RELEASE OF KALLIKREIN BY THE ISOLATED PERFUSED RAT KIDNEY

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Renal kallikrein is believed to regulate electrolyte and water transport in the nephron and blood flow in the kidney. The physiological control of the release of renal kallikrein is uncertain in spite of many in vivo studies (see Lauar, Shacklady & Bhoola, 1982). The aim of the present experiments is to examine the effect of changes in arterial perfusion pressure and to determine the action of arginine-vasopressin (AVP) on the release of kallikrein by the isolated rat kidney.

Male rats weighing (350-450g) were anaesthetised with urethane (1.75g/kg body wt). The right kidney was prepared for perfusion in situ by cannulating the ureter, renal vein and renal artery in sequence. Heparin (0.2ml, 200mu) was injected prior to cannulating the renal vein. Perfusion was commenced in vivo and continued until the venous effluent was free of blood. The kidney was transferred to the perfusion chamber and perfused at 37°C with a modified Krebs buffer solution (mM/l: NaCl 94.8, KcL 4.69, $MgSO_4$ $7H_2O$ 1.18. $CaCl_2$ 2.52, $NaHCO_3$ 25.0, KH_2PO_4 1.2, glucose 11.6, Na pyruvate 4.9, Na fumarate 5.3, Na glutamate 4.9; pH 7.4), to which was added bovine serum albumin (1g/100ml, lyophilised, Sigma No. 7638), Creatinine (0.2mg/ml) and Ficoll 70 (4g/100ml). The Krebs buffer solution was gassed with 95% O_2 and 5% CO_2 and the aeration continued throughout the perfusion. The Krebs perfusion buffer solution was recycled in a closed system at flow rates that varied from 6 to 15 ml/min. The mean arterial perfusion pressure was maintained at 120 mm Hg in control experiments. Samples were taken from the venous line (1ml) and the ureter at half hourly intervals after the initial perfusion period of 60 min. Enzymic activity of kallikrein was measured on the chromogenic peptide substrate, H-D-Val-Leu-Arg pNa (Kabi Vitrum, S2266). Measurements of the active enzyme were made in the presence of soya bean trypsin inhibitor (SBTI 100µg/ml). Total enzymic activity was determined by prior incubation of samples with trypsin (5µg/ml) for 15 min at 37°C, followed by inhibition with SBTI (40µg/ml) prior to measurement. Both creatinine (Sigma Kit No. 555) and lactate dehydrogenase (LDH, Sigma Kit No. 500) were measured in order to assess functional viability of the perfused kidney.

The amount of active, inactive or total kallikrein cleared by the isolated, perfused kidney was calculated from the venous and urine samples. Increasing the mean arterial perfusion pressure from 120 to 160mm Hg resulted in a significant increase in the 'clearance' of total kallikrein. A similar increase was found with arginine-vasopressin ($4x10^{-10}$M; bacitracin 50µg/ml and dithiothreitol 6µM/l); however if the perfusion pressure was maintained at 120mm Hg after the addition of AVP, the increase in total kallikrein was no longer observed. An interesting difference was that in the AVP experiments the kallikrein cleared was mainly in the active form. Our results suggest that the increase in kallikrein recorded with AVP may be due primarily to changes in arterial perfusion pressure. Furthermore, AVP seems to alter the ratio of active to inactive kallikrein.

Namir Lauar is a CAPES (Brazil) Fellow.

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GASTRIC DAMAGE IN THE RAT PRODUCED BY THE HERBICIDE DIQUAT: SIMILARITIES TO ASPIRIN-INDUCED DAMAGE

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The herbicide diquat (N, N'-ethylene 2, 2'-bipyridilium) is moderately toxic to animals. Deaths occurring within the first 3 days after administration of an oral LD_{50} dose to rats may be due to fluid redistribution, associated with a rapid accumulation of fluid in the gastrointestinal tract and dehydration of other tissues. (Crabtree et al, 1977). We have examined morphological changes in rat gastric mucosa following administration of diquat to rats, as part of a study undertaken to investigate the mechanism of fluid loss into the gastrointestinal tract.

Male Wistar-derived rats (150-200 g) were fasted for 24 hours before dosing orally by gavage with 900 μmol/kg or subcutaneously with 90 μmol/kg diquat in sterile saline. At specified intervals after dosing (½, 1, 2, 4, 8, 12, 18, 24 hours, 3, 8 and 15 days), gastric tissue samples were taken from groups of 4 rats. These samples were processed for histopathological and ultrastructural examination and sections of mucosa were also stained histochemically (PAS/Alcian Blue) for the assessment of gastric mucins.

Oral administration of diquat resulted in some erosion of the pyloric gastric mucosa, occurring 1-3 days after dosing. The changes observed included almost total loss of surface mucin-secreting epithelium, necrosis of cells in the gastric pits, haemorrhage, inflammation and submucosal oedema. Mild changes in the fundus of the stomach preceded these changes, characterised by minor erosions of superficial mucin-secreting cells, vacuolation and desquamation of parietal cells, occurring within 4-8 hours of dosing. Ultrastructural examination showed disruption of the canaliculi of parietal cells and the appearance of fluid-filled vacuoles in these cells. Chief cells were normal. There was loss (as judged by histochemical staining) of acid mucins within 30 minutes of dosing, leaving only PAS-positive neutral mucin in the superficial mucosa.

Subcutaneous administration of diquat also produced damage to the gastric mucosa. The changes were similar to those seen after oral dosing but were less severe. Again, changes in the pyloric region were the most marked, with focal cell necrosis, loss of surface mucin-secreting cells and acid mucins. The changes were preceded by vacuolation of cells in the fundus, and electron microscopy showed that both parietal cells and chief cells were affected. There was also an early (2-4 hours after dosing) loss of acid mucins from the fundus and hyperplasia of superficial mucin-secreting cells was seen by 24 hours after dosing.

Our results show that diquat damages the gastric mucosa, and this may in part explain the loss of fluid into the gastrointestinal tract. This effect is not simply due to local irritant effect following oral administration, since changes are also seen following subcutaneous diquat. This may indicate a specific effect of diquat on one or more cell types in the stomach. The changes seen are very similar to those reported for aspirin (St. John et al, 1973). In particular, the early damage seen in parietal cells is similar to that reported to be a principal factor in the development of aspirin-induced gastric damage. (Rainsford & Brune, 1978).

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TREATMENT OF AN EXPERIMENTAL ARTHRITIS WITH LIPOSOME-ENTRAPPED METHOTREXATE

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Methotrexate (MTX) has been administered intra-articularly in an attempt to control the synovitis in arthritic joints, though with disappointing results, possibly because of the rapid clearance of the drug from the joint (Bird et al, 1977; Wigginton et al, 1980). The retention of MTX in the joints can be greatly enhanced by administering the drug entrapped in liposomes (Foong & Green, 1983), and we report here on the efficacy of intra-articular (i.a.) liposomal MTX in treating an experimental arthritis.

An allergic bilateral arthritis was induced in the knee joints of Old English rabbits using the procedure described by Consden et al (1971). The chronic arthritis produced involves both a type III (Arthus) and a type IV (delayed) hypersensitivity reaction which histopathologically closely resembles human rheumatoid arthritis (Hunneyball & Stanworth, 1981). Inflammation was assessed by measuring changes in joint diameter and skin temperature over the joint. Multi-lamellar liposomes containing MTX were prepared as previously described (Foong & Green 1983).

