# THE METABOLISM OF PROSTAGLANDIN $D_2$ AFTER INHALATION AND INFUSION IN NORMAL MEN

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Prostaglandin $D_2$ ($PGD_2$) is a putative inflammatory mediator released by immunological stimuli from human mast cells. In order to identify its fate in man we have administered $PGD_2$ to normal volunteers by intravenous infusion or inhalation. The study was approved by the Southampton Hospitals ethics committee and certified under the Medicines Regulations (1978).

Eight subjects (29 ± 4 yr) received a 20 min intravenous infusion of 86 ± 4.7 $\mu$Ci [$^3H_7$]-$PGD_2$. Blood samples were taken during, and for 180 min after the infusion. A further six subjects (28 ± 1 yr) received an inhalation of 20-40 $\mu$Ci $PGD_2$ from an Inspiron nebuliser. Blood samples were taken for 90 min after inhalation. In both studies cell free plasma was prepared by centrifugation at 4°C and extracted on C18 Sep Paks. Extracted plasma was analyzed by C18 reversed phase h.p.l.c. using a mobile phase of 32.8% acetonitrile in 0.017M phosphoric acid. Radioactivity was monitored by scintillation spectrometry. Samples taken during the infusion period contained a maximum of 34.5% of unchanged $PGD_2$ (relative retention time, $t_r$ 1.00), together with metabolites with $t_r$ values of 0.560 ± 0.003 (M1) and 1.12 ± 0.002 (M2) (n=29). Metabolite M2 was the more abundant, accounting for a maximum of 64.4% of the recovered radioactivity. There was no evidence of the formation of $PGF_{2\alpha}$ and its metabolites. Only small quantities (<5%) of 13,14-dihydro-15-keto-$PGD_2$ were observed. On termination of the infusion, the $PGD_2$ peak declined rapidly ($t_{½}$ 2.1 min) but the less polar metabolite M2 was more persistent, accounting for 23.2%. of the radioactivity at the end of the infusion. The disappearance of these initial metabolites was associated with the formation of uncharacterized polar metabolites. After inhalation, $PGD_2$ rapidly appeared in the circulation, reaching a maximum of 24.7% at 1 min but declining rapidly thereafter. This decline was paralleled by increases in M1 to 7.5% and M2 to 47% of the total recovered radioactivity 7 min after inhalation. Again, no $PGF_{2\alpha}$ or any of its metabolites were detected. By comparison of pooled plasma samples with materials prepared by total chemical synthesis, M1 was tentatively identified as 9$\alpha$,11$\beta$-$PGF_2$, whilst M2 coeluted with 13,14-dihydro-15-keto-9$\alpha$,11$\beta$-$PGF_2$.

These results confirm that the likely route of $PGD_2$ metabolism in man is via initial stereoselective reduction of the C-11 ketone function to produce the biologically active metabolite 9$\alpha$,11$\beta$-$PGF_2$ (Liston and Roberts, 1985). Our present data also support our previous report that inhalation of $PGD_2$ does not lead to the formation of $PGF_{2\alpha}$ metabolites (Hardy *et al.*, 1986). The formation of a 13,14-dihydro-15-keto metabolite is consistent with the view that further metabolism of 9$\alpha$,11$\beta$-$PGF_2$ may proceed by the 15-hydroxyprostaglandin dehydrogenase pathway (Bacon *et al.*, 1987).

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# OBSERVATIONS ON THE METABOLISM OF PROSTAGLANDIN $D_2$ AND 9α11β-PROSTAGLANDIN $F_2$ IN HUMAN LUNG

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Prostaglandin $D_2$ ($PGD_2$) is a mast cell derived bronchoconstrictor mediator (Beasley *et al.*, 1987). An important reaction in its metabolism is the 11-ketoreductase (11-KR) dependent stereoselective reduction of the C-11 keto function to produce 9α,11β-$PGF_2$ (Liston and Roberts, 1986). This metabolite is itself bronchoactive and may be further metabolised by a combination of 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and $\Delta^{13}$-reductase ($\Delta^{13}$R) (Bacon *et al.*, 1987). We now report on the metabolism of $PGD_2$ and 9α,11β-$PGF_2$ in human lung.

Fresh, grossly normal, human lung was obtained from patients undergoing resection of bronchial carcinoma, and cytosolic supernatants were prepared by homogenization and ultracentrifugation. Metabolism was studied by h.p.l.c. with liquid scintillation counting detection of radioactivity following incubation at 37°C with 2 μg $ml^{-1}$ substrate and 0.1 μCi tritiated PG (Bacon *et al.*, 1987). Reactions were supplemented with 4mM $NAD^+$ for studies with 15-PGDH, or an NADPH generating system for studies on 11-KR.

In the presence of the NADPH generating system, metabolism of $PGD_2$ proceeded slowly in the 100 000 x g supernatant fraction, with a reaction rate of 0.30 ± 0.03 pmol $min^{-1}$ $mg^{-1}$ protein (n=4 lungs). The rate of reaction was linear over 60 min. Analysis of the extracted supernatant by h.p.l.c. demonstrated the formation of 9α,11β-$PGF_2$ as metabolite, this being fully resolved from the 11α epimer ($PGF_{2\alpha}$) by reversed phase h.p.l.c. with a mobile phase of 32.8% acetonitrile in 0.017M phosphoric acid. In the presence of $NAD^+$ or absence of NADPH, $PGD_2$ was not metabolised enzymatically, although there was some chemical dehydration of the parent molecule to a less polar compound(s).

In 9 out of 10 lungs tested, 9α,11β-$PGF_2$ was slowly metabolised in cytosolic supernatants. The rate of reaction was 1.46 ± 0.3 pmol $min^{-1}$ $mg^{-1}$ protein and was linear over 60 min. The major metabolite formed in this reaction had a retention time of 1.33 ± 0.01 (9α,11β-$PGF_2$ = 1.00, n=15 determinations) on a Nucleosil C18 column. Electron impact GC/MS of the methoxime, methyl ester, trimethylsilyl ether derivative indicated the following fragment ions: 539 ($M^+$), 508, 449, 418 and 328, consistent with the presence of one keto function and 2 hydroxyl groups in the molecule and its possible identity as 15-keto-9α,11β-$PGF_2$. When incubations were performed with $PGF_{2\alpha}$, the 15-keto-$PGF_{2\alpha}$ metabolite was the most abundant product.

Our results thus demonstrate that 11-KR and 15-PGDH type pathways exist in human lung for $PGD_2$ and 9α,11β-$PGF_2$. A full kinetic analysis will be one step necessary to investigate the potential significance of these reactions *in vivo*.

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# THERMOREGULATORY RESPONSES OF ENDOTOXIN-RESISTANT MICE TO POLY I:C AND ENDOTOXIN

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Endotoxin induces fever in most mammalian species, however, whether fever is produced in mice is open to question as hypothermia as well as hyperthermia have been observed, however Milton et al. (1982) showed mice could respond to prostaglandin $E_2$ which produced a rise in deep body temperature when it was injected directly into the pre-optic area of the anterior hypothalamus (PO/AH). Polyinosinic:polycytidylic acid (Poly I:C), a synthetic double-stranded polyribonucleotide, which induces interferon production is pyrogenic in rabbits. Rotondo et al. (1987) have shown that effects of this interferon inducer on body temperature is blocked by the antipyretic drug ketoprofen. This suggests that both lipopolysaccharide endotoxin and Poly I:C have a common final pathway in the production of fever. Poly I:C 5.0 µg/kg i.v. to 1.0 mg/kg had no significant effect on rectal temperature whereas 8mg/kg i.v. caused a significant hypothermia. Rectal temperature began to fall one hour after drug administration, reached a minimum after two hours and returned to pre-treatment level within four hours, ($\Delta$T max -0.94 $\pm$ 0.056). Sterile saline (0.2 ml i.v.) had no significant effect on deep body temperature, ($\Delta$T max 0.01 $\pm$ 0.086). Lipopolysaccharide (LPS 1 mg/kg i.v.) produced a significant hypothermia, rectal temperature began to fall one hour after drug administration, reached a minimum after two hours and returned to pre-treatment level within four hours, ($\Delta$T max -0.94 $\pm$ 0.058). Ketoprofen when given 5 minutes prior to either Poly I:C or lipopolysaccharide prevented the development of hypothermia. Rabbit interleukin 1 prepared from monocyte/lymphocytes produced a significant hyperthermia. The hyperthermia produced by rabbit interleukin 1 was completely abolished by the administration of ketoprofen (3 mg/kg i.v.) 5 minutes prior to the administration of the interleukin 1 solution. In contrast mouse endotoxin stimulated monocyte/lymphocyte preparation had no significant effect on rectal temperature. The results reported in the study show that MF1 endotoxin-resistant mice do not develop a fever when given Poly I:C in doses which provoke very marked increases in body temperature in rabbits, similar observations were made using lipopolysaccharide (endotoxin). In contrast in the rabbit, a prominent febrile response is seen with 5 µg/kg Poly I:C and 50 nanograms/kg lipopolysaccharide (Abul et al. 1987). Of particular importance is the observation that just as the febrile responses in the rabbit to these two pyrogens are blocked by the non-steroidal anti-inflammatory agent ketoprofen so are the hypothermic responses in the mouse. This would suggest that the hypothermic responses in the mouse involve prostaglandins. That endotoxin-resistant MF1 mice are capable of developing a fever is shown by the experiments in which interleukin 1 prepared from rabbit was given intravenously resulting in a significant fever and also by previous observations (Milton et al. 1982) that endotoxin-resistant mice respond to prostaglandin $E_2$ with a hyperthermia. Since no hyperthermic response was obtained with the supernatant prepared from mouse lymphocytes/monocytes it would appear that these cells do not liberate mouse interleukin 1 when stimulated with lipopolysaccharide and this is consistent with the results first reported by Milton et al. (1982).

This research was supported by the Wellcome Trust.

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# EFFECTS OF INTRAVENOUS AND ORAL ADMINISTRATION OF THE PROSTACYCLIN-MIMETIC RS 93427 IN BABOONS

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Male Baboons (Papio, papio, 10-20kg) were used, either lightly sedated (diazepam,Roche,0.3-0.5mg/kg, i.m.) and restrained in a chair, or else conscious, unrestrained, but sedated (as before) and anaesthetized with ketamine (Bristol Labs; 5-10mg/kg) immediately prior to blood withdrawal or dosing. RS93427 (Calcium salt) was dissolved in Tris Buffer (0.01M, pH8) for intravenous use, diluted in 0.9% NaCl solution. Prostacyclin ($PGI_2$, Sigma) was also dissolved just prior to use in the Tris Buffer, kept on ice and fresh dilutions made immediately prior to use. Blood pressure was determined by sphygmomanometry using a semiautomated device (Digitronics, Model 100-046) and heart rate measured by palpation. Both prostacyclins were administered intravenously via a catheterized femoral vein by stepwise 15 min intravenous infusions of 32-1,000ng/kg/min, and decay of effect followed for up to 2 hours. For oral administration of RS93427 only ($PGI_2$ is not orally active), the compound (0.3,0.6 and 1.2mg/kg) was administered by gavage and blood samples taken just prior to and at 2 and 4h after dosing. Platelet aggregation procedures were as described previously (Willis, et al, 1986; 1987). Samples of baboon platelet-rich plasma (BPRP) were prepared from blood withdrawn into sodium citrate (final concentration of 0.3% w/v). The BPRP was mixed 1:1 with baboon platelet-poor plasma (BPPP) and examined for turbidometric aggregation response to ADP (Sigma; 1.6$\mu$g/ml). In contrast to this "homologous ex-vivo aggregation" procedure the "heterologous ex vivo aggregation" response was determined in similar mixtures of BPPP and human platelet-rich plasma prepared from blood withdrawn from healthy human donors into sodium citrate (0.38% final concentration). By both oral and intravenous routes, marked decreases (approaching 100%) in platelet aggregation (homologous and heterologous) were produced that increased with drug infusion rate. However (as also seen in vitro) baboon platelets were approximately 10-fold less sensitive than human platelets to RS93427 although very sensitive to $PGI_2$. For RS93427 administered by i.v. infusion (n=7), maximal inhibition of platelet aggregation was accompanied by a mean reduction ($\pm$ s.e. mean) of 41.5 $\pm$ 4.5 mm Hg in estimated diastolic b.p. with a mean increased heart rate of 44.4 $\pm$ 4.4 b.p.m. with little effect on systolic b.p. Results were similar for intravenous infusions of $PGI_2$ (n=5): mean diastolic b.p. was decreased by 45.1 $\pm$ 2.9 mm Hg, and mean heart rate increased by 55.5 $\pm$ 6.3 b.p.m. In contrast to the almost immediate disappearance in effects of intravenously infused $PGI_2$, those of RS93427 were sustained during the 2h follow up period.

Similar effects of RS93427 were seen following oral administration of RS93427, which was almost immediately absorbed via the intragastric route (Willis et al, 1987) and produced peak effects within 2h with effects sustained to 4h.

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# EFFECTS OF PHENIDONE, INDOMETHACIN AND DEXAMETHASONE ON ZYMOSAN INDUCED PERITONEAL INFLAMMATION IN THE RAT

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Intraperitoneal injection of zymosan in mice or rabbits causes an acute inflammatory response which is associated with the production of eicosanoids (Doherty et al 1987, Forrest et al 1986). We have characterised the inflammatory response induced by zymosan in the rat peritoneal cavity and have studied the effects of a variety of drugs which interfere with arachidonic acid metabolism on the response.

Charles River CD rats (250g) were given an i.v. injection of pontamine sky blue (50mg/kg) to label plasma proteins 3 minutes prior to an i.p. injection of 2ml of a 5mg/ml zymosan suspension. Groups of 7 rats were killed at various times from 7.5 min to 24 hours later and the peritoneal cavity washed out with 5ml phosphate buffered saline. Aliquots of each sample were taken for total and differential cell counts, absorbance measurements of dye labelled plasma proteins ($A_{610}$) and measurements of prostaglandin and leukotriene levels by RIA.

Intraperitoneal injection of zymosan caused an increase in vascular permeability to plasma proteins which was maximal at 3 hours ($A_{610}$ = 0.75±0.09) and an influx of inflammatory cells which was maximal at 6 hours (cell numbers increased from 24.5±6.1x$10^6$/rat at 7.5min to 75.5±22.2x$10^6$/rat, PMN's accounted for 82% of the cells which made up this increase). The inflammatory response was associated with the production of $LTB_4$ (25.4±4.7 ng/ml at 7.5min) $LTD_4$ (16.7±8.7ng/ml at 7.5min) and 6-oxo $PGF_1\alpha$ (86.5±21.6ng/ml at 7.5min).

The effect of drugs was studied on the inflammatory response at 30 minutes and 6 hours. Drugs were either dosed orally one hour prior to zymosan (indomethacin and phenidone) or subcutaneously (dexamethasone) 3 hours prior to zymosan. Phenidone (50mg/kg), a selective lipoxygenase inhibitor, substantially inhibited dye extravasation at 30 minutes but was less effective at 6 hours and did not affect inflammatory cell influx at this time (Table). Indomethacin (3mg/kg), a selective cyclooxygenase inhibitor, had little antiinflammatory activity at either time point. Dexamethasone (10mg/kg) had no effect on production of eicosanoids and did not influence dye extravasation at 30 minutes. However, dexamethasone significantly inhibited dye extravasation and inflammatory cell influx at 6 hours.

| Treatment (mg/kg) | Dye 30 min $A_{610}$ | Dye 6 hrs $A_{610}$ | Cells 6 hrs x$10^6$/rat | $LTB_4$ 30 min ng/ml | 6-oxo-$PGF_1\alpha$ 30 min ng/ml |
|---|---|---|---|---|---|
| Vehicle | 0.33±0.16 | 0.42±0.11 | 66.8±36.0 | 4.8±0.9 | 22.0±7.1 |
| Phenidone (50) | 0.10±0.02* | - | | 0.6±0.3* | 21.4±9.5 |
| Phenidone (100) | - | 0.25±0.13* | 68.9±25.8 | - | - |
| Vehicle | 0.43±0.13 | 0.47±0.13 | 82.0±21.7 | 4.3±0.6 | 14.6±3.5 |
| Indomethacin (3) | 0.31±0.11 | 0.52±0.04 | 84.1±22.6 | 4.2±0.6 | 2.0±1.2* |
| Vehicle | 0.34±0.11 | 0.44±0.13 | 75.7±32.6 | 3.8±0.6 | - |
| Dexamethasone (10) | 0.41±0.06 | 0.21±0.09* | 34.1±7.3* | 4.2±1.2 | - |

Results show mean ± sd, n = 7 *p <0.05 Student's t-test.

These results suggest that leukotrienes are important mediators of the early phase of vascular permeability produced by zymosan but they do not contribute significantly to the later inflammatory events.

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# THE PROSTACYCLIN MIMETIC RS 93427: SELECTIVE INHIBITION OF PLATELET MITOGEN RELEASE FROM HUMAN PLATELETS

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We have postulated dual anti-atherosclerotic activity of prostacyclin ($PGI_2$) or some mimetics via inhibition of both vascular cell accumulation of cholesterol and release of mitogens from platelets and other cells (Willis et al, 1986). A mixture of several mitogens and co-mitogens is released from activated platelets (Bowen-Pope, et al, 1984), thus a mitogenic assay remains the most suitable way of examining their release. We here report on comparative effects of RS93427 in inhibiting ADP-induced aggregation of human platelets and inhibiting release of mitogenic activity.

Platelet-rich plasma (PRP) was prepared by low speed centrifugation of human blood (50-100ml) withdrawn into 0.1 vol of sodium citrate (0.38% final concentration) from young healthy drug-free volunteers. The PRP was stirred (1,000 r.p.m. 37°C) in the light path of a dual channel aggregation module (Payton) for 3 min with various concentrations of RS93427 (Calcium salt) and then aggregation stimulated by ADP (1.6μg/ml). After a further 3 min, each PRP sample was then twice sequentially centrifuged (13,000 g for 1 min, Eppendorf microfuge) and the supernatant platelet-free plasma frozen (<-20°C) until tested for mitogenic activity. An aliquote of each sample was added to lightly seeded human fibroblasts in culture and the increased number of cells directly counted after 2-3 days.

As can be seen from Table I, there was considerable individual variation between concentrations of RS93427 producing 50% inhibition of aggregation and mitogen release, but that mitogen release was inhibited at considerably lower concentrations than those required for inhibition of aggregation ($P < 0.05$). These data further support a potential anti-atherosclerotic role for prostacyclins acting like RS93427.

Table I: $IC_{50}$ (ng/ml) Values for RS93427

| EXPT. # | MITOGEN RELEASE | AGGREGATION | RATIO AGG/MR |
|---|---|---|---|
| 1 | 0.31 | 2.4 | 7.7 |
| 2 | 0.28 | 1.3 | 4.6 |
| 3 | 0.04 | 2.3 | 57.5 |
| 4 | 0.50 | 10.0 | 20.0 |
| 5 | 0.45 | 6.5 | 14.4 |
| 6 | 4.00 | 6.0 | 1.5 |
| 7 | 0.15 | 1.25 | 8.3 |
| MEAN | 0.81 ± 0.54 | 4.25 ± 1.26 | |

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# SMS 201-995 MAY ACTIVATE THE $K^+$ CHANNELS OF VASCULAR SMOOTH MUSCLE

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Somatostatin has been shown to depress hormone secretion in a number of endocrine systems by increasing $K^+$ channel conductance, and, in some cases, by decreasing $Ca^{2+}$ channel conductance (Lewis et al, 1986; Pace & Tarvin, 1981). As both of these conductances are present in smooth muscle, the effects of the somatostatin analogue, SMS 201-995 (Sandostatin; SMS) on the contraction of rat mesenteric resistance vessels (mrv), and on $^{45}Ca$ influx and $K^+$ currents in cultured rat aortic smooth muscle cells (smc) were examined. Whole cell voltage clamp with 2-10 MΩ electrodes (containing (mM): KCl,145; EGTA,10; HEPES,10) was used to monitor current-voltage relationships in cultured rat aortic smc. Cells were clamped at -40mV and successively hyper- or depolarized in 10 mV increments to potentials of -90 and +60 mV, respectively. $^{45}Ca$ influx was measured by prelabelling the smc with $^{45}Ca$ (1 μC; 30 s) before challenging them with $K^+$ (55 mM) for 5 min. Cells were preincubated with SMS for 1 min before labelling. Isometric tension responses of rat mrv (internal diameter ~125 μm) were monitored using a myograph. The mrv were contracted by 10 min applications of noradrenaline (NA; $10^{-5}$M), $K^+$ (80 mM), [Arg$^8$]vasopressin (AVP; $10^{-8}$M), or by 80 mM $K^+$ for 5 min with a subsequent, additional contraction induced by the application of $10^{-5}$M NA (10 min).

SMS ($10^{-6}$M) increased the magnitude of the outward currents induced by depolarising pulses in the cultured smc. A bath application of $Ba^{2+}$ (1 mM), $Cs^+$ (1 mM), $Co^{2+}$ (2.5 mM) and TEA (20 mM) inhibited the SMS-induced increase in the outward currents. 55 mM $K^+$ promoted a 3-fold uptake of $^{45}Ca$ into the smc; SMS ($10^{-8}$ and $10^{-6}$M) had no inhibitory effect on this increased uptake. In isolated rat mrv, SMS inhibited NA-induced tension but not 55 mM $K^+$- or AVP- induced tension. The maximal inhibition of NA-induced tension by SMS was 34 ± 1% with its half maximal effect at 0.3 ± 0.1 nM, n=5. SMS failed to inhibit NA-induced tension in the high $K^+$ depolarised tissues. These results suggest that SMS activates the $K^+$ channels of vascular smooth muscle.

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# CHRONIC EFFECTS OF ATENOLOL IN SPONTANEOUSLY HYPERTENSIVE RATS

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Previous work has indicated that following chronic atenolol administration, changes in end-organ response are accompanied by altered presynaptic mechanisms. (Carr *et al*, 1983, Draper *et al*, 1986) The present study investigates this phenomenon in the spontaneously hypertensive rat (SHR).

