values (concentration to produce 50% maximum inhibition) of calcium entry blocker-induced depression of indirect twitch tension were: 60±3 μM,verapamil, 725±18 μM,nifedipine,and 176±9 μM,for diltiazem (means±S.E.,n=6,$P < 0.001$). The mean IC50 values for tubocurarine-induced depression of twitch tension in the absence and presence of verapamil (10 μM), nifedipine (50 μM) and diltiazem (20 μM) were: 19±2 μM, 4.2±0.1 μM, 12.3±0.5 μM, and 9.4±0.1 μM respectively (n=6,$P < 0.001$).

Based on the IC50 values of the calcium entry blockers,it is apparent that verapamil was about 9 or 3 times as potent as nifedipine or diltiazem respectively.Furthermore,when interacted with tubocurarine,verapamil was 3 or 2 times as potent as nifedipine or diltiazem in potentiating tubocurarine-induced blockade produced at the rat neuromuscular junction.

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EFFECT OF LOCAL ANAESTHETICS ON CHOLINERGIC RESPONSES IN THE CHICK ISOLATED TRACHEAL SMOOTH MUSCLE

F.A. WALI. Anaesthetics Unit,The London Hospital Medical College,Whitechapel, London E1 1BB.

Local anaesthetics,in particular inhaled lignocaine,have been employed in the treatment of some respiratory disorders,e.g. bronchial asthma (Griffin,McFadden, Ingram & Pardee,1982 ;Cross,Guz,Jain,Archer,Stevens & Reynolds,1971).

In the present investigation,the effects of 5 different local anaesthetics on tone and contractility,and on cholinergic responses of the chick isolated tracheal smooth muscle were studied to see if local anaesthetics increase the tone while inhibiting the cholinergic responses in the tracheal smooth muscle in vitro.

Trachea from freshly killed chicks were cut longitudinally along the ventral wall and stripped from mucosa (Karlsson & Persson,1984; Bach-Dieterle,Holden & Junod, 1983). The preparation was set up under an initial tension of 0.5 g,in an organ bath containing 20 ml of Krebs-Henseleit solution maintained at 38± 2°C and bubbled with 5% carbon dioxide in oxygen. Tracheal preparations were electrically stimulated,by field stimulation with pulses of 0.2 msec at a maximal voltage producing twitch responses which were completely blocked by tetrodotoxin (1 µM).

The contractile responses produced by electrical field stimulation in the presence of indomethacin (2.8 µM),and guanethidine (10 µM),were abolished by atropine (1 µM). Atropine (1 µM) also blocked the isometric contractions produced by bath application of acetylcholine (ACh , 88 µM).

Lignocaine,prilocaine,etidocaine,mepivacaine and bupivacaine,in low concentrations, all reduced the contractions produced both by electrical field stimulation and by exogenous ACh (see Table 1, col. 1 & 2). Moreover,the local anaesthetics,in high concentrations,produced marked contractures (0.5-1.4 g of tension) in the isolated tracheal smooth muscle (Table 1, col. 3 & 4).

Table 1. Effects of local anaesthetics on tone and contractility and cholinergic responses of chick isolated tracheal smooth muscle.

	(1) IC50 (µM) for Field Stimultn (*) Mean±S.E.	(2) IC50 (µM) for Applied ACh Mean±S.E.	(3) EC50 (µM) for Contracture Mean±S.E.	(4) Maximum Contracture(g) Mean±S.E.
Lignocaine	88±6	105±11	700±130	1.2±0.3
Prilocaine	250±10	312±16	1170±102	0.8±0.2
Etidocaine	256±18	340±22	1600±180	0.5±0.1
Mepivacaine	118±10	140±11	800±22	1.0±0.2
Bupivacaine	61±9	73±6	535±18	1.4±0.1

(*): n=6. IC50/EC50 : concentrations to produce 50% of maximum inhibition/contraction of tracheal smooth muscle responses.

The results indicated that the local anaesthetics studied modified (reduced) the cholinergic responses due to field stimulation and to ACh application.Furthermore, the anaesthetic drugs increased the tone and produced a contracture in the chick tracheal smooth muscle.

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FMLP-INDUCED CONSTRICTION OF AIRWAY PERFUSED GUINEA-PIG LUNGS

R.Budhram, S.Clements-Jewery, A.M.Johnston and T.A.Thomson (introduced by P.Miller). Roussel Laboratories Limited, Swindon, Wiltshire, U.K.

The chemotactic peptide, N-formyl-methionyl-leucyl-phenylalanine(FMLP), has been shown to be a potent myotropic agent on the guinea-pig lung parenchymal strip preparation in addition to causing bronchoconstriction "in vivo" (Hamel *et al.* 1984). Arachidonic acid metabolites generated from both cyclo-oxygenase and lipoxygenase pathways were implicated in these responses.

In the present studies, the effects of FMLP were investigated in unsensitised guinea-pig (male, Dunkin Hartley) half-lung preparations (Clay *et al.* 1985). The half-lungs were perfused via the airways at a constant flow (4ml/min) with Kreb's bicarbonate buffer at 37°C, gassed with 5% CO_2 in O_2. The buffer also contained 10^{-5}M mepyramine, 10^{-5}M atropine and $5x10^{-6}$M indomethacin; added in order to abrogate the influences of histamine, acetylcholine and cyclo-oxygenase products on airway tone. Under these conditions, a dose-response study revealed that FMLP induced a dose-related increase in perfusion pressure showing a maximal increase of 30.7 $\pm$ 4.3 (n=7) mmHg at a dose of 30ug. Doses of 30ug FMLP were then administered to paired half-lung preparations from the same animal and the responses compared. Regression analysis of the paired data produced a correlation coefficient of 0.68 ($p < 0.001$) at n=20; thus allowing one half lung from a single animal to be used to control for the other half lung in drug inhibition studies. The increase in perfusion pressure induced by 30ug FMLP was inhibited by the constant perfusion of the B_2-adrenoceptor agonist, salbutamol, and compounds reportedly active as either 5-lipoxygenase inhibitors or as a leukotriene D_4 antagonist (Table).

Table	IC_{50} micromolar
Salbutamol	0.05
FPL 55712	2.3
Benoxaprofen	10.0
Phenidone	11.5
NDGA	14.5

Under the conditions tested, the results suggest that FMLP induces a bronchospasm which may be primarily mediated by lipoxygenase products. It therefore appears that this system may be used to study compounds which inhibit the synthesis or antagonise the effect of leukotrienes in the guinea-pig lung.

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EFFECT OF COTTON DUST EXTRACTS ON GUINEA PIG PERFUSED LUNG.

N. El-Mahdy and P.J. Nicholls, Welsh School of Pharmacy, UWIST, PO Box 13, Cardiff, CF1 3XF.

Inhalation of cotton dust or of aerosols of its aqueous extracts induces a reversible bronchoconstriction in both byssinotic textile workers and healthy subjects (Bouhuys, 1984). These effects may be related to a smooth-muscle contractor activity in the dust (Davenport and Paton, 1962) or to dust-mediated release of bronchoactive agents (Evans and Nicholls, 1974). The present work has examined the action of cotton dust extracts on perfused guinea pig lung. The airways of isolated lungs of adult female animals were perfused with Krebs-bicarbonate saline at 32°C via the trachea. Perfusion rate was 5ml.min^{-1}. Extracts and various pharmacological agents were injected into the perfusion fluid and bronchoconstriction was assessed by measuring changes in perfusion pressure.

The dust extracts (10-100mg) caused a reversible bronchoconstriction of slower onset and longer duration than that elicited by histamine or carbachol. The time-course of the response of dust extracts was similar to that produced by the calcium ionophore A-23187. The effects of the extracts were dose-related and were unaffected by atropine (0.34µg/ml), mepyramine (0.34µg/ml), methysergide (1µg/ml) and indomethacin (6µg/ml). However, the responses were reduced by at least 50% in the presence of nordihydroguaiaretic acid (3µg/ml), FPL 55712 (1µg/ml) and diethylcarbamazine (4mg/ml). As the dust itself does not contain a leukotriene-like agent, it is possible that the dust-induced effect is mediated by a release of arachidonic acid metabolite(s) of the lipoxygenase pathway in view of the action of these various agents on the dust response. This suggestion would be consistent with the previously reported ability of cotton dust to release arachidonic acid metabolites from alveolar macrophages (El-Mahdy et al., 1985).

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LEUKOTRIENE INVOLVEMENT IN GUINEA PIG ANAPHYLACTIC CUTANEOUS VASCULAR PERMEABILITY

H.J. Barton and T.P. Cole (introduced by W. Dawson), Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey, GU20 6PH.

Changes in cutaneous vascular permeability can be elicited by a range of mediators. This study demonstrates that the microvascular protein extravasation resulting from cutaneous anaphylaxis is mediated in part by histamine and leukotrienes.

Cutaneous anaphylactic reactions were elicited with intradermal injections of ovalbumin into the shaved abdominal skin area of ovalbumin-sensitized guinea pigs. Animals were sensitized by ovalbumin injection 1 mg s.c. and 1 mg i.p. and were ready for use 21 days later. All compounds were dissolved in 0.9% saline. Three dose levels of ovalbumin (5, 1 and 0.1 μg/site) were used in this study. The extravasation of protein was quantitated using Evans blue dye (35 mg/kg i.v.) injected 5 minutes before ovalbumin challenge.

Animals were sacrificed 30 minutes post-challenge, the skin sites excised and the dye extracted and quantitated using a modification of the method described by Katayama et al (1978).

Intradermal administration of ovalbumin (0.1, 1 and 5 μg) to sensitized guinea pigs resulted in marked and dose-related protein extravasation. Mepyramine (2 mg/kg s.c.) given 30 minutes before ovalbumin challenge reduced the protein extravasation at the ovalbumin 5 and 1 μg sites by 30%, and at the 0.1 μg site by 60%. A combination of mepyramine and FPL 55712 (infused at 1 mg/kg/min i.v.) further reduced the permeability change to ovalbumin by 50% ($p < 0.01$) at the 5 μg site (Student "T" test), 74% ($p < 0.001$) at the 1 μg site and 62% ($p < 0.001$) at the 0.1 μg site, compared with mepyramine-alone treated animals. In addition, indomethacin (10 mg/kg i.p.) given 30 minutes before challenge, in combination with mepyramine, consistently potentiated the protein extravasation to ovalbumin at all dose levels by a mean of 50%.

In conclusion, the large reduction observed with mepyramine alone and the further reduction obtained with concomitant administration of FPL 55712 together with the potentiation elicited by indomethacin suggest involvement of both a histamine and a leukotriene component in the skin anaphylactic permeability change to ovalbumin.