The i.a. injection of a single dose of 1 mg free MTX at time of challenge produced a 20-30% reduction in swelling (Table 1) and surface temperature of the treated joint which persisted for approx 6 weeks. A similar, but slightly greater response was produced by the intra-articular injection of 0.1 mg liposomal MTX. In joints challenged with antigen 1 week previously, 0.1 mg liposomal MTX again caused a 20-30% reduction in inflammation, but 1 mg free MTX was ineffective. Neither 1 mg free MTX or 0.1 mg liposomal MTX were effective in suppressing arthritis which had been induced 3 weeks prior to treatment.

Table 1. Diameter of knee joints injected with 1 mg MTX at time of challenge compared with untreated joint (*P <0.05, paired t-test).

Days after induction of arthritis	0	7	14	28	56
Untreated knee (mm)	18.6±0.3	23.1±0.6	23.0 ±0.8	21.8±0.4	22.3±0.8
Treated knee (mm)	18.6±0.3	22.4±0.5*	21.5 ±0.8*	20.8±0.3*	21.6±0.8

Antigen-induced arthritis in rabbits is very resistant to treatment (Hunneyball & Stanworth, 1981), but it is disappointing that liposomal MTX was ineffective in suppressing the established arthritis, despite the prolonged retention of MTX in the joint when administered entrapped in liposomes (Foong & Green, 1983). The liposomes presumably act as a storage depot from which the drug is slowly released, so that only a fraction of the retained MTX is available in an active form at any one time. Nevertheless, 0.1 mg liposomal MTX was at least as effective as 1 mg of the free drug in suppressing the development of arthritis, and we are currently investigating the possibility of suppressing established arthritis with larger and multiple doses of liposomal MTX.

This work was supported by a grant from the Nuffield Foundation. We thank Lederle for generous supplies of methotrexate.

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EFFECT OF AMBROXOL ON MUCOCILIARY TRANSPORT IN THE GUINEA-PIG

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Impaired mucociliary clearance is a common feature of such diseases as chronic bronchitis, cystic fibrosis and bronchial asthma, and occlusion of airways by mucus plugs is characteristic in asthma death (Dunnill, 1971). Although the effects of mucolytic agents (e.g. S-carboxymethylcysteine and N-acetyl-cysteine) on mucus rheology are well established from *in vitro* studies, the effect of these drugs on mucociliary clearance *in vivo* is not as well defined. Mucociliary transportation rates can be measured in the trachea of small laboratory animals by monitoring the transit of a bolus of 32-phosphorus (32-P) labelled erythrocytes (Morley and Sanjar, 1983). This technique has been used to ascertain the effect of the mucolytic ambroxol in guinea-pigs.

Animals were anaesthetised with urethane (1.4 g/kg), the trachea exposed, and a 2 ul aliquot of 32-P labelled erythrocytes injected into the distal end of the trachea using a 25 ul Hamilton syringe. Particle transportation was timed using a collimated solid state device to detect radioactivity 15 mm from the point of application, and amplified signals from the detector logged by a microcomputer at 10 sec intervals. Tracheal transportation of erythrocytes was calculated as the mean of at least three observations in each animal.

Acute intravenous administration of ambroxol (20 mg/kg) significantly ($p < 0.005$) increased the rate of transport from 5.12 ± 0.35 mm/min to 7.29 ± 0.63 mm/min (n=10). Injection of 10 mg/kg ambroxol increased mucociliary clearance in 2 of 5 animals. When animals were pretreated for 5 days with ambroxol (2 and 20 mg/kg p.o.), and mucociliary clearance measured 24 hrs after administration of the final dose, there was no difference in mucociliary transportation in these animals compared with controls which received distilled water alone (control, 4.56 ± 0.25 mm/min (n=11); 2 mg/kg ambroxol, 4.96 ± 0.41 mm/min (n=11); 20 mg/kg ambroxol, 4.36 ± 0.2 mm/min (n=9)).

The present study demonstrates an increase in mucociliary clearance in the intact trachea following intravenous administration of ambroxol. This increase may result from a direct effect of the drug on ciliary beat frequency (Iravani and Melville, 1974) or an action on mucus secretion. It has been reported that the rate of clearance of minimicrospheres from the upper airways of patients with chronic obstructive lung disease was significantly increased by oral pretreatment with ambroxol, although whole lung clearance did not increase significantly (Weiss et al., 1981). The absence of a similar effect in the guinea-pig following oral pretreatment with this drug cannot be attributed to insensitive or imprecise measurement, since pilocarpine increases mucociliary transportation in normal animals, and since intravenous ambroxol achieves a pronounced stimulatory effect. Animals with impaired mucociliary clearance have yet to be investigated.

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EFFECT OF MUCOLYTIC AGENTS ON FROG MUCOCILIARY TRANSPORT

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Mucolytic agents are used in diseases characterised by an over- production of mucus with altered physicochemical properties. Although effects of these drugs on mucus rheology *in vitro* are well documented, effects on mucociliary clearance *in vivo* are not clear (Wanner, 1977).

We have used an *in situ* frog oesophageal preparation (Morley and Sanjar, 1983) to compare the effect of acute topical application of a range of mucolytic compounds on the rate of transportation of lead particles.

Frogs (Rana temporaria) were pithed and dissected so as to display the oesophagus from the ventral aspect. The ciliated lumen was exposed by an incision along the midline of the oesophagus which was then pinned using sufficient tension to flatten the luminal surface *in situ*. The preparation was irrigated at regular intervals with frog Ringer's solution to prevent drying out. Lead filings were placed at the oral end of the oesophagus, and the mean transportation rate of at least ten particles was measured along a 5 mm path with the aid of a video recording system (Morley and Sanjar, 1983). This allows repeated and reproducible measurement of the rate of transportation in mm/sec. Drugs were dissolved in frog Ringer's solution and applied topically in a fixed volume (2 ml).

Ambroxol (0.1-1 mg/ml) caused a dose-related increase in the rate of particle transportation (Table 1). As mucociliary clearance in the frog is sensitive to acceleration by cholinergic stimuli (Morley and Sanjar, 1983), the dose response curve to ambroxol was repeated after pretreatment with atropine (1 ug/ml). The increase in mucociliary transportation resulting from application of ambroxol was not altered by atropine at a concentration known to inhibit the effects of vagal stimulation (Table 1). Ambroxol may exert this effect as a consequence of increased ciliary beat frequency, as has been observed in isolated rat airways (Iravani and Melville, 1974); alternatively it may act directly by altering mucus rheology. Two other mucolytic agents, N-acetyl-cysteine and S-carboxymethylcysteine, at similar concentrations, did not affect the rate of particle transportation in this preparation (Table 1).

Table 1. Effect of mucolytics on frog mucociliary clearance

Drug	n	Drug Concentration (mg/ml)			
		Control	0.1	0.5	1.0
Ambroxol	8	0.27 ± 0.02	0.28 ± 0.02	0.6 ± 0.05	0.81 ± 0.04
Ambroxol + Atropine (1 ug/ml)	3	0.26 ± 0.01	0.25 ± 0.01	0.53 ± 0.060	0.77 ± 0.08
N-acetyl-cysteine	4	0.31 ± 0.03	0.24 ± 0.03	0.27 ± 0.02	0.25 ± 0.02
S-carboxymethyl cysteine	4	0.40 ± 0.02	0.32 ± 0.04	0.34 ± 0.02	0.36 ± 0.02

Results are expressed as mean ± s.e. mean.