Atenolol (50mg/Kg) was administered orally in 5% polyethylene glycol (PEG) to male SHR's (300-350g); control results were obtained from animals dosed with vehicle alone. Blood pressure was measured in the conscious rat by the "tail-cuff" technique using the schedule described previously. (Kingsbury *et al*, this meeting) Twenty-four hours after the last dose of atenolol or PEG the *in situ* blood-perfused mesentery was prepared according to the method of Jackson and Campbell (1980). Vasoconstricor responses to exogenous noradrenaline and periarterial electrical stimulation were assessed as previously described. (Kingsbury *et al*, this meeting)

After the first day of dosing with atenolol, blood pressure in the conscious rat was significantly ($P<0.001$) reduced from $214.5\pm3.4$mmHg in control animals to $165.3\pm5.2$mmHg in atenolol-treated animals. After 5 days the mean blood pressure of the control (PEG), group was $227\pm3.2$mmHg while that of the atenolol-treated group was $165.3\pm5.2$mmHg. This reduction was maintained throughout the dosing period; after 19 days atenolol-treated animals had a mean blood pressure of $185.3\pm3.7$mmHg compared to $226\pm5.1$ in controls. Results from the *in situ* mesentery showed that treatment with atenolol for 7 days resulted in increased vasoconstriction in response to higher doses of noradrenaline. After 21 days this potentiation had disappeared, and was replaced by a significant ($P<0.001$) reduction in response to lower doses of noradrenaline. Response to periarterial electrical stimulation was unchanged after 7 days atenolol treatment. This was in contrast to the highly significant ($P<0.001$) decrease in response after 21 days atenolol treatment. (fig 1)

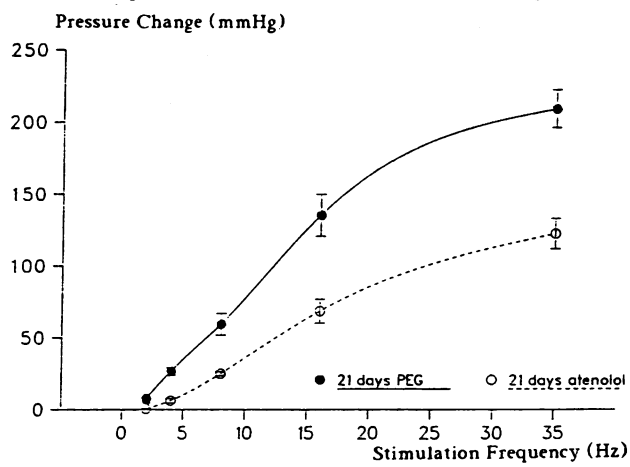


Fig 1: Effect of 21 days Atenolol p.o. on Electrical Stimulation.

These results indicate that chronic administration of atenolol caused a reduction in the blood pressure in the conscious SHR which reached a maximum after 3 days, and was maintained for at least 18 days thereafter. Although this reduction was highly significant, blood pressure in the SHR was still higher than in untreated normotensive animals. Results from the *in situ* mesentery indicate that after 21 days atenolol administration, there is a significant reduction in transmitter release from the presynaptic terminal, as evidenced by the reduced response to periarterial electrical stimulation. The time course and size of this effect suggests that it may be important in the antihypertensive effect of atenolol observed clinically.

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# CHRONIC EFFECTS OF ATENOLOL IN WISTAR RATS

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We have previously shown that after long-term administration of β-adrenoceptor blocking drugs in the rat, complex changes are produced quite distinct from the blockade of post-synaptic β-adrenoceptors. (Carr *et al*, 1983, Draper *et al*, 1986) These effects are more consistent with the time course of the antihypertensive action of β-adrenoceptor blocking drugs. The present study investigates further the effect of chronic β-adrenoceptor blockade on adrenergic neurotransmission using an *in situ* preparation to avoid complications arising from possible "wash-out" of antagonist following tissue removal.

Atenolol (50mg/Kg) was administered orally to normotensive male Wistar rats (300-350g) in 5% polyethylene glycol (PEG). Control results were obtained from animals dosed with vehicle alone. Blood pressure was measured in the conscious rat by the "tail-cuff" technique and was recorded daily before and 2h after dosing. Twenty-four hours after the last dose of atenolol or PEG the degree of blockade was assesed by constructing dose response curves to isoprenaline injected as a bolus i.v. The *in situ* blood-perfused mesentery was prepared according to Jackson and Campbell (1980). Vasoconstrictor responses to exogenous noradrenaline (20-2000ng) and periarterial electrical stimulation (15v rectangular pulses of 1ms duration for 20sec. 2-35Hz) were obtained 24h after the last dose.

During the first week of dosing with atenolol, blood pressure in the conscious rat was significantly reduced. After 5 days the mean blood pressure of the control (PEG), group was 153±2.9mmHg while that of the atenolol treated group was 128.5±2.8mmHg ($P<0.001$). This reduction was maintained throughout the dosing period; after 19 days, atenolol-treated animals had a mean blood pressure of 130.1±2.0mmHg compared to 151.8±1.7mmHg in controls. ($P<0.001$)

| | ISOPRENALINE (μg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | .0025 | .005 | .01 | .02 | .05 | .1 | .2 |
| 7d PEG | 3.8±2.5 | 8.9±2.8 | 20±2.5 | 28.8±7.6 | 88.8±13.6 | 110±11.2 | 140±12.2 |
| 7d atenolol | 2.5±2.2 | 8.8±5.1 | 25±7.5 | 30±10.6 | 50±18.1 | 60±15.8* | 70±15** |
| 21d PEG | 0 | 3.1±1.5 | 18.9±5.9 | 35.6±9.9 | 88.1±4.8 | 118.1±4.3 | 158.8±8 |
| 21d atenolol | 0 | 7.5±4.1 | 17.5±7.4 | 30±8.8 | 35±7.7*** | 55.0±8.3*** | 58.8±6.7*** |

Table 1 :- Mean Increase in Heart Rate (beats/min)±SEM, n=4 atenolol, n=8 PEG. *=$P<0.05$, **=$P<0.01$, ***=$P<0.001$

Results from the *in situ* mesentery showed that treatment with atenolol for 7 days resulted in increased vasoconstriction in response to higher doses of noradrenaline. This trend increased after 21 days treatment. In contrast, responses to higher frequencies of periarterial electrical stimulation were reduced after 21 days treatment with atenolol.

These results indicate that chronic administration of atenolol caused a reduction in blood pressure which reached a maximum after five days, and was maintained for at least 16 days thereafter. β-adrenoceptor blockade was established within 7 days and was maintained up to 21 days of dosing with atenolol (Table 1). Results obtained from the *in situ* mesentery further suggest that atenolol exerts a presynaptic action apparent after 21 days but not after 7 days drug treatment.

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# EFFECT OF FIBRINOPEPTIDES A AND B ON HUMAN AND RAT PLATELET AGGREGATION

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Thrombin-induced proteolysis of fibrinogen which occurs as a natural consequence of haemostasis, thrombosis or inflammation, results in the sequential formation of fibrinopeptide A (FpA) and fibrinopeptide B (FpB). These peptides contain 16 and 14 amino acids respectively and occur naturally in the plasma of healthy humans (Nossel et al., 1974). Although FpB constricts the pulmonary vascular beds of several species (Bayley et al., 1967) and increases rat blood pressure (Osbahr et al., 1967) there have been no reports of their effect on platelet aggregation.

In the present study, blood obtained by venepuncture of human volunteers and cardiac puncture of ether anaesthetised rats (male, Sprague-Dawley, 250-300g) was anticoagulated (1:9 v/v with 3.8% trisodium citrate) and centrifuged (300g, 15 min) to prepare platelet rich plasma (PRP). Platelet aggregation was measured turbidometrically using a Payton dual channel aggregometer.

FpA mimicked the effect of ADP on human platelet aggregation. Low concentrations (0.65-6.5μM) caused a monophasic, reversible wave of platelet aggregation while, at a higher concentration (30μM), biphasic platelet aggregation was observed. At concentrations in excess of 65μM, addition of FpA resulted in irreversible platelet aggregation. FpA was approximately 10 times more potent than ADP. Fibrinogen (minimally effective concentration, 0.6-0.9μM) also aggregated human platelets. Preincubation of PRP (37°C, 1 min) with p-bromophenacyl bromide (100uM) indomethacin (10μM) or dazoxiben (10μM) prevented the second wave of aggregation in response to maximally effective concentrations of FpA (65μM), fibrinogen (5μM) and ADP (10μM). At lower concentrations, which had no direct platelet aggregatory activity, FpA (0.01-0.1μM), potentiated platelet aggregation due to ADP and collagen in both species. Neither FpA (0.1-65μM) nor fibrinogen (0.1-50μM) aggregated rat platelets.

In contrast, FpB at concentrations up to 1 mM had no detectable effect on human or rat platelet aggregation and did not potentiate ADP- or collagen-induced responses. However, addition to the cuvette of FpB (0.65μM and 6.5μM) did reduce the potency and the maximal inhibitory effect of $PGI_2$ and $PGD_2$ in PRP from both species. For example, $PGI_2$ (0.9nM) inhibited ADP-induced platelet aggregation in human PRP by 56.5 ± 7.6% (n=8) in the absence and 17.8 ± 2.3% (n=8, $P < 0.01$, Student's t test) in the presence of FpB (6.5μM). The threshold concentration of FpB required to antagonise the effect of $PGI_2$ on human platelets was 0.65μM. FpB (up to 65μM) did not influence the platelet anti-aggregatory effect of adenosine in human PRP. Neither FpA nor fibrinogen, at concentrations which did not aggregate human or rat platelets directly, affected the platelet inhibitory activity of $PGI_2$ or $PGD_2$ in human or rat PRP in response to ADP or collagen.

Thus, FpA and FpB released during blood coagulation may, by different mechanisms, exacerbate platelet aggregation thus accelerating the formation of a platelet plug and indirectly the production of a thrombus.

We would like to thank the Wellcome Trust for financial support.

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# INVESTIGATION OF THE ACTION OF NEUROPEPTIDE Y IN THE ISOLATED HUMAN CORONARY ARTERY

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We have previously reported that neuropeptide Y (NPY), infused into the left anterior descending coronary artery (LADC) of patients, produces a pronounced decrease in coronary blood flow and ischaemia, concomitant with ECG ST elevation, but with no change in epicardial coronary artery diameter (Clarke et al, 1987). The present experiments were undertaken as part of a study to determine the site of action of NPY in the human coronary arterial circulation.

Segments of LADC were obtained from eleven patients undergoing transplant operations, eight for cardiomyopathy, two for myocarditis and one for cystic fibrosis. Spiral strips were prepared from these segments and suspended in Krebs solution at 37°C, gassed with $95\%O_2/5\%CO_2$, in 2ml baths. 1g tension was applied to the tissues and changes in tension were recorded isometrically. Cumulative dose-response curves to nor adrenaline (NA; $10^{-7}$ to $3x10^{-5}$M) were prepared in the absence and presence of NPY ($10^{-7}$ and $5x10^{-7}$M).

Arterial strips could not be prepared from two patient's tissues because of severe atherosclerosis. Arterial strips from four of the patients (10 preparations) were found to be unresponsive to both NA and NPY. The arterial strips from the remaining five (eight preparations) patients were also found to be unresponsive to NPY at concentrations of $5x10^{-8}$ to $5x10^{-7}$M. In addition NPY was without effect on the dose-response curves to NA. The lack of response to NA was unrelated to patients' age, pathology of the tissues or the patients' drug therapy.

This work supports the contention that NPY acts predominantly in resistance vessels in the human coronary circulation.

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# COMPARISON OF AFFINITY OF 8-SUBSTITUTED XANTHINES FOR ADENOSINE RECEPTORS IN ATRIA FROM RATS AND GUINEA-PIGS

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Comparative studies of the affinity of xanthine derivatives at A1 binding sites in C.N.S. membranes from the rat and guinea-pig have indicated that affinity is often significantly higher for the rat (Ukena et al, 1986). In the present study we have compared the apparent affinity of some alkyl-xanthines in atria from rats and from guinea-pigs in order to determine whether a species difference can be detected, at functional adenosine receptors.

Atrial pairs from rats and guinea-pigs were mounted for isometric tension recording in organ baths containing Krebs solution (dipyridamole 10 μM, 37°C). Dose-response curves were constructed for the bradycardic effects of 2-chloroadenosine and carbachol or acetylcholine. Dose-response curves were repeated after exposure to 8-phenyltheophylline (8PT), 8-(4-(2-aminoethyl) aminocarbonylmethyloxyphenyl)-1,3-dipropylxanthine (XAC), 8-(4-carboxy methyloxyphenyl)-1,3-dipropylxanthine (XCC), 8-cyclopentyl-1,3-dipropylxanthine (CPX). The tissues were incubated with the antagonists until equilibrium was judged to have occurred (generally 1 hour).

All of the xanthines behaved as competitive antagonists in atria from both species. None of the xanthines altered dose-response curves to muscarinic receptor agonists in the atria. The data was exposed to Schild analysis and the results are presented below.

Antagonist data for 8-substituted xanthines in atria from rat and guinea-pig

| Antagonist | Species | $pA_2$ (95% Confidence interval) | Slope | Concentration range (μM) | n |
|---|---|---|---|---|---|
| 8PT | Rat | 7.16(6.54-7.95) | −0.95±0.12 | 0.1-3 | 13 |
| | G.Pig | 6.48(5.89-7.23) | −0.86±0.12 | 0.1-10 | 14 |
| XAC | Rat | 7.82(6.94-9.0) | −0.88±0.11 | 0.1-10 | 20 |
| | G.Pig* | 7.22(6.68-7.87) | −1.03±0.09 | 0.1-10 | 11 |
| XCC | Rat | 7.93(7.06-9.08) | −0.81±0.09 | 0.1-10 | 14 |
| | G.Pig* | 7.40(7.0 -7.85) | −1.12±0.06 | 0.1-10 | 15 |
| CPX | Rat | 8.24(7.33-9.42) | −0.93±0.13 | 0.01-1 | 13 |
| | G.Pig | 8.14(7.67-8.65) | −0.91±0.06 | 0.01-1 | 15 |

* From Collis et al (1987)

Although $pA_2$ values in the rat atria tended to be higher than those in guinea-pig atria, these differences were not significant, and were small in comparison with those reported in ligand binding studies (Ukena et al, 1986). The results of this study do not support the concept that there are marked species differences in the affinity of 8-substituted xanthines in atria from guinea-pigs and rats.

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# SOURCES OF CALCIUM FOR CONTRACTION OF RAT FEMORAL VEIN TO $\alpha_1$- AND $\alpha_2$-ADRENOCEPTOR STIMULATION

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Pressor responses of pithed rats to $\alpha_2$-adrenoceptor agonists are invariably sensitive to calcium entry blockers while those to $\alpha_1$-adrenoceptor agonists are generally resistant. It has been suggested (Timmermans et al, 1987) that this is due to the dual nature of the utilization of $Ca^{2+}$ by these agents: $\alpha_1$-agonists producing $Ca^{2+}$ release and entry, $\alpha_2$-agonists Ca2+ entry only. However, it has also been suggested that sensitivity to calcium entry blockers is not related to the subtype of adrenoceptor activated, but rather to characteristics of the agonists. One such suggestion is that it is the intrinsic activity (i.a.) of the agonist that determines sensitivity to calcium entry blockers such that agonists with low i.a. are susceptible while those with high i.a. are not (Ruffolo et al, 1984). Observations in vitro in support of either hypothesis are equivocal. In the present study, we have examined these hypotheses in the rat femoral vein in vitro, a preparation which possesses both $\alpha_1$- and $\alpha_2$- postjunctional adrenoceptors (Downing et al, 1986).

Ring segments of femoral veins from male Wistar rats (200-250g) were mounted under 0.5g tension between two parallel fine steel wires in physiological salt solution (PSS) at 37°C gassed with 95% $O_2$, 5% $CO_2$. In some experiments responses were elicited following a 2 min incubation in a calcium free PSS containing 0.2mM EGTA (the EGTA-resistant response). Table 1 shows maximal contractile responses to the non-selective adrenoceptor agonist noradrenaline (NA), the $\alpha_1$-selective adrenoceptor agonist cirazoline (CIR) and the $\alpha_2$-selective adrenoceptor agonist BHT-920 (BHT), in the presence of EGTA and following 30 minutes incubation in PSS containing $10^{-5}$M verapamil.

Table 1. Contractile responses of Rat Femoral Vein, % control.

| Agonist | i.a. | EGTA-resistant Response* | Response* in presence of $10^{-5}$M verapamil |
|---|---|---|---|
| NA | 1.0 | 36.0±3.5 | 78.0±2.1 |
| CIR | 0.7 | 42.8±4.0 | 79.8±4.7 |
| BHT | 0.7 | 0 | 34.0±1.0 |

* Results expressed as % of control response to each agonist in normal PSS (mean±sem, n≥6).

These results show that the $\alpha_2$-selective adrenoceptor agonist BHT, which has a similar i.a. to CIR, was incapable of producing an EGTA-resistant contraction whereas stimulation of $\alpha_1$-adrenoceptors by CIR produced a clear EGTA-resistant contraction. Furthermore, BHT was much more susceptible than either NA or CIR to inhibition by the calcium entry blocker verapamil. These observations provide in vitro support for the hypothesis of Timmermans and co-workers (Timmermans et al, 1987) that contractions of vascular muscle mediated via stimulation of $\alpha_1$-adrenoceptors utilise intracellular and extracellular sources of $Ca^{2+}$ whereas contractions mediated by $\alpha_2$-adrenoceptor stimulation utilise extracellular $Ca^{2+}$ only.

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# THE INFLUENCE OF HYPOXIA ON ACETYLCHOLINE-INDUCED RELAXATION IN A RANGE OF RABBIT ARTERIES

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It is nov generally accepted that acetylcholine (ACH) produces relaxation of vascular smooth muscle by causing release of endothelium-derived relaxing factor (EDRF), while the finding that a fall in $PO_2$ reversed the ACH-induced relaxation in rabbit aortae that were pre-contracted with noradrenaline (NA), led to the proposal that the production of EDRF is attenuated by hypoxia (Furchgott & Zawadki, 1980). Further, measurement of Ca uptake in rabbit aorta led to the proposal that EDRF released by ACH causes relaxation by inhibiting NA-induced Ca influx, as well as by influencing processes which are independent of extracellular Ca (Ratz et al, 1987). In the present study we have investigated these proposals in a range of blood vessels with different dependencies upon extracellular Ca for NA-induced contraction.

Segments of the thoracic aorta (TA), common carotid (CA), femoral (FA) and superior mesenteric (MA) arteries were removed from rabbits under pentobarbitone anaesthesia (45mg/kg i.v.) and mounted between parallel wires at 37°C for recording of tension. Contractions to NA ($ED_{80}$) were elicited during normoxia ($PO_2$=102.4±0.5, n=507) before and 5 minutes after exposure to Ca-free Krebs' (with EGTA 0.5mM) and during hypoxia ($PO_2$=7.1±1.2, n=338). Further, at peak contraction induced by NA in normoxia, ACH (1μM) was added to produce relaxation, then the tissue was made hypoxic.

| | Ca free | H | ACH | ACH+H |
|---|---|---|---|---|
| TA | 74.5±2.9(7) | 33.8±7.2(7) | 36.3±7.7(6) | 48.6±9.8(6) |
| CA | 63.9±4.7(5) | 76.9±22.8(5) | 47.6±7.0(6) | 99.9±11.2(6) |
| FA | 52.9±4.8(7) | 5.6±3.0(7) | 50.6±14.2(5) | 46.8±21.5(5) |
| MA | 71.7±12.7(6) | 47.6±13.6(6) | 44.4±14.0(6) | 105.3±21.2(6) |

Table 1 Magnitudes of NA-induced contractions in aorta (TA), carotid (CA), femoral (FA) and mesenteric (MA) arteries as % of normoxic control in the absence of Ca (Ca free), in hypoxia (H), in the presence of ACH in normoxia (ACH) and in hypoxia (ACH+H).

These results suggest that the dependency of a given vessel upon extracellular Ca for NA-induced contraction is not obviously correlated with the extent of relaxation induced (i) by hypoxia (cf Marriott & Marshall, 1987) (ii) by ACH in normoxia nor (iii) with the ability of hypoxia to reverse ACH-induced relaxation. It has recently been suggested that EDRF is identical with nitric oxide (Moncada et al, 1987). The present finding, that hypoxia attenuated ACH-induced relaxation in CA and MA, but not in TA and FA suggests that either EDRF and/or the pathway for its production is not identical in all vessels.

Supported by the British Heart Foundation.

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# THE CHRONOTROPIC PROPERTIES OF EPANOLOL IN THE ANAESTHETISED DOG

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Epanolol (ICI 141292, [N-[-2-(3-o-cyanophenoxy-2-hydroxypropylamino)ethyl]-4-hydroxyphenylactamide) is a new cardioselective $\beta_1$-adrenoceptor partial agonist which has been developed to assess the role of modest partial agonism on the heart particularly in angina pectoris (Bilski et al. 1987). The partial agonist properties of the compound were evaluated in two series of experiments in the dog.

The potency and partial agonist activity were firstly determined in pentobarbitone anaesthetised vagotomised dogs which had been depleted of catecholamines by pretreatment with syrosingopine (5 mg $kg^{-1}$s.c., 24 hours prior to experimentation). Heart rate and blood pressure were monitored and blood gasses, pH and body temperature were maintained within physiological limits. Chronotropic dose response curves were determined first to isoprenaline (in the range 0.01-10μg $kg^{-1}$) and then to epanolol (0.3-1444μg $kg^{-1}$). In a further series of experiments; the ability of epanolol to modulate the response of the heart to sympathetic nerve stimulation was determined using anaesthetised dogs. In these, the stellate ganglia were located and crushed and the vagal nerves sectioned in the neck. Frequency - response curves relating changes in heart rate to right ansa subclavia stimulation were obtained in the absence or presence of increasing intravenous concentrations of epanolol (5-50μg $kg^{-1}$). The antagonist potency of epanolol, expressed as an effective dissociation constant, $K_B$ was determined using the method of Bilski et al. 1979.

In the catecholamine-depleted dog the agonist potency of epanolol, as determined by its $ED_{50}$, was found to be 5.2±0.9 μg.$kg^{-1}$ (n=4). Its partial agonist activity was 22.3± 1.5 % of the maximum isoprenaline response. In the further experiments, epanolol acted as a classical partial agonist in that it raised the resting heart rate in accordance with its agonist properties and displaced the frequency - response curves to the right demonstrating β-adrenoceptor antagonist activity. Its potency as a β-adrenoceptor antagonist, $K_B$, was found to be 4.6±0.6μg $kg^{-1}$, a value similar to its agonist $ED_{50}$. A feature of this experiment was the inability of the sympathetic nerves even at high rates of stimulation to release enough noradrenaline to overcome the antagonism produced by epanolol. Consequently with increasing doses of epanolol, the magnitude of the sympathetic response to nerve stimulation was decreased. The overall effect was to limit the response to sympathetic stimulation to a level close to the partial agonist activity of the compound viz 22%.