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THE ELECTROPHYSIOLOGICAL EFFECTS OF SK&F 94120 A SELECTIVE PDE III INHIBITOR ON SHEEP PURKINJE FIBRES

R.W. Gristwood, D.A.A. Owen and A.L. Rothaul, Smith Kline and French Research Ltd., The Frythe, Welwyn, Hertfordshire, U.K.

SK&F 94120 is a novel agent possessing both positive inotropic and vasodilator activity and has been shown to be a highly selective inhibitor of phosphodiesterase type III (Gristwood, et al., 1985). SK&F 94120 has been demonstrated to increase the plateau height of normal action potentials (5.9 mM $[K]_o$) and to enhance slow response action potentials (27 mM $[K]_o$) in preparations from human ventricular tissue (Cameron, et al., 1985). Both of these observations are consistent with an increase in the second, calcium carried, inward current (Isi).

The purpose of the present study is to further explore the electrophysiology of this highly selective agent by considering it's effects on specialised conducting (Purkinje) tissue.

Purkinje fibres (false tendons) were excised from sheep hearts obtained from an abbatoir. The tissues were superfused at 6 cm^3 min^{-1} at 37.5°C with Krebs solution aerated with 95% O_2 5% CO_2. The tissues were paced with supra-maximal stimuli at 1Hz and were allowed to stabilise for 1 hour. Action potential measurements were made using standard microelectrode techniques. Dose-response curves were constructed by the cumulative addition of drug. Measurements were made from the maintained impalement of a single cell throughout each experiment. The parameters measured include: resting membrane potential (RP mV); action potential amplitude (APA mV); action potential duration at both 50 and 90% levels of repolarisation (APD_{50} and APD_{90} ms); maximal rate of repolarisation of phase 0 (MRD Vs-1); and effective refractory period (ERP ms).

RMP was not effected by SK&F 94120 in any experiment, mean control value was 92 ± 1 mV N=5. The effects of the drug on other parameters are shown in the table below:-

Conc. SK&F 94120 M	N	APA	APD_{50}	APD_{90}	MRD	ERP
0	5	115±6	131±15	234±16	532±22	221±20
10^{-7}	4	118±5	137±14	230±21	533±25	219±22
10^{-6}	5	118±5	121±12	229±17	533±22	216±22
$3X10^{-6}$	5	119±5	125±9	228±15	532±23	214±20
10^{-5}	5	114±2	123±14	227±18	529±23	213±20
$3X10^{-5}$	5	112±3	122±14	223±18	532±22	210±22
10^{-4}	5	116±3	122±15	230±16	533±22	224±23

Thus, SK&F 94120 failed to significantly alter any of measured action potential characteristics. This would suggest that inhibition of PDE III is unlikely to effect specialised conducting tissue in vivo.

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THE EFFECT OF IDAZOXAN ON BLOOD PRESSURE, AND PLASMA CATECHOLAMINE CONCENTRATIONS OF SPONTANEOUSLY HYPERTENSIVE RATS

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

Acute i.v. administration of the α_2-adrenoceptor antagonist idazoxan to anaesthetised or conscious normotensive rats results in a large increase in plasma noradrenaline (NA) concentration without a concomitant change in blood pressure (Brown & Harland, 1984; 1985). These results are consistent with the hypothesis that antagonism of prejunctional α_2-adrenoceptors can modulate release of NA from peripheral sympathetic nerves (Majewski et al., 1983). In this study we have investigated the acute effects of idazoxan on blood pressure and plasma catecholamine concentrations of spontaneously hypertensive rats (SHR).

Male SHR, 17-18 weeks old anaesthetised with Inactin (thiobutobarbitone sodium, 100 mg/kg i.p.) received a 5 min i.v. infusion of idazoxan 300 μg/kg, or saline (1 ml/kg). Mean arterial pressure (MAP) and heart rate (HR) were measured 0, 10, 30, 60 and 120 min after the end of the infusion. Concentrations of NA and adrenaline (A) in plasma of arterial blood samples (350 μl) were measured at -10, 0, 30 and 120 min. In a separate group of SHR, cumulative i.v. doses of the directly acting vasodilator hydralazine (12.5-200 μg/kg) were administered to assess baroreceptor-mediated changes in HR and plasma NA following a fall in MAP.

MAP fell by 20.3±4.5 mmHg ($P<0.01$, n=6) during infusion of idazoxan 300 μg/kg, and remained below pre-idazoxan levels for the duration of the experiment. HR rose by 17±7 beats/min during the infusion. Plasma NA rose to 1.143±0.08 ng/ml from a basal value of 0.563±0.06 ng/ml (n=6) immediately after idazoxan. Plasma A rose from 0.121±0.03 ng/ml to 0.354±0.06 ng/ml. Both plasma NA and A concentrations changed significantly with time over the period of the experiment ($P<0.01$ in each case, repeat measures analysis of variance), and were still greater than pre-idazoxan levels 120 min after idazoxan administration. Saline (1 ml/kg i.v.) had no significant effect on MAP, HR or plasma NA and A at any time point. Reflex activation of the sympathetic nervous system by hydralazine (12.5-200 μg/kg i.v.) caused increases in HR and plasma NA which were linearly related to the fall in MAP produced by the drug ($y=15.6+0.39x$, $r=0.752$ and $y=17.4+5.87x$, $r=0.79$ respectively, $P<0.005$ in each case). The increase in plasma NA concentration following idazoxan 300 μg/kg i.v. was much greater than that produced by an equihypotensive dose of hydralazine (580±90 pg/ml compared with 151±21 pg/ml, n=6 in each case). The tachycardia caused by idazoxan in SHR was no greater than that which could be accounted for by stimulation of the baroreceptor reflex.

These results suggest that idazoxan causes a greater increase in plasma NA concentration than that which can be attributed to baroreceptor stimulation. Blockade of prejunctional α_2-adrenoceptors by idazoxan may therefore increase release of NA from peripheral sympathetic nerves of anaesthetised SHR. In addition, vascular α_2-adrenoceptors appear to be involved in maintenance of blood pressure in intact anaesthetised SHR.

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REPERFUSION-INDUCED ^{86}RUBIDIUM EFFLUX IN THE ISOLATED RAT HEART. THE ROLE OF FREE RADICALS.

C.P. Blackwell, B. Woodward and M.N.M. Zakaria, Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY

The effects of the free radical scavengers glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and mannitol (MAN) on reperfusion-induced rubidium ($^{86}Rb^+$) efflux were studied to trace the movement of potassium (K^+) ions in isolated perfused rat hearts subjected to left anterior descending coronary artery ligation (CAL). $^{86}Rb^+$ efflux in the presence of the free radical generating system xanthine/xanthine oxidase was studied in non-ligated hearts. Hearts were loaded with $^{86}Rb^+$ (20 μCi) over 10 min. After a 35 min washout period, perfusate samples were collected for scintillation counting every 2 min. Drug perfusion commenced 5 min prior to CAL and continued throughout the experiments. CAL was maintained for 10 min. During the first 3 min of reperfusion (REP) perfusate samples were collected every 30 s then every 2 min for the remainder of the experiment. In experiments using the free radical generating system hearts were perfused with xanthine (0.1mM) after the initial washout period. 5 min after xanthine perfusion commenced a bolus injection of xanthine oxidase (0.65 iu) was administered into the perfusion system. Perfusate samples were collected each min after xanthine perfusion.

Table 1 **Effects of GSH and SOD + CAT + MAN on $^{86}Rb^+$ efflux rate constant (erc) during CAL and REP of isolated rat hearts**

DRUG	n	MEAN PEAK $^{86}Rb^+$ erc (min^{-1})±s.e.mean		
		CAL	REP	
Control	20	0.032±0.001	0.078±0.010	t
GSH (0.01mM)	3	0.031±0.0003	0.063±0.002	t
GSH (0.1mM)	3	0.031±0.001	0.046±0.006	
GSH (1.0mM)	8	0.033±0.002	0.043±0.003	m
SOD(10iu/ml)+CAT(100iu/ml)+MAN(20mM)	3	0.032±0.002	0.074±0.011	

t P<0.05:paired t-test (CAL v REP). m P<0.05:Mann Whitney U-test (vControl REP).

Table 1 shows the concentration dependant attenuation by GSH of the reperfusion-induced rise in peak erc which reaches statistical significance at 1mM. Although peak erc was not reduced on REP by the mixture SOD+CAT+MAN total $^{86}Rb^+$ efflux was reduced over the period of REP studied (data not shown). In the study using xanthine/xanthine oxidase, peak $^{86}Rb^+$ erc rose on addition of xanthine oxidase to a value of 0.086±0.017 min^{-1} from a control value measured during xanthine perfusion of 0.045±0.003 min^{-1} (P<0.05; n=5). Elevation of the erc was completely prevented by deactivation of xanthine oxidase by boiling and was greatly reduced (0.050±0.001min^{-1}) by pretreatment with the SOD+CAT mixture.

These results indicate that superoxide radical generation by xanthine/xanthine oxidase increases K^+ efflux and this provides evidence of a free radical-induced perturbation of cardiac cell membrane structure and/or function as suggested by Meerson et al (1982). We conclude that the depressant effect of free radical scavengers on reperfusion-induced K^+($^{86}Rb^+$) efflux could contribute to their antiarrhythmic action which has previously been reported (Woodward and Zakaria, 1985).

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REAL TIME ANAYLSIS OF INTRACELLULAR CARDIAC ACTION POTENTIALS USING A MOTOROLA 68000

Catlow, C.J., Gwilt, M., Higgins, A.J. and King, R.C. Pfizer Central Research, Sandwich, Kent CT13 9NJ.

The analysis of cardiac action potentials (APs) by hand from photographs is tedious, time consuming and inaccurate. Virtually all existing computer methods rely on analogue differentiation (Fusi et al., 1984) or on replaying previously recorded data from magnetic tape (Millar and Vaughan Williams, 1982). A system using on-line digital differentiation has been described previously (Elharrar and Lovelace, 1979) using an 8-bit machine. We have developed a system for real time on-line analysis of APs using a Motorola 68000 microcomputer with 12 bit resolution.

The program is written in 68000 assembler for speed of execution. In our system it runs on a satellite computer (128K memory) linked to a mainframe Motorola 68000 (875K memory). Thirty two on-line experiments can be run simultaneously in this way. For running only one experiment, only the computer with the smaller memory is necessary.