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ALDOSTERONE AND RENAL MAGNESIUM EXCRETION: AN ACUTE CLEARANCE STUDY IN ADRENALECTOMIZED RATS

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The role of aldosterone in the renal handling of magnesium is unclear. It has been suggested that there is a difference in the acute and chronic effects of mineralocorticoids on urinary magnesium excretion (Massry et al, 1968). Thus while chronic studies have indicated an action by aldosterone to increase urinary magnesium excretion (Hanna & McIntyre, 1960; Horton & Biglieri, 1962), the results of acute administration of aldosterone in adrenalectomized dogs did not confirm these effects (Massry et al, 1967). Lemann et al (1970), in an acute study in man, failed to show any effect of aldosterone on urinary magnesium excretion. The present study was undertaken to investigate the effects of acute administration of aldosterone in adrenalectomized rats on the renal clearance of electrolytes including magnesium.

Male Wistar rats were bilaterally adrenalectomized via flank incisions under ether anaesthesia, 6-8 days before clearance experiments were performed. These animals were given 1% NaCl ad libitum as the sole drinking fluid. Effective adrenalectomy was verified 3 days after surgical removal of the adrenals, by the water excretion test (water loading to 5% body weight). Only those animals which excreted less than 50% of the water load within 2 hours were regarded as adrenalectomized. The adrenalectomized rats were given maintenance doses of glucocorticoid (dexamethasone, 1 mg i.p. daily) for 3 days. On the morning of the clearance experiments, the effectiveness of the glucocorticoid replacement therapy was assessed by the water excretion test. Only those animals which excreted more than 50% of the water load on this occasion, were used for clearance experiments.

Clearance experiments were performed on rats anaesthetized with Inactin (100 mg/kg i.p.). A modified Ringer solution was infused (30 ml kg^{-1} h^{-1}). The left ureter was cannulated. GFR was measured using (^{3}H)-inulin. After 100 minutes equilibration, two 15-minute control urine collections were made. D-Aldosterone (20 µg/100 g) was then administered as a bolus injection. 75 minutes later, two experimental 15-minute urine collections were made. A series of time-control experiments were carried out in 6 adrenalectomized animals, following a similar protocol but without administration of aldosterone. Statistical analysis was by paired Students t test.

The time-control experiments showed that no significant changes occurred in the fractional excretion (FE) of either sodium, potassium or magnesium over the period of investigation in adrenalectomized rats. Administration of aldosterone (20 µg/100 g) caused a significant reduction in FE_{Na} ($p<0.05$) but had no effect on FE_{Mg}. Aldosterone also caused a significant increase in FE_K/FE_{Na} ratio ($p<0.05$) but did not alter the FE_{Mg}/FE_{Na} ratio.

These results provide further evidence that acute administration of aldosterone does not alter the renal clearance of magnesium. Previously reported chronic effects of mineralocorticoids on renal magnesium excretion may be due to indirect actions.

Supported by the Medical Research Council of Ireland.

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KINETIC MODELLING OF IN VIVO EXCHANGE OF MAGNESIUM IN RAT TISSUES

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Although magnesium (Mg) is second only to potassium as the main intracellular cation and is a required co-factor in many biological processes, relatively little is known about the in vivo exchange kinetics of Mg in different tissues. The main reason for this lack of information relates to the short $t_{\frac{1}{2}}$ (21.4 h) of the radioisotope ^{28}Mg and its expense and non ready availability. We investigated the exchange of Mg in rat tissues in vivo using ^{28}Mg and kinetic modelling.

Male Wistar rats were given an i.v. injection of ^{28}Mg (1.75 μCi/100 g). Rats were killed at intervals of 0.5 to 30 h after injection. Samples of blood, cardiac ventricular muscle, soleus muscle and thymus were removed. Blood was separated into plasma, red blood cells and lymphocytes. Lymphocytes and thymocytes were isolated on a ficoll 400-sodium metrizoate gradient. ^{28}Mg was measured in a γ counter and all counts were corrected to zero time. Total Mg was determined by atomic absorption spectrophotometry. Specific activity and relative specific activity were calculated.

The plasma specific activity was best described by a biexponential decay curve with $t_{\frac{1}{2}}$ values of 0.5 h adn 32.03 h for the respective decay constants. The tissue experimental data was described by a linear multicompartment model consisting of a plasma compartment and several tissue compartments. The tissue compartments were divided into two groups: (A) those which contributed to the initial rapid decline in the plasma decay curve and (B) those corresponding to the slower second phase of the plasma decay curve. The following assumptions were included in the modelling process: (1) non-radioactive Mg was in the steady state, (2) a fraction (ϕ) of non-radioactive intracellular Mg was exchangeable with the plasma Mg, (3) intracellular binding of ^{28}Mg was taken to be so slow as to be negligible during the time course of the experiment. The experimental data was fitted to the model by means of an iterative least squares regression program, NONLIN (Metzler, 1974). The value of the multiple correlation coefficient (R^2) indicates the goodness of fit. Values for ϕ, Mg exchange rate, and R^2 are shown in table 1.

Table 1. Magnesium kinetics in rats tissues in vivo.

Tissue	Type	Exchange (m.mol kg^{-1} min^{-1})	ϕ	R^2
Soleus muscle	B	0.055	0.91	0.977
Thymus	B	0.066	0.95	0.983
Thymocytes	B	0.089		0.880
Lymphocytes	B	0.109	0.80	0.947
Red blood cells	B	0.009	0.77	0.960
Cardiac ventricular muscle	A	0.530	1.17	0.734

From the table, it can be seen that the agreement between the model and experimental data was excellent for tissue type B. A very slow exchange was evident in red blood cells. The exchange was extremely rapid in cardiac ventricular muscle and the agreement between experimental data and model was not very good for this tissue. Inclusion in the model of active processes may be necessary to describe the kinetics of Mg in ventricular muscle.

The work was supported by the Medical Research Council of Ireland.

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INVESTIGATION OF A LOOP DIURETIC-SENSITIVE ION TRANSPORT SYSTEM IN RAT THYMOCYTES

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Isolated perfused tubule experiments indicate that loop diuretics inhibit active chloride transport in the thick ascending limb of the loop of Henle (Burg & Stoner, 1976). Recent studies indicate that several cell model systems have a chloride-dependent Na-K cotransport system which is sensitive to loop diuretics (Palfrey et al, 1980; Aiton et al, 1981; Ellory & Stewart, 1982). We have investigated the effects of bumetanide, a loop diuretic, on ^{86}Rb (used as an analogue of K) influx in rat thymocytes.

Thymus glands were removed from male Wistar rats (100-150 g) and thymocytes were isolated by mincing, aspiration and separation at 4°C through gauze. Cells (1×10^8/ml) were incubated at 37°C in medium 199 containing 20% foetal calf serum. ^{86}Rb was added at time zero and triplicate samples (200 µl) were removed at time intervals up to 90 min. After sampling, cells were rapidly separated from medium by centrifugation through di-n-butylphthalate. Flux rates were calculated by the procedure of Segel & Lichtman (1976) using our own estimates of cell potassium and water.