In conclusion epanolol is a potent β-adrenoceptor partial agonist which should stabilise heart rate at a level close to the agonist activity of the compound.

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# INTERACTION OF HIMBACINE WITH ACETYLCHOLINE AT MUSCARINIC RECEPTORS OF RABBIT AORTA

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Muscarinic receptors in heart and smooth muscle are known to belong to different subtypes. Recently, a plant alkaloid himbacine has been shown to have potent competitive antimuscarinic activity with at least 10 times greater affinity for cardiac receptors than for those in ileum, uterus and trachea (Gilani & Cobbin, 1986). In these smooth muscle preparations, muscarinic receptors mediate excitatory responses whereas in atria they mediate inhibitory responses to cholinomimetics. Rabbit aorta responds to acetylcholine by relaxation (inhibition) provided that the preparation has undamaged endothelium (Furchgott & Zawadzki, 1980). This communication describes the interaction of himbacine with muscarinic receptors of rabbit aorta to study whether himbacine has selectivity for inhibitory muscarinic receptors other than those in the heart.

Rabbit aorta ring preparations were suspended in Krebs solution taking care not to damage endothelial surface (Furchgott & Zawadzki, 1980). Krebs solution was maintained at 37°C and aerated with 5% $CO_2$ in $O_2$. Each tissue was allowed to equilibrate for 2 h under the resting tension of 2 g and phenylephrine was then added to bring the tissue to a moderate tone. Acetylcholine (ACh) was administered in the absence and presence of antagonist using 40 min antagonist exposure time. Antagonist affinities were assessed using the method of Arunlakshana & Schild (1959).

ACh (10 nM - 1 uM) produced concentration-dependent inhibition of phenylephrine-induced contraction of aorta. Pretreatment with himbacine (0.3 - 3.0 uM) caused concentration-related and parallel displacement of the concentration-response curves to ACh to the right. Schild plot of the data yielded a $pA_2$ value of 7.13 (Table 1) with slope of the plot not significantly different from unity ($P > 0.05$). Affinity of himbacine for muscarinic receptors in aorta was similar to that in ileum ($P > 0.05$), but 12 times less than that obtained in atria. In contrast, atropine had similar affinities for muscarinic receptors in all three preparations.

Table 1 Antagonist affinities ($pA_2$) of himbacine and atropine in cardiac and smooth muscle preparations

| Tissue | Response to cholinomimetic | $pA_2$ values* | |
|---|---|---|---|
| | | Himbacine | Atropine |
| Guinea-pig ileum | Excitatory | 7.27 ± 0.15 (15) | 9.10 ± 0.14 (14) |
| Guinea-pig atria | Inhibitory | 8.22 ± 0.10 (24) | 9.08 ± 0.11 (13) |
| Rabbit aorta | Inhibitory | 7.13 ± 0.15 (15) | 9.20 ± 0.19 (12) |

*Values shown are mean ± s.e.mean, together with number of observations in parentheses. Values other than aorta are taken from Gilani & Cobbin (1986).

These results suggest that himbacine does not differentiate between excitatory and inhibitory muscarinic receptors rather it exhibits selectivity for cardiac versus smooth muscle muscarinic receptors.

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# DETERGENT ABOLISHES CARBACHOL VASODILATATION AND CHANGES PRESSURE/ FLOW RELATIONS IN SITU IN RAT SUPERIOR MESENTERIC ARTERIAL BED

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Endothelium-dependent relaxation to acetylcholine occurs in the isolated superior mesenteric arterial bed of the rat perfused with Krebs-Henseleit solution (Byfield et al, 1986; Burdet et al, 1986; Hiley et al, 1987). In this preparation the endothelium can be removed by perfusion with detergents which include deoxycholate and CHAPS (3-[(3-cholamido-propyl)-dimethyl-ammonio]-1-propane sulphonate). Recently, it has been proposed that endothelium derived relaxing factor (EDRF), which is the mediator of endothelium-dependent relaxation to acetylcholine, is released tonically and modulates the resistance properties of small arterioles (Griffith et al, 1987). In this paper we report an investigation of the relaxant effects of carbachol on the *in situ* blood perfused superior mesenteric arterial bed and on the effects of perfusion of this preparation with CHAPS.

Male Wistar rats (270-350g; Bantin & Kingman, Hull) were anaesthetised with 120mg/kg sodium thiobutabarbitone (Inactin, BYK), given i.p., and prepared for *in situ* blood perfusion of the superior mesenteric arterial bed, at a rate of 2ml/min, essentially as described by Jackson & Campbell (1980) except that a trapped air system, to reduce pressure fluctuations, and a heat exchanger, to reheat blood to body temperature, were included in the extracorporeal circuit. Noradrenaline (Koch Light) and carbachol (Sigma) were administered close arterially into the extracorporeal circuit; the effects of carbachol were assessed as the reductions in the pressor response to a standard dose of noradrenaline (1μg, giving an increase in perfusion pressure of 135±9mmHg, n=14) co-administered with the test dose of carbachol (see Hiley et al, 1987). CHAPS was administered by perfusing the mesenteric vascular bed with an 0.3% solution in 0.9% NaCl for 150s at 2ml/min in place of blood.

Before perfusion with CHAPS, carbachol (1ng-3μg) gave dose related reductions in the pressor response to coadministered noradrenaline with an ED50 of 25.5±6.0ng and a maximal reduction of 51.8±1.9% (n=8). After CHAPS, carbachol was without any significant relaxant effect and the mean reduction of the noradrenaline response with 3μg was 10.7±6.4% (n=7). After perfusion with CHAPS the pressor response to noradrenaline (164±16mmHg; n=6) was not significantly different from control. Also, the relaxant response to 10μg sodium nitroprusside (Koch Light) was not affected by CHAPS; before treatment it was 47.3±3.4% and afterwards it was 45.5±2.4% (n=6 for both). Central arterial pressure, heart rate and haematocrit were not changed by the CHAPS perfusion.

Pressure/flow relations were established in control preparations and those treated with CHAPS using flow rates from 0.4-3.5ml/min. In the controls, the regression of pressure on rate gave a mean slope of 9.0±0.8mmHg.min/ml and a mean elevation ($\bar{y}$) of 34.7±1.6mmHg (n=6) whereas in the CHAPS perfused preparations the mean slope was 15.0±2.1mmHg.min/ml and $\bar{y}$ was 57.9±3.2mmHg. (n=6). Analysis of covariance showed both variables to be significantly different in the control and the CHAPS preparations with $P<0.01$.

Perfusion with CHAPS abolished the relaxant response of carbachol which is consistent with the functional destruction of the endothelium as seen *in vitro*. This treatment also increased the resistance to flow which may indicate an *in vivo* role for the endothelium in modulating vascular resistance.

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# EFFECTS OF ENALAPRIL ON CHANGES IN CARDIAC OUTPUT AND ITS DISTRIBUTION IN PITHED RATS WITH HYPOXIA AND HYPERCAPNIA

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We have previously shown that a low artificial ventilation volume (AVV) of 15ml/kg in air-ventilated pithed rats induces moderate acidosis, hypoxia and hypercapnia (MacLean & Hiley, 1988a). This induces an increase in cardiac output which is inhibited by propranolol. Angiotensin II increases cardiac output in pithed rats (Kaufman & Vollmer, 1985) and beta-adrenoceptor blockers inhibit renin release in rats (Oates et al, 1978). Here we investigate the possibility that the increased cardiac output induced by lowering AVV is mediated by angiotensin II.

Male Wistar rats (250-300g; Bantin & Kingman) were pithed under halothane anaesthesia. Both femoral arteries were cannulated to allow withdrawal of blood (at 0.5ml/min) during the microsphere injection and continuous monitoring of blood pressure (BP). When BP had stabilised after pithing, the rats were given either 0.5ml saline or 2mg/kg enalapril (Merck,Sharp & Dohme) i.v. For the next 10min the rats received an i.v. infusion of saline (0.1ml/min) after which 60000-80000 $^{113}$Sn-labelled microspheres (15μm diameter; NEN) were injected through a cannula into the left cardiac ventricle. Cardiac output and organ blood flows were determined by the method of McDevitt & Nies (1976). Rats were artificially ventilated at either 15ml/kg or 20ml/kg (54 cycles/min). Statistical comparisons were by analysis of variance; n=6 for each of the 3 groups.

Low AVV induced moderate acidosis (pH=7.34), hypoxia ($PO_2$=53.7mmHg) and hypercapnia ($PCO_2$=46.9mmHg) compared with controls (pH=7.47, $PO_2$=75mmHg, $PCO_2$=29.8mmHg). Low AVV increased cardiac output from 12.4±0.7 to 17.2±1.1 ml/min/100g body weight ($P<0.01$) and this increase was inhibited by enalapril ($P<0.05$), cardiac output being reduced to 14.1±0.9 ml/min/100g body weight. Enalapril decreased mean arterial pressure from 38.5±1.9mmHg to 23.0±2.4mmHg ($P<0.001$) and both low AVV and enalapril reduced total peripheral resistance ($P<0.01$) from 3.3±0.2mmHg.min/ml.100g body weight to 2.4±0.2 and 1.6±0.1mmHg.min/ml.100g body weight, respectively. Low AVV increased cardiac output distribution to the heart (33%) and epididimides (46%) and increased blood flow through the heart (82%), liver (42%), spleen (76%), testes (47%), epididimides (92%), gastrointestinal tract (43%) and skeletal muscle (47%). All of these effects of low AVV, except that on skeleletal muscle were inhibited by enalapril. We have found that decreases in cardiac output induced by enalapril at an AVV of 20ml/kg are reversed by an infusion of angiotensin II (MacLean & Hiley, 1988b).

The results suggest that the increase in cardiac output, the changes in its distribution and consequential changes in organ blood flows, induced by low AVV in air-ventilated pithed rats are mediated by endogenous angiotensin II.

This work was supported by a grant from The Wellcome Trust.

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# EFFECTS OF FLOSEQUINAN, A NOVEL ARTERIOVENOUS DILATING AGENT, ON cGMP LEVELS IN RAT ISOLATED AORTIC STRIPS

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Flosequinan (F) (7-fluoro-1-methyl-3-methylsulphinyl-4-quinolone), a novel arteriovenous dilating agent (Cowley et al 1984), is on clinical trial in hypertension and heart failure. There are similarities between the cardiovascular responses to F and to sodium nitroprusside (SNP) in man (Kessler and Packer 1987), animals (unpublished) and in vitro (Yates 1988). The vasodilator effects of SNP and atrial natriuretic factor (ANF) are similar (Winquist et al 1984) and associated with increased cGMP levels. We therefore decided to determine if the relaxant activity of F is also associated with increased cGMP levels and have compared the three dilators at equiactive relaxant concentrations. The effect of the cGMP phosphodiesterase inhibitor M & B 22948 (MB) was also examined.

For determination of equiactive relaxant concentrations, spirally cut rat aortic strips from the thoracic aorta of male Wistar rats (200-250g) were suspended with an initial resting tension of 1g in 15ml organ baths containing Krebs bicarbonate buffer gassed with 95% $O_2$/5% $CO_2$ at 37°C (K). Vessels were contracted with noradrenaline $10^{-8}$M (NA) to achieve a sustained tone, 81% of maximal. Relaxant drugs were then added to the bath and changes in tension recorded over 5 min. Concentrations of F, SNP and ANF producing approximately 90% relaxation of the NA-induced contraction were determined.

For the measurement of cGMP levels, aortic strips were mounted on rigid nylon threads and suspended in K. The tissues were then transferred to K + NA for 600 secs and then to K + NA + the dilator agent at 90% relaxant concentration and incubated for 30, 90 or 300 secs. Control (C) tissues were incubated in K + NA. At the end of incubation the tissues were removed, rapidly freeze clamped and frozen in liquid nitrogen before being powdered and extracted in 10% w/v trichloroacetic acid. Lyophylised extracts were resuspended in deionised water and an aliquot acetylated for the determination of cGMP by radioimmunoassay (NEN RIA Kit). cGMP is expressed as fmol/mg protein.

SNP 5 x $10^{-8}$M produced maximal elevation of cGMP at 90 secs (C: 76 ± 26 fmol/mg (mean ± SEM), SNP: 510 ± 90 fmol/mg, n=7 $p<0.01$). ANF 5 x $10^{-8}$M produced maximal elevation of cGMP at 300 secs (C: 88 ± 16 fmol/mg, ANF: 1320 ± 314 fmol/mg, n=7 $p<0.001$). F $10^{-3}$M produced maximal elevation of cGMP at 90 secs (C: 82 ± 23 fmol/mg, F: 295 ± 110 fmol/mg, n=7 $p<0.01$). Each treatment produced significant elevations in cGMP ($p<0.01$) at 30, 90 and 300 secs. In another experiment MB, 5 x $10^{-4}$M, the maximum practical concentration, caused only a 30% relaxation of the tissue but was associated with a marked increase in cGMP (C: 36 ± 11 fmol/mg, MB: 216 ± 36 fmol/mg, n=6, $p<0.001$). Increase in cGMP preceded development of maximal relaxation after SNP, ANF, MB and F.

We conclude that the mode of vasodilator action of F may be associated with an increase of cGMP but F may differ from SNP and ANF since the increase in cGMP after F was less for equivalent relaxant concentrations. The weak relaxant activity of MB is associated with marked increases in cGMP.

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# EFFECTS OF FLOSEQUINAN, A NOVEL ARTERIOVENOUS DILATING AGENT, ON ISOLATED SMOOTH MUSCLE

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Flosequinan, a novel hypotensive agent (Sim et al 1988), is currently under clinical trial for use in both hypertension and heart failure. Studies in animals (unpublished) and man (Cowley et al 1985) suggest that it is an orally effective agent dilating both arteries and veins and having much in common with sodium nitroprusside (Kessler and Packer 1987). The work presented here was undertaken with the object of elucidating its effect on isolated smooth muscle.

Isolated thoracic aortae from male Charles River Wistar rats weighing 175-200 g were spirally cut and mounted under 1 g tension in Krebs bicarbonate buffer at 37°C gassed with 95% $O_2$; 5% $CO_2$ (K). Contractions were recorded with isotonic transducers on Lectromed recorders.

Tissues were contracted with noradrenaline (NA) $10^{-8}$M or KCl 30 mM to give contractions of 80.9 ± 1.4% and 81.3 ± 0.9% of maximum respectively. Drugs were given cumulatively to produce relaxation. Table 1 shows the concentrations required for 50% relaxation ($IC_{50}$).

Table 1 IC50 Values against noradrenaline and KCl contractions

| Drug | Noradrenaline $10^{-8}$M | KCl 30 mM |
|---|---|---|
| Flosequinan (F) | $8.7 \times 10^{-5}$M | $2.3 \times 10^{-4}$M |
| Sodium nitroprusside (NP) | $2.3 \times 10^{-9}$M | $9.7 \times 10^{-9}$M |
| Atriopeptin II (ANP) | $4.7 \times 10^{-9}$M | $2.8 \times 10^{-8}$M |
| 8-Br cyclic GMP (BrGMP) | $5.5 \times 10^{-5}$M | $4.0 \times 10^{-4}$M |
| Nifedipine (Ni) | $1.9 \times 10^{-7}$M | $9.1 \times 10^{-9}$M |
| Diazoxide (D) | $1.8 \times 10^{-5}$M | $5.6 \times 10^{-5}$M |

All drugs except Ni were more potent in relaxing NA than KCl induced contractions. Greater concentrations of NA or KCl resulted in higher IC50 values for F, NP, ANP, BrGMP and D suggesting antagonism of the mechanism of contraction. In calcium free K with EGTA ($1 \times 10^{-3}$M) + NA $10^{-8}$M, IC50 values for F or NP remained similar to those obtained in K + NA $10^{-8}$M. Using K+ Ni ($1 \times 10^{-7}$M) + NA $10^{-8}$M, IC50 values for F were similar to those obtained in K+ NA $10^{-8}$M. Against cumulative dose responses to NA, $10^{-4}$M F produced a shift to the right but $10^{-5}$M F produced only a small rightward shift. However, in depolarising K (38 mM KCl) cumulative contractions to $CaCl_2$ were not antagonised by F $10^{-4}$M. In addition, unlike slow calcium channel antagonists such as Ni or diltiazem, both F and NP produced no inhibition of $CaCl_2$ dose response curves in guinea-pig taenia caeci at concentrations up to $10^{-3}$M and $10^{-3}$M respectively.

These in vitro studies on smooth muscle provide further support for the similarity between F & NP; furthermore, contractions with greater dependence on involvement of internal, as opposed to external, Ca++ seem to be more sensitive to these agents.

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# EFFECT OF CHRONIC TREATMENT WITH ATENOLOL ON DEPRESSOR RESPONSES TO PRAZOSIN IN THE ANAESTHETIZED RAT

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We have previously shown that the depressor response to prazosin in the anaesthetized rat is enhanced following acute administration of propranolol or ICI 118,551 (Chess-Williams and Winn, 1987). In the present study we have examined whether this enhanced depressor response also occurs following acute administration of the $\beta_1$-selective antagonist atenolol and also if this effect is maintained after chronic pretreatment with this antagonist.

Male Wistar rats (250-350g) were anaesthetized with sodium pentobarbitone (60mg $kg^{-1}$ i.p.). Catheters were inserted into the carotid artery for the measurement of diastolic blood pressure (DBP) and heart rate (HR) and also into the jugular vein for the administration of drugs.

In the first group of rats (n=7) prazosin (100µg $kg^{-1}$ i.v.), alone, was administered, and changes in DBP and HR were recorded over the subsequent 30 min. In the second group of animals (n=8), atenolol (1mg $kg^{-1}$, i.v.) was administered 30 min before prazosin (100µg $kg^{-1}$). The final group of rats (n=7) were treated with one dose of atenolol (10mg $kg^{-1}$ s.c.) daily for 7 days before undergoing the procedure described for group 2.

Prazosin, alone, caused a significant ($P<0.01$) fall in DBP (38.6 $\pm$ 4.1 mmHg) within one minute of its administration, with a slight increase in HR. Although DBP recovered slightly over the next 30 min, it remained below resting levels. In the second group of rats, atenolol produced a fall ($P<0.001$) in HR of 71.5 $\pm$ 8.1 b $min^{-1}$ and a significant fall in DBP of 31.9 $\pm$ 9.2 mmHg ($P<0.05$), although DBP had returned to resting levels after 30 min, HR remained depressed ($P<0.001$). Subsequent administration of prazosin produced a fall ($P<0.001$) in DBP of 63.5 $\pm$ 4.6 mmHg. This was significantly greater than that produced by prazosin alone ($P<0.01$). Although DBP recovered over the next 30 min, pressure remained below resting levels. Whilst there was a slight increase in HR observed with prazosin alone, following atenolol, prazosin produced a significant bradycardia ($P<0.01$) of 19.0 $\pm$ 3.4 b $min^{-1}$.

In animals which had received atenolol for 7 days prior to the measurement of blood pressure the i.v. administration of atenolol when the rats were anaesthetized resulted in a fall in HR (44.0 $\pm$ 4.8 b $min^{-1}$; $P<0.01$), with no significant change in DBP. The bradycardia produced by atenolol under these conditions was significantly smaller ($P<0.05$) than that seen in animals not chronically pretreated with atenolol. The subsequent administration of prazosin caused a reduction in DBP (47.7 $\pm$ 3.6 mmHg; $P<0.001$) which was significantly smaller ($P<0.05$) than the hypotension produced in rats given prazosin in the presence of acute atenolol administration. However, the fall in pressure was not significantly different from that obtained after the administration of prazosin alone. Prazosin produced a bradycardia of 24.6 $\pm$ 5.2 b $min^{-1}$ ($P<0.05$) in rats given chronic atenolol. This was not significantly different from the bradycardia seen in the presence of acute atenolol treatment.

One explanation of these data is that a marked $\alpha_1$-adrenoceptor-mediated vasoconstriction occurs following acute atenolol administration, which is not maintained during chronic treatment with this β-blocker.

RCW is a Bristol-Myers lecturer

Chess-Williams, R. & Winn, M.J. (1987) Br. J. Pharmac. 91: 386P.

# SK&F 104078 DOES NOT SELECTIVELY ANTAGONISE $\alpha_2$-ADRENOCEPTORS IN THE RAT

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Despite their different anatomical locations, most experimental evidence suggest that pre- and postjunctional $\alpha_2$-adrenoceptors are similar pharmacologically. However, Ruffolo et al. (1987) have recently described the pharmacological profile of SK&F 104078 (SK&F), as $\alpha_2$-adrenoceptor antagonist which is significantly more effective at postjunctional $\alpha_2$-adrenoceptors than $\alpha_2$-receptors located prejunctionally. We have studied the effects of this compound at pre- and postjunctional $\alpha_2$-adrenoceptors and postjunctional $\alpha_1$-adrenoceptors both *in vitro* and *in vivo* to assess its reputed selectivity for postjunctional $\alpha_2$-receptors.

All experiments were performed using Sprague Dawley male rats (250-350 g). *In vitro* antagonist activity at $\alpha_2$-adrenoceptors was assessed against p-aminoclonidine (PAC) in the vas deferens preparation and at $\alpha_1$-adrenoceptors against phenylephrine in rings of the abdominal aorta. Experiments were performed as described by Doxey et al. (1983) and were controlled by BBC microcomputers. Concentrations of SK&F were used which evoked 3-20 fold shifts of the control agonist concentration-response curves. The specificity of SK&F for α-receptors was assessed by studying its effects on the responses to 5-HT in the rat aorta preincubated with prazosin (70 nM). *In vivo* antagonist effects of SK&F (1-5 mg/kg i.v.) were assessed against UK-14,304 induced inhibition of stimulation-evoked contractions of the vas deferens (pre $\alpha_2$), UK-14,304 induced pressor responses (post $\alpha_2$) and cirazoline induced pressor responses (post $\alpha_1$) in pithed rats as described by Doxey et al. (1983). Saline pretreated pithed rats were used as controls and groups consisted of 5-7 rats. Agonist potencies were expressed as $ED_{50}$ values (cumulative doses inhibiting the vas deferens by 50% or raising the diastolic blood pressure by 50 mmHg).