After loading the program, the operator calibrates the system. This is done by using keyboard commands to input OmV and a 60mV pulse (superimposed on the recording trace by a separate millivolt source). After this the program is ready to accept data.

Following a keyboard command, 500 ms of data containing an AP is digitised (20KHz) and digitally filtered. Resting potential, AP amplitude, maximum rate of depolarisation (Vmax) and action potential durations at 20%, 50% and 90% repolarisation (APD20, APD50 and APD90) are calculated and displayed instantaneously on a VDU. The 20KHz 12-bit A/D converter presently in use allows accurate differentiation of upstrokes as fast as 500 Vs^{-1}. A 50KHz converter is about to be installed, which will allow differentiation of faster upstrokes, i.e. those of Purkinje fibres. To check for artefacts, the AP itself and an extended sweep of its upstroke are also displayed. A second keyboard command stores accepted data in computer memory for subsequent printing. Headings can be inserted at any time. Hard copy plots of selected APs are also available. At the end of an experiment the results are printed out and hard copies are plotted. Results are stored on a Winchester hard disk (64M capacity). Each set of results is identified by a file number so that experiments can be subsequently pooled for statistical analysis, which is done using separate programs run on the same computer.

This system has several advantages:
1. Instantaneous on-line analysis.
2. Flexibility - programs can be written for a variety of needs.
3. Cheap - microcomputer based system.
4. Graphical and tabular hard copy are available.
5. Statistical analyses can be performed from results stored on disk.

The on-line analysis of action potential data saves considerable time. The very low cost of this machine makes it available to most institutions.

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COMPARATIVE METABOLISM OF CINNAMIC ACID IN RATS AND MICE AND ITS VARIATION WITH DOSE

J. Caldwell & B.P. Nutley, Department of Pharmacology, St. Mary's Hospital Medical School, London, W2 1PG.

The cinnamyl ($C_6H_5CH=CH-$) moiety occurs in the structures of many flavour and fragrance materials, both natural and synthetic. These include cinnamyl alcohol, cinnamaldehyde, cinnamic acid and numerous esters. Cinnamic acid is important both in its own right and as a common metabolite of the other substances. Since little metabolic and pharmacokinetic information is available to aid the safety evaluation of this group of compounds, we have studied the fate of cinnamic acid in rats and mice with both radio- and stable-isotope techniques.

Male Fischer 344 rats and CD-1 mice received [3-^{14}C,ring-d_5]-cinnamic acid (p.o. and i.p. respectively, at doses of 0.5μmol-2.5mmol/kg) and their urine and faeces were collected daily for 3 days. Excretion of ^{14}C was determined by liquid scintillation counting and urinary metabolites separated and characterized by TLC, HPLC and GC-MS.

Excretion of ^{14}C was essentially the same in both species, and was not influenced by dose size. A total of >90% was recovered in 3 days, predominantly in the urine (>85%) with 5% in the faeces: <1% of administered ^{14}C was found in the carcasses, indicating the completeness of excretion. Cinnamic acid underwent extensive side-chain cleavage, to yield benzoic acid. This was excreted mainly as its glycine conjugate, hippuric acid (>50%) together with much smaller amounts of benzoyl glucuronide (ca.5%) and free benzoic acid (ca.2%). This cleavage occurred by beta-oxidation, since the obligatory intermediates 3-hydroxy- and 3-keto-3-phenylpropionic acids (2% of each; the latter detected as its decarboxylation product, acetophenone, in the mouse only) were found. Mice excreted far greater quantities of cinnamoyl glycine (20% cf.2% in rat), with correspondingly less hippuric acid present, but the excretion of cinnamoyl glycine fell with increasing dose. The hydroxylation product, p-coumaric acid (rat 0.2%, mouse 1%) and unchanged cinnamic acid (0.5%) were also excreted.

Although the metabolic pathways of cinnamic acid were essentially the same in both species, the mouse excreted relatively more metabolites with the intact 3-carbon side chain than did the rat. The dose size had relatively little effect over the range studied on the fate of cinnamic acid in the rat, but in the mouse the ratio of cinnamoyl glycine to hippuric acid increased with increasing dose. These data show the rapid and virtually quantitative metabolism and excretion of cinnamic acid, by rodent species widely used in toxicity testing, to metabolites which would not be expected to exert toxic effects, and also provide a reference point for studies on the metabolic precursors of cinnamic acid i.e. the aldehyde, alcohol and esters.

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DISPOSITION AND IMMUNOGENICITY OF BENZYLPENICILLIN

G. Christie, J.W. Coleman, N.R. Kitteringham, B.K. Park and J.H.K. Yeung, Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, L69 3BX.

The high incidence of allergic reactions to penicillin has been attributed to its chemical reactivity with proteins leading to formation of a hapten recognisable by the immune system. The nature (endogenous or foreign) of the protein carrier has not been defined and is still a matter of debate. The purpose of this study was twofold, 1) to quantify drug-protein conjugation of penicillin, 2) to examine the humoral immune response to penicillin and to penicillin conjugated to a foreign protein.

^{3}H-Benzylpenicillin (BP) (9.3 or 930 mg kg^{-1}; 20 μCi) was administered i.v. to urethane anaesthetized male Wistar rats and blood samples were taken. Plasma, separated immediately, was diluted (0.3 ml to 1 ml) with phosphate buffer (pH 7.4; 0.05M) and proteins precipitated with acetone (3 ml). BP, covalently bound to plasma proteins, was measured by equilibrium dialysis (Park et al., 1982). BP, in the acetone extraction, was measured by liquid scintillation counting after separation by h.p.l.c. (C_{18}, 10μ column: eluent 50% methanol/50% ammonium phosphate buffer; pH 2.3, 0.05M). The reaction between BP (15 μg, 0.35 μCi) and rat serum proteins (0.25 ml) in vitro was investigated using the same analytical methods. The immunogenicity of BP was assessed after chronic administration (i.v. and i.m.) of BP (9.3 and 93 mg kg^{-1}; 4 days), at monthly intervals to rats (n = 7). Serum obtained 7 and 14 days after each series of injections, was tested for IgG anti-benzylpenicilloyl (BPO) activity by enzyme-linked immunosorbent assay (Coleman et al., 1986) (incorporated BPO-ovalbumin antigen coated on to the solid phase).

The clearance of BP from plasma was dose-dependent, as indicated by the AUC_0^{3h} for free BP and for total plasma radioactivity (table 1). However, after the higher dose, there was no significant increase in covalent binding at 3h, which represented < 6 X 10^{-3}%/ml of the dose administered. Incubation of BP with rat serum proteins in vitro for 3 hours resulted in 2.2 ± 0.6% covalently bound.

Table 1 Total radioactivity, free penicillin and covalent binding 3h after administration of [^{3}H]-penicillin to rats (mean ± SD)

Dose	n	Total Radioactivity AUC (μg/ml.h)	Free Penicillin AUC (μg/ml.h)	Covalent binding (ng/mg protein)
9.3 mg/kg	(4)	20.1 ± 9.8	3.0 ± 1.2	1.64 ± 0.95
930 mg/kg	(7)	2795 ± 1032	1580 ± 625	260 ± 131

Serum IgG antibodies directed against BPO were not detected in all blood samples after administration of free BP. In contrast, administration of a single dose (500μg/kg) of BP-Keyhole limpet haemocyanin conjugate at monthly intervals produced a measurable antibody response (titres > 1000).

These studies show that penicillin is cleared rapidly after i.v. administration and that covalent binding to plasma proteins leads to small amounts of circulating autologous protein-hapten conjugates. However, conjugation in vivo is insufficient to generate a functional immunogen in the rat.

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DISPOSITION AND IMMUNOGENICITY OF DINITROPHENYLATED AUTOLOGOUS PROTEINS IN THE RABBIT

J.W. Coleman, P.S. Grabowski, N.R. Kitteringham, B.K. Park* and M.D. Tingle, Department of Pharmacology and Therapeutics, Liverpool, L69 3BX, U.K.

The formation of immunogenic hapten-protein conjugates is thought to be a key step in drug hypersensitivity (Amos & Park, 1985). We have therefore investigated the relationship between the immunogenicity and disposition of the mild-arylating agent dinitrofluorobenzene (DNP-F). In this respect DNP-F may be considered a model drug hapten, since it forms conjugates, via lysine groups, with proteins in vivo and is detoxified by conjugation with glutathione (Maggs et al., 1986). Thus, chronic i.v. administration of DNP-F (0.5 mg kg^{-1}; 1.5 µCi kg^{-1}; in polyethylene glycol 200, 0.1 ml kg^{-1}; daily for 9 days) to male rabbits resulted in a specific IgG anti-DNP antibody response from day 7 onwards.

The range of antibody titres (serum dilution required for 50% maximum response), determined by enzyme linked immunosorbent assay (ELISA; Coleman et al., 1986) was 2160 - 11400 on day 15. Analysis of plasma samples (Maggs et al., 1986) showed an accumulation of circulating DNP-plasma protein conjugates. Concentrations of conjugated DNP reached a maximum (0.17 ± 0.03% dose ml^{-1}; epitope density 0.014 ± 0.002) on day 6 and declined, after day 9, with an elimination half-life of 5.3 ± 2.0 days.

To assess the immunogenicity of particular haptenated proteins, DNP-conjugates were prepared from autologous serum proteins (RSP) and also from isolated (Travis et al., 1976) autologous albumin (RSA), essentially as described (Kitteringham et al., 1985). The epitope density (degree of conjugation in terms of albumin equivalents) of the various synthetic conjugates was determined by radiometric analysis. The absence of low molecular weight impurities was established by h.p.l.c. and t.l.c.

Administration (i.v.) of a single dose (50 mg kg^{-1}, 20-30 µCi; in saline, 1 ml kg^{-1}) of autologous RSP-DNP conjugates (epitope densities 0.5, 2, 15, 30) resulted in a specific IgG anti-DNP response in all rabbits in each group (n = 4). The range of RSP antibody titres (day 14) observed in each group were; $DNP_{0.5}$, 31-199; DNP_{2}, 28-135; DNP_{15}, 46-581; DNP_{30}, 42-104. The specificity of the response was confirmed by hapten inhibition (> 50%) with N-acetyl-lysine-DNP (10µg ml^{-1}). DNP conjugates derived from autologous albumin were less immunogenic. There was no detectable anti-DNP response after i.v. administration of a single dose (50 mg kg^{-1}; 20 µCi in saline 1 ml kg^{-1}) of either $RSA\text{-}DNP_{0.5}$ or $RSA\text{-}DNP_{5}$ conjugates but there was a detectable response (titre range 43-338) to $RSA\text{-}DNP_{15}$. Furthermore, analysis of plasma indicated rapid, immune clearance of the $RSA\text{-}DNP_{15}$ conjugate between days 7 and 11.