Total ^{86}Rb influx rate was 7.79 ± 0.36 f.mol $cell^{-1}$ h^{-1}. Ouabain (1 mM) reduced this to 2.41 ± 0.06 f.mol $cell^{-1}$ h^{-1} indicating that approximately 70% of the total influx in rat thymocytes is sensitive to ouabain at an extracellular potassium concentration of 5.37 mM. All experiments with bumetanide were carried out in the presence of ouabain (1 mM). Bumetanide significantly decreased the ouabain-insensitive component of ^{86}Rb influx and a dose-response relationship was established for this action of bumetanide. The IC_{50} value was 5×10^{-6} M. At maximal doses, the influx rate was reduced by 0.65 ± 0.06 f.mol $cell^{-1}$ h^{-1}.

In attempts to further characterize the bumetanide-sensitive and ouabain-insensitive ion transport system, experiments were carried out in high K (16.11 mM) medium. Although the total ouabain-insensitive flux rate was increased in 16.11 mM K, no stimulation of the bumetanide-sensitive component was detected. The stimulation of total ouabain-insensitive flux in this cell system may be due to competition between ouabain and K for binding to the Na-K-ATP'ase (Jones et al, 1981). Addition of either 8-Br-cAMP (1 mM) or sodium fluoride (10 mM) did not stimulate the bumetanide-sensitive component of ^{86}Rb transport.

In conclusion we have demonstrated the presence of a loop diuretic sensitive component of ^{86}Rb influx in rat thymocytes. This transport system does not appear to be linked to adenylate cyclase in contrast to the system described in the avian erythrocyte (Palfrey et al, 1980).

This work was supported by the Irish Heart Foundation.

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INVESTIGATION OF CARDIOPROTECTION BY β-ADRENOCEPTOR ANTAGONISTS IN CLINICAL AND EXPERIMENTAL MYOCARDIAL INFARCTION

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Recent studies, including the Norwegian Multicenter Study Group (1981) demonstrating that timolol reduced mortality and reinfarction in patients surviving acute myocardial infarction, have stimulated interest in the cardioprotective action of β adrenoceptor antagonists. There is good evidence as reviewed by Lehr (1981) that alterations in Mg levels are involved in acute myocardial infarction. A protective action of high Mg concentrations in cardioplegic solutions has been advocated (Hearse et al, 1978). The effects of β adrenoceptor antagonists on intracellular Mg levels requires investigation as loss of intracellular Mg and K may play an important role in triggering post-infarction arrhythmias. We studied the effect of β adrenoceptor antagonists on Mg and K levels in patients with myocardial infarction and in an experimental model of myocardial infarction. Assay of serum myoglobin was used for detection of myocardial infarction (Stone et al, 1975).

Patients admitted to the coronary care unit with acute myocardial infarction were studied. Plasma samples, taken within two hours of admission, were analyzed for myoglobin by radioimmunoassay and also for Mg and K. Values in 19 healthy subjects were compared to those in patients both with and without β adrenoceptor antagonist treatment prior to myocardial infarction. The myoglobin levels (160 ± 36.1 ng/ml) in 5 patients without prior treatment with β adrenoceptor antagonists were significantly ($p<0.01$) higher than levels (52.3 ± 9.25 ng/ml) obtained in 4 patients on treatment. Plasma Mg values (0.84 ± 0.04 mM) also tended to be higher in patients not treated with β adrenoceptor antagonists compared to values (0.79 ± 0.05 mM) in treated patients. Plasma K values were increased from control subjects to a similar extent in both groups of patients.

The experimental model of myocardial infarction was that described by Nayler (1981). Isolated rat hearts were set up by the nonrecirculation Langendorff technique. Hearts were perfused for 30 min to allow equilibration and then ischaemia and experimental infarction was produced by occlusion of coronary flow for 60 mins. At reperfusion, timed samples were collected and assayed for myoglobin, Mg and K. The effects of inclusion of nadolol (1×10^{-7} M), a β adrenoceptor antagonist without membrane stabilizing activity or intrinsic sympathomimetic activity, in the perfusion fluid throughout the period of infusion or at reperfusion were investigated. At the onset of reperfusion, when isolated hearts which had been ischaemic for 60 min, were perfused with normal Kreb's solution significant ($p<0.001$) increases were detected in myoglobin, Mg and K. Addition of nadolol throughout the entire perfusion sequence prevented the post-infarction increase in perfusate Mg and significantly attenuated the increase in myoglobin. Addition of nadolol only during the reperfusion phase was not as successful in attenuating the increases in perfusate Mg and myoglobin.

These results provide additional evidence that β adrenoceptor antagonists exert a cardioprotective role during myocardial infarction. The prevention of loss of intracellular Mg may be an important effect of β adrenoceptor antagonists. Experimental studies have shown that Mg is important in the maintenance of cardiac muscle K. (Ryan et al, 1973).

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COMPARATIVE ABSORPTION OF OESTROGEN CONJUGATES FROM THE GASTRO-INTESTINAL TRACT OF THE RAT: EFFECT OF AMPICILLIN

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Oestrone (E_1), oestrone glucuronide (E_1G) and oestrone sulphate (E_1S) are endogenous oestrogens. E_1 is a biologically active steroid; E_1S is the major circulating oestrogen and E_1G is a major excretory product both in bile and urine. These steroids may be present in the gastrointestinal tract (g.i.t.) as a result of biliary excretion or in the case of E_1S after administration in hormone replacement therapy. Since the steroids exhibit marked differences in their physicochemical properties (e.g. polarity and lipophilicity) we have investigated their absorption profiles.

Female Wistar rats (180-240g) were used in two studies. After starving overnight rats were anaesthetized with urethane, the bile duct cannulated and the appropriate part of the g.i.t. (see below) isolated by ligatures. In study 1, rats were divided into 9 groups and the absorption of [^{3}H]E_1 (3.7 μM; 10 μCi/kg; in pH 6.5 phosphate buffered saline, PBS:ethanol, 9:1 v/v), [^{3}H]E_1G (in PBS) and [^{3}H]E_1S (in PBS:ethanol, 9:1 v/v) was determined from the stomach, small intestine and caecum. In study 2, the absorption of the oestrogen conjugates from the proximal small intestine (PSI), distal small intestine (DSI) and caecum of control and ampicillin pretreated (200 mg/kg/day for 2 days) rats was determined. In both studies, after injecting the drug (0.1 ml/100 g.b.w.) into the isolated segment of gut, bile was collected at regular intervals to 5h and the radioactive content determined.

Biliary recovery of steroid is a good reflection of absorption (Back et al., 1981). Percentage excretion in bile (at 5h) was least after administration of each drug into the stomach (E_1 22.4 ± 5.6; E_1G 6.0 ± 4.0; E_1S 10.7 ± 4.0; mean ± S.D.). This correlates well with the lipophilicity of the drugs as shown by their relative partition coefficients between n-octanol and pH 6.5 PBS (E_1 >> E_1S > E_1G). There was no significant difference in biliary excretion profile when the drugs were given into the caecum (at 5h, E_1 46.3 ± 9.1; E_1G 42.2 ± 14.5; E_1S 39.9 ± 7.1). The similarity, despite differences in physicochemical properties, suggested hydrolysis of the conjugates to the parent steroid. In contrast, after administration into the small intestine, excretion of E_1 was very rapid and was maximal (72.5 ± 8.0) at 1h; E_1G showed a near linear excretion rate (1h, 14.4 ± 3.0; 5h, 80.0 ± 11.7) whereas in comparison E_1S excretion was low (1h, 12.1 ± 2.4; 5h, 36.9 ± 2.7).