SK&F had $pA_2$ values of 6.47±0.15 (n=3) and 6.76±0.15 (n=3) at pre $\alpha_2$- and post $\alpha_1$-adrenoceptors in the rat vas deferens and aorta respectively. However, SK&F was slightly more potent at 5-$HT_2$ receptors in the rat aorta with a $pA_2$ value of 7.35±0.04 (n=3). In pithed rats, SK&F did not antagonise the effects of UK-14,304 in the vas deferens (UK-14,304 $ED_{50}$ values after saline and 5 mg/kg SK&F were 2.3±0.2 and 3.0±0.4 µg/kg). Higher doses of SK&F could not be tested. SK&F (1 mg/kg) produced a 2.2 fold shift of the UK-14,304 pressor response curve (UK-14,304 $ED_{50}$ values after saline and SK&F were 5.5±0.6 and 12.1±1.5 µg/kg $P<0.01$ Students t-test). Increasing the dose of SK&F to 3 mg/kg did not further antagonise the pressor responses to UK-14,304. At $\alpha_1$-adrenoceptors, SK&F produced a dose-related antagonism of cirazoline (cirazoline $ED_{50}$ values after saline, SK&F (3 and 5 mg/kg) were 0.7±0.1, 1.3±0.2 and 1.9±0.2 µg/kg).

In contrast to the results of Ruffolo et al. (1987), we have demonstrated that SK&F was able to antagonise prejunctional $\alpha_2$-adrenoceptors *in vitro*. *In vivo* there was relatively little difference between the antagonist effects of SK&F at post- junctional $\alpha_1$- and $\alpha_2$-adrenoceptors. *In vitro* data showed that SK&F is a slightly more potent antagonist of 5-$HT_2$ receptors than either $\alpha_1$- or $\alpha_2$-receptors. These results suggest that SK&F is not a selective antagonist at post $\alpha_2$-adrenoceptors in rats.

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# CENTRAL CARDIOVASCULAR EFFECTS OF NEUROPEPTIDE Y AND CLONIDINE IN THE RAT

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The rostral ventrolateral medulla (RVLM), a vasopressor region, and a vasodepressor area of the caudal ventrolateral medulla (CVLM) are thought to play an important role in the regulation of tonic and baroreflex mediated changes in blood pressure (Granata et al, 1986). Neuropeptide Y-immunoreactive terminals have been localised in these areas and this peptide coexists with adrenaline and noradrenaline in some perikarya of the RVLM and CVLM respectively (Chronwall et al, 1985; Everitt et al, 1984). The aim of this study was to determine the cardiovascular effects of NPY and clonidine, an alpha2-adrenergic agonist, following microinjection into these areas and to examine the possibility of a functional interaction.
Stereotaxic microinjections of drug or vehicle (0.9% saline) in a volume of 0.1ul, were administered to separate groups of male Wistar-Kyoto rats (250-320g), anaesthetised with a mixture urethane (1g/kg; i.p.) and alpha-chloralose suspension (60mgs/kg; i.p). Blood pressure (BP) and heart rate (HR) were measured for 2 hours post-injection via an indwelling intra-aortic cannula and treatments were compared using ANOVA (with the Bonferroni test for multiple comparisons). Following each experiment the brain was fixed in Faglu and 50um sections cut for verification of the injection site.

NPY (25pmol and 50pmol) microinjected into the CVLM resulted in a decrease in BP and HR but in the RVLM had no significant effects (table 1). Clonidine (10nmol) evoked a significant fall in BP and HR in both regions examined (table 1). Microinjection of saline into CVLM and RVLM produced no significant cardiovascular effects.
Submaximal doses of clonidine and NPY coadministered in the CVLM produced a reduction in BP and HR which was greater than with either drug alone but similar in magnitude to their sum.

Table 1 Changes in BP (mmHg) and HR (b/min) - 30 minutes postinjection. Results are means + sem, n=6-12 per group.

| DRUG | NPY(25pmol) | NPY(50pmol) | CLON(10nmol) | CLON(10nmol) +NPY (25pmol) | CLON(10nmol) +NPY (50pmol) |
|---|---|---|---|---|---|
| CVLM | | | | | |
| BP | -7.4± 2.2 | -12.3± 3.0* | -18.5± 3.9* | -23.5± 3.3* | -30.3±2.6* |
| HR | -87.2±21.2* | -73.9 16.5 | -103.0± 9.1* | -109.0±13.6* | -163.8±8.0* |
| RVLM | | | | | |
| BP | -5.1±4.1 | -1.7±3.1 | -21.6± 5.9* | -16.7± 4.6* | |
| HR | 2.1±5.6 | 1.7±5.8 | -54.6±12.2* | -68.8±20.1* | |

* p< 0.05 compared to saline

In conclusion, the cardiovascular effects of NPY appear to be restricted to the CVLM while clonidine has depressor effects in both the RVLM and CVLM. There does not appear to be a functional interaction between these two agents.

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Supported by the M.R.C., S.H.H.D. and B.H.F.

# ISOPRENALINE RESPONSES ON THE GUINEA-PIG ATRIA AND THE EFFECT OF ENDOGENOUS ADENOSINE

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It has been suggested that in the heart the response to catecholamines is attenuated by the increased levels of endogenous adenosine formed as a consequence of stimulation by the amine (Schrader et al. 1977). This effect of endogenous adenosine has been demonstrated in both the rat heart and atria (Dobson 1983, Dobson et al. 1986). However, we have been unable to show this effect on the guinea-pig Langendorff heart (Broadley and Wilson 1985). Atrial muscle is considerably more sensitive to the effects of adenosine than ventricular (Böhm et al. 1984), so it was decided to see if any effects of endogenous adenosine could be detected in guinea-pig isolated atria.

Isolated left (L.A.) and right atria (R.A.) were set up in Krebs-bicarbonate solution at 38°C gassed with 5% $CO_2$/95%$O_2$. Tension responses of left atria paced at 2Hz (5ms, threshold voltage + 50%) and rate responses of spontaneously beating right atria were recorded. Cumulative concentration-response curves to isoprenaline (ISO) were determined before and during incubation with adenosine deaminase (0.3U/ml (DEAM), dipyridamole ($2.0\times10^{-6}$M) (DiPY) or deoxycoformycin ($1.5\times10^{-7}$M) (DEOX). Pretreatment curves were corrected using control experiments.

| Treatment | Control EC50 for ISO ($10^{-8}$M) | | Treated EC50 for ISO ($10^{-8}$M) | | Effect of Treatment Alone | |
|---|---|---|---|---|---|---|
| | R.A. | L.A. | R.A. | L.A. | R.A. | L.A. |
| DEAM | 1.35 | 6.18 | 1.3 | 3.53* | 0 | + |
| DiPY | 4.84 | 2.29 | 7.55 | 3.04 | - | 0 |
| DEOX | 9.24 | 3.3 | 7.19 | 3.17 | 0 | + |

*=Significant difference between treated and control ($P<0.05$) +/- = significant +ve or -ve effect on basal tension or rate when compared to untreated controls ($P<0.05$).

Although minor but significant changes in the cumulative concentration-response curves were detected with some of the treatments used, only in the case of DEAM treatment of L.A. did this cause any significant change in the EC50 and even this was a leftward shift of less than 2 fold! Depression of maximum response to ISO was only observed with DiPY treatment of R.A. and this is probably due to the direct effect of DiPY itself rather than endogenous adenosine.

Therefore it would seem that even in atrial muscle endogenous adenosine does not markedly attenuate responses to catecholamines. These experiments add further evidence that in the normoxic guinea-pig heart the modulation of the whole heart response to catecholamines by endogenous adenosine is of little significance.

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# THE EFFECTS OF ADENOSINE AND NECA UPON NORADRENALINE AND POTASSIUM CONTRACTED RABBIT AORTA

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Noradrenaline (NA) and high concentrations of potassium chloride (KCl) induce sustained contractions of rabbit aortic spirals, which are dependent upon Ca++ influx through Receptor Operated Channels (ROCs) and Voltage Operated Channels (VOCs), respectively (Karaki 1987). Nitro vasodilators and organic calcium antagonists, for example sodium nitroprusside (NaNP) and verapamil (Ver), selectively relax NA and KCl contracted tissues respectively (Karaki 1987). This study examined the effects, upon NA and KCl contracted aortic strips, of adenosine (Ad) and the adenosine agonist 5'-N-ethylcarboxamidoadenosine (NECA) and compared them with the NaNP and Ver.

Helical strips of aorta (40x5mm) from male New Zealand Rabbits (1.5-3kg) were stripped of endothelium and suspended in Krebs-bicarbonate solution at 37.5°C, under a maintained 1g tension, gassed with 5% $CO_2$ in $O_2$. Sub-maximal doses of $10^{-6}$M NA and 3.71g/l KCl (51mM K+) were used throughout.

NA and KCl produced sustained contractions of 2.37±0.12g (n=22) and 2.44±0.19g (n=23) respectively, which were not significantly different. NaNP produced dose-dependent relaxations of NA and KCl contracted tissues. The maximum relaxation was significantly greater ($P<0.05$) for the NA (2.4±0.29g, $7.6\times10^{-5}$M, n=6) than for the KCl contracted tissues (1.78±0.16g, $7.6\times10^{-5}$M, n=6). The geometric mean concentration of NaNP producing a 1.5g relaxation of NA ($1.3\times10^{-7}$M, n=5) was significantly lower ($P<0.05$) than that required in KCl contracted tissue ($4.4\times10^{-7}$M, n=4). These two facts together indicate the greater sensitivity to NaNP of the NA contracted tissues. Ver also relaxed NA and KCl contracted tissues in a dose-dependent manner. The maximum relaxation was significantly greater ($P<0.01$) for the KCl (2.8±0.46g, $1.3\times10^{-5}$M, n=5) than for the NA contracted tissue (1.06±0.16g, $1.3\times10^{-5}$M, n=5). The geometric mean concentration of Ver producing a 0.5g relaxation of KCl ($8.3\times10^{-8}$M, n=5) was significantly lower ($P<0.01$) than that in NA contracted tissue ($2.4\times10^{-6}$M, n=5). Together, these two facts indicate a greater sensitivity to Ver of the KCl contracted tissue.

NECA dose-dependently relaxed the NA contractions, with the maximum response (1.7±0.17g, n=5) at $1.95\times10^{-5}$M, but failed to relax the KCl contractions at concentrations up to $1.95\times10^{-5}$M (n=6). Ad also relaxed the NA contractions dose-dependently with the maximum response (1.81±0.34g, n=5) at $2.3\times10^{-3}$M. Ad produced a small but significant ($P<0.01$) dose-dependent relaxation of the KCl contractions with a maximum (0.44±0.13g, n=6) which was significantly less ($P<0.01$) than the maximum of NA contracted tissue. The geometric mean concentrations of Ad producing a 0.25g relaxation of NA ($5.8\times10^{-5}$M, n=5) and KCl contractions ($1.23\times10^{-3}$M, n=4) were significantly different ($P<0.01$) and together with the greater maximum response in NA contracted tissues this indicates that the NA contractions are more sensitive to Ad. Ad in the presence of dipyridamole ($10^{-5}$M) failed to relax KCl contractions at concentrations up to $2.3\times10^{-3}$M. This suggests that an intracellular Ad sensitive site was responsible for the relaxation of KCl contracted tissue by Ad. This was backed up by the failure of NECA, not a substrate for purine transport (Collis et al. 1983), to relax KCl contracted tissues. Both Ad and NECA selectively relaxed NA contractions, and in this respect resembled NaNP more closely than Ver. We therefore postulate that in the rabbit aorta Ad and NECA selectively inhibit influx of Ca++ through ROCs.

Supported by a SERC Case Award with SK & F Research Ltd.

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# EFFECTS OF VASODILATORS ON TONE, CYCLIC GUANOSINE MONOPHOSPHATE LEVELS AND PHOSPHATIDYLINOSITOL HYDROLYSIS IN RAT AORTIC RINGS

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Isolated blood vessels contracted with noradrenaline (NA) or other agonists can be relaxed by acetylcholine (ACh) or sodium nitroprusside (SNP). Both the endothelium-dependent and the endothelium-independent vascular relaxation produced by ACh and SNP respectively, are mediated *via* stimulation of soluble guanylate cyclase and increased levels of cyclic guanosine monophosphate (cGMP) (Rapoport & Murad, 1983). The ability of ACh or SNP to relax vascular smooth muscle may be dependent on the nature of the agonist used to raise the tone. There is evidence that NA-induced contraction of isolated aortic rings is associated with hydrolysis of phosphatidylinositol (PI) and that this PI response and the associated contraction can be inhibited by vasodilators that elevate cGMP levels in vascular smooth muscle (Rapoport, 1986) but KCl-induced contractions, which are not associated with PI hydrolysis, are less sensitive to such vasodilators (Furchgott, 1983).

This study sought to determine whether the relaxation of rat aortic rings is affected by the nature of the contractile agonist used and the intracellular coupling mechanisms involved.

Aortic rings (2-3 mm in length), prepared from the descending thoracic aorta of male Wistar rats (200-250 g) were suspended, with an initial resting tension of 2 g, between wire hooks in 25 ml organ baths, containing Krebs bicarbonate buffer gassed with a mixture of 95% $O_2$/5% $CO_2$. Isometric tension was measured with Statham force displacement transducers and displayed on a Linseis recorder. Tone was raised with either NA or KCl ($EC_{50}$ concentrations) and, subsequently, at various times after adding the relaxant drug (ACh, SNP) tissues were frozen in liquid nitrogen, then homogenised and extracted in trichloroacetic acid 5% w/v. The cGMP content of an aliquot of each extract was determined by radioimmune assay. In separate experiments the rate of PI hydrolysis was monitored by measuring the levels of phosphatidic acid (PA) using the lipid extraction method of Lloyd et al (1972).

In tissues contracted with KCl, the ability of ACh ($10^{-5}$ M) to induce vascular relaxation was significantly less than in tissues contracted with NA (NA: 96.9 ± 2.3%; KCl: 21.2 ± 5.3%; mean ± S.E. mean, n ≤ 6, P<0.01). SNP ($10^{-6}$ M) relaxed KCl-contracted tissues but again the degree of relaxation was significantly less than that observed when the tone was raised with NA (NA:100.0 ± 1.6%; KCl: 75.5 ± 3.9%; n ≤ 6, P<0.01). The maximum cGMP level associated with ACh-induced relaxation in KCl-contracted tissues was significantly lower than that obtained with NA-contracted tissues (NA: 324.1 ± 80.4 pmol.$g^{-1}$; KCl: 28.3 ± 9.5 pmol.$g^{-1}$; mean ± S.E. mean, n ≤ 6, P<0.05). A similar effect was observed with SNP ($10^{-6}$ M) (NA: 3350.9 ± 435.3 pmol.$g^{-1}$; KCl: 649.5 ± 198.4 pmol.$g^{-1}$, n ≤ 6, P<001). Both ACh and SNP reduced the rate of NA-induced PI hydrolysis but neither ACh nor SNP reduced the low level of PI hydrolysis associated with KCl-induced contractions.

K.J.M. is an M.R.C. Research Student.

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# INVOLVEMENT OF GUANYLATE CYCLASE IN OVERFLOW OF RADIOACTIVITY IN RAT ATRIA INCUBATED IN [$^{3}$H]-NORADRENALINE AND [$^{14}$C]-CHOLINE

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Complex interactions occur between neurotransmitters in atria where the sympathetic and parasympathetic nerve terminals are in close apposition (Ehinger et al, 1970). Auto-inhibition of noradrenaline (NA) and acetylcholine (ACh) release is accompanied by neuromodulation of ACh release by NA and of NA release by ACh (Boyle & Pollock, In Press). There is evidence that increased adenylate cyclase activity is associated with enhanced release of NA (Wikberg, 1979) and increased guanylate cyclase activity is associated with inhibition of NA release (Pelayo, 1978). This study re-examined the involvement of these enzymes in regulating transmitter release from autonomic nerves in atria. Field stimulation-induced release of radioactivity from atria previously incubated in ($^{3}$H)-NA and ($^{14}$C)-choline was examined in the presence of drugs that selectively stimulate these enzymes or prevent the breakdown of the cyclic nucleotides they synthesise.

Paired, spontaneously-beating atria from male Wistar rats (200-250 g) were incubated in ($^{3}$H)-NA (500 nM, 43 Cimmol$^{-1}$, 30 min, 37°C) and/or ($^{14}$C)-choline (550 nM, 43 Cimmol$^{-1}$, 60 min, 37°C) and inserted into silver ring electrodes in 2 ml organ baths, containing Krebs bicarbonate buffer, gassed with $O_2$ 95%/$CO_2$ 5% and containing eserine ($10^{-6}$ M) and hemicholinium ($10^{-5}$ M). Atria were stimulated with supramaximal voltage, 1 ms pulses and trains of 60 pulses at 2 Hz, if incubated in ($^{3}$H)-NA alone or trains of 525 pulses at 5 Hz, if incubated in ($^{3}$H)-NA and ($^{14}$C)-ACh. Krebs buffer bathing the atria was collected during and between stimulations and the radioactivity in each sample was counted.

Forskolin ($10^{-5}$ M) increased $^{3}$H overflow by 71 ± 3% (mean ± SE mean, n = 4, 0.01>P>0.001) in atria previously incubated in ($^{3}$H)-NA. This enhancement was not greater than that produced by the vehicle, dimethylsulphoxide (1% v/v), which increased $^{3}$H overflow by 86 ± 13% (n = 5, 0.01>P>0.001). Sodium nitroprusside ($10^{-5}$ M, SNP) increased $^{3}$H overflow by 56 ± 6% (n = 5, 0.01>P>0.001). Overflow of $^{3}$H was also increased by the permeant cyclic nucleotide, 8 bromo-cyclic guanosine monophosphate ($10^{-5}$ M) by 31 ± 5% (n = 4, 0.01>P>0.001), by isobutylmethylxanthine ($10^{-5}$ M) by 47 ± 5% (n = 6, 0.05>P>0.01) and by atropine ($10^{-6}$ M) by 50 ± 8% (n = 4, 0.01>P>0.001). The combination of SNP ($10^{-5}$ M) and atropine ($10^{-5}$ M) did not enhance $^{3}$H overflow above the level produced by either drug alone. SNP ($10^{-6}$ M) inhibited $^{14}$C oveflow by 47 ± 3% (n = 5, 0.01>P>0.001) in atria previously incubated in ($^{14}$C)-choline. These results do not support the view that increased NA release is associated with enhanced adenylate cyclase activity but suggest instead that increased guanylate cyclase activity may be involved. The ability of SNP to increase $^{3}$H release may be indirect, involving guanylate cyclase in cholinergic nerves, resulting in removal of ACh-mediated restraint on NA release.

S.J.B. is a University of Glasgow Postgraduate Scholar.

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# INFLUENCE OF α-ADRENOCEPTOR RESERVE AND [$K^+$] ON THE INHIBITORY EFFECTS OF CROMAKALIM AND NITRENDIPINE IN DOG SAPHENOUS VEIN

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It has been proposed that the relative potency with which $Ca^{2+}$-antagonists inhibit contractions produced by receptor agonists can be greatly affected by the number of spare receptors in the tissue (Ruffolo et al, 1984). We have studied the influence of the alpha-receptor reserve on the inhibitory effects of the $K^+$-channel opener cromakalim,(CROM:BRL 34915; Buckingham et al, 1986; Weir and Weston, 1986) using phenylephrine (PHE)-induced contractions in dog saphenous vein after $alpha_1$-receptor occlusion with phenoxybenzamine (PBZ). Rings of dog saphenous vein (SV), were denuded of endothelium and set up under 2g tension in Krebs'bicarbonate containing propranolol (1uM) at 37°C . $Log_{10}$ concentration response curves to PHE were obtained before and after increasing concentrations of PBZ (1nM-1 uM; 30 min incubation followed by 30 min wash). The receptor reserve (q, % receptors remaining after PBZ) was calculated from $K_A$ plots using the methods of Furchgott and Bursztyn (1967). Concentration response curves to PHE were also made before and after 30min incubation with CROM or nitrendipine (NTR) in control tissues and after PBZ treatment.
PBZ non-competitively antagonized the contractile responses to PHE and progressively removed the $alpha_1$-receptor reserve (Table 1). Response curves to PHE were shifted to the right of controls by CROM (0.1-30 uM) with little depression of Emax. After progressive receptor alkylation the displacement by CROM (1uM) of PHE-response curves (calculated at $EC_{50}$) was markedly increased (Table 1). In contrast the contractile effects of PHE were resistant to antagonism by NTR and removal of the alpha-receptor reserve with PBZ failed to modify the inhibitory activity of NTR (Table 1). Increasing the $[K^+]_{Ext}$ to 22.5 mM prevented the inhibitory effects of CROM but not NTR after PBZ.

Table 1.Influence of receptor reserve on the inhibitory effects of CROM or NITR in dog saphenous vein.

| | CROM (1uM) | | | NITRENDIPINE (1uM) | | |
|---|---|---|---|---|---|---|
| | CR | q (%) | %Δ Emax | CR | q (%) | %Δ Emax |
| Control | 4.3 | 100.0 | -14 ± 2 | 4.0 | 100.0 | -34.2± 4 |
| PBZ(nM) 1 | 4.2 | 36.8 | -8 ± 2 | - | - | - |
| 10 | 56.2 | 18.6 | -23 ±11 | 7.4 | 17.3 | -33.2± 2 |
| 100 | 105.0 | 4.2 | -14 ± 4 | 12.0 | 6.1 | -39.2± 6 |
| 1000 | 30.4 | 0.6 | -59 ± 2 | 0 | 0.7 | 0 |

CR,Conc-ratio at $EC_{50}$;q % receptors remaining after PBZ,n=4-10/group.

In conclusion PHE- induced contractions in SV are not dependent on $Ca^{2+}$-entry through dihydropyridine calcium-channels. Futhermore the increased inhibitory effects of CROM against PHE after receptor occlusion with PBZ is not simply explained by removing the alpha-adrenoceptor reserve, since this phenomenon was prevented by raising $[K^+]_{Ext}$ to 22.5 mM. Under the same conditions, the weak inhibitory effects of NTR were not modified by PBZ.