These studies indicate that, while the presence of a chemically reactive agent in blood may lead to a specific IgG anti-hapten response, conjugates derived from albumin, are unlikely to be potent immunogens.

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THE METABOLISM OF RANITIDINE BY GUINEA PIG LIVER MICROSOMES

G.D. Ogg, J.R. Paterson, I.H. Stevenson and J. Feely[1], Department of Pharmacology and Clinical Pharmacology, University of Dundee, Dundee, DD1 9SY and [1]Department of Pharmacology and Therapeutics, Trinity College, Dublin 2, Ireland.

The in vitro metabolism of ranitidine and other H_2-antagonists has been reported previously (Oldham and Chenery, 1985; Paterson et al, 1985). Since in these studies, substrate-dependent oxygen consumption was measured polarographically, no data were obtained on either the nature of the metabolites formed or their relative rates of formation. Using a HPLC method we have now investigated the metabolism of ranitidine by guinea-pig liver microsomes with a view to identifying the metabolites formed and the extent of oxidation by cytochrome P_{450} and non-P_{450} dependent systems.

Ranitidine (5 mM) was incubated in the presence of guinea-pig liver microsomal protein (1 mg/ml), NADPH (0.5 mM), n-octylamine (1 mM, an inhibitor of cytochrome P_{450} activity) and phosphate buffer (0.1 M) pH 7.4 or 8.0. In a further series of incubations n-octylamine was omitted. Following incubation the mixtures were ultra-filtered through an Amicon YMT membrane filter and the filtrate assayed using essentially the method of Carey and Martin (1979). Only one major metabolite was formed corresponding in retention time to ranitidine N-oxide, the major metabolite of ranitidine in most species (Martin et al, 1981). Although other minor peaks were obtained, they did not correspond to the known S-oxide N-desmethyl or furoic acid metabolites. Rates of formation were calculated assuming the major metabolite peak to be ranitidine N-oxide. These are presented in Table 1. The rates of formation were found to be linear up to an incubation time of 20 min. No significant difference in reaction velocity was recorded in the absence or presence of n-octylamine. However, the rate of formation of the tentatively identified N-oxide was significantly greater at pH 8.0 than pH 7.4. This could indicate the involvement of flavin-containing monooxygenase, an enzyme which has a pH optimum of 8.4. Further studies are underway to fully characterise this metabolite of ranitidine.

Table 1 - Rate of Formation of Ranitidine N-Oxide

(n moles/min/mg microsomal protein)

pH 8.0		pH 7.4	
+n-octylamine	-n-octylamine	+n-octylamaine	-n-octylamine
2.55	2.726	1.16	1.33
1.433	1.404	0.749	0.9013
1.041	0.6775	0.545	0.545
1.68 ± 0.45	1.60 ± 0.6	0.818 ± 0.18	0.925 ± 0.23

Mean ± S.E.M.

We gratefully acknowledge the gift of ranitidine and metabolites from Glaxo Group Research plc.

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STUDIES ON THE EFFECTS OF GLIPIZIDE ON MICROSOMAL DRUG METABOLISM

A.H. El Debani & I.H. Stevenson, Department of Pharmacology and Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee, DD1 9SY

Although the second generation oral sulphonylurea drug glipizide (GPZ) is well established in the treatment of non insulin-dependent diabetes and has been shown to be extensively eliminated by hepatic metabolism both in man and experimental animals (Tommasini, 1975) there is little or no information regarding the effects of GPZ on drugs metabolised by the hepatic mixed function oxidase system. The objective of this study was to characterize the binding spectrum of GPZ to hepatic microsomes and to investigate the effects of GPZ on drug metabolism in male rats, using particularly pentobarbitone and aminopyrine as substrates.

The addition of GPZ (5 mM) to microsomal suspensions produced a binding spectrum characteristic of modified type II compounds with a trough of maximum at 386 nm and a peak of maximum at 416 nm. The in vitro metabolism of pentobarbitone measured as the rate of appearance of metabolites (mg^{-1} g^{-1} liver, per 45 min incubation time, Kuntzman et al, 1967) was significantly ($P < 0.001$) reduced (from 1.94 ± 0.3 to 1.74 ± 0.01) following sub-chronic administration of GPZ (10 mg kg^{-1} i.p.) twice daily for 4 days. Similarly acute administration of GPZ (10 mg kg^{-1} i.p.) 2h prior to assay also resulted in a significant decrease ($P < 0.05$) in the in vitro metabolism of pentobarbitone (from 1.94 ± 0.03 to 1.59 ± 0.09). The duration of pentobarbitone sleeping time following a single dose (40 mg kg^{-1} i.p.) was significantly ($P < 0.05$) increased (from 46.0 ± 2.2 to 53.8 ± 2.4 min) by sub-chronic pretreatment with GPZ However, the effects of GPZ on aminopyrine demethylase activity determined by the Nash reaction for formaldehyde were complex with, surprisingly, a significant increase ($P < 0.001$) occurring 2h after a single dose of GPZ (20 mg kg^{-1} i.p.).

The results suggest that GPZ appears to inhibit pentobarbitone metabolism by a mechanism possibly involving the competition of the two drugs for the same cytochrome P-450 system which mediate their metabolism. Results with aminopyrine demethylase were difficult to interpret.

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INFUSION OF GENTAMICIN INCREASES RENAL CLEARANCE OF CALCIUM AND MAGNESIUM IN FISCHER 344 RATS.

J.E. Foster and E.S. Harpur, MRC Mechanisms of Drug Toxicity Research Group, Pharmaceutical Sciences Institute, Aston University, Birmingham B4 7ET

Disturbance of electrolyte homeostasis may be a frequent occurrence in patients treated with gentamicin (Zaloga et al., 1984). Hypercalciuria and, to a lesser extent, hypermagnesiuria, occur in response to administration of gentamicin to the rat (Chahwala and Harpur, 1983; Harpur et al., 1985). The present study was designed to determine whether or not such increases in electrolyte excretion are a consequence of altered handling of cations by the kidney.

Male Fischer 344 rats (190-310 g) were anaesthetised with Inactin (120 mg/kg, i.p.). Saline, 0.9% (100 µl/min), was infused via a cannula in the left jugular vein; blood pressure was recorded via a cannula in the right carotid artery which was also used to sample arterial blood. Urine was collected continuously through a catheter inserted in the bladder. After a 3 h equilibration period ten 30 min urine collections were made with mid-point collections of 150 µl arterial blood. Saline alone was infused during the first four collection periods and a total of 50 or 100 mg/kg gentamicin (Cidomycin, Roussel Laboratories Ltd.) in saline was infused during the final six collection periods. Urine volumes were estimated by weight and calcium (Ca) and magnesium (Mg) concentrations in plasma and urine were determined by atomic absorption spectrophotometry. From these values the renal clearances (CL) of Ca and Mg were determined (Table 1).

Table 1. Changes in renal clearance of Ca and Mg induced by gentamicin (GEN).

	CL_{Ca} (ml/min)		CL_{Mg} (ml/min)	
Dose of GEN (mg/kg)	50 (n = 6)	100 (n = 7)	50 (n = 6)	100 (n = 8)
Control period	0.083±0.005	0.065±0.008	0.474±0.042	0.429±0.013
Drug period	*0.150±0.009	*0.190±0.008	0.517±0.033	*0.511±0.018

Values for the four 30 min clearances during the initial saline infusion (Control period) and for the last four 30 min clearances during the gentamicin infusion (Drug period) were averaged to give one experimental result for each period in each animal. Results are expressed as mean ± s.e.m. of n animals. * significantly different from control, $p < 0.01$.

CL_{Ca} was increased by infusion of gentamicin in a dose-related manner. CL_{Mg} was also increased but only at the higher dose of gentamicin. The increases in CL occurred during the first 30 min of the gentamicin infusion, i.e. in the case of Ca after a dose as small as 8.3 mg/kg. The increases in CL remained stable for the remainder of the gentamicin infusion.

In separate experiments it was established that the higher dose of gentamicin caused no change in GFR ($[^3H]$-inulin clearance). Furthermore, the drug had no effect on the proportion of ultrafilterable Ca in plasma. Thus there was no increase in the filtered load of electrolytes and the increases in CL_{Ca} and CL_{Mg} can be interpreted as increases in fractional excretion of these cations.

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IN VITRO ELECTROPHYSIOLOGICAL STUDIES WITH PARAQUAT DICHLORIDE

A.A. Round*, D.I. Wallis*, and D.B. Webb, Department of Renal Medicine, Cardiff Royal Infirmary, Newport Road, Cardiff, *Department of Physiology, University College, Cardiff.

Paraquat has chemical similarities with the bisquaternary amine ganglion blockers, a common feature being two quaternary amine groups separated by similar intramolecular distances. Gastric ileus, urinary retention, hypotension and vasodilation may all be seen in both paraquat poisoning and ganglion blockade. Pulmonary fibrosis which is well recognised in paraquat poisoning is also seen after therapy with ganglion blockers. Because of these similarities between the compounds and the clinical implications of any ganglion blocking activity of paraquat, the ganglion-blocking potential of paraquat was examined in vitro.

Superior cervical ganglia were taken from adult New Zealand White rabbits of either sex killed by air embolus. The ganglia were desheathed in ice-cold Krebs solution with the aid of a dissecting microscope. Ganglia were superfused at 37° C with paraquat dichloride in Krebs solution at concentrations ranging from 10 to 50 mg/L ($4x10^{-5}$-$2x10^{-4}$ M) for up to 2.5 hours. The superfusate was then changed to one containing hexamethonium bromide at a concentration of 36.2 mg/L (10^{-4} M). The pre- and postganglionic trunks of the ganglion were laid over sets of platinum electrodes in a double mineral oil bath (Brimble & Wallis, 1974). Electrical stimuli (0.1 to 0.5 msec pulses, 10 - 100 V) were applied to the preganglionic nerve trunk to produce an orthodromic response. Single stimuli were used or, in a few experiments, trains of stimuli at a frequency of 10 or 25 Hz for 1 sec. Either the postganglionic or ganglionic compound action potential were monitored on a digital storage oscilloscope. The output from the digital store was averaged using a Digitimer Averager (Neurologue NL 750). A permanent record of the average of four responses was made on a Gould 3054 chart recorder. A series of control responses was recorded before superfusing with paraquat.