Study 2 helped to elucidate the involvement of hydrolytic enzymes, present in the gut lumen, in the absorption of the conjugates from the small intestine and caecum. Ampicillin pretreatment reduced the absorption of E_1G from PSI and DSI (by approximately 50%) but had no effect on absorption of E_1S. However, pretreatment reduced the absorption of both conjugates (greater with E_1S) from the caecum.

This study gives further evidence that E_1S is absorbed intact in the small intestine but is hydrolysed in the caecum (Sim et al., 1983). In addition, it has been shown that quantitative absorption of E_1G from the intestinal tract requires hydrolysis of the conjugate. These conclusions were supported by an *in vitro* study which showed that ampicillin pretreatment abolished the hydrolysis of E_1S by caecal content but only partially reduced the hydrolysis of E_1G. The presence of mammalian glucuronidase enzyme may account for this difference.

SMS is supported by the Mersey Regional Health Authority and is in receipt of an ORS award.

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FORMATION OF PGI_2, PGE_2 AND TXB_2 IN WHOLE BLOOD IN VITRO AND ITS PHARMACOLOGICAL MODIFICATION

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Intravascular platelet activation may play an important role in the pathogenesis of diseases associated with injuries of the vessel wall. One possibility to prevent excessive platelet activation is to inhibit the formation of platelet-derived pro-aggregatory arachidonic acid (AA)-metabolites, such as thromboxane (TX) A_2. This may be obtained by agents with different mechanisms of actions, including inhibitors of the cyclooxygenase (CI), TX-synthetase (TSI) or prostacyclin mimetics. In this study the CI indomethacin (IND), the TSI dazoxiben (DAZ) and the chemically stable prostacyclin mimetic ZK 36 374 (ZK) were compared with respect to their potency to inhibit TX-formation in human whole blood in vitro. The biosynthesis of two other AA-metabolites, PGE_2 and 6-oxo-$PGF_{1\alpha}$ (degradation product of PGI_2) was also measured simultaneously in the same samples.

Fasted human males rested in supine position for 30 min. Blood was then drawn from the antecubital vein and collected into plastic tubes containing the drug or respective solvent (10:1, v:v). The blood was allowed to coagulate for 30 min at 37oC. Immunoreactive TXB_2, PGE_2 and 6-oxo-$PGF_{1\alpha}$ were determined in serum by specific RIA.

In solvent-treated control sera, the concentrations of TXB_2, PGE_2 and 6-oxo-$PGF_{1\alpha}$ amounted to 101 + 29, 2.1 $\pm$ 0.3 and 2.4 $\pm$ 0.2 nM, respectively (n = 10). The generation of 6-oxo-$PGF_{1\alpha}$ was confirmed by TLC using two different solvent systems. IND produced a dose-dependent inhibition of all 3 products (IC_{50}: 0.2 - 0.5 µM). In contrast, DAZ dose-dependently inhibited TXB_2 but stimulated PGE_2 for about the same amount (Figure 1). There was little change in 6-oxo-$PGF_{1\alpha}$, amounting to 2.6 $\pm$ 0.3 nM in absence and 7.7 $\pm$ 1.3 nM in presence of 370 µM DAZ (n = 6, $P < 0.01$). ZK inhibited both PGE_2 and TXB_2 (IC_{50}: 2-5 nM), whereas 6-oxo-$PGF_{1\alpha}$ was slightly enhanced from 2.2 $\pm$ 0.2 to 3.0 $\pm$ 0.5 nM after treatment with 2.8 µM ZK (n=10, $P<0.01$).

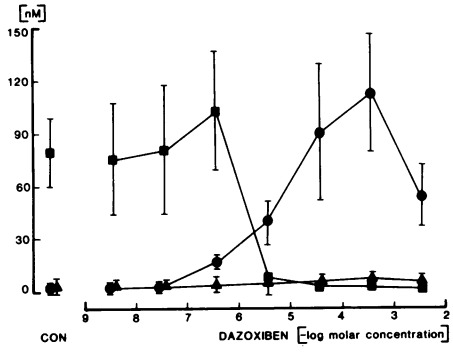

Figure 1 Modification of PGE_2 (●), TX (■) and 6-oxo-$PGF_{1\alpha}$ (▲) (ordinate) by DAZ (abscissa) in clotted whole blood.

These data on DAZ agree well with earlier observations by Needleman et al (1977) with imidazole on washed human platelets. The present study shows that the bulk of TX-precursor is now available for enzymatic or non-enzymatic transformation into PGE_2 (45-fold above control), although a small increase in 6-oxo-$PGF_{1\alpha}$ (4-fold above control) is also evident. The latter finding agrees with investigations of Defreyn et al (1982) on collagen stimulated citrated blood. From a theoretical point of view, our data suggest that prostacyclin mimetics, such as ZK 36 374, may be superior to both CI and TSI compounds. Those agents not only prevent TX biosynthesis, being 2-3 orders of magnitude more potent than DAZ or IND, but in addition do not inhibit PGI_2 formation. IND blocks both TX and PGI_2. DAZ leads to an apparently equimolar accumulation of PGE_2 which in turn may stimulate platelet aggregation rather than to inhibit it (Kloeze, 1969; Andersen et al, 1980).

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DIETARY FAT INFLUENCES THE INHIBITORY EFFECT OF ALCOHOL ON HUMAN PLATELET AGGREGATION

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Alcohol intake is implicated by epidemiological studies as a protective factor in coronary heart disease (Klatsky et al, 1977). We have previously suggested (Fenn & Littleton, 1982) that the marked inhibitory effect of alcohol on human platelets *in vitro* when these contain high proportions of saturated membrane phosphlipids could be of relevance to these epidemiological findings. Here we report an interaction *in vivo* between ethanol and dietary fat which suggests that this may be the case.

Fifteen healthy male volunteers gave blood at 10 am and 1 pm and platelet aggregation to collagen and ADP was measured in platelet-rich plasma with acid citrate: dextrose as anticoagulant. Each individual gave blood twice on six separate days; on one no food or alcohol was given between taking blood samples, on another alcohol alone (1 bottle of white wine i.e. c 70 g alcohol) was given and on the others a meal high in saturated fat (hard cheese, bread and butter) or unsaturated (smoked mackerel, bread and margarine) was given with or without alcohol. The effects of these treatments on platelet aggregation were assessed by comparing the concentration of aggregating agent required to produce full secondary aggregation in the afternoon with that required in the morning. (Aggregation ratio, AR; values given are those for ADP and are means ± SD of 15 observations). Plasma lipids, platelet membrane phospholipids and blood alcohol concentration were measured by gas liquid chromatography (Fenn & Littleton, 1982).

Significant increases in the saturated fat content of plasma and of platelet phospholipids were obtained after the meal high in saturated fats and after fasting. These changes were not prevented by the concomitant administration of alcohol and neither did food intake significantly affect the concentration of alcohol (c 25 mM) obtained in blood. A meal high in unsaturated fat produced a small increase in the unsaturated fat content of platelet membrane phospholipids.