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# EFFECT OF INHIBITORS OF ION TRANSPORT ON 5-HT INDUCED INCREASES IN SHORT CIRCUIT CURRENT IN ISOLATED JEJUNUM

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5-HT has been reported to evoke a transient increase in short circuit current (SCC) in the rat jejunum in vitro which has been attributed to a stimulation of electrogenic $Cl^-$ secretion (Hardcastle et al, 1981). In rabbit ileum in vitro 5-HT has been shown to produce a similar transient increase in SCC and a sustained stimulation of non-electrogenic coupled NaCl secretion (Donowitz et al, 1980). We have used piretanide (Zeuthen et al, 1978), amiloride (Cuthbert et al, 1979) and acetazolamide (Waygood, 1955) as inhibitors of chloride, sodium and bicarbonate transport respectively to further investigate the ionic nature of the SCC response of rat jejunum to 5-HT.

Tissues were obtained from male Wistar rats (200-250g) killed by cervical dislocation and exsanguination. Stripped preparations of rat jejunum were mounted in a modified Ussing chamber and bathed with a Krebs' bicarbonate solution at 37±0.5°C, gassed with 5% $CO_2$ in $O_2$. The mucosal solution contained mannitol in place of glucose. Measurements were made of the changes in SCC evoked by 5-HT alone and following a 15 min incubation with acetazolamide, piretanide or amiloride. 5-HT ($10^{-6}$M) added serosally 30 minutes after tissue mounting caused a sub-maximal, transient increase in SCC of 21.7±3.8 $\mu amps.cm^{-2}$ (n=6) which reached a peak within 1-1.5 min. This response to 5-HT was unaffected by mucosal addition of amiloride ($10^{-3}$M) or by serosal addition of piretanide ($10^{-3}$M). This concentration of piretanide completely reversed the response evoked by $4x10^{-3}$M theophylline, a potent stimulant of $Cl^-$ secretion (Frizzell & Schultz, 1979). However, bilateral addition of the carbonic anhydrase inhibitor acetazolamide ($10^{-3}$M) significantly reduced the 5-HT response to 10.1±3.0 $\mu amps.cm^{-2}$ (n=6) but had no effect upon the response to theophylline. ($4x10^{-3}$M).

The results of the present study using pharmacological inhibitors of ion transport are not consistent with the suggestion that the transient increase in SCC evoked by 5-HT in the rat jejunum is mediated via a stimulation of $Cl^-$ secretion but are consistent with a stimulation of bicarbonate secretion.

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# INVESTIGATION OF ALUMINIUM NEUROTOXICITY USING RAT BRAIN REAGGREGATION CULTURES

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The central neurotoxicity of aluminium has been associated with various types of dementia. Four primary neurodegenerative conditions of late adult life, including Alzheimers Disease, exhibit neurofibrillary degeneration of cortical neurones which also contain elevated aluminium levels (see Crapper McLachlan & van Berkum, 1986). In Dialysis Encephalopathy aluminium is similarly associated with neurofilament abnormalities in cortical neurones but the neuropathological profile differs from that of Alzheimers Disease (Scholtz et al, 1987). Since cholinergic dysfunction is implicated in Alzheimers Disease and aluminium has been reported to inhibit central cholinergic function (Johnson & Jope, 1986) we have investigated whether cholinergic neurotoxicity occurs in rat brain reaggregate cultures. We have recently demonstrated specific cholinergic lesions in these cultures using another cholinergic neurotoxin, ECMA (Atterwill et al, 1988).

Whole brain reaggregates were cultivated from 16-17 day rat foetuses as previously described (Atterwill et al, 1984). Cells were grown for 9 days *in vitro* (9 DIV) in a serum - supplemented DMEM medium (10% FCS) at 37°C (9% $CO_2$/humidified air) with constant rotation at 70-80 rpm. 0.1 or 0.01 mM $AlCl_3$ was added at 9 DIV and again at 11 DIV. Reaggregates were harvested at 2, 48, 72 and 96h after initial treatment and assayed for ChAT activity, protein and neurofilament protein by an Elisa assay using an anti-neurofilament McAb.

Exposure to 0.1 or 0.01 mM $AlCl_3$ for up to 48 hours produced no change in ChAT activity in the brain reaggregates when compared to untreated control cultures (ChAT activity in the controls was similar to previously reported values of around 50 pmols/min/mg protein). However, after 72 and 96 hours both concentrations produced 30-40% losses of enzyme activity ($p<0.01$). No significant change in the content of neurofilament protein was observed although there were slight increases especially at 96 hours.

Despite the apparent connection between aluminium and dementia it was reported that central cholinergic activity was minimally affected in rabbits with aluminium-induced neurofibrillary tangles (Wisniewski et al, 1980). However, Johnson and Jope (1986) demonstrated inhibition of choline uptake, glucose metabolism and carbachol-stimulated phosphoinositide hydrolysis in synaptosomes. Our data support an inhibitory action of aluminium on intact, cultured cholinergic neurones. The specificity of this lesion is not yet known, nor whether cholinergic cell loss or direct inhibition of ChAT activity occurs. Nevertheless, it is clear that a substantial loss of neurones does not occur since neurofilament protein levels were not significantly reduced.

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# INTERACTION OF AMINOGLUTETHIMIDE AND SKF 525-A IN THE MOUSE

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Several studies have demonstrated that aminoglutethimide (AG) is metabolized partly by N-acetylation and partly by various hydroxylations in man (Foster et al. 1984) and mouse (Seago et al. 1985). The acetylation follows a polymorphic pattern (Adam et al. 1984). However, there is no information regarding the nature of the enzymes involved in the oxidative pathway, and the present preliminary in vivo experiment was conducted to clarify this aspect. The study involved the effect of the cytochrome P-450 inhibitor, SKF 525-A, on the sleeping time response to AG in mice.

Male albino mice (23-25g) were given either saline or SKF 525-A (either 20, 30 or 50mg/kg ip) 30 min before AG (250mg/kg suspended in 0.75% carboxymethylcellulose, ip). Pentobarbitone sodium (30mg/kg ip; metabolized) and barbitone sodium (200mg/kg ip; non-metabolized) were used in place of AG as controls. For each animal, duration of absence of the righting reflex was recorded. Plasma levels of AG were determined at 3, 6, 9 and 12h after dosing by a reverse-phase hplc method.

Table. Effect of SKF 525-A on hypnosis in mice induced by AG, pentobarbitone and barbitone.

| | Saline (control) | SKF 525-A (mg/kg) 20 | 30 | 50 |
|---|---|---|---|---|
| AG | 141 ± 16 | 158 ± 16 | 202 ± 23* | 374 ± 15* |
| Pentobarbitone | 22 ± 3 | 71 ± 11* | 84 ± 12* | 77 ± 10* |
| Barbitone | 98 ± 18 | 108 ± 15 | 103 ± 22 | 112 ± 31 |

Values are hypnosis time (min) mean ± s.e.mean, n=10, *P<0.05 compared with saline

The results in the Table show that, as anticipated, while SKF 525-A did not affect barbitone-induced hypnosis, it significantly (P<0.05) prolonged the effect of pentobarbitone. The action of AG was also significantly prolonged by SKF 525-A at 30 and 50mg/kg but not at 20mg/kg, indicating a different sensitivity compared with the effects on pentobarbitone. In addition, pretreatment of mice with the P-450 inhibitor metyrapone (29mg/kg ip) caused a two-fold increase in AG-induced hypnosis. In mice pretreated with SKF 525-A (50mg/kg), the plasma levels of AG were significantly (P<0.05) elevated above control values at all times studied. Pharmacokinetic parameters derived from these (STRIPE programme; Johnston & Woolard, 1983) showed that SKF 525-A was without effect on the apparent volume of distribution ($V_d$) of AG but significantly (P<0.05) prolonged plasma $t_{\frac{1}{2}}$ and decreased total clearance ($Cl_T$). Control and test values (mean ± s.e.mean, n=4) were: for $V_d$ 57±4 and 43±3ml, for $t_{\frac{1}{2}}$ 7.9±0.6 and 15.6±0.6h, and for $Cl_T$ 0.08±0.002 and 0.032±0.001ml/min respectively. In contrast, SKF 525-A (20mg/kg) did not influence the pharmacokinetics of AG.

The data are consistent with AG being metabolized in part by a P-450 system. The differential sensitivity of SKF 525-A towards AG and pentobarbitone may indicate that either different P-450 isozymes are involved in the oxidation of these two substrates or non-P-450 pathways have a dominant role in AG metabolism.

Financial assistance was received from Ciba-Geigy, Horsham.

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# THE IRREVERSIBLE BINDING OF DRUGS TO PROTEIN IN THE PRESENCE OF CHLORINE

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It has been suggested that the antimicrobial agent chlorhexidine may become irreversibly bound to protein in the presence of chlorine and hence render the protein immunogenic (Layton et al, 1986). Hapten formation is thought to proceed via a protein-reactive N-chloro derivative. The purpose of this study was to investigate whether the postulated mechanism of N-chlorination is applicable to other drugs. The drugs investigated were [$^{14}$C]chlorhexidine, [$^{14}$C]amodiaquine, [$^{3}$H]mianserin, [$^{3}$H]paracetamol, [$^{3}$H]sulphanilamide, [$^{3}$H]sorbinil and [$^{3}$H]ethinylestradiol.

Radiolabelled drug was pre-incubated with various concentrations of hypochlorous acid (0-100ppm, final concentration) in acetate buffer, pH 6.5, for 5 minutes before the addition of protein (human serum albumin). The reaction was stopped after 20 minutes by the addition of diethyl ether and the degree of irreversible binding determined by liquid scintillation counting after exhaustive solvent extraction.

Table 1. Irreversible binding of drugs to human serum albumin in the presence of chlorine (MEAN ± S.D.; N=3)

| DRUG | | DRUG:HSA (mmol: mol) | | |
|---|---|---|---|---|
| | $[Cl_2]$(PPM) | 0 | 10 | 100 |
| Chlorhexidine | | 920.0 ± 75.0 | 1132.0 ± 149.0 | 2632.0 ± 299.0 |
| Amodiaquine | | 8.2 ± 2.4 | 91.6 ± 8.3 | 586.6 ± 170.5 |
| Mianserin | | 0.3 ± 0.0 | 18.0 ± 3.0 | 327.0 ± 95.0 |
| Paracetamol | | 10.7 ± 0.8 | 140.3 ± 11.5 | 941.1 ± 48.8 |
| Sulphanilamide | | 28.0 ± 13.0 | 171.0 ± 44.0 | 209.1 ± 7.1 |
| Phenytoin | | 0.0 ± 0.00 | 1.3 ± 0.2 | 2.7 ± 0.8 |
| Ethinylestradiol | | 2.8 ± 0.2 | 3.2 ± 1.3 | 5.6 ± 1.1 |

Irreversible binding was detected for all the drugs, and was dependent upon the chlorine concentration. Although ethinylestradiol does not possess a nitrogen atom, it would appear that chlorine is able to oxidise the drug to a protein-reactive species. It has been reported that oestrogens become covalently bound to DNA in the presence of iodine (Blackburn et al, 1985), and chlorine, a more powerful oxidising agent than iodine, may act in a similar fashion. Agner (1972) demonstrated that sulphanilamide may be N-chlorinated by the myelo-peroxidase (MPO) system found in neutrophils. Generation of reactive species by chlorine may also occur when drugs are taken with drinking water that is heavily chlorinated for sterilisation purposes; normal chlorine concentration 0-2 ppm. N-chlorination may therefore provide a mechanism for the formation of immunogenic hapten-protein conjugates and cytotoxic intermediates.

This work was supported by ICI Pharmaceuticals.

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# KETOCONAZOLE TOXICITY IN RAT HEPATOCYTE SUSPENSIONS

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10% of patients on ketoconazole (KCZ) show transient abnormalities of liver function tests, but the incidence of severe, symptomatic hepatotoxicity is far lower ( $\sim$ 1 in 15,000) (Lewis et al., 1984). Buchi et al. (1986) demonstrated dose-dependent toxicity in the range 56-132uM KCZ in 24h primary cultures of rat hepatocytes. The toxicity was decreased by pretreatment of the animals with phenobarbitone (PB), but was unaffected by piperonyl butoxide pretreatment. Since primary cultures of rat hepatocytes exhibit differential changes in the levels of the isozymes involved in drug activation and detoxification by 24h, the mechanism(s) responsible were investigated using suspensions of freshly isolated rat hepatocytes.

KCZ in 0.05 M HCl was added to suspensions of hepatocytes ($10^6$ cells $ml^{-1}$) in Krebs Hepes buffer, pH 7.4, at a final concentration of 50-200uM and incubated at 37°C in 95% $O_2$/5% $CO_2$ for 3½ h. KCZ exhibited reproducible, dose dependent toxicity, manifested by a decrease in cell viability, preceded by depletion of intracellular reduced glutathione (GSH). After 3h hepatocyte viability was significantly reduced to 36 $\pm$ 14%, 11 $\pm$ 7% and 0% of initial viability at 140, 160 and 190uM KCZ respectively, compared with >80% viability of control suspensions. PB pretreatment markedly increased the toxicity of KCZ over the concentration range 50-200uM, in contrast to Buchi et al., 1986. Piperonyl butoxide pretreatment significantly decreased the toxicity of KCZ (160uM), increasing the viability at 3h from 11 $\pm$ 7 to 60 $\pm$ 5%.

GSH depletion was accompanied by an increase in oxidised glutathione (GSSG) in the medium. When hepatocytes were pre-incubated with diethylmaleate (DEM, 1.2mM for 3 min) to deplete GSH, there was an increase in toxicity. Pre-incubation with both DEM and buthionine sulphoximine (5mM) for 30 min resulted in a further increase in toxicity, with viability at 2h falling from 64 $\pm$ 7% in control suspensions to 2% in the presence of 160uM KCZ. At this concentration of KCZ, there was a 60% decrease in GSH conjugation with 1-chloro-2, 4-dinitrobenzene.

After 1h incubation GSSG reductase was slightly increased in the presence of 160uM KCZ (126 compared with 109 nmoles NADPH $min^{-1}$ $(10^6$ cells$)^{-1}$). Under the same conditions the elevation of Se-dependent GSH peroxidase was more marked. This suggests an increase in oxygen radical concentration.

N, $N^1$-diphenyl-p-phenylenediamine (DPPD) and dithiothreitol (DTT) are potent antioxidants. When DPPD (1uM) or DTT (5mM) were added to rat hepatocyte suspensions in the presence of KCZ, there was a marked increase in toxicity. At 3h viability of cells incubated with 120uM KCZ was 73 $\pm$ 5%, compared with 29 $\pm$ 9% when co-incubated with DPPD. A less marked effect was observed in the presence of DTT.

Compounds such as DPPD have been shown to undergo redox cycling via the formation of cation free radicals (Mason, 1982). Incubation of cells with DPPD and KCZ could therefore result in a further depletion of GSH compared with KCZ alone. Oxygen radical generation by redox cycling of KCZ could therefore be the determinant factor resulting in the dose dependent toxicity observed with KCZ in vitro. It is possible that this could account for idiosyncratic toxicity in individuals with compromised peroxidative enzymes or antioxidant systems.

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# TOXICITY OF MENADIONE AND MITOZANTRONE IN HUMAN HEP G2 HEPATOMA CELLS

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The cytotoxicity of the model quinone, menadione (M), and the anticancer quinone drug, mitozantrone (MZ), was measured in the human Hep G2 hepatoma cell line. The cytotoxic properties of quinones are thought to be mediated through one-electron reduction to semiquinone radicals catalysed mainly by NADPH cytochrome c reductase. Semiquinones are readily re-oxidised under aerobic conditions and participate in deleterious redox cycling which produces reactive oxygen species, causes oxidation of reduced glutathione (GSH) and ultimately results in cell death. In contrast, two-electron reduction catalysed by DT-diaphorase is considered to be a detoxifying reaction.

The cytotoxicity of M and MZ was measured in suspensions of Hep G2 cells ($10^6$/ml) incubated in Krebs-Henseleit buffer, pH 7.4, containing 12.5 mM Hepes. Toxicity was assessed by loss of viability (Trypan blue exclusion) and depletion of GSH. In addition, the ability of the two quinones to inhibit cell growth was measured by exposing monolayer cultures to the drugs for 4h, washing the cells and allowing the cells to grow for 48h before determining the cell number and protein content per flask. In some experiments specific enzyme inhibitors were present to investigate the mechanisms responsible for toxicity. Dicoumarol (30μM) was used to inhibit DT-diaphorase, aminotriazole (5mM) to inhibit catalase and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, 75μM) to inhibit glutathione reductase.

At concentrations above 50μM M caused a concentration dependent loss in viability within 120 min preceded by a rapid depletion of GSH which occurred within 5-10 min. The toxicity of M was exacerbated by the presence of dicoumarol, BCNU or aminotriazole indicating that the enzymes DT-diaphorase, glutathione reductase and catalase protect the cells and that M-induced toxicity involves one-electron reduction. MZ, at concentrations up to 200μM, did not alter the viability or GSH content of Hep G2 cell suspensions when incubated for 4h. However, in the presence of MZ plus dicoumarol the viability declined to 36.7 ± 5.1% (n=11) within 4h compared with 82.7 ± 1.2% (n=5) in controls. DT-diaphorase therefore detoxifies MZ. This decline in viability was not preceded by GSH depletion. MZ was not toxic in the presence of BCNU or aminotriazole indicating that this quinone was not undergoing significant one-electron reduction.

Growth inhibition studies showed that MZ was a more potent inhibitor than M. Dicoumarol potentiated the growth inhibition induced by M but not by MZ (Table 1).

Table 1 Inhibition of cell growth by M and MZ

| Drug | Cell numbers (% control) | Protein (% control) |
|---|---|---|
| 10μM M | 80.3 ± 12.3 (4) | 80.9 ± 9.7 (3) |
| 25μM M | 19.0 ± 5.4 (7) | 40.8 ± 3.5 (4) |
| 25μM M + Dicoumarol | 0 ± 0 (4) | 13, 13 |
| 10μM MZ | 11.7 ± 2.2 (4) | 58.3, 53.1 |
| 25μM MZ | 8.3 ± 0.9 (4) | 46.9, 53.7 |
| 25μM MZ + Dicoumarol | 16.4 ± 4.0 (4) | 58.2, 75.9 |

Values are means ± SEM, with the number of experiments in parentheses. Where only two experiments were carried out both values are given. Drug effects on both cell numbers and protein are expressed as % of control values.

MZ toxicity is therefore not mediated through the one-electron reduction pathway which is accepted for menadione.

# THE ANTI-INFLAMMATORY ACTIVITY AND DISTRIBUTION OF INDOMETHACIN FOLLOWING ORAL AND TOPICAL ADMINISTRATION

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The successful use of non-steroidal anti-inflammatory drugs, such as indomethacin, in the treatment of arthritis is limited by their frequent induction of gastro-toxic symptoms and by their failure to completely control the inflammatory reaction. Attempts to reduce the gastro-toxicity of these drugs by the use of formulations designed to decrease gastric mucosal contact of the drug have not proved entirely satisfactory as ulceration has been shown to occur even with rectally administered indomethacin indicating that this effect is mediated via the circulating drug rather than a simple local irritant effect (Taylor et al 1968). The question arises, therefore, whether the anti-inflammatory activity of these drugs may also be due to an effect in the circulation rather than directly in the tissues.

In this study the distribution and activity of indomethacin were determined following either the topical application of 300mg of a 1% hydro-alcohol gel to each hind paw or the oral administration of an aqueous suspension (6mg/kg). The drug preparations were administered to groups of 5 rats 30 minutes before the sub-plantar injection of 0.1ml of 1% carrageenan. Paw volumes were measured with a mercury plethysmograph and the percentage inhibition of swelling determined by comparison with untreated animals. Serum and paw levels of indomethacin were determined by a modified version of the method of Skellern and Salole (1975).

Table 1 The anti-inflammatory effect and paw and serum levels of indomethacin.

| Time after carrageenan (min) | 30 | 60 | 120 | 240 |
|---|---|---|---|---|
| Oral administration | | | | |
| Inhibition of swelling (%) | 27 | 24 | 39* | 51** |
| serum drug level (μg/ml) | 7.04±2.58 | 11.46±3.9 | 11.79±7.48 | 4.53±1.73 |
| paw drug level (μg/paw) | 4.62±3.21 | 3.20±0.98 | 4.77±0.82 | 3.99±1.35 |
| Topical administration | | | | |
| inhibition of swelling (%) | 0 | 15 | 20 | 39** |
| serum drug level (μg/ml) | 1.48±0.82 | 2.46±1.51 | 2.97±1.39 | 4.41±1.71 |
| paw drug level (μg/ml) | 11.56±3.95 | 3.52±1.52 | 5.71±2.28 | 5.46±1.85 |

The Figures are the mean (±) the standard deviation. *P<0.05 ** P<0.005

Despite the fact that the groups of rats treated with a topical preparation of indomethacin achieved consistently high paw levels of the drug the anti-inflammatory activity of the preparation was considerably inferior to that found when the drug was given orally. A 39% reduction in paw oedema was seen two hours after the carrageenan injection in the orally treated rats, but significant anti-inflammatory activity was not detected until 4 hours after carrageenan in the rats given topical indomethacin corresponding to a point where the serum drug level had risen to 4.41μg/ml. These results suggest that the anti-inflammatory activity of indomethacin may be expressed in the circulation rather than in the tissues.

The support of Merck Sharp & Dohme Ltd. is gratefully acknowledged.

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# CONTRIBUTION OF ARTERIAL CHEMORECEPTORS TO SALICYLATE - INDUCED HYPERVENTILATION IN ANAESTHETIZED RATS

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In previous experiments on salicylate-induced hyperventilation in anaesthetized rats (Birrell et al, 1987) we found that, whereas central actions accounted for most of the respiratory response in the later stages of a 4mgkg$^{-1}$ min$^{-1}$ i.v. infusion of sodium salicylate, there was some evidence for involvement of arterial chemoreceptors in the hyperventilation seen during the early stages of salicylism. We have used a lower dose of salicylate in the present study in order to obtain a better separation between the peripheral and central actions of salicylate on respiration, and have also compared the effects of intravenous injections of salicylate with those of infusions.

Male Wistar rats (240-360g) were anaesthetized with a long-acting thiobarbiturate (Inactin, 115mgkg$^{-1}$ i.p.) and respiration measured using a pneumotachograph and electrospirometer (see Bond et al, 1982). Sodium salicylate was administered intravenously by infusion (0.5mgkg$^{-1}$ min$^{-1}$), or by bolus injection (10-240mgkg$^{-1}$) administered at 3 min intervals. Arterial plasma salicylate concentration was estimated by colorimetery. Input to the CNS from functional arterial chemoreceptors was abolished by cutting both carotid nerves 15 min before starting to administer salicylate. Loss of chemosensitivity was verified by lack of response to injections of the peripheral chemoreceptor stimulant NaCN (10- 50µg i.v.), which contrasted with the reflex hyperventilation evoked in intact animals.