In 8 ganglia no diminution in the compound action potential in response to a single stimulus was detected, nor were the action potentials altered in latency or shape. All ganglia subsequently showed very substantial blockade when superfused with hexamethonium for 30 minutes. Trains of stimuli were employed because the degree of response decrement during repetitive orthodromic stimulation is readily affected by agents having a presynaptic action on ganglionic transmission. During trains of stimuli at 10 and 25 Hz there was a substantial decrement in response amplitude during the course of the train, especially at the higher frequency. However, paraquat at these concentrations had no apparent effects on responses to repetitive stimulation.

It is concluded that paraquat does not have ganglion blocking properties at concentrations which correspond to the plasma levels observed in cases of severe poisoning. The sequelae of paraquat intoxication which mimic ganglion blockade would seem to be due to non-specific metabolic effects.

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INDUCTION OF MIXED FUNCTION OXIDASE ACTIVITY OF RAT AND MOUSE LIVER BY AMINOGLUTETHIMIDE.

Z. Damanhouri and P.J. Nicholls, Welsh School of Pharmacy, UWIST, PO Box 13, Cardiff, CF1 3XF.

Aminoglutethimide (AG) is an established drug for the treatment of advanced oestrogen dependent breast cancer in postmenopausal women (Santen et al. 1974). It is capable of inhibiting several cytochrome P-450-mediated steroid hydroxylation steps and it is effective in patients by inhibiting peripheral aromatase, so reducing oestrogen synthesis. There is evidence in man that AG stimulates its own metabolism (Murray et al. 1979) and increases the clearance of coumarin anticoagulants (Lonning et al. 1984). The present work studies the ability of AG to induce hepatic mixed function oxidases in the female rat and male mouse after an oral dosing schedule of AG 60mg/kg daily for 3 days.

In rats (n=19), AG significantly lowered ($P<0.05$) plasma levels of dicoumarol at 4 and 6h after oral dosing (8mg/kg) from 25.9±1.3 and 21.1±1.3µg/ml (control) to 19.4±1.0 and 17.2±1.4µg/ml (AG) respectively. Sleeping time was significantly ($P<0.05$) reduced by AG pre-treatment in rats (n=10) receiving hexobarbitone (135mg/kg i.p.) from 205±16 min to 102±16 min and in mice (n=10) receiving pentobarbitone (45mg/kg i.p.) from 44.8±5.3 min to 11.2±2.8 min. In this latter species (n=6), the zoxazolamine (120mg/kg)-induced paralysis time was significantly ($P<0.001$) reduced from 30.1±3.6 min (control) to 8.8±1.6 min (AG). Also in mice (n=8) the rate of $^{14}CO_2$ exhalation following i.p. administration of (N-dimethyl-^{14}C) antipyrine (Houston et al. 1981) was significantly ($P<0.001$) altered by AG pretreatment, the exhalation $t_{\frac{1}{2}}$ decreasing from 22.8±1.0 min to 14.7±0.6 min.

Electronmicroscopy of the liver from AG-pretreated rats revealed considerable proliferation of the endoplasmic reticulum. The metabolism of 4-nitroanisole and of dicoumarol by the 10,000 x g supernatant of liver homogenates from AG-pretreated rats was significantly increased over control values. AG-pretreatment of rats also resulted in significant elevation of the levels of microsomal protein, cytochrome P-450 and NADPH-cytochrome c reductase in the liver.

The data demonstrate that in rat and mouse AG is a potent inducer of hepatic mixed function oxidation. This supports suggestions that in man AG exhibits inducing activity and focuses attention of the phenomenon as a potential site of drug interaction with this agent.

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EFFECT OF CHRONIC ETHINYLESTRADIOL PRETREATMENT ON PARACETAMOL IN THE RAT.

P.N. Bennett, Karen Fordyce and Lidia J Notarianni
School of Pharmacy and Pharmacology, University of Bath, BA2 7AY

Chronic use of oral contraceptive steroids (OCS) has been shown to increase paracetamol clearance due to induction of the glucuronidation and oxidation pathways although sulphation is not affected (Miners et al, 1983). The component of the OCS that is responsible for this induction is not known. We report on the effect of the estrogenic element, ethinylestradiol (EE_2) on paracetamol (P) metabolism in the rat.

Female Wistar albino rats (Bath University Strain) weighing between 200-220 g were treated orally for 40 days with either 1 ml of 5% ethanol (controls, n=12) or EE_2 (11 µg in 5% ethanol, n=12). On day 29 of the treatment each animal received a single oral dose of paracetamol (50 mg/kg) and urine collected from 0-8 and 8-24 h. On day 40, a second oral dose of paracetamol was administered (50 mg/kg) and the animals bled by cardiac puncture at intervals over eight hours. Each animal was bled up to 4 times. Paracetamol was analysed in blood and urine by HPLC. Paracetamol glucuronide (PG) was assayed as paracetamol following enzyme hydrolysis and PS assayed per se.

The EE_2-treated rats showed a significant increase in the amount of PG excreted in the 0-8 h urine when compared to the control rats suggesting induction of glucuronidation. However, PS and total P excreted was not significantly altered by EE_2. In the 8-24 h urine, PS was found to be significantly lower in EE_2-treated rats and consequently, since PS is the major metabolite, total P excreted was also lower (Table 1). Plasma paracetamol concentrations were lower in the EE_2-treated rats, the difference becoming greater after 3 h. The area under the time-concentration curve (AUC) was lower in the treated animals. Paracetamol half-life was similar in two groups.

Table 1 Paracetamol Kinetics in Control and EE_2-Treated Rats

	Controls	EE_2-Treated Rats
P excreted in 0-8 h urine	1.8±0.1	2.0±0.2
P excreted in 8-24 h urine	1.3±0.3	1.1±0.3
PS excreted in 0-8 h urine	40.9±3.5	45.1±3.5
PS excreted in 8-24 h urine	19.1±2.2	12.3±2.2*
PG excreted in 0-8 h urine	11.1±1.1	16.0±1.7*
PG excreted in 8-24 h urine	2.9±0.5	2.8±0.6
AUC (0-8 h) mg min/L	1412.8	1092.8

Figures represent % mean ± sem as P equivalents; *$p<0.05$

Chronic EE_2 treatment induced paracetamol glucuronidation in the rat. Although PG is not the major metabolite in the rat, this effect was sufficient to result in a lower AUC in the treated animals. PS excretion was also decreased after 8 h. This may be due to depletion of available sulphate or possible by competitive inhibition of sulphotransferase.

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Miners et al (1983) Br. J. Clin. Pharmacol. 16; 503-509

CHRONIC GUANETHIDINE DENERVATION REVEALS DOPAMINE HAS MIXED ACTIONS ON THE RAT VAS DEFERENS

M.A.K. Lafi & L.D. Leake, Department of Biological Sciences, Portsmouth Polytechnic, King Henry I Street, Portsmouth, PO1 2DY, Hants. U.K.

Dopamine (DA) is a normal constituent of most adrenergically innervated tissues (Bell, et al., 1984). Although noradrenaline (NA) is considered to be the primary transmitter, recent reports suggest the existence of peripheral dopaminergic nerves (for review see Bell, 1982). Both NA and DA are discharged by transmural stimulation from the rat vas deferens (Bell, et al., 1984). Bell et al., (1984) conclude that DA co-exists and is co-released with NA in the rat vas. By the use of specific antagonists, postsynaptic DA receptors have also been demonstrated in the rat vas (Simon and Van Maanen, 1976; Tayo, 1979; Badia, et al., 1982), but the role of DA in this tissue is not clear. In this study, using chronically denervated vasa, we show that DA has a mixed action: presynaptic, causing the release of endogenous NA, and a direct postsynaptic action.

Vasa deferentia from Sprague-Dawley rats (200-300g) were set up in Mg^{2+}-free Tyrodes bubbled with 95% O_2: 5% CO_2. Some rats received chronic treatment with guanethidine (injected i.p. 25mg/kg per day 5 days a week for 6 weeks) in order to destroy adrenergic nerves (Burnstock, et al., 1971). Controls received 0.85% saline injections.

Exogenous NA and DA produced concentration-dependent contractions of the vas with a 50% maximal contraction being produced by 3 μM NA and 17.5 μM DA. The antagonists phentolamine (0.01 μM), metoclopramide (50 μM), sulpiride (10 μM) and deprenyl (5 μM), all antagonised responses to both amines although the inhibitory effects were significantly greater against DA than NA (in each case $p<0.01$). Chronic guanethidine pretreatment markedly potentiated responses to NA, but had little effect on responses to DA. Half-maximal contractions in the denervated vas were produced by 0.15 μM NA and by 15 μM DA. Both amines were inhibited to the same extent by phentolamine (0.01 μM), metoclopramide (50 μM), sulpiride (10 μM) and deprenyl (5 μM). Further, whereas chronic denervation did not affect the maximal height of NA-induced contractions but shifted the dose-response curve to the left by at least a factor of 10, the dose-response curve for DA was shifted to the right and the maximal height of contraction was reduced by approximately 30%.

All these results indicate that DA action is partly presynaptic, mediated by release of endogenous NA. If DA were only acting postsynaptically it would also be potentiated by denervation to the same extent as is NA, but this does not happen. These results also show that α-adrenoceptor blockers have neuronal uptake blocking (i.e. a presynaptic) action in addition to their postsynaptic action, thus resulting in reduced antagonism of responses to exogenous NA. However, as DA action is partly mediated by releasing endogenous NA, then both the post and presynaptic actions of α-adrenoceptor antagonists will block DA action. Thus an enhanced antagonism of responses to exogenous DA will result. Caution should, therefore, be taken when using α-adrenoceptor antagonists purely as postsynaptic blockers.

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EVIDENCE THAT LOWERED WHOLE BLOOD PLATELET-ACTIVATING FACTOR ACCOMPANIES GESTATION IN NORMOTENSIVE AND HYPERTENSIVE RATS

J.C.Neill & M.P.Seed. Pharmacology Group, University of Bath, Bath BA2 7AY. U.K.

Hypotension in pregnancy is known to be common in both normotensive and hypertensive rats (Terragno et.al.,1982). A plasma transferable hypotensive factor has been observed which is released from the kidney during pregnancy (Kubota & Yamada, 1981. Takeda 1964). Circulating platelet-activating factor (PAF) also originates from the kidney in the rat (Caramelo et.al.,1982). Since PAF is rapidly deacylated to lyso-PAF in serum (Pinckard et.al.,1979), we have assayed circulating lyso-PAF in male and virgin or pregnant female Wistar rats (WRs) and Okamoto hypertensive rats (SHRs).