Platelets showed a reduced tendency to aggregate after fasting (AR = 1.25 ± 0.53) and this was affected relatively little by alcohol. Intake of food, either saturated (AR = 0.98 ± 0.24) or unsaturated (AR = 1.13 ± 0.30), prevented the reduction of platelet aggregability associated with fasting. The concomitant intake of alcohol with the saturated fat meal reduced aggregability of platelets to approximately the same degree as that associated with fasting (AR = 1.41 ± 0.62). Intake of alcohol with the meal high in unsaturated fat did not influence platelet aggregability.

These results suggest that the inhibitory action of alcohol on human platelet aggregation is greatest when the saturated fat content of the diet is high. If this mechanism is relevant to the protective effect of alcohol in coronary thrombosis then this is also likely to be greatest in populations which have a high dietary intake of saturated fats.

Acknowledgements. These experiments were supported by the Medical Research Council and the Brewers Society.

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HYPER-REACTIVITY TO NORADRENALINE IN ISOLATED ARTERIES FROM RABBITS WITH PERINEPHRITIS HYPERTENSION

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An increased reactivity to noradrenaline has been shown in vivo in rabbits with perinephritis hypertension (Berthelot et al, 1981). We have performed experiments using large and small arterial preparations to see if this hyper-reactivity also occurs in vitro. Non-receptor-mediated changes in reactivity were examined using potassium chloride. The importance of the neuronal uptake of noradrenaline was also examined since Mulvany et al (1980) have reported that in spontaneously hypertensive rats there is an increased neuronal uptake of noradrenaline which masks any increase in noradrenaline sensitivity. Rabbits were either sham operated or one kidney was wrapped in cellophane to induce perinephritis hypertension. Animals were used 4-6 weeks after operation by which time the hypertensive group possessed a mean blood pressure of 126±4mm Hg compared to 81±3mm Hg in the sham group. Segments of the thoracic aorta and the first generation vessel off the superior mesenteric artery were set up as ring preparations and isometric tension recorded. The terminal 5 cm of the ileal artery was cannulated and its arterial bed perfused with Krebs-Henseleit solution at 37°C and perfusion pressure measured. The results are summarised in Table 1.

Table 1 EC_{50} values (mean± s.e.mean) for noradrenaline (NA) and potassium chloride (KCl) in sham operated and hypertensive rabbits

		NA (μM) n = 8-10	NA+cocaine 10μM (μM) n = 5-6	KCl (mM) n = 7-10
Aorta	Sham	0.086 ± 0.015	0.052 ± 0.006	-
	Hypertensive	0.066 ± 0.006	0.038 ± 0.005	-
Mesen. Rings	Sham	12.9 ± 2.9*	1.4 ± 0.3*	46.9 ± 3.3
	Hypertensive	6.3 ± 1.0	0.75 ± 0.16	46.5 ± 3.6
Mesen. Perfusion	Sham	15.7 ± 3.0	2.42 ± 0.35	72.4 ± 6.9
	Hypertensive	12.1 ± 2.2	2.07 ± 0.33	73.4 ± 3.8

* $p = 0.05$

Noradrenaline concentration-response curves in all three preparations from hypertensive rabbits were shifted to the left (1.3-2 fold) of those from normotensive rabbits. This effect was only significant for the mesenteric arterial ring preparations, $p = 0.05$ (Analysis of Variance). The noradrenaline maximum response was unchanged. The hyper-reactivity was not further increased by cocaine in either the densely innervated mesenteric preparations or the more sparsely innervated aorta, suggesting that neuronal uptake is not an important factor in this model of hypertension. In contrast to noradrenaline, the sensitivity to potassium chloride was identical in preparations from normotensive and hypertensive animals.

These results suggest that in rabbits with perinephritis hypertension there is a small degree of hyper-reactivity to noradrenaline, but not to potassium chloride. This hyper-reactivity is not further increased when neuronal uptake of noradrenaline is blocked.

This work was supported by the University of Strathclyde R & D Fund.

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COMPARISON BETWEEN THE NEUROMUSCULAR EFFECTS OF KETAMINE AND METHOHEXITONE

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In a previous report (Wali,1983) it was shown that the intravenous anaesthetic drugs methohexitone,thiopentone,di-isoprofol,althesin and etomidate all potentiated the amplitudes of the twitch contractions produced by repetitive nerve stimulation at 0.2 Hz, reduced the contractures produced by acetylcholine(ACh),while they increased those produced by tetraethylammonium(TEA) in the isolated chick biventer cervicis nerve-muscle preparation.

In the present report,the effect of ketamine,a water-soluble intravenous anaesthetic agent,on the contractile responses produced by repetitive nerve stimulation and by drug action were studied and compared with those obtained with methohexitone,pentobarbitone and phenobarbitone in the same preparation.

Ketamine (50-500 $\mu g.ml^{-1}$) reduced the amplitude of the twitch contractions produced by nerve stimulation at 0.2 Hz with 5V and 0.5 ms pulse duration by 50-70% in 5-6 min.Recovery of the responses was achieved in about 10 min.Low concentrations of ketamine(1-10 $\mu g.ml^{-1}$) had no significant effect on the amplitude of the twitch contractions(control 1.8* 0.05 g,n=6).Ketamine (100 $\mu g.ml^{-1}$) reduced the contractures produced by ACh(250 $\mu g.ml^{-1}$) by 26* 2.3% (control 4.5* 0.2 g,n=6,$P < 0.02$). The contractures produced by TEA(2.4 $mg.ml^{-1}$) were also reduced in ketamine by 38* 1.9%(control 0.8* 0.01 g,n=6,$P < 0.001$).

Repetitive nerve stimulation at 20 Hz with 5V and 0.5 ms pulse duration produced tetanic contractions which were only slightly reduced in ketamine(100 $\mu g.ml^{-1}$) (control 2.9* 0.1,n=6).

Pentobarbitone (120 $\mu g.ml^{-1}$),like methohexitone(25 $\mu g.ml^{-1}$), increased the amplitude of the twitch contractions by 33* 1.4%(n=6).Pentobarbitone reduced the contractures produced by ACh(250 $\mu g.ml^{-1}$) by 32* 0.7% whereas it increased those produced by TEA(2.4 $mg.ml^{-1}$) by 67* 3.1% (n=6,$P < 0.02$,$P < 0.001$).

Phenobarbitone (60 $\mu g.ml^{-1}$) increased the amplitudes of all the contracture and contraction responses in the chick muscle.These responses were increased by 36* 1.1% , 33* 1.5% and 63* 1.1 % respectively(n=6,$P < 0.02$,$P < 0.001$).

The pharmacology,pharmacokinetics and the clinical uses of ketamine (Ghoneim & Korttila,1977; Clements & Nimmo,1981) and methohexitone (Sunshine et al.,1966 ; Whitwam,1972; Breimer,1976) have been studied but little is known about their actions and interactions at the vertebrate neuromuscular junctions.

In the present study,ketamine,unlike methohexitone,reduced the amplitudes of all the contractile responses in the chick biventer cervicis muscle,suggesting that it may depress transmission at the chick neuromuscular junction.

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ATRACURIUM REDUCES THE PRESYNAPTIC RELEASE OF ACETYLCHOLINE AT THE VERTEBRATE NEUROMUSCULAR JUNCTION

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In a previous report(Wali & Flynn,1983) it was suggested that atracurium,a new competitive neuromuscular blocking agent,may act presynaptically to reduce the spontaneous and evoked release of acetylcholine (ACh) in the isolated chick biventer cervicis (BVC) nerve-muscle preparations.