Infusion of salicylate in intact (control) rats caused a significant hyperventilation with respiration increasing significantly ($P < 0.05$, t test) above basal values (120 ± 5ml min$^{-1}$, mean ± s.e.m., n = 5) from 45 min onwards, reaching a peak of 235 ± 8ml min$^{-1}$ at 115 min. In rats with sectioned carotid nerves ventilation did not increase significantly above basal values (88 ± 11ml min$^{-1}$, n = 4) until 75 min after starting the infusion, and the peak value was 170 ± 35ml min$^{-1}$ at 115 min. Bolus injections of sodium salicylate also revealed significant differences between intact and denervated rats. A marked hyperventilation occurred during the first 30s following 150mg (cumulative dose) or more of sodium salicylate in intact animals, whereas no such response could be obtained at any dose in denervated rats. Central effects of salicylate appeared to be responsible for the delayed increase in basal R.M.V. which began 3-5 min after the injection of higher doses in both groups. Mean plasma salicylate levels did not differ significantly in the two groups.

The results from these experiments provide further evidence that salicylate initially increases respiration by stimulating peripheral arterial chemoreceptors; as the salicylate levels in the blood rise central actions of the drug also cause hyperventilation. Thus, in the early stages of salicylism symptoms such as hyperventilation appear secondary to activation of peripheral chemoreceptors.

This work was supported by a project grant from S.H.E.R.T.

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# THE EFFECT OF SULPHASALAZINE AND ITS METABOLITES ON COLLAGEN ARTHRITIS IN THE MOUSE

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Interest has recently been revived in the potential of sulphasalazine (SASP) as a disease-modifying anti-rheumatic drug and this has resulted in the identification of the metabolite sulphapyridine as the active moiety in rheumatoid arthritis (Pullar et al, 1985; Neumann et al, 1986). Experimental research into the mechanism of action underlying the anti-arthritic activity of SASP is hampered by the paucity of animal models of arthritis capable of detecting the activity of the compound. A recent report (Holmdahl et al, 1986) suggesting efficacy of SASP in mouse collagen arthritis offered the possibility of a suitable model for studying the effects of SASP and led us to investigate whether sulphasalazine itself or one of its metabolites, sulphapyridine or 5-amino salicylic acid might be responsible for activity in this model.

Arthritis was induced in male DBA/1 mice by injection of 100 µg of type II collagen in Complete Freund's Adjuvant into the tail and of 6-9 $x10^6$ BCG cells intraperitoneally. Mice received an i.p. booster injection of type II collagen and BCG cells 21 days later leading to development and progression of arthritis in the days immediately following. Compounds were administered orally, at molar equivalent dose levels, to groups of 8 mice, starting on the day of booster injection and continuing once daily for 3 weeks. Arthritis was assessed in terms of a subjective visual score of swelling and erythema, using a scale from 0 = normal to 4 = severe, for each paw. Summation of the scores for the four paws provided an individual total score for each animal from which group means were calculated. As a biochemical marker of the acute phase response to the inflammation blood samples were taken at the end of the experiment and their serum amyloid P content was determined using rocket immunoelectrophoresis.

Sulphasalazine (50, 100 and 200 mg/kg) reduced paw inflammation slightly at all doses but this reduction was not statistically significant as judged by the Mann-Whitney 'U' test. However, serum amyloid P levels were reduced in a dose-dependent manner compared with vehicle-treated controls (38.6% reduction at 200 mg/kg, $P<0.01$, Student's 't' test). At the end of the experiment the only statistically significant effect seen in the groups treated with sulphapyridine (32.5, 65 and 130 mg/kg) and 5-amino salicylic acid (20, 40 and 80 mg/kg) was at the highest dose of sulphapyridine, where there was a reduction in paw inflammation from a visual score of 11.1 in the control group to 7.8 in the treated group.

We conclude that SASP has only modest efficacy in reducing mouse collagen arthritis and that the active moiety is probably the intact SASP molecule. This would concur with recent findings in a model of arthritis in the mouse induced by the intra-articular injection of methylated bovine serum albumin (Hunneyball et al, 1986) and suggests that murine models of arthritis do not detect the clinically relevant activity of sulphasalazine, namely that of its metabolite sulphapyridine.

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# CHARACTERIZATION OF THE FORM(S) OF CYTOCHROME P-450 INVOLVED IN OESTROGEN 2-HYDROXYLATION

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The endogenous steroid oestradiol ($E_2$) and the synthetic steroid ethinyloestradiol ($EE_2$) are extensively metabolised to the catechols 2-hydroxy $E_2$ ($2\text{-}OHE_2$) and 2-hydroxy $EE_2$ ($2\text{-}OHEE_2$) by the cytochrome P-450 dependent mixed function oxygenase system in both rat and man (Ball & Knuppen, 1980; Maggs et al., 1983). We have attempted to characterise the selectivity of the form(s) of cytochrome P-450 towards the endogenous and synthetic oestrogens by using isozymes of cytochrome P-450 isolated from rats treated with phenobarbital (PB) or 3-methylcholanthrene (3-MC) (Wolf et al., 1986).

Incubations contained cytochrome P-450 isolated enzyme (2 nmols/incubation), cytochrome P-450 reductase (10,000 units/incubation), substrate ($E_2$ or $EE_2$, 25μM), ascorbic acid (1.0 mM), NADPH (0.6 mM) and 20 mM Tris HCl (pH 7.4; 3.0 ml). Incubations were terminated by ether extraction after 30 min. $2\text{-}OHE_2$ and $2\text{-}OHEE_2$ were quantified by hplc using a modified method of Purba et al. (1986).

The table shows oestrogen 2-hydroxylase activity of the purified forms of cytochrome P-450. The results are the average of 2 determinations.

TABLE $E_2$- and $EE_2$-hydroxylase activity of purified enzymes

| Purified Enzymes | $EE_2$ 2-hydroxylase (nMole $min^{-1}$ nMole $P\text{-}450^{-1}$) | $E_2$ 2-hydroxylase (nMole $min^{-1}$ nMole $P\text{-}450^{-1}$) |
|---|---|---|
| $PB_{1_a}$ | 0.51 | 0.00 |
| $PB_{1_b}$ | 0.20 | 0.12 |
| $PB_{2_a}$ | 0.18 | 0.46 |
| $PB_{2_b}$ | 0.06 | 0.30 |
| $PB_{3_a}$ | 0.01 | 0.04 |
| $PB_{3_b}$ | 0.05 | 0.65 |
| $MC_{1_a}$ | 0.00 | 0.49 |
| $MC_{1_b}$ | 0.02 | 0.47 |

$PB_{3_b}$ exhibited the highest $E_2$ 2-hydroxylase activity but in contrast showed low activity towards $EE_2$. $PB_{1_a}$ possessed the highest $EE_2$ 2-hydroxylase activity but had no activity towards the endogenous oestrogen. $E_2$ 2-hydroxylation was catalysed by both PB and 3-MC inducible forms of cytochrome P-450 whereas $EE_2$ appears to be metabolised by the PB inducible $PB_1$ gene family.

In conclusion, although certain isozymes show overlapping substrate specificity (eg $PB_{1_b}$, $PB_{2_a}$) there are major differences in the substrate selectivity of the PB and MC inducible cytochrome P-450s towards the endogenous and synthetic oestrogen.

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# THE INFLUENCE OF SOME LABORATORY ANAESTHETICS ON RENAL AND HEPATOSPLANCHNIC BLOOD FLOW IN THE RAT: A MICROSPHERE STUDY

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Pharmacokinetic data is often obtained from the anaesthetised laboratory animal. Anaesthetic-induced alterations in the pharmacokinetics of thiamine (Pipkin and Stella 1982), carboxyfluorescein (Woolfrey et al. 1985) and p-aminohippurate (Gumbleton et al. 1987) have been reported. The aim of this present study was to investigate the possible differential effect that some commonly used injectable anaesthetics may have upon regional haemodynamics in the rat, the significance of which may be relevant to laboratory pharmacokinetic studies.

The study was performed using male Wistar rats (270±19g) and the following anaesthetic regimens: (H) - Fentanyl & fluanisone (0.26 & 8.3mg/kg, Hypnorm) in combination with midazolam (4.16mg/kg, Hypnovel) given i.p.; (U) - i.p. urethane (1.75mg/kg); (P) - i.p. pentobarbitone (67mg/kg); (K) - i.p. ketamine (80mg/kg) in combination with i.p. midazolam (5mg/kg); (S) - i.v. alphaxalone & alphadolone (9 & 3mg/kg, Saffan). After the animals had reached a sufficient depth of anaesthesia the right carotid artery was catheterised, the tip of the catheter was manipulated into the left ventricle and 60000-80000 Sn-113 labelled 15 um microspheres were injected. Simultaneously blood was withdrawn from the left femoral artery at a constant rate of 0.43ml/min for 90 sec after microsphere injection. Cardiac output and regional haemodynamics were calculated as described by McDevitt and Nies (1976).

Table 1 Anaesthetics and Regional Haemodynamics (mean ± sd, n=6)

| | H | P | U | K | S | DMRT |
|---|---|---|---|---|---|---|
| Cardiac output (ml/min/100g BW) | 32.36 ±6.50 | 22.82 ±2.93 | 17.42 ±1.43 | 19.27 ±3.74 | 24.36 ±2.52 | H S P K U |
| Renal blood flow (RBF) (ml/min/g tissue) | 2.84 ±0.54 | 3.19 ±0.41 | 1.99 ±0.26 | 3.24 ±0.56 | 3.90 ±0.58 | S K P H U |
| Hepatosplanchnic blood flow (HBF) (ml/min/g liver) | 1.23 ±0.15 | 1.11 ±0.17 | 0.65 ±0.19 | 0.95 ±0.95 | 1.26 ±0.28 | S H P K U |

DMRT - analysis of variance and Duncan's test. The groups are ranked left to right in decreasing magnitude and those jointly underlined are not significantly different (P>0.05) from each other.

Using microsphere techniques a RBF of 4.7ml/min/g tissue (Idvall, 1980) and a HBF of 1.9ml/min/g tissue (Seyde and Longnecker, 1984) have been reported in chronically-catheterised conscious rats. Our results demonstrate that both RBF and HBF with S were almost twice that found with U anaesthesia whilst H, K and P gave intermediate values which nevertheless were significantly greater than U. Although cardiac output was noted to be significantly different between some anaesthetics RBF and HBF were found not to correlate with cardiac output. Such haemodynamic differences may result in anaesthetic-induced alterations in pharmacokinetics.

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# AMINOGLUTETHIMIDE AND THYMUS WEIGHT IN THE RAT

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In adult male rats the thymus undergoes progressive atrophy throughout life, and can be regenerated after orchidectomy (Fitzpatrick et al. 1985). Regeneration can be inhibited by treatment with either testosterone or oestradiol, and studies with the steroidal aromatase inhibitor 1,4,6-androstatriene-3,17-dione suggest that aromatisation of androgens to oestrogens may be at least partly responsible for involution of the thymus (Mander et al. 1986). In this study the effects on thymus weight of the non-steroidal aromatase inhibitor aminoglutethimide (AG) were studied in immature rats, where the thymus is well-developed.

Female rats, weighing approximately 50g at the start of treatment, were allocated into groups of 5 and treated daily for between 9 and 21 days with one of the following: 1 or 10mg testosterone (as mixed esters) in 0.1-0.2ml arachis oil s.c., 0.1 or 1mg oestradiol benzoate in 0.2ml arachis oil s.c., 0.2ml arachis oil s.c. alone; testosterone s.c. plus 2, 20 or 50mg/kg AG orally in distilled water containing 0.75% (w/v) sodium carboxymethylcellulose; AG alone. At the end of the treatment period the rats were killed by cervical dislocation under ether anaesthesia and the thymus dissected out, blotted dry, and weighed. In some experiments blood was collected by cardiac puncture for measurement of hormone concentrations.

Testosterone reduced the mean thymus weight from 3.1±0.1 (s.e. mean, n=15) mg/g body weight in solvent-treated rats to 2.5±0.1mg/g (n=10) and 1.4±0.1mg/g (n=5) in rats treated with 1 or 10mg respectively. Oestradiol (0.1 or 1mg) had a comparable effect on thymus weight to the 10mg dose of testosterone. The lowest dose (2mg/kg) of AG partially reversed the effect of 10mg testosterone (mean thymus weight in treated rats 2.0±0.3mg/g, n=5), but no dose of AG was able to reverse the effect of 1mg testosterone. The higher doses of AG caused an increase in thymus weight (20mg/kg: 4.3±0.3mg/g, n=5; 50mg/kg: 3.8±0.2mg/g, n=5).

These results confirm earlier findings (Mander et al. 1986) that both oestradiol and testosterone reduce thymus weight in rats. AG was however much less effective at reversing this than the steroidal aromatase inhibitor used by Mander et al. One factor responsible for this may be a feedback increase in gonadotrophin secretion due to inhibition by AG of oestrogen synthesis. This would stimulate ovarian steroidogenesis, thereby counteracting the effect of AG. Measurements of plasma oestradiol showed that concentrations in rats treated with 20 or 50mg/kg AG alone (0-370pM) were similar to those in control rats, whereas in rats treated with 2mg/kg oestradiol concentrations were less than 30pM. The steroidal aromatase inhibitor used by Mander et al. may be weakly androgenic, and thus suppress the rise in gonadotrophins due to aromatase inhibition. This may partly explain its more pronounced effect on thymus weight.

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# RESISTANCE OF HUMAN COLORECTAL CANCER CELLS TO THE ANTIMETABOLITE METHYLGLYOXAL BIS(GUANYLHYDRAZONE)

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The polyamines, putrescine, spermidine and spermine, are naturally occurring polycations which are essential for cell growth and differentiation. Intracellular polyamine concentrations and their biosynthetic enzyme activities are typically high in rapidly proliferating cells including certain neoplastic tissues. For example, in human colorectal cancer tissue, the polyamine content and biosynthetic enzyme activities are increased several fold over that of normal surrounding mucosa (Kingsnorth et al, 1984; Porter et al, 1987). Methylglyoxal bis(guanylhydrazone) (MGBG), a structural analogue of spermidine, is a potent, reversible inhibitor of polyamine biosynthesis known to inhibit the growth of many cell types at 10-20 μM concentrations (Warrell et al, 1983). Due to their high intracellular polyamine content it seems likely that human colorectal cancer cells would be sensitive to growth inhibition by this drug.

The aim of this study was to determine the effect of a range of concentrations of MGBG on the growth and polyamine content of human colorectal cancer cells.

HT29/219 cells were grown in culture in the presence or absence of MGBG (100 nM-150 μM). The drug was added at the time of plating. After 48h, the cells were harvested and the protein content, polyamine and MGBG concentrations determined as described previously (Wallace and Cameron, 1986).

The results shown represent the mean ± SD (n = 3); nd: not detected; *p < 0.05; **p < 0.01 by student's t-test.

| Additions | Protein Content (mg/plate) | Total Polyamine Content (nmoles/mg protein) | MGBG Content (nmoles/mg protein) |
|---|---|---|---|
| None | 0.80 ± 0.07 | 7.26 ± 0.71 | nd |
| 50 μM-MGBG | 0.44 ± 0.01* | 5.41 ± 0.18* | 10.80 ± 0.72 |
| 100 μM-MGBG | 0.36 ± 0.01** | 3.12 ± 0.42** | 12.93 ± 1.02 |

Unlike other cell types, HT29/219 cells were found to be resistant to MGBG with a concentration of 100 μM (5-10 fold higher than that used in other cells) causing only 55% inhibition of cell growth at 48h. This inhibition was accompanied by an almost equal (57%) decrease in the intracellular polyamine content. The apparent resistance was not due to the inability of cells to take up the drug since treatment with 100 μM MGBG resulted in an intracellular drug content of 13 nmoles/mg protein. This represents approximately a 14 fold intracellular accumulation of the drug suggesting active transport into the cells. This value is however markedly lower than in other cell lines e.g. in BHK-cells a 300-500 fold accumulation of MGBG has been observed (Nuttall and Wallace, 1987).

HT29/219 cells appear to be almost unique in being resistant to this drug. Resistance may be related to intracellular accumulation, the lack of which could be the result of increased metabolism or excretion.

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# INHIBITION OF FAST AXONAL TRANSPORT BY NERVE COMPRESSION IN THE GALACTOSE-FED RABBIT IS PREVENTED BY ICI 128463 (STATIL)

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Streptozotocin-induced experimental diabetes in the rat leads to an increased susceptibility of fast axonal transport to inhibition by acute nerve compression (Dahlin et al., 1986). This may be the result of intraneural accumulation of the sugar alcohol sorbitol, since the effect is prevented by administration of the aldose reductase inhibitor ICI 128436 (Statil) (Dahlin et al., 1987). The following experiments were carried out to determine if similar effects are produced in rabbits following galactose feeding, which leads to increased intraneural galactitol, increased nerve water content and, in rat sciatic nerve, elevated endoneurial fluid pressure (Myers et al., 1979).

Rabbits were fed a diet containing 50% galactose for 21 days over a 29 day period. One group of galactose-fed rabbits received the aldose reductase inhibitor Statil at a dose of 25mg.kg$^{-1}$ daily throughout the experiment. Of two groups of control rabbits fed normal diet one was treated with Statil and one was untreated. Rabbits were then anaesthetised and one nodose ganglion injected with 25μCi (5μl) $^{3}$H-L-leucine. Two hours later, the same vagus nerve was exposed and a 15mm length of nerve compressed with a chamber that maintained a pressure of 20mm Hg for a further 2 hours. The animal was killed and radiolabelled nerves were cut into 2.5mm pieces and processed for liquid scintillation counting (Dahlin et al., 1984). Contralateral nerves were assayed for myo-inositol, galactitol and total nerve water content.

The inhibition of fast axonal transport produced by compression was measured in terms of the amount of radiolabelled proteins that accumulated in the nerve proximal to the compression zone, relative to the amount of radiolabelled proteins in an unaffected piece of nerve (Dahlin et al., 1984). This ratio had a mean value ($\pm$ S.D.) of 0.93 $\pm$ 0.61 (n=10) in control animals; this was similar to the ratio in nerves subjected to no compression. After galactose feeding the ratio was increased to 12.97 $\pm$ 20.31 (n=5). The difference from controls was statistically significant at $P<0.02$. In galactose-fed animals treated with Statil the ratio was 0.85 $\pm$ 0.74 (n=5), which was not different from that in controls. Galactose feeding led to significant increases in nerve galactitol and water content but no change in nerve myo-inositol. Both increases were markedly reduced after treatment with Statil.

The results indicate that, as in experimental diabetes, the effects of mild compression can be additive with the osmotic changes produced by elevated nerve polyols to lead to an impairment of fast axonal transport.

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# THE EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES ON THE IN VITRO BLOOD N-ACETYLTRANSFERASE ACTIVITY OF THE ADULT MALE SPRAGUE-DAWLEY RAT

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We have previously shown that the mean in vitro blood N-acetyltransferase (NAT) activity of human diabetic (Type 2) subjects is significantly higher than that of healthy volunteers and the enzyme activities of both groups may be elevated by increasing the incubation glucose concentration (Lindsay et al, 1985). The objective of this study was to investigate the effect of drug induced diabetes on the in vitro blood NAT activity of the rat to determine if the activities in diabetic and non-diabetic animals displayed a similar pattern to that observed with human samples.

Streptozotocin in citrate buffered isotonic saline (pH 4.5) was administered (50 mg/kg i.v.) to 4 adult male Sprague-Dawley rats. Blood samples were collected one week later from these animals and from 6 untreated rats by piercing the heart after stunning and cervical dislocation. These samples were collected into potassium-EDTA anticoagulant containers and used immediately. Plasma glucose concentrations were determined enzymatically. In vitro blood NAT activity was determined using an initial sulphamethazine (SMZ) concentration of 0.18 mmol/l as previously reported (Baty et al, 1986). In a further set of experiments, the incubation glucose concentration was increased by 50 mmol/l.

The in vitro blood NAT activity of rats treated with streptozotocin was significantly ($p<0.02$) higher than that of untreated animals. Increasing the in vitro glucose concentration of blood samples from both groups significantly ($p<0.01$) increased the amount of acetyl-SMZ produced (Table 1).

Table 1

| Animals studied (number) | Mean (range) plasma glucose, mmol/l | Increase in incubation glucose concentration, mmol/l | Mean (range) amount of acetyl-SMZ produced, nmol |
|---|---|---|---|
| Non-diabetic (6) | 6.8 ( 4.5 - 7.8) | 0 | 2.20 ( 1.50 -3.48) |
| | | 50 | 4.74 ( 3.17 -8.35) |
| Diabetic (4) | 26.1 (22.6 -29.3) | 0 | 3.76 ( 2.95 -4.45) |
| | | 50 | 8.44 ( 6.95 -9.74) |

The comparative in vitro NAT activities and the effect of added glucose on this enzyme activity in blood samples from diabetic and non diabetic rats (Table 1) parallels the results obtained using human blood samples (Lindsay et al, 1985). This study therefore supports the hypothesis that elevated blood glucose concentrations increase in vitro drug acetylation. The significance of these results with respect to in vivo acetylation of SMZ, particularly the reported increased acetylation capacity of human diabetic subjects (Shenfield et al, 1982) will depend on both the contribution of blood NAT activity to the total acetylating capacity of the body and whether the levels of acetyl-CoA at other sites of NAT activity are limiting.

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# THE EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES ON THE IN VIVO ACETYLATION CAPACITY OF THE ADULT MALE SPRAGUE-DAWLEY RAT

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The objective of this study was to investigate if induction of experimental diabetes in the rat using streptozotocin significantly changed the in vivo acetylation capacity. The reported increased frequency of rapid acetylators amongst human diabetic patients (Shenfield et al, 1982) has been disputed (Ladero et al, 1982). An alternative explanation for the increased in vivo acetylation of sulphamethazine (SMZ) by diabetic subjects is that their higher blood glucose levels increases blood levels of acetyl-CoA, the acetyl group donor for the reaction. The apparent change in acetylator phenotype distribution could therefore be a biochemical artefact (Shenfield et al, 1982).