Single samples of whole blood were taken by cardiac puncture under halothane anaesthesia from the males and virgin females. For repeated sampling over gestation two groups for each strain were mated and samples taken on different days in order to reduce the sample load for each animal. Blood (0.5ml) was immediately dropped into 2ml ice-cold acetone and the lyso-PAF extracted and assayed according to the method of Parente and Flower (1985). The precipitated proteins were removed by centrifugation and the lipids were extracted into 2ml chloroform. After centrifugation the chloroform phase was evaporated under nitrogen and the residue acetylated by overnight incubation at room temperature with 0.1ml acetic anhydride in 0.1ml dry pyridine. The acetylating agents were then evaporated under nitrogen and the samples were washed twice with 1ml chloroform, and again taken to dryness. The acetylated lipid extract (ALE) was reconstituted in 200ul Tris buffer (25 mM, pH 8.0) with 0.25% BSA. The ALE was bioassayed as ng PAF equivalents/ml blood by the induction of rabbit platelet aggregation. The bioactivity was destroyed on incubation with phospholipase A-2, co-chromatographed with PAF on TLC (chloroform: methanol: water, 65:35:6) and failed to aggregate platelets specifically desensitised to PAF. Significance was calculated using ANOVA and Mann Whitney U test.

Circulating lyso-PAF in male WRs (37.9 ± 7.8 ng/ml) was significantly higher ($p<0.05$, n=9) than virgin females (18.1 ± 2.9 ng/ml, n=10), however no differences were found between the male and female SHRs (30.9 ± 3.4, n=10 and 25.3 ± 4.7 ng/ml, n=11 respectively). Strain related differences were not seen in the non-pregnant state.

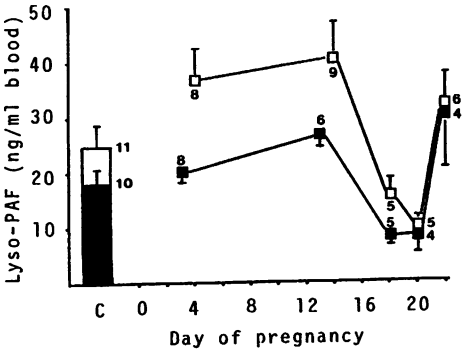

Figure 1. The effect of pregnancy on blood levels of lyso-PAF in WRs (closed symbols) and SHRs (open symbols) with the non-pregnant controls (Histogram C). Points show mean ± s.e.mean (N°. on the bars).

During pregnancy the amount of lyso-PAF increased from non-pregnant levels (see Figure 1) reaching significance in the case of SHRs at day 14 ($p<0.05$). Up to day 14 SHR blood lyso-PAF was consistently higher than WRs ($p< 0.05$). The profound fall at day 18 ($p<0.01$) seen with both strains was completely reversed on day 22 (post partum).

If lyso-PAF does reflect the release of PAF into the circulation, then this data shows that PAF does not play a role in the hypotensionseen at term. The reduction is suprising and requires further investigation, but could reflect a requirement for reduced levels of a potent smooth muscle relaxant at a time of heightened uterine contractility.

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ADRENAL BLOOD FLOW AND CORTICOSTERONE PRODUCTION FOLLOWING SPINAL (T7-T13) STIMULATION OF THE PITHED RAT

A. Gibson, S.L. Hart, & T. Wright[1], Department of Pharmacology, Kings College London, Chelsea Campus, London SW3 and [1]Department of Biological Sciences, Napier College, Collington Road, Edinburgh EH10.

Corticosterone secretion may vary independently of adrenocorticotrophin (ACTH), and it has been suggested that this may result from altered activity of autonomic nerves to the adrenal cortex (Doell et al., 1981 ; Wilkinson et al., 1980). Initial studies with the pithed rat showed that stimulation of spinal outflows T7 - T13 elevated plasma concentrations of corticosterone, but not of ACTH (Gibson et al., 1984). The present study examines the effect of spinal stimulation on adrenal blood flow, and on its content and secretion of corticosterone.

Male Wistar rats (300 - 400 g) were pretreated with betamethasone (2.5 mg/kg ; 24 h). 2 h prior to pithing under ether anaesthesia the rats were given slow-release ACTH (Synacthen 1 : 10 ; 1 ml/kg ; sc). This produced a steady, elevated plasma corticosterone concentration. Following pithing, the right adrenal was removed and the left adrenal vein was cannulated. Heparin (500 U ; iv) was administered and adrenal venous blood collected under free-flow. Fluid volume was replaced by heparinised saline. Corticosterone was measured fluorimetrically.

During spinal stimulation (T7 - T13 ; 10 Hz ; 10 min) adrenal blood flow was elevated (by 40% ; $P < 0.02$; n = 9). Overall secretion of corticosterone was unaltered, but adrenal corticosterone content was reduced (by 27% ; $P < 0.05$; n = 10). Immediately after stimulation both adrenal blood flow and corticosterone secretion were reduced (by 42% ; $P < 0.01$; n = 9, and by 43% ; $P < 0.02$; n = 7 respectively), and adrenal corticosterone content returned to pre-stimulation levels.

Some rats did not receive ACTH. In these, basal adrenal blood flow and corticosterone secretion were reduced (by 43% ; $P < 0.01$; n = 8, and by 96% ; $P < 0.001$; n = 8). Spinal (T7 - T13) stimulation produced no change in adrenal blood flow or corticosterone secretion, but reduced adrenal corticosterone content (by 60% ; $P < 0.02$; n = 10). Further, adrenal corticosterone content did not return to pre-stimulation levels at the end of the stimulation period.

The main findings are : 1) the presence of ACTH is necessary for the increase in adrenal blood flow associated with spinal stimulation ; 2) spinal stimulation reduces adrenal corticosterone content, and it is possible that hormone secretion during stimulation is maintained at the expense of content ; 3) spinal stimulation did not alter corticosterone secretion. It appears that the adrenal gland is not involved in the previously observed elevation of arterial plasma corticosterone concentration during spinal stimulation.

At the time of this work, TW was an MRC Scholar.

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EFFECTS OF MODIFICATION OF ADRENERGIC TRANSMISSION ON HYPOTHALAMO-PITUITARY-ADRENOCORTICAL (HPA) RESPONSES TO MORPHINE AND NALOXONE

Julia C. Buckingham and Teresa A. Cooper, Academic Department of Pharmacology, Royal Free Hospital School of Medicine, London NW3 2PF.

Opiate drugs stimulate the secretion of corticotrophin releasing factor (CRF) in the rat. It has been suggested that this action is effected by a reduction in the activity of the noradrenergic neurones which inhibit tonically the production of the releasing factor (Buckingham & Cooper, 1984). In order to investigate this possibility the concentrations of corticotrophin (ACTH, Alaghband-Zadeh et al, 1974) corticosterone (Al-Dujaili et al, 1981) in the plasma and CRF (Buckingham & Hodges, 1977a) in the hypothalamus were determined before and after stress (ether anaesthesia) at various times after a single injection of morphine or naloxone in rats (Sprague-Dawley) pre-treated with either α-methyl-para-tyrosine (α-MPT) or prazosin. The ability of hypothalami removed from these rats to secrete CRF in vitro was also assessed (Buckingham & Hodges, 1977b).

Both α-MPT (25-50mg/100g i.p.) and prazosin (10-100μg/100g i.p.) stimulated HPA activity. The concentrations of CRF in the hypothalamus and ACTH in the plasma were increased in a dose-dependent manner ($P<0.01$, Duncan's test, 5 animals/group) as also were the pituitary-adrenocortical responses to stress ($P<0.01$, Duncan's test, 5 animals/group). In addition there was a five-fold increase in the spontaneous secretion of CRF in vitro by hypothalami removed from animals treated with α-MPT (50mg/100g i.p.).

The rises in plasma ACTH and corticosterone concentration and the exaggeration in stress-induced pituitary-adrenocortical activity which morphine (1-2mg/100g, i.p.) normally causes were significantly ($P<0.01$, Duncan's test, 5 animals/group) less in α-MPT-treated (50mg/100g i.p.) rats. The morphine-induced (10^{-8}-10^{-6}M) secretion of CRF by hypothalami in vitro was also reduced significantly ($P<0.01$, Duncan's test, 5 animals/group) by the drug treatment.

Naloxone (25-100μg/100g, i.p.), which did not affect resting HPA activity, not only antagonized the stimulatory effects of morphine but also significantly ($P<0.01$, Duncan's test, 6 animals/group) depressed, in a dose-dependent manner, the stress-induced secretion of ACTH and corticosterone. The ability of naloxone to attenuate the stress response was reduced by prazosin (100μg/100g, i.p.) treatment. At a dose-level of 100μg naloxone/100g the stress-induced release of ACTH was attenuated by 60±6 and 34±1% (n=6) in control and prazosin-treated rats respectively.

The results support the suggestion that opioid substances stimulate ACTH production by depressing the activity of the noradrenergic neurones which, in the rat, inhibit tonically the secretion of CRF.

This work was supported by the Medical Research Council. We are also grateful to Pfizer Ltd. and Dupont (U.K.) Ltd. for prazosin and naloxone respectively.

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THE DIFFERENTIAL ACTIONS OF CALCITONIN GENE RELATED PEPTIDE ON THE VASCULAR AND CAPSULAR SMOOTH MUSCLE OF THE DOG SPLEEN

P.G. Withrington, Dept. of Pharmacology, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.

Calcitonin Gene Related Peptide (CGRP) is a potent vasodilator in many species including Man (Brain et al., 1985). In the present experiments the actions and potency of the peptide were assessed following intra-arterial injection into the isolated, blood perfused dog spleen. This organ contains vascular and extra-vascular (capsular) smooth muscle, both richly innervated by the sympathetic nervous system.

Experiments were performed on dogs anaesthetised with a mixture of chloralose (50 mg/kg) and urethane (500 mg/kg). The spleen was isolated, placed in a plethysmograph and perfused with arterial blood. Splenic arterial blood flow (SABF) and perfusion pressure (SAPP) were recorded continuously to indicate changes in vascular tone, whilst spleen volume was recorded to indicate changes in capsular activity.