The present experiments were designed to investigate the effect of atracurium on the release of ACh in 3 different nerve-muscle preparations,the frog gastrocnemius, the chick biventer cervicis and the rat hemidiaphragm.

The preparations were loaded with radioactive choline (tritiated choline, ^{3}H-methyl choline of specific activity of 15 Ci.mmol^{-1} and final bath concentration of 2 μCi. ml^{-1}) in an organ bath for 2 h (Rand,Story & Wong-Dusting,1982). Aliquot samples and samples from the digested preparations (by hyamine hydroxide, 1 M) were taken for measurement of radioactivity in a liquid scintillation counter. In 50% of the preparations,atracurium was added after 105 min incubation to give an initial concentration of 1 μg.ml^{-1}. During the remaining 15 min this concentration will have been reduced to 0.8 μg.ml^{-1}, on the basis of exponential decline with a half-life of 20 min. The latter concentration,however,would still produce more than 50% reduction in the single twitch response (Weatherley,Williams & Neill,1983). In 50% of the preparations,the motor nerve was stimulated at a repetitive rate of 0.2 Hz with 5V and 0.5 ms pulse duration before and after the application of atracurium.

In the absence of repetitive nerve stimulation,atracurium reduced the amount of radioactivity (measured in counts min^{-1}, CPM),loaded in the control preparations, by 35-45% (Table 1). During repetitive nerve stimulation,atracurium produced similar reductions in the radioactivity taken up by the control preparations.

Table 1 Effect of atracurium on the amount of radioactivity loaded in the control frog,chick and rat nerve-muscle preparations.

	Frog	Chick	Rat	
Controls	1195 ± 75	11186 ± 892	1056 ± 53	(mean ± SEM)
Atracurium	765 ± 36	5660 ± 209	684 ± 16	" " "
% decrease	36 ± 1.7	49 ± 2.2	35 ± 1.3	" " "
P <	0.01	0.01	0.01	
n	8	12	8	

The present results show that atracurium reduces the amount of radioactivity loaded in the control preparations of the frog,chick and rat nerve-muscle preparations,suggesting that it may reduce the spontaneous and evoked release of ACh. Experiments,using intracellular recording and voltage-clamp techniques,are required to fully investigate the possible presynaptic effect of atracurium at the vertebrate neuromuscular junction.

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THE EFFECTS OF CADMIUM ON RAT HEPATIC MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION

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Cadmium is a highly toxic trace metal, having a long half-life in man. Following ingestion, cadmium is distributed predominantly in the liver and kidneys, although significant amounts are also found in other soft tissues (Webb, 1975). In laboratory animals, cadmium has been shown to produce a range of toxic effects, including testicular necrosis, proteinuria, and both structural and functional damage to the liver (Friberg et al, 1974; Fox, 1982).

Measurement of mitochondrial oxygen consumption gives a sensitive indicator of the effects of toxic substances on the synthesis of high energy compounds within the cell. We have investigated the effects of cadmium on mitochondrial oxidative phosphorylation and ATPase activity, in an attempt to further elucidate the mechanisms by which this ion exerts its toxic effects.

Tightly coupled rat hepatic mitochondria exhibited the phenomenon of respiratory control, that is when the mitchochondria were respiring in the presence of substrate, phosphate & oxygen (State 4), the addition of ADP elicited a stimulation of respiration (State 3) which returned to control levels when the ADP had been converted to ATP.

In the presence of cadmium (3.3-33.3μM) a concentration dependent inhibition of State 3 respiration was observed for the NADH-linked oxidation of glutamate (5mM) plus malate (5mM), with a maximum inhibition of 85.9+/-5.3% (n=5) occurring at a cadmium concentration of 33.3μM. The IC_{50} (concentration required to give 50% inhibition of the maximal rate) value for the effect of the stimulatory rate was found to be 1.92+/-0.26μM (n=5). Over the same concentration range (3.3-33.3μM) cadmium failed to release oligomycin (1-5μg) inhibited State 3 respiration, or to significantly change the State 4 rate.

When glutamate plus malate were replaced by succinate (5mM), a concentration dependent inhibition of State 3 respiration (55.6+/-7.6 to 7.4+/-1.4 ng atom O_2 consumed/min/mg protein, n=5) was observed in the presence of cadmium (3.3-33.3μM). A maximum inhibition of 88.6+/-1.2% occurred at a metal concentration of 33.3μM, and an IC_{50} value of 8.34+/- 0.83μM (n=5) was obtained.

Evidence for the possible energy dependent transport of cadmium into mitochondria, in a manner similar to calcium uptake (Carafoli 1977), was obtained when low concentrations of cadmium (3.3 to 13.3μM) were observed to increase State 4 respiration from 27.4+/-4.5 to 50.6+/-5.4 ng atom O_2/min/mg protein. This significant ($p<0.05$) stimulatory effect gave an EC_{50} value of 6.38+/-1.22μM (n=5). At concentrations above 13.3μM, the rate was observed to return to control levels.

The inability of cadmium (0.01-1mM) to cause loss of respiratory control, or to stimulate mitochondrial ATPase (E.C.3.6.1.4) activity, indicates that cadmium does not act as an uncoupling agent. The IC_{50} values for State 3 respiration, and its failure to release oligomycin inhibited respiration, indicate that the prime site of action of cadmium is the inhibition of electron transport by its interaction with the NADH-dehydrogenase enzyme complex.

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EVIDENCE THAT α-TOCOPHEROL PROTECTS AGAINST HYPOXIA IN GUINEA-PIG ATRIA BY MEMBRANE STABILISATION

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α-Tocopherol (Vitamin E) protects cardiac tissue and maintains the rate and force of contraction in perfused hearts and in isolated atria in hypoxia (Guarnieri et al., 1978; Kelly and Richardson, 1981). Two possible mechanisms have been proposed for the action of α-Tocopherol i) free radical scavenging and ii) membrane stabilisation. Since these actions may be produced by different parts of the molecule, we have investigated the actions of α-tocopherol; the chromane ring (6-hydroxy-2,5,7,8 tetra methyl chromane-2 carboxylic acid) and the hydrocarbon chain (phytol) on the force and rate of guinea-pig isolated atria during hypoxia. Guinea-pig isolated atria were suspended in organ baths containing Krebs-Henseleit solution at 32°C bubbled with 95% O_2 and 5% CO_2. Spontaneous contraction rate and force were recorded before, during and after a 20 min period of hypoxia, induced by bubbling the solution with 95% N_2 and 5% CO_2. After 15 min, α-tocopherol (2.3 -697μM), the chromane ring (69.7 μM), phytol (69.7 μM) or dimethyl sulphoxide vehicle (1.5%) were added and after a further 5 min the hypoxia, normoxia cycle was repeated. Comparisons between the percentage inhibition of rate and force by hypoxia, in the presence and absence of drug, were made by paired t-test. Results are expressed as means with standard errors in parenthesis. None of the compounds tested produced any effect on the force or rate of contraction in normoxia. Hypoxia decreased the rate and force by 54 (9)% and 83 (2)% respectively, an effect which was repeatable in non-treated tissues. α-tocopherol produced a dose related inhibition of the effects of hypoxia on rate (ED_{50} 69.7 μM). The maximum effect was produced by 100 μM α-tocopherol, when hypoxia reduced the rate to 100 (15) bpm compared with 43 (10) bpm in the control period. Phytol at the same dose produced a similar effect. The chromane ring of α-tocopherol (69.7 μM) produced a slight [5.2(2.5)%] potentiation of the hypoxic inhibition of atrial rate.