Sodium sulphamethazine in isotonic saline was administered (40 mg/kg p.o.) to 5 adult male Sprague-Dawley rats (Trial 1). Urine was collected between 0-4 h, 4-8 h, 8-12 h, 12-24 h, 24-32 h and 32-48 h . The concentrations of SMZ and it's acetylated metabolite in these samples were determined by HPLC (Baty et al, 1986). This experiment was repeated using the same animals one week later to determine the extent of intra-individual variation in acetylation capacity (Trial 2). After a further week, streptozotocin in citrate buffered isotonic saline (pH 4.5) was administered (50 mg/kg i.v.) to the animals which were then orally dosed with SMZ for a third time one week later (Trial 3).

Diabetes was successfully induced as evidenced by weight loss, diuresis, glucosuria and elevated plasma glucose levels. The mean (±S.E.M.) values of percentage acetylation by the rats on Trials 1-3 are shown in Table 1. Intra-individual variation of the in vivo acetylation of SMZ was insignificant ($p>0.05$). The correlation between the urinary values of percentage acetylation on Trials 1 and 2 was 0.99. Induction of experimental diabetes using streptozotocin significantly ($p<0.001$) reduced the extent of SMZ acetylation. The total amount of sulphonamide excreted renally was also significantly ($p<0.05$) reduced by this treatment.

Table 1

| Time interval of urine collection,h | Mean (±S.E.M.) percentage acetylation of SMZ | | |
|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 |
| 0 - 4 | 49.3 ± 2.2 | 49.4 ± 2.6 | 44.8 ± 6.2 |
| 4 - 8 | 59.7 ± 4.4 | 58.8 ± 3.5 | 53.5 ± 2.8 |
| 8 -12 | 60.3 ± 4.0 | 58.9 ± 3.8 | 47.6 ± 2.2 |
| 12 -24 | 57.8 ± 4.7 | 58.8 ± 4.4 | 47.4 ± 4.2 |
| 24 -32 | 49.5 ± 7.2 | 50.3 ± 6.2 | 36.2 ± 6.7 |
| 32 -48 | 32.2 ± 5.4 | 32.1 ± 6.4 | 21.3 ± 6.3 |

In conclusion, the results of this study do not support the hypothesis that the onset of diabetes increases the in vivo acetylation capacity of the rat.

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Shenfield, G.M. et al (1982) Diabetologia 22: 441-444.

# ISOLATED STRIPS OF HUMAN ENDOCERVICAL MUCOSA - A TISSUE FOR INVESTIGATING MYOFIBROBLAST DRUG SENSITIVITY

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The myofibroblast is a unique mesenchymal cell possessing dual properties of contractility and collagen production, i.e. those of the myoblast and fibroblast (Guber and Rudolph, 1978). It is considered to be responsible for various tissue reactions ranging from contraction of granulation tissue to Dupuytren's contracture (Rudolph, 1980) as evidenced from morphological studies (Optical/electron microscopy). Investigations of the physiology/pharmacology of myofibroblasts in human granulation tissue is limited as for obvious reasons it is under ethical restraint. This communication draws attention to a readily available, ethically acceptable and unexplored source of human myofibroblasts - the endocervical mucosa. The latter undergoes a progressive granulomatous change related to age and parity. Many human uteri have to be removed in the treatment of various gynaecological conditions, for example pelvic floor repair and from these tissues can be obtained samples of cervical mucosa.

Human uteri were obtained following hysterectomies, bisected and a strip, 18-22mm in length, 4-6mm in width and 2-3mm in thickness of the endocervix was removed, placed in chilled Krebs solution (4°C) and used either immediately (n = 6) or after overnight storage at 4°C (n = 40). They were then placed in a superfusion system using a flow rate of 2ml $min^{-1}$ and equilibrated for 1 hour. Tissues were placed under a resting isometric tension of 2g. Response heights or areas were calculated. Agonists administered were barium chloride (0.01-10mg), oxytocin (0.01-1iμ), 5HT (1-100μg) and mepyramine (0.01-1mg).

The tissue samples studied showed a range of different types of spontaneous activities. These were classified into five types, ranging from no activity to 0.1Hz contractions of up to 0.5g change of tension. All the preparations contracted to the agonists used but the threshold sensitivities, types and duration of responses were variable except for mepyramine which produced clearly defined effects in all the tissues studied. The order of sensitivity was mepyramine>oxytocin>5HT>barium ions. The type of response to an agonist was not predictable from the type of spontaneous activity a tissue possessed.

Despite some differences in these responses as compared with animal in vitro models of myofibroblasts, there are some similarities. Although barium ions contracted some of the preparations the sensitivity was low as compared with those needed for 'pure' smooth muscle preparations. In addition the lack of repeatable effects with 5HT and oxytocin but the reproducible effects with mepyramine suggests a similarity to myofibroblasts in the rat testicular capsule (Lal and Naylor, 1985). These results when taken in combination with the histological appearance of the tissue suggest that myofibroblasts in the human endocervical mucosa have a similar responsiveness to that found in rat tissues. Studies to elucidate the contractile mechanisms of the cell are in progress.

The authors thank the Yorkshire Clinic and the attending consultant gynaecologists for making the tissue samples available for this study.

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# THE ACTIONS OF ATRIOPEPTIN III IN MODELS OF CHRONIC RENAL FAILURE

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Recently we showed that DOCA-salt hypertensive rats exhibited an enhanced natriuretic and diuretic response to atriopeptin III at doses which had minimal blood pressure and renal haemodynamic effects (Johns & Rutkowski, 1987). In the present study we have assessed the sensitivity of the kidney to atriopeptin III in two models in which renal function is severely reduced chronically.

Male Sprague Dawley rats were used throughout. Chronic renal failure was induced by feeding a diet containing adenine (0.75%) and renal insufficiency was precipitated by combined unilateral nephrectomy and removal of 3/5 of the remaining cortex under pentobarbitone anaesthesia. Four-five weeks later, rats were anaesthetized with sodium pentobarbitone (60 mg/kg, i.p.) and prepared for renal function measurements (Johns, 1985). Fifteen minute clearance periods were used, two before and two after a period in which atriopeptin III was given as a bolus dose at the beginning of the collection. Three doses of atriopeptin III were given to each animal, 125, 250 and 500 ng/kg, in random order.

Chronic renal failure animals had lower renal blood flow and glomerular filtration rate compared to control animals, 7.5±1.1 versus 12.9±1.1 ml/min/kg ($p<0.01$) and 0.4±0.1 versus 2.4±0.2 ml/min/kg ($p<0.001$), respectively, whereas blood pressure, urine flow and fractional sodium excretion were higher, 164±14 versus 122±6 mmHg ($p<0.05$) and 75.2±13.4 versus 38.3±6.7 µl/min/kg ($p<0.05$) and 15.0±3.5 versus 1.7±0.3% ($p<0.001$), respectively. At all doses atriopeptin III was without effect on blood pressure and renal blood flow but filtration rate increased by 8% ($p<0.05$) at 500 ng/kg in the control rats and by 22% ($p<0.05$) at 250 and 500 ng/kg in the renal failure rats. Absolute sodium excretion was increased by 2.5±0.8, 2.4±0.6 and 3.4±1.0 µmol/min/kg (all $p<0.01$) in the control rats and by 1.7±0.5, 2.9±0.7 and 3.1±0.6 µmol/min/kg (all $p<0.01$) in the failure rats with increasing doses of atriopeptin III while fractional sodium excretion rose by 0.6±0.2, 0.9±0.2 and 0.83±0.3% (all $p<0.05$) in control and 1.84±0.8, 1.64±0.8 and 2.2±0.8% (all $p<0.05$) in the failure rats. Compared to unilateral nephrectomised control rats, those with renal insufficiency had a lower glomerular filtration rate, 0.8±0.1 versus 3.5±0.4 ml/min/kg ($p<0.01$), higher blood pressure, 165±5 versus 140±5 mmHg ($p<0.01$), urine flow, 80±18 versus 41±7 µl/min/kg ($p<0.01$) and fractional sodium excretion, 7.0±1.6 versus 1.0±0.3% ($p<0.01$). Atriopeptin III had no consistent effect on blood pressure or renal haemodynamics in either group, however, increasing doses of atriopeptin III increased absolute sodium excretion by 3.2±0.9, 3.8±1.8 and 7.1±1.9 µmol/min/kg (all $p<0.05$) in the insufficiency animals and by 2.6±0.7, 3.7±1.1 and 4.2±1.6 µmol/min/kg (all $p<0.05$) in the control rats, while fractional sodium excretion rose by 2.5±0.7, 3.08±1.3 and 3.3±1.2% (all $p<0.05$) in the insufficiency animals and by 0.6±0.2, 0.6±0.2 and 0.7±0.2% (all $p<0.05$) in control rats.

The results of this study showed that in chronic renal failure or insufficiency there was a greatly reduced filtration rate but water and sodium output was comparable to normal rats, consequently fractional sodium excretion was greatly elevated. Under these conditions, the natriuretic effect of the atriopeptin III was still evident and appeared to be somewhat enhanced.

This work was supported by the British Heart Foundation.

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# STUDIES ON THE RELAXANT EFFECTS OF PHORBOL DIACETATE ON GUINEA-PIG AIRWAYS SMOOTH MUSCLE

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Phorbol esters are known to stimulate the enzyme protein kinase C (Castagna et al., 1982) and have been shown to cause a contraction of various smooth muscle preparations (Spedding, 1987) including airways (Park and Rasmussen, 1985). These findings have lead to a view that activation of this enzyme may be implicated in mediation of the tonic component of muscle contraction (Park and Rasmussen, 1985). Other studies have shown a relaxant effect of phorbol esters on airways smooth muscle (Menkes et al., 1986) and a reduction in intracellular $Ca^{++}$ in carbachol stimulated canine trachealis muscle (Murray et al., 1987). We have investigated further the relaxant effects of phorbol diacetate (PDA) on guinea pig tracheas.

Helical strips of trachea were bathed in Krebs solution at 37°C and studied under isometric conditions. Half maximal contractions induced by carbachol ($1.6 \times 10^{-7}$M) were relaxed consistently by PDA ($EC_{50}$ $1.7 \times 10^{-7}$M). These responses were unaffected by the β-adrenoceptor antagonist propranolol ($10^{-6}$M) or by the cyclooxygenase inhibitor indomethacin ($10^{-6}$M). Preparations contracted by the $Na^{+}$ $K^{+}$ ATPase inhibitor ouabain ($10^{-5}$M) were relaxed by PDA with a similar $EC_{50}$ ($3 \times 10^{-7}$M) to that seen with carbachol contracted tissues.

To determine whether relaxation was mediated by the formation of cyclic AMP (cAMP), carbachol contracted tissues were relaxed with PDA ($3 \times 10^{-6}$M - $10^{-5}$M) and frozen rapidly in liquid nitrogen. Cyclic AMP was extracted from pulverised tissues using methanol and measured by a protein binding assay (Amersham). Resting levels of cAMP (18.5 ± 4.6 pmol/100mg tissue) were reduced significantly after the tissues were contracted with carbachol (8.9 ± 0.92 pmol/100mg tissue). Relaxation of the tissues with PDA ($3 \times 10^{-6}$M) to baseline was accompanied by a small elevation of cAMP (14 ± 3.3 pmol/100mg tissue), whereas higher concentrations of PDA ($10^{-5}$M) relaxed tissues below baseline tension and increased significantly cAMP content (23 ± 4.2 pmol/100mg) above carbachol control levels. Isoprenaline behaved in a similar manner to PDA and only increased cAMP content (20 ± 3.2 pmol/100mg tissue) at a dose ($10^{-6}$M) which reduced muscle tone below baseline.

In conclusion, it is unlikely that PDA relaxes guinea pig tracheas through the release of catecholamines or prostaglandins, or through activation of the $Na^{+}$ $K^{+}$ ATPase pump. Although the cAMP content of tracheas was elevated after PDA, greater effects were seen on muscle relaxation.

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# DOPAMINE SUPPRESSES THE NON-CHOLINERGIC CONTRACTION OF ELECTRICALLY STIMULATED BOVINE IRIS SPHINCTER MUSCLE

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Isolated strips of bovine iris sphincter muscle display biphasic frequency dependent contractions in response to electrical field stimulation (500μs pulse width, 1.25 to 40 Hz). The response has typically two clearly identifiable components, a fast atropine-sensitive contraction which commences immediately on stimulation and reaches a peak within 5 to 10 seconds, declining rapidly upon cessation of the stimulus, and a second, slower contraction which continues to develop after cessation, reaching its maximum up to 60 s later and relaxing slowly to the baseline. Abolition of the fast contraction with 10-6 M atropine reveals a small transient relaxation followed by the onset of the second component which is frequency-dependent, from 2.5 to 40 Hz, and unaffected by the presence of 10-5M atropine.

In the rabbit sphincter pupillae Butler et al (1980) and Zhang et al (1984) have shown a similar non-muscarinic component to be mediated through 'antidromic' transmission in primary afferent nerves of trigeminal origin, analagous to that occurring in the Triple Response in the skin, and have proposed that the mediator released from the peripheral terminals of these bipolar neurones is a substance P-like neuropeptide. It is not unreasonable to suppose that a similar mechanism operates in these two species and indeed in both rabbit and cow iris sphincter muscles exogenous substance P (SP) produces dose-dependent contractions comparable in profile to the slow component of this response to field stimulation. The present study sought to identify any naturally occurring substances which would antagonise this antidromic sensory effect, either by inhibiting the release of the putative mediator or its action on the sphincter muscle.

Of several neurohumoral mediators or modulators examined, dopamine (DA) 5 x 10-8 to 10-6 M produced a most striking inhibition of the maximum non-cholinergic response to field stimulation, with a corresponding shift of the log. frequency-response curve to the right, 10-6 M DA reducing by 90 per cent the maximal contraction to 40 Hz. In contrast the magnitude of the cholinergic component of the response to electrical stimulation was unaffected by 10-5 DA, as was the log. dose-response curve to carbachol (10-8 to 10-6 M). Contractions obtained with a maximal dose of SP (2.6 x 10-6 M) were, however, reduced by up to 50 per cent in the presence of 10-6 M DA, and a significant shift of the log. dose-effect curve to the right indicates that in achieving this effect DA acts at a post-junctional site where it may inhibit peptide-receptor interaction but does not interfere with muscarinic mechanisms.

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# 5-HT INDUCED RELAXATION IN ISOLATED HUMAN BRONCHUS

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Ergometrine has been reported to precipitate bronchospasm in a few patients. Following experience of such a case we investigated the effects of ergometrine in isolated human bronchus. This study (Hill et al., 1987) revealed that ergometrine had no direct constrictor effect and, at high concentrations, produced relaxation. We therefore decided to pursue this further, on the assumption that this action of ergometrine might be due to interaction with 5-HT receptors.

Human lung was obtained following lobectomy for carcinoma. Bronchi (internal diameter 3-6mm) were dissected out. Rings of bronchus were suspended between stainless steel hooks at a tension of 1-2g. Changes in tone were measured isometrically. The segments were suspended in Krebs-Ringer buffer, aerated with 95% $O_2$ / 5% $CO_2$. After equilibration all rings were challenged with depolarising Krebs buffer (120 mM KCl). Rings where contraction was observed were studied further. After washout the re-equilibration, all rings were treated with indomethacin ($10^{-5}$M) and then histamine (1-3μM). In each experiment 1-2 rings were used to construct concentration-response curves to 5-HT ($10^{-9}$-$10^{-5}$M). Other segments were exposed to the following drugs: methysergide (1μM or 10μM), ketanserin (10μM), 1-alprenolol (10μM) or spiroxatrine (10μM) before cumulative addition of 5-HT. Other rings were treated cumulatively with the central $5\text{-HT}_{1A}$ agonists 8-OH DPAT and ipsapirone ($10^{-9}$-$10^{-5}$M). Results below are expressed as geometric means with 95% confidence limits.

In 7 rings of bronchus from 5 individuals 5-HT produced concentration-dependent relaxation after histamine preconstriction. The $pD_2$ for 5-HT was -7.46 (± 1.0) and the maximal relaxation 151.4% (± 2.7) of the histamine preconstriction. The relaxation was not antagonised by any of the above drugs (n = 3-4). The maximum relaxation to 5-HT was 64.0% (±2.5) of that induced by forskolin (10μM). At very high concentrations (100μM) only, both 8-OH DPAT and ipsapirone induced relaxation, comparable in magnitude to the maximum 5-HT response.

Our findings extend and largely confirm those of Raffestin et al. (1985). They too noted that the response could not be blocked by the non-selective antagonist methysergide or the $5\text{-HT}_2$ antagonist ketanserin. We have found, in addition, that the $5\text{-HT}_{1A}$ antagonist spiroxatrine and 1-alprenolol, a beta-adrenoceptor blocker with $5\text{-HT}_{1A}$ and $5\text{-HT}_{1B}$ antagonist properties, were similarly ineffective. Although the $5\text{-HT}_{1A}$ agonists did produce relaxation, the concentrations required were too high to allow any conclusions to be drawn regarding receptor selectivity.

In conclusion, 5-HT relaxes isolated human bronchial smooth muscle. Its action may not be mediated by the "5-HT-like" receptors described by Feniuk et al. (1983), since the relaxant response is not blocked by methysergide. Further investigations are needed to characterise this response, particularly in terms of agonist (e.g. 5-carboxamidotryptamine) selectivity.

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# PHYSIOLOGICALLY ANTAGONISTIC $H_1$ AND $H_2$ RECEPTOR SUBTYPES IN RABBIT SECONDARY BRONCHI SMOOTH MUSCLE

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In vitro, histamine produces highly variable effects on respiratory smooth muscle depending upon the tone, region of the airways and species. In this study we have examined the contribution of $H_1$ and $H_2$ receptor subtypes in rabbit secondary bronchi smooth muscle using diphenhydramine ($H_1$ antagonist) and ranitidine ($H_2$ antagonist).

Male New Zealand White rabbits (2.0-2.5 kg) were sacrificed by cervical dislocation and exsanguination. The lungs were removed en bloc and secondary bronchi dissected free from extraneous tissue. Isometric tension was recorded from rings (2-3mm diameter) suspended under 1g initial tension in a 30ml organ bath containing Krebs-Henseleit solution aerated with 5%$CO_2$ in $O_2$ at 37°C. The rings were washed every 15 min over a period of 60-90 min. A submaximal concentration of histamine (30μM) was added to the organ bath to test the responsiveness of the smooth muscle rings to histamine. After washing and recovery of resting tension, a first cumulative concentration response curve (CCRC) to histamine (0.03μM-1000μM) was made on all preparations after which untreated preparations were used as time controls and others exposed to diphenhydramine or ranitidine for 30 min before subsequent histamine CCRCs. In a second series of experiments tone was induced with histamine (30μM) and then ranitidine (0.1μM) was added.

Time controls (for the diphenhydramine experiments) showed a slight shift to the right of the CCRC to histamine ($pD_2$: 1$^{st}$ CCRC, 5.58 (0.04); 2$^{nd}$ CCRC, 5.44 (0.05); 3$^{rd}$ CCRC, *5.15 (0.11) : mean(s.e.m.) ) but no significant change in the maximum tension. Diphenhydramine (0.001μM, 0.01μM and 0.1μM ) caused a rightward shift in the CCRC ($pD_2$: 5.40 (0.10) ; *5.16 (0.08) and *4.52 (0.24) respectively ) with no significant change in the maximum tension. After correction for the time controls the slope of the Schild plot for diphenhydramine was significantly less than unity (0.68) and the $pA_2$ was 7.90. Time controls (for the ranitidine experiments) showed small but non-significant shift to the right ($pD_2$ : 1$^{st}$ CCRC 5.54 (0.08); 2$^{nd}$ CCRC 5.54 (0.06); 3$^{rd}$ CCRC 5.38 (0.10) ). Ranitidine ( 1μM and 10μM ) did not cause a significant shift in the $pD_2$ values for the CCRCs to histamine ($pD_2$ : 5.58 (0.09) and 5.46 (0.10) respectively ) but produced a significant increase in the maximum tension. In the second series of experiments, the addition of ranitidine (10μM) to preparations with histamine-induced tone showed a 9% (2%) increase in tension.
(* $p<0.05$ compared to first control values).

The data indicates that rabbit secondary bronchi smooth muscle has both $H_1$ and $H_2$ receptor subtypes. At low histamine concentrations the $H_1$ receptor mediates contraction and this response can be blocked noncompetitively with diphenhydramine. However at high histamine concentrations, in addition, $H_2$ receptors, which can be blocked by ranitidine, mediate relaxation. Thus, at high histamine concentrations the contraction is the net result of excitatory $H_1$ and inhibitory $H_2$ influences. Consequently an increase in the " maximum " tension can be observed with ranitidine.

# CALCIUM DEPENDENCY OF THE HISTAMINE-INDUCED CONTRACTION IN RABBIT SECONDARY BRONCHI SMOOTH MUSCLE

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In airway smooth muscle, for example, guinea pig trachealis muscle, contraction induced by histamine and $PGF_{2\alpha}$ is considered to be dependent mainly upon extracellular calcium (Creese and Denbourough, 1981) whereas contraction induced by ACh is more dependent upon intracellular calcium (Ahmed et al ., 1985). In the present study we have investigated the calcium dependence of contraction to histamine in rabbit secondary bronchi smooth muscle and the effects on this of nifedipine and BAY-K 8644.

Male New Zealand White rabbits (2.0-2.5 kg) were sacrificed by cervical dislocation and exsanguination. The lungs were removed en bloc and secondary bronchi dissected free from extraneous tissue. Isometric tension was recorded from rings (2-3mm diameter) suspended under 1g tension in a 30ml organ bath containing Krebs-Henseleit solution aerated with 5% $CO_2$ in $O_2$ at 37°C. Calcium-depleted medium was made by omitting $CaCl_2$. The rings were washed every 15 min over an initial equilibration period of 60-90 min. A submaximal concentration of histamine (30μM) was added to the organ bath to test the responsiveness of the smooth muscle rings to histamine. After washing and recovery of resting tension, three washes at 5 min intervals were made with calcium-depleted Krebs-Hensleit solution. Paired rings were used as untreated controls or were exposed to nifedipine or BAY-K 8644 for 10 min before adding histamine (30μM). A cumulative calcium concentration-effect relationship was then obtained by a stepwise (x2) increase in $[CaCl_2]$ from 0.039mM to 5.0mM. Contractile responses were expressed as percentages of the response to the initial contraction to histamine (0.03mM) and $-\log EC_{30}$ values were calculated on this basis.