Human CGRP (Bachem) was administered as bolus doses (1.0 fmol-50 pmol) into the splenic artery. The only splenic vascular response observed was an increase in SABF indicating a fall in splenic arterial vascular resistance and vasodilatation. The threshold dose was less than 10 fmol. The peptide had a vasodilator action on the splenic vasculature which was of longer duration (Fig. 1) than to either isoprenaline (ISO) or Vasoactive Intestinal Peptide (VIP). In individual experiments the dose-response curves to CGRP, VIP and ISO were parallel although the maximum response to CGRP (and VIP) were less than the maximum to ISO. The ED_{50} for CGRP on the splenic arterial vascular bed was less than 1.0 pmol in all experiments. Accompanying the marked splenic vasodilatation to CGRP was a small increase in spleen volume, probably passive in nature. The time-course and magnitude of the dilator response to CGRP was unaffected by the iv administration of the selective β_2 adrenoceptor antagonist, ICI 118,551 (0.5 mg/kg iv). CGRP is the most potent splenic arterial vasodilator yet examined but with only minimal actions on the smooth muscle of the spleen capsule.

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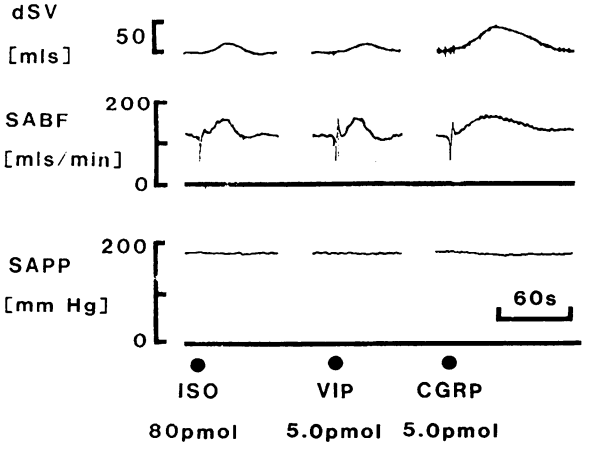

Fig. 1. Changes in spleen volume (dSV) and splenic arterial blood flow (SABF) in response to intra-arterial bolus injections of isoprenaline, VIP and CGRP.

Spleen = 152 g

VASOCONSTRICTION OF THE PERFUSED RAT CAUDAL ARTERY IS INDEPENDENT OF LONGITUDINAL TENSION AND FLOW RATE.

J.Atkinson, A.K. Fouda and M. Sonnay, Institut de pharmacologie de l'Université, rue de Bugnon 21, 1005 Lausanne, Switzerland.

The isolated caudal artery of the rat is being increasingly used as a model for the study of changes in the vasculature induced by hypertension (Hicks et al., 1984; Fouda et al., 1985), old age (Fouda et al., 1986), etc. As differences in longitudinal and cross-sectional tension can alter the sensitivity of isolated vascular tissue (Jones, 1981; Price et al., 1981; Moulds, 1983; Arjamaa, 1984) we studied the effects of changes in longitudinal tension and flow rate on electrically-induced vasoconstriction.

Proximal segments (2 cm) of the caudal artery were removed from 3 month old, male Wistar rats under pentobarbital anesthesia (50 mg/kg, I.P.). They were cannulated at both ends and perfused/superfused with Krebs bicarbonate solution at 1 ml/min. A weight of 0.5 g was attached to the free end of the artery. Flow rate was gradually increased to 4 ml/min over the next hour. Following this, in the first series of 6 experiments (n=2/experiment) weight was kept constant and arteries were perfused at 2, 4 or 6 ml/min and electrically stimulated (ES, 1-256 Hz for 10 sec, 0.3 msec pulse duration, supramaximal voltage). Twelve arteries were subjected to the six possible permutations. Vasoconstriction was estimated from the change in perfusion pressure. Flow rate was then increased or decreased and after 15 min stabilisation, ES repeated. There was then a third and final change in flow rate and repitition of ES. Basal perfusion pressure was taken after each stabilisation period before any stimulation. There was no cross-over effect so results at each flow rate (n=12/flow rate) were pooled. In a second series of experiments flow rate was maintained at 4 ml/min and weight was varied (0.2, 0.5, 1 g). ED_{50} and the slope of the dose-response curve were determined after "logit % vasoconstriction versus $\log_{10}$ frequency ES" transformation.

Table: Effect of changes in flow rate and longitudinal tension of the vasoconstrictor responses to electrical stimulation in the isolated perfused/superfused rat caudal artery.

Flow rate (ml/min)	Longitudinal tension (g)	Basal perfusion pressure (mmHg)	Maximal vaso-constrictor (mmHg)	ED_{50} (Hz)	Slope (logit/log)
2	0.5	36±3	156±17	22±4	5.6±0.4
4	0.5	75±4	200±12	16±1	5.2±0.4
6	0.5	125±7	176±13	15±2	5.3±0.4
4	0.2	66±4	222±13	11±3	4.7±0.2
4	0.5	67±4	223±14	15±3	5.1±0.1
4	1.0	66±5	230±17	11±2	4.7±0.2

As can be seen from the table changes in longitudinal tension from 0.2 to 1 g had no effect on vasoconstriction induced by ES. A change in flow rate from 2 to 6 ml/min produced a proportional change in basal perfusion pressure but no change in responsiveness to ES.

In conclusion the sensitivity of the isolated rat caudal artery preparation to ES appears to be relatively independent of flow rate or longitudinal tension.

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A METHOD FOR THE STUDY OF ENDOTHELIAL DERIVED RELAXING FACTOR (EDRF) IN THE ISOLATED PERFUSED RAT MESENTERY

R.A. Byfield, G.T.G. Swayne and T.J. Warner (introduced by D.A.A. Owen), Department of Pharmacology, Smith Kline and French Research Ltd., The Frythe, Welwyn, Hertfordshire, U.K.

Acetylcholine (Ach) induced vasodilatation of pre-contracted rabbit aorta strips and rings is dependent on the presence of endothelial cells (EC) (Furchgott et al, 1980; 1984). EC were removed in these preparations mechanically or with collagenase, for this reason work on EDRF has been limited to large blood vessels. However, in whole vascular beds, arterioles determine the effective perfusion resistance but have not been studied to date due to the absence of a suitable method for the removal of microvessel EC in situ.

The isolated perfused rat mesenteric bed was prepared by the method of McGregor (1965) using heparinised animals, (100 U. $100g^{-1}$ bw). The isolated bed was perfused at constant flow with an oxygenated physiological buffer which contained 5g l^{-1} of bovine serum albumin at 37°C. Vascular tone was increased by the addition of methoxamine (10^{-4}M) to the perfusion bath which caused the perfusion pressure to rise from approx. 20mmHg to between 50-60mmHg. Compounds were then administered as a bolus (0.01ml) via an injection port proximal to the mesenteric artery. Removal of the EC was achieved by perfusion with a 1 or 3 mg ml^{-1} solution of sodium deoxycholate, in saline, for 30 secs via a parallel perfusion circuit. Sodium deoxycholate (1.5%) has previously been used to remove epithelial cells from isolated renal tubules (Welling et al. 1972).

Ach, carbachol, histamine and ATP produced EC dependent, dose related, falls in perfusion pressure. The doses of these agents which produce a half max. (ED_{50}) fall in perfusion pressure before and after removal of EC are shown in Table 1.

Table 1 Effect of removal of EC on vasodilatation

	n	ED_{50} (g) Unstripped	Stripped	Ratio
Ach	6	3.2×10^{-9}	ND at 1×10^{-5}	>3000
Carbachol	3	1.1×10^{-8}	2% at 1×10^{-6}	>100
ATP	5	6.3×10^{-8}	Vasoconstr. at 1×10^{-5}	-
Histamine	4-6	1.4×10^{-6}	6.3×10^{-4}	450

Isoprenaline (n=5) at 3 doses (1-10µg) caused a mean fall in perfusion pressure before and after removal of EC of 23±2.2% and 21±2.2% respectively. In a separate series of expts. Papaverine (10µg, n=3), an endothelial independent vasodilator (Furchgott et al, 1984), produced a fall in perfusion pressure of 30±3.3% and 16±3.5% before and after stripping respectively, while a near maximal response of Ach (100ng), was converted to a vasoconstriction after deoxycholate treatment. Preliminary histological studies demonstrated that, amongst other vessels, arterioles of approx. 100µm in diameter were also devoid of intact EC. In this initial series of experiments the existence of EDRF in a whole vascular bed, containing microvascular resistance vessels, has been demonstrated.

We would like to acknowledge the histological investigation performed by Dr. G. Betton at Smith Kline and French.

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DIFFERENT POTENCIES OF CALCITONIN GENE-RELATED PEPTIDES (CGRP) ON MESENTERIC VASCULATURE CONSTRICTED BY NORADRENALINE OR POTASSIUM

S.J. Al-Kazwini, R.K. Craig, J.J. Holman & I. Marshall*, Department of Pharmacology, The Middlesex Hospital Medical School, London W1P 7PN.

Human and rat α-CGRP are vasodilators in the rat isolated perfused mesenteric vascular preparation (Al-Kazwini et al, 1985). The present experiments have examined the effect of human α- and β-CGRP and rat α-CGRP in the perfused mesenteric vasculature and in rings of mesenteric artery. The perfusion pressure or the tone was raised either by adding noradrenaline to the Krebs solution or by substituting a high concentration of potassium for sodium.

The rat mesenteric vasculature was perfused with Krebs solution containing noradrenaline, 10^{-5} M (pressure 101 ± 10 mm Hg, mean ± s.e. mean). The three peptides (3×10^{-11} - 3×10^{-9} moles; Cambridge Research Biochemicals) evoked dose-dependent falls in pressure. The geometric mean dose to evoke a fall of 35 mm Hg (ED_{35}) was 6.9×10^{-10}, 6.1×10^{-11} and 5.5×10^{-11} moles for human α-, β- and rat α-CGRP respectively. When the perfusion pressure was raised by potassium chloride, 6×10^{-2} M (with prazosin 300 nM present, pressure 108 ± 13 mm Hg) none of the three peptides evoked falls in perfusion pressure of 35 mm Hg, even at 10^{-8} moles. The vasodilator sodium nitroprusside had an ED_{35} of 8.5×10^{-11} moles in the presence of noradrenaline 10^{-5} M and 1.2×10^{-8} moles in the presence of potassium, 6×10^{-2} M.

In the rat mesenteric artery rings (in Krebs solution at 37°C, 0.5 g resting tension), noradrenaline (10^{-7} M) evoked a tension of 222 ± 67 mg. This tone was relaxed by acetylcholine and by sodium nitroprusside (both 10^{-9} - 3×10^{-8} M) with similar potencies. These two drugs were more potent and had steeper concentration-effect curves than the CGRPs (10^{-9} - 3×10^{-7} M). The noradrenaline tension (after 10^{-7} M) was relaxed by 44 ± 11%, 54 ± 7% and by 61 ± 8% for human α- and β-CGRP and rat α-CGRP at 3×10^{-7} M respectively. In similar experiments rings of rat mesenteric artery were contracted by potassium 3×10^{-2} M (with prazosin 300 nM present). Sodium nitroprusside (3×10^{-9} - 3×10^{-6} M), acetylcholine (3×10^{-9} - 10^{-6} M) and the three CGRPs (10^{-8} - 10^{-6} M) were less potent when the tone was raised with potassium as opposed to noradrenaline. For example, the three peptides, even at 10^{-6} M, relaxed the potassium evoked tension by an average of 20% or less.

In conclusion, compared with sodium nitroprusside, the CGRPs are more potent as vasodilators in the perfused mesenteric vasculature than as relaxants of rings of mesenteric artery. In these vascular preparations the peptides are more potent against noradrenaline than against potassium, which is the opposite of that found for the calcium antagonists (Cauvin et al, 1983).

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COMPARISON OF THE VASODILATOR ACTIVITIES OF HUMAN αCGRP, HUMAN βCGRP, RAT αCGRP AND SALMON CALCITONIN

S.D. Brain, I. MacIntyre[1] & T.J. Williams, Department of Vascular Biology, MRC Clinical Research Centre, Harrow, Middlesex HA1 3UJ and [1]Department of Clinical Pathology, Hammersmith Hospital, London, W.12 OHS.

Since the description of the predicted sequence of rat αCGRP (Amara et al, 1982), several similar sequences have been described. Both rat and human αCGRP (Morris et al, 1984) have been shown to have potent and persistent vasodilator activity (Brain et al, 1985). A second calcitonin/CGRP gene has now been proposed to give rise to a second form of CGRP, βCGRP, in both rat (Rosenfeld et al, 1984) and man (Steenbergh et al, 1985). We have investigated whether human βCGRP exhibits vasodilator activity. Human calcitonin is not structurally related to CGRP, however salmon calcitonin does exhibit some structural similarity to CGRP and was tested for activity.

To investigate the structure/activity relationships of the different forms of CGRP we have determined their vasodilator potencies using a ^{133}Xenon clearance method to measure local blood flow changes induced in rabbit skin following 0.1ml intradermal injections (Williams, 1979). Results were expressed as % increased blood flow at test sites compared to saline-injected sites. βCGRP (Peninsula) increased blood flow in a dose-related manner (CGRP 10^{-13} mol/site, 17.1 ± 3.4% increase mean ± s.e.m, n=5 rabbits; 10^{-12} mol/site, 43.9 ± 8.8% increase; 10^{-11} mol/site, 58.7 ± 8.9% increase. In 4 experiments where the vasodilator activity of αCGRP (10^{-13} - 10^{-11} mol/site, Bachem) was compared with that of βCGRP the two compounds exhibited identical activity. Previously we have shown that human and rat αCGRP exhibit comparable activity in rabbit skin (Brain et al, 1985). Salmon calcitonin (CT) was inactive when tested at doses up to 10^{-10} mol/site.

Table 1: The relative vasodilator potencies and structural similarities are shown below. Dashes (-) indicate residues identical to those in human αCGRP. The vasodilator potency is that compared to human αCGRP (100%).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
human αCGRP	NH_2-Ala	Cys	Asp	Thr	Ala	Thr	Cys	Val	Thr	His	Arg	Leu	Ala	Gly	Leu	Leu	Ser	Arg	Ser	Gly
human βCGRP	NH_2	-	-	Asn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
rat αCGRP	NH_2-Ser	-	Asn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
rat βCGRP	NH_2-Ser	-	Asn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
salmon CT	NH_2-Cys	Ser	Asn	Leu	Ser	-	-	-	Leu	Gly	Lys	-	Ser	Gln	Glu	-	His	Lys	Leu	Gln

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37		Potency
human αCGRP	Gly	Val	Val	Lys	Asn	Asn	Phe	Val	Pro	Thr	Asn	Val	Gly	Ser	Lys	Ala	Phe	-Amide	100%
human βCGRP	-	Met	-	-	Ser	-	-	-	-	-	-	-	-	-	-	-	-	Amide	100%
rat αCGRP	-	-	-	-	Asp	-	-	-	-	-	-	-	-	-	Glu	-	-	Amide	100%
rat βCGRP	-	-	-	-	Asp	-	-	-	-	-	-	-	-	-	-	-	-	Amide	not tested
salmon CT	Thr	Tyr	Pro	Arg	Thr	Asn	Thr	Gly	Ser	Gly	Thr	Pro	-Amide						0.1%

Salmon calcitonin, like human calcitonin, had no detectable vasodilator activity at the doses tested. The different forms of CGRP tested so far all exhibit similar vasodilator activity despite differences in structure at amino acid positions 1, 3, 22, 25 and 35.

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VASODILATOR EFFECT OF CALCITONIN GENE RELATED PEPTIDE IN MAN

P. Goldberg[1], A. Hughes*, D. Redman[1], P. Sever, S. Thom. [1]Dept. of Anaesthetics and Dept. of Clinical Pharmacology, St. Mary's Hospital, London, W.2.

Calcitonin gene related peptide (CGRP) is a recently described peptide. It has been identified in perivascular nerves (Mulderry et al., 1985) and also shown to be a potent skin vasodilator and hypotensive agent in several species including man (Editorial, Lancet, 1985). In this study we have investigated the effect of intraarterial infusion of CGRP on the forearm vascular bed.

Four healthy male volunteers (aged 23-40) participated in studies for which St. Mary's Hospital Ethical Committee approval was obtained. Forearm blood flow (FBF) was measured by venous occlusion plethysmography using temperature compensated mercury in rubber strain gauges (Whitney, 1953). The hand circulation was excluded during measurement of FBF by inflation of wrist cuffs to supra-arterial pressure. The brachial artery of one arm (study) was cannulated to allow intra-arterial infusion of agents and intraarterial blood pressure monitoring. The other arm served as a control throughout. CGRP was diluted appropriately in 1:1 0.9% sterile saline : haemaccel for infusion. CGRP (10, 30, 100 ng/min; i.a.) was infused for 5 minutes at each dose level and FBF was measured during the last 3 minutes of each infusion when consistent FBF responses were achieved.

CGRP produced a dose related increase in FBF in the study arm. Study arm FBF increased from a control value of 3.3 $\pm$ 0.2 ml/100 ml forearm/min, to 3.4 $\pm$ 0.3, 5.0 $\pm$ 0.7 and 8.9 $\pm$ 1.1 ml/100 ml forearm/min (means $\pm$ s.e.m.; n = 4) at 10, 30, 100 ng/min respectively; a net increase of 173% at the highest dose. Marked flushing of the skin of the study arm was also noted during infusion of CGRP. FBF in the control arm was not significantly altered during infusion of CGRP, suggesting insignificant systemic effects of these doses of CGRP.

We recently reported that CGRP potently relaxes isolated human arteries, including brachial, by an endothelium dependent mechanism (Hughes et al., 1985). This study confirms that CGRP is a vasodilator in man in vivo, probably acting by a local mechanism. Nevertheless, the role of this peptide in human circulatory physiology has yet to be established.

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CARDIOVASCULAR EFFECTS OF HA1004 IN ANAESTHETISED AND PITHED RATS

E. Donato Di Paola, P.E. Hicks and Janine Lorrain, Laboratoire d´Etudes et Recherches Synthélabo, rue de la Glacière, 75013 Paris, France.

HA1004,(N-(2-guanidinoethyl)-5-isoquinolinesulfonamide), (Asano and Hidaka; 1985) is a novel intracellular calcium antagonist with vasodilatator properties but little effect on the heart, in addition HA has no affinity for ^{3}H nitredipine binding sites (Schoemaker et al; 1986). In this study we evaluate the effects of HA on blood pressure in anaesthetised rats, and on vasoconstrictor responses in pithed rats.

Male normotensive rats(230-250 g)were anaesthetised with pentobarbitone(60mg/kg i.p.)respired artificially and prepared for the aortic blood pressure and heart rate recordings. Some experiments were carried out in bivagotomized, pithed rats. The effects of several doses of HA(1-10mg/kg i.v. infused over 15min) were studied in intact rats or after pretreatment with chlorisondamine(0.5 mg/kg)with blood pressure supported by a continuous infusion of vasopressin (7mU/kg/min). Vasoconstrictor response curves to i.v. cirazoline(0.1-102.4µg/kg), UK-14,304(5-1280µg/kg), 5HT(5-40µg/kg), vasopressin(40mU/kg), tyramine(25 and 250µg/kg) or electrical stimulation(60V,1 ms,0.125-4Hz) through the spinal cord were determined in separate groups of pithed rats during 15min i.v. infusions of either saline(0.4ml/kg) or HA(0.3-3mg/kg). HA(1-10mg/kg) or diltiazem(1mg/kg) were also studied on the tachycardia evoked by cumulative doses of isoprenaline.

Infusions of HA(1,3 and 10mg/kg i.v.)produced maximal decreases in blood pressure of 13.6 $\pm$2.9 ; 45.4 $\pm$4.6 and 64.6 $\pm$7.5mmHg respectively, which rapidly returned to predose levels on stopping the drug. A significant bradycardia(94.4 $\pm$ 25.2b/min)occurred after the 10mg/kg dose of HA. In ganglion blocked vasopressin supported rats the maximal fall in blood pressure(45.2 $\pm$4.1mmHg)to HA (3mg/kg)was of the same magnitude but of longer duration than in intact animals. In pithed rats vasoconstrictor-response curves to UK-14,304 were progressively antagonized by HA(0.3,1 and 3mg/kg)with a decrease in the Emax. The effects of cirazoline were only antagonized by HA at 3 mg/kg i.v., but HA significantly antagonized the responses to tyramine and low, but not high frequency stimulation of the spinal cord at 1mg/kg i.v.. At this dose, HA did not block the tachycardia to tyramine or electrical stimulation. HA(1mg/kg)antagonized the pressor responses to 5HT or vasopressin in pithed, but not syrosingopine (5mg/kg i.p. 18h before)pretreated rats. The heart rate responses to isoprenaline were inhibited by infusions of diltiazem(1mg/kg)but not HA(1-10mg/kg).

These results show that HA has short acting vasodilatator properties in anaesthetised rats with little effect on heart rate. HA preferentially blocks α_2-receptor mediated, with less effect on α_1-receptor mediated vasoconstrictor responses, although the pressor effects mediated by endogenous release of NA were also antagonised. Unlike diltiazem, HA did not antagonize isoprenaline-induced tachycardia, and therefore shows considerable vascular selectivity.

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