Addition of nifedipine or BAY-K 8644 did not affect the resting tension. When histamine (0.03mM) was added in $Ca^{2+}$-depleted medium there was an initial transient response followed by a small maintained elevation of baseline tension. In controls (for the nifedipine experiments) the readdition of $Ca^{2+}$ produced a concentration-related response reaching a maximum at 2.52mM $Ca^{2+}$ ($-\log EC_{30}$ : 3.90 (0.22); mean (s.e.m.)). Nifedipine (0.03μM, 0.3μM & 3.0μM) caused a concentration- related rightward shift in the concentration response curve to $Ca^{2+}$ ($-\log EC_{30}$ : 3.42 (0.16); *2.67(0.10); and *2.43 (0.09), respectively) . In controls (for the BAY-K 8644 experiments) a similar response was observed as described above ($-\log EC_{30}$ : 3.52 (0.23)). BAY-K 8644 (0.1μM) caused a leftward shift in the concentration response curve to $Ca^{2+}$ ($-\log EC_{30}$ : 4.26 (0.12)). (* $p < 0.05$ compared with control value ).

Thus the major part of the histamine-induced contraction of rabbit secondary bronchi smooth muscle was dependent upon extracellular calcium. Since these $Ca^{2+}$ - dependent contractions were inhibited by nifedipine and facilitated by BAY-K 8644 these results are consistent with histamine acting through the opening of voltage-dependent $Ca^{2+}$-channels.

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# IS FLUSPIRILINE A POTENTIAL LIGAND FOR THE SITE OF ACTION OF CLASS III CALCIUM-ANTAGONISTS?

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Lipophilic diphenylalkylamines (flunarizine, pimozide, lidoflazine etc) have been grouped as "Class III" calcium-antagonists on the basis of a variety of functional tests (Spedding, 1985). The inhibitory effects of these agents are not reversed by the calcium-channel activator, Bay K 8644 and there is therefore doubt as to whether these drugs interact with the $Ca^{2+}$ channel (Spedding, 1985). Lazdunski's group (Qar et al, 1987) have proposed that fluspiriline has very high affinity (<1 nM) for the dihydropyridine binding site in skeletal muscle and we have investigated its profile as a calcium-antagonist.

Fluspiriline (0.1 - 10 µM) shifted concentration-response curves to $Ca^{2+}$ (0.1 - 10 mM) to the right in parallel in $K^{+}$ (40 mM)-depolarised taenia preparations from the guinea-pig caecum (Spedding & Berg, 1984). The compound had an apparent pA2 of 7.5 +/- 0.1 (slope 1.1) (n=4). Fluspiriline (1-1000 nM) was a potent and non-competitive antagonist of the contractile effects of Bay K 8644 (1-3000 nM) in these preparations, when Bay K 8644 was added cumulatively in the presence of 0.1 mM $Ca^{2+}$. Fluspiriline was a more potent antagonist of Bay K 8644 than of $Ca^{2+}$, and in this respect resembles other class III calcium-antagonists.

In ligand binding experimants, fluspiriline was potent (IC50 51 nM n=4) at displacing [$^{3}$H]-PN200-110 from its binding site on rat cortical membranes. Analysis of the displacement curves gave rise to Hill slopes consistently less than unity (nH = 0.52). Scatchard transformations of the saturation isotherms produced over the [$^{3}$H]-PN200-110 concentration range 0.01 - 1.00 nM indicated binding to a single site, an equilibrium dissociation constant of 0.05 nM and a binding site density (Bmax) of 215 fmol/mg protein. In the presence of 100 nM fluspiriline the Bmax was reduced to 134 fmol/mg protein with an apparent increase in the Kd of [$^{3}$H]-PN200-110 to 0.14 nM. These results are consistent with the view that fluspiriline binds to a site distinct from the [$^{3}$H]-PN200-110 binding site (non-competitive) but sufficiently close to influence dihydropyridine binding in a negative allosteric manner.

Fluspiriline, thus, appears to have high affinity for sites on the $Ca^{2+}$ channels in smooth muscle and brain, although the compound is less potent than in skeletal muscle. The compound appears to act as a "Class III" calcium-antagonist (Spedding, 1985).

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# DIFFERENTIAL EFFECTS OF CALCIUM-ANTAGONISTS ON INSULIN SECRETION

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Secretion of insulin from the pancreatic B-cell has been shown to be a $Ca^{2+}$ dependent process. However while inhibition of insulin secretion by calcium entry blockers (CEB) can be demonstrated in vitro (Semple et al. 1988), the effects of CEB therapy on glucose tolerance in man remain controversial (Kanatsuna et al, 1985). Calcium antagonists have been classified into 3 subgroups on the basis of in vitro studies (Spedding, 1985). This study has investigated the effects of these different subgroups on insulin secretion in vitro and examines the effects of diclofurime, a recently classified Class II CEB (Spedding,et al 1987).

Rat (female Sprague Dawley 200 - 300g) pancreatic islets were isolated using a collagenase digestion technique. After a 30 min preincubation period in 3mM glucose, batches of 5 islets were incubated in a shaking water bath ($37^{o}C$) and oxygenated (95% O2 : 5% CO2) for 60 min in the presence of 10mM or 18mM glucose with or without compound. Aliquots of the incubation medium were retained for insulin determination by RIA. Insulin secretion under these conditions was glucose dependent (3mM glucose = 11.5 +/- 0.8; 10mM glucose = 46.7 +/- 1.5; 18mM glucose = 60.9 +/- 2.5 uIU/islet/hr). The Class I dihydropyridine nicardipine ($10^{-8}$ - $10^{-5}$ M) caused a concentration dependent inhibition of insulin secretion (pIC50 6.90 (6.77 - 7.08) 18mM glucose). 100% inhibition was achieved at $10^{-5}$ M as previously reported by Semple et al.,(1988). The Class II antagonists diltiazem and verapamil ($10^{-7}$ - $10^{-3}$M) were less effective in this study. Maximum inhibition was 85.2% and 66.2% of control with 18mM glucose stimulation. Transdiclofurime was similar in potency to verapamil producing maximum inhibition of 50.9 +/- 17.4% at $10^{-5}$ M in the 18mM glucose stimulated group. The cis isomer of diclofurime was ineffective. The potencies of the Class II compounds were not significantly altered by stimulation with 10mM glucose. The Class III antagonist flunarizine ($10^{-8}$ - $10^{-5}$ M) also inhibited insulin secretion. However, the inhibitory potency was higher in the 18mM stimulated group than in 10mM (pIC50 6.55 (6.37 - 6.88) and 5.45 (5.32 - 5.64).

These results show that in contrast to the high potency of Class I and Class III antagonists, Class II antagonists are less effective in inhibiting insulin secretion in this model. This data suggests that the diltiazem /verapamil site or coupling to the $Ca^{2+}$ channel in B-cells may be different to that described in other tissues and this together with the peripheral vasosensitivity of the Class I antagonists may contribute to the difference between in vitro and in vivo activity of CEBs on normal islet function.

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# NEURONAL AND NON-NEURONAL 5-HT RECEPTORS CONTROL ELECTROLYTE SECRETION IN ISOLATED ILEAL MUCOSA

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5HT stimulates electrolyte secretion in rat small intestine in vitro by promotion of electrogenic anion secretion which can be measured as increases in short-circuit current (Hardcastle et al, 1981). The present study attempts to classify the 5HT receptors that mediate stimulation of electrolyte secretion using a range of 5HT receptor antagonists.

Male Wistar rats were killed by cervical dislocation and the ileum was removed. The mucosa was dissected from the overlying muscle layers and mounted in Ussing chambers, bathed with Krebs-Henseleit solution, maintained at 37°C and gassed with 95% $O_2$/5% $CO_2$. The mucosa was voltage clamped at zero potential and the resultant short-circuit current (SCC) was continuously recorded.

Under resting conditions ileal mucosa produced an SCC of $109\pm3\mu Acm^{-2}$ (mean ± SEM; n=6). When added in a cumulative manner to the serosal bathing solution, 5HT (0.03 to 10μM) increased SCC with an $EC_{50}$ value of 1.7 (1.2-2.3)μM (mean, 95% confidence limits), and a maximum increase in SCC of $53\pm8\mu Acm^{-2}$ (n=6). The antagonists ketanserin (0.3μM), methysergide (3μM) and cyanopindolol (3μM) did not inhibit responses to 5HT suggesting that $5HT_2$ and $5HT_1$-like receptors were not involved. Tetrodotoxin (TTX; 0.3μM) had no effect on responses to low concentrations of 5HT (<1μM), but inhibited responses to higher concentrations of 5HT (>1μM), producing a reduction in the 5HT maximum response of 23%. Similar effects were seen with the $5HT_3$ receptor antagonists GR38032F (0.3μM) and ICS 205-930 (0.001 and 0.01μM) which reduced the 5HT maximum response by 41%, 28% and 39% respectively. Metoclopramide which also has $5HT_3$ receptor antagonist activity (Ireland and Tyers, 1987) reduced the 5HT maximum response by 30% when tested at 1μM, but at a higher concentration (10μM) metoclopramide also produced a tenfold shift of the lower portion of the 5HT concentration response curve. The antagonists did not affect SCC responses to $PGE_2$ (0.01-10μM) suggesting that they were selective antagonists of 5HT-induced secretion.

These studies are consistent with the view that stimulation of electrogenic anion secretion in rat isolated ileal mucosa is mediated by two 5HT receptors. Responses to high concentrations of 5HT (>1μM) appear to be mediated by neuronal $5HT_3$ receptors, since they are inhibited by TTX, GR38032F, ICS 205-930 and metoclopramide. Responses to lower concentrations of 5HT (<1μM) are only inhibited by metoclopramide and therefore this receptor type may resemble the benzamide-sensitive 5HT receptor reported to modulate electrolyte secretion in rat colonic mucosa (Ball et al, 1988).

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# INVESTIGATION OF THE 5-HT RECEPTOR TYPE MEDIATING SECRETION IN RAT COLON

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Cisapride inhibits 5HT-induced secretion in preparations of intestinal mucosa from the rat and guinea-pig as measured by changes in short circuit current (SCC) (Allbee & Gaginella, 1985; Cooke & Carey, 1985). In guinea-pig ileum the response to 5HT is also inhibited by both tetrodotoxin (TTX) and the $5HT_3$-receptor antagonist, ICS 205-930 (Baird & Cuthbert, 1987), suggesting that cisapride was antagonising neuronal $5HT_3$ receptors. In contrast, in rat colon the secretory response to 5HT was not inhibited by TTX (Zimmerman & Binder, 1984), although it was still inhibited by cisapride (Allbee & Gaginella, 1985). In the present study we have further investigated the 5HT receptor type mediating the antagonist activity of cisapride in rat colon, and the effect of cisapride has been compared with a closely related benzamide structure, metoclopramide, and also with a number of standard 5HT receptor antagonists.

Male Wistar rats, 100-150g, were killed by cervical dislocation and the descending colon removed. The mucosa was dissected from the overlying muscle layers and mounted between the two halves of an Ussing chamber. The tissues were bathed with Krebs-Henseleit solution and gassed with 95% $O_2$/5% $CO_2$. The potential difference across the mucosa was held at zero by application of external current, and this SCC was continuously recorded.

Under resting conditions the tissues produced an SCC of 51.4±10.8μA $cm^{-2}$ (mean ± S.E.M., n=8). Addition of 5HT (0.1-300μM) to both sides of the mucosa stimulated a concentration dependent increase in SCC with an $EC_{50}$ value of 5.4 (3.1, 9.4)μM (95% confidence limits) and a maximum increase in SCC of 181.1±28.1μA $cm^{-2}$ (n=8) at 100μM 5HT. This response was not inhibited by TTX (0.3μM) or a $5HT_3$ receptor antagonist, GR38032F (0.3μM) (Brittain et al., 1987) indicating that neuronal $5HT_3$ receptors were not involved. In addition, the SCC response to 5HT was not mediated by $5HT_1$-like receptors since it was not inhibited by methiothepin (0.3μM) or methysergide (1μM), nor by $5HT_2$ receptors since it was not inhibited by ketanserin (1μM). However, both cisapride and metoclopramide inhibited the SCC response to 5HT in an unsurmountable manner with depression of the 5HT maximum; cisapride, 0.1 and 1μM, inhibited the 5HT maximum by 32.8±8.6% and 46.0±9.6% respectively and metoclopramide, 3 and 10μM, inhibited the 5HT maximum by 30.5±4.3% and 44.2±11.1% respectively (n=4 for each value). Cisapride (1μM) and metoclopramide (10μM) did not inhibit the resting SCC or the response to $PGE_2$ (0.01-3μM) indicating that both compounds were selective antagonists of 5HT-induced secretion.

These results show that cisapride and metoclopramide are antagonists of 5HT in rat colonic mucosa, but that the receptor type cannot be designated $5HT_1$-like, $5HT_2$ or $5HT_3$ (Bradley et al., 1984).

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# CALCIUM ANTAGONISM BY BENZODIAZEPINES IN GUINEA-PIG ILEUM

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Benzodiazepines (BDZs) reduce contractile responses of intestinal smooth muscle to a range of stimuli, including acetylcholine, potassium and calcium (Leeuwin et al., 1975; Hullihan et al., 1983). We have compared the effects of BDZs with those of calcium antagonists.

Segments of guinea-pig mid-ileum were suspended in calcium-free, depolarising (80mM $K^+$) Krebs-Henseleit solution at 37°C, the solution being gassed with 95% $O_2$, 5% $CO_2$ and containing atropine (0.3μM). Changes in muscle tension were monitored. Initially the ability of calcium antagonists or BDZs to relax submaximal contractions to $CaCl_2$ (4mM) was assessed, the order of relaxant potency ($IC_{50}$±SEM, n≥6) being: nifedipine (1±0.05nM); verapamil (27±1nM); cinnarizine (64±7nM); diltiazem (120±2nM); trifluoperazine (3±1μM); midazolam (9±1μM); flurazepam (10±1μM); diazepam (16±2μM); Ro5-4864 (chlorodiazepam, 17±2μM); chlordiazepoxide (20±2μM). Despite marked differences in potency, the characteristics of relaxation induced by BDZs and by diltiazem were similar (immediate onset, rapid relaxation, ready reversal on washing) and differed from those for nifedipine and verapamil (immediate onset, slow relaxation, ready reversal) and for cinnarizine and trifluoperazine (slow onset, slow relaxation, poor reversal).

Tissues were incubated for 2 min with low concentrations of midazolam (3μM), nifedipine (0.1nM), verapamil (10nM) and diltiazem (30nM), either individually or in combination. Alone, no compound significantly reduced a subsequent maximal contraction to $CaCl_2$ (10mM) compared with preceding control responses (n≥6). Incubation with midazolam + diltiazem or midazolam + nifedipine caused inhibitions of maximal responses to $Ca^{2+}$ (by 27±2, 19±3% respectively) which were greater ($p<0.05$) than the inhibition (by 9±4%) obtained with midazolam + verapamil. The combination diltiazem + nifedipine induced inhibition (by 41±2%) which was greater ($p<0.05$) than that observed with diltiazem + verapamil or nifedipine + verapamil (5±2, 12±6% inhibition, respectively).

Using unmodified Krebs-Henseleit solution, all compounds were assessed for their ability to inhibit submaximal phasic contractions induced by acetylcholine (Ach, 40nM), γ-aminobutyric acid (GABA, 100μM), or transmural stimulation (TMS, 0.1Hz, 0.2ms, supramax voltage): n≥6 for each stimulant. Responses to GABA and TMS were abolished by tetrodotoxin (0.1μM), whilst atropine (0.3μM) abolished contractions to all three stimuli. Responses to GABA and TMS were more sensitive ($p<0.01$) to inhibition by BDZs (e.g. midazolam $IC_{50}$ 6±3, 7±2μM for GABA, TMS responses respectively) than were contractions to exogenous Ach (midazolam $IC_{50}$ 77±13μM). Conversely, all three responses were similarly sensitive to verapamil, nifedipine and diltiazem (e.g. diltiazem $IC_{50}$ 8±2, 16±8, 5±0.3μM for Ach, GABA, TMS responses respectively).

Thus, the calcium-antagonism by BDZs was dissimilar to that by cinnarizine and trifluoperazine, compounds having marked intracellular effects (Spedding, 1983) and most closely resembled that by diltiazem, which is similar in structure to BDZs. BDZs inhibited cholinergic nerve-mediated effects in intestine, unlike the other calcium-antagonists studied.

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# TETANIC FADE AND RECOVERY PROFILE OF ATRACURIUM-INDUCED NEUROMUSCULAR BLOCKADE IN THE RAT DIAPHRAGM PREPARATION

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Electrical stimulation of the phrenic nerve, in the rat, e.g., at 50 Hz for 1s duration, produces a tetanic tension in the hemidiaphragm, and this tension is usually maintained, for several minutes, in the absence of a non-depolarizing muscle relaxants, e.g., atracurium. However, in the presence of atracurium, the tetanic tension is not maintained, i.e, it fades away, probably due to an effect on presynaptic mechanism, of transmitter release (Bowman & Webb, 1976; Gibb & Marshall, 1986). Tetanic fade has been observed in both man and experimental animals (Madden, Hughes & Payne, 1983; Wali, Suer, Dark & McAteer, 1987), and it is **usually** used as an index for the degree of neuromuscular block by a skeletal muscle relaxant.

In the present investigation, we studied tetanic fade and peak tetanic tension depression, in the rat hemidiaphragm preparation, in the presence of a blocking concentration of atracurium (1 or 10 μM), and we also followed the pattern of revery from neuromuscular blockade by atracurium following removal of the drug by washing it out with Krebs-Henseleit solution.

The preparation was set up in an organ bath (80 ml) containing Krebs solution maintained at 38±2°C and bubbled with oxygen and 5% carbon dioxide. The pH of the solution was 7.4. The phrenic nerve was stimulated at 50 Hz for either 0.5 or 1.0 s duration, with 5-10 V (supramaximal) and 0.2 ms pulse duration. Tetanic tension (peak and sustained tensions) was measured (in gram, g) and fade was determined by subtracting the sustained from the peak tension. This was often expressed as a percentage difference between the peak tetanic tension (Tp) and sustained or end tetanic tension (Te).

The results showed that atracurium reduced the tetanic tension (peak and sustained) and produced a tetanic fade in the diaphragm with time. The control tetanic tension (peak) was 5.6± 0.4 g (mean±s.e., n=8), and the control sustained tension was 4.8±0.2 g, giving rise to a control tetanic fade of 14±0.1%. A full tetanic fade occurred within 28±3.1 s, and at this stage, the peak tetanic tension was depressed by only 40% of the control peak tetanic tension. A full peak tetanic tension depression occurred within 3.75±0.9 min. After removal of atracurium by washing the preparation in Krebs solution (3-4 times) and waiting for several min (2-3 min), the tetanic fade was reversed and the sustained tension recovered within 30 s. However, the peak tetanic tension returned to control value in several minutes (3.5±0.1 min), indicating that the two phenomena were due to different mechanisms.

We concluded that atracurium had a rapid onset of action and an intermediate duration of action (as judged by progression of rapid tetanic fade and slower Tp recovery). The mechanism(s) of tetanic fade and peak tetanic tension were **n o t** analysed, but it is possible that the former is due to a presynaptic mechanism, while the latter is attributed to a postsynaptic mechanism. Other possibilities, e.g ion channel blockade, may be involved, especially in the latter mechanism (see the references cited above).

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# THE EFFECT OF GLYCOPYRROLATE ON NEUROMUSCULAR TRANSMISSION IN THE RAT PHRENIC NERVE-DIAPHRAGM PREPARATION

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Glycopyrrolate (Glycopyrronium, Robinul), is a quaternary ammonium compound, used as a substitute for atropine (a tertiary amine) to reverse (with neostigmine) the residual neuromuscular blockade produced by non-depolarizing muscle relaxants (Ramamurthy, Shaker & Winnie, 1972; Mirakhur, Dundee & Clarke, 1977; Baraka, Yared, Karam & Winnie, 1980). We studied the effect of glycopyrrolate (a muscarinic antagonist) and neostigmine on atracurium-induced neuromuscular blockade in the rat isolated phrenic nerve-diaphragm preparation, to see if glycopyrrolate intensified the neuromuscular blockade produced by atracurium in this preparation.

The phrenic nerve was stimulated repetitively at either 0.2 Hz or at 1 Hz, with 5-10 V (supramaximal) and 0.2-0.5 ms pulse duration. Mechanical responses of indirectly-elicited twitch tension were recorded isometrically. The effect of glycopyrrolate (10 μM), atracurium (1-100 μM) and neostigmine (1 μM), either alone, or in a mixture of two or three drugs, were studied on indirectly-elicited twitch responses, and sometimes on directly-elicited twitch tension, in the same and different preparations.

Atracurium had a rapid onset of blockade, reaching a maximum block in 30-40 s. Glycopyrrolate had no significant effect on indirectly-or directly elicited twitch (0.2 Hz) tension, whereas it significantly ($P < 0.001$) enhanced atracurium-induced depression of twitch tension, and it also shortened the time required to completely block the twitch tension by about 10 s. The control twitch tension was $1.3 \pm 0.11$ g (mean $\pm$ s.e., n=6), and this was $0.2 \pm 0.01$ g in the presence of atracurium (1.6 μM) in 40 s exposure. Glycopyrrolate (10 μM), had little effect on twitch tension (11% decrease in control tension), but significantly increased the atracurium-induced depression of twitch tension. Atracurium, alone, depressed the twitch tension by $46 \pm 1.2$%, and in the presence of glycopyrrolate, the twitch was depressed by $72 \pm 3.0$%. Neostigmine enhanced the twitch tension by $29 \pm 1.6$%, lasting for 1 min, thus, delaying atracurium-induced depression of twitch tension. Glycopyrrolate+neostigmine had less antagonistic effect of atracurium blockade than had neostigmine alone. The mean $IC_{50}$ values (i.e concentration of atracurium to produce 50% maximum block of twitch tension) were: $1.6 \pm 0.1$, $0.3 \pm 0.1$, $4.8 \pm 0.2$ and $2.7 \pm 0.1$ μM, for atracurium, alone, for atracurium+glycopyrrolate, atracurium+neostigmine and for atracurium+glycopyrrolate+neostigmine, respectively. The results were significantly different ($P < 0.001$) from the control atracurium value.

We concluded that glycopyrrolate had little effect on twitch tension, but it significantly enhanced the neuromuscular blockade produced by atracurium. The mechanism of glycopyrrolate action was not further analysed, but it is possible it may antagonize transmitter release or act by some other unknown mechanism at the rat neuromuscular junction.

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