	Atrial rate (b.p.m.)			
Compound (69.7μM)	Control (Normoxic)	Control (Hypoxic)	Hypoxia + Drug	n
Vehicle	112(6)	51(10)	50(12)	8
α-Tocopherol	109(6)	47(7)	77(12)*	4
Phytol	163(6)	47(4)	73(11)*	4
Chromane Ring	140(5)	50(3)	43(5) *	8

*Significantly different from hypoxic control $p<0.05$.

In addition, both α-tocopherol and phytol produced small but significant reductions of the effect of hypoxia on force of contraction.

These experiments confirm the effects of α-tocopherol on hypoxic atria (Kelly et al, 1981). The proposal that it is the antioxidant properties of α-tocopherol that protect the heart in hypoxia is not supported by these experiments, because the antioxidant properties reside in the chromane ring not the phytyl side chain. Since both α-tocopherol and phytol produce similar effects we suggest that membrane stabilisation may exert some protection in the hypoxic heart.

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m-IODOBENZYLGUANIDINE : AN ADRENAL IMAGING AGENT

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A number of radiopharmaceuticals have been developed as adrenal imaging agents. It is claimed that such agents are selectively accumulated in the adrenal tissue and can remain trapped within the storage vesicles for some days allowing adrenal imaging at a time when plasma levels of the agent have decreased to very low levels (Sisson et al., 1981). In association with the abnormally high plasma and urine catecholamine levels such compounds make the diagnosis of phaeochromocytoma easier. One of the more useful imaging agents is meta-iodo(I^{131})benzylguanidine (MIBG), an analogue of guanethidine. Although in regular use in several centres its basic pharmacology has not been investigated thoroughly but has rather been inferred from a knowledge of the actions of guanethidine. Using a number of test systems we have examined the actions of MIBG.

In the rat anococcygeus muscle MIBG acts in an identical manner to guanethidine. At low doses (10^{-6} M) there is a transient potentiation of contractions produced by nerve stimulation, presumably due to the inhibition of neuronal accumulation of noradrenaline. However within 5-10 min the neurone blocking action becomes apparent and the effect of nerve stimulation is inhibited. At higher doses (10^{-5} M) of MIBG this neurone blocking action is accompanied by a large release of noradrenaline producing a sustained contraction of the anococcygeus. As with guanethidine, nerve stimulation now produces an inhibitory response. There was no evidence from its actions on exogenous noradrenaline that MIBG acted as an α-adrenoceptor antagonist. Confirmation that MIBG interferes with neuronal accumulation was obtained in vitro. Slices of rat ventricle were incubated in the presence of I^{131}-MIBG. The accumulation of MIBG was decreased by incubation at 0 °C and by incubation in the presence of guanethidine, noradrenaline or desiprimine. Conversely, MIBG inhibited the accumulation of ^{3}H-noradrenaline in rat ventricular slices (Table 1). Once taken up the location of the MIBG cannot be determined precisely but using a perfused slice preparation it is possible to demonstrate a Ca^{++} dependent release on exposure to high $[K^{+}]$. This is suggestive of storage within vesicles in noradrenergic nerve endings with subsequent exocytotic release.

From the above information it would not be anticipated that MIBG would be selectively accumulated in adrenal tissue but should be widely distributed in most noradrenergic nerve endings. However MIBG at the clinical level has proved extremely successful in the imaging of phaeochromocytoma with very few false positives. It must be assumed then that tumour tissue has peculiar properties allowing the selective accumulation of MIBG, a point which is difficult to test experimentally since surgical removal of phaeochromocytoma tumour is now a relatively rare means of treatment.

Table 1 Uptake of ^{3}H-labelled noradrenaline in the presence of drugs

Drug	Concentration	% of control
Control		100
Guanethidine	10^{-5} M	43.3
Desmethylimipramine	10^{-6} M	56.8
Phenoxybenzamine	10^{-5} M	60.0
Phentolamine	10^{-5} M	108.6
Meta-iodobenzylguanidine	4.3×10^{-6} M	42.7
	4.3×10^{-7} M	75.0
	8.6×10^{-8} M	80.0
	4.3×10^{-8} M	96.0

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EVIDENCE FOR THE PRESENCE OF TWO CALCIUM TRANSPORT SYSTEMS IN MITOCHONDRIA ISOLATED FROM GUINEA-PIG LIVER, USING VERAPAMIL

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The observation that mitochondrial calcium accumulation in hypoxic heart muscle was prevented by verapamil (Nayler et al, 1978) has led us to examine the mode of action of this drug on mitochondrial activities, including ATP synthesis and calcium movements.

Succinate oxidation, catalysed by guinea-pig liver mitochondria and stimulated by ADP or calcium, was measured using an oxygen electrode. Calcium movements across the mitochondrial inner membrane were followed with a calcium-specific electrode. Both reactions were carried out at 25°C in 0.25 M sucrose and 3.4 mM tris-HCl buffer, pH 7.4. Calcium influx was followed in the presence of 10 - 100 μM calcium chloride; the reaction was initiated by the addition of guinea-pig liver mitochondria (5 mg protein). Calcium efflux was monitored after the mitochondria had been allowed to accumulate calcium from the reaction medium.

Verapamil inhibited calcium influx in a concentration-dependent manner (IC50 = 61 ± 7 μM; n = 5). In contrast, the efflux of calcium was promoted by verapamil (EC50 = 45 ± 3 μM; n = 5). The two actions did not require significantly different concentrations of verapamil. It was concluded that neither effect was likely to be due to an action on mitochondrial respiration, since ADP-stimulated respiration required 106 ± 19 μM verapamil for 50% inhibition, and calcium-stimulated respiration required 583 ± 42 μM for 50% inhibition (n = 5 in both cases).

We considered the possibility that the inhibition of calcium influx by verapamil was a consequence of the calcium releasing action of the compound. To test this idea, we examined the effect of verapamil on calcium transport in the presence of bongkrekic acid (1 μM), a substance known to protect mitochondria against the loss of accumulated calcium (Peng et al, 1977). Under these conditions, the concentration of verapamil required to promote efflux of calcium was increased to 155 ± 22 μM; n = 5 (significantly different from controls, $P = < 0.01$, in the presence of verapamil alone). In contrast, when calcium influx was studied, bonkrekic acid reduced the concentration of verapamil required to inhibit the process (IC50 = 33 ± 1.5 μM; n = 5: significantly different from controls, $P = < 0.01$). Thus, even though verapamil-stimulated calcium efflux is reduced when bongkrekic acid is included in the reaction medium, the potency of verapamil on calcium influx is increased, and the two effects are significantly different ($P = < 0.001$). Inhibition of influx cannot therefore be secondary to promotion of efflux.

We conclude that verapamil has two distinct actions on mitochondrial calcium transport: one on a component mediating calcium influx, the other on a second component responsible for efflux of the cation. Although relatively high concentrations are needed to produce these effects, modification of calcium transport in mitochondria cannot be excluded as a possible target for the protective action of verapamil, since the drug has been shown to accumulate in a variety of cell types (Pang and Speralakis, 1983).

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AN ALTERNATIVE HUMAN SMALL AIRWAY PREPARATION

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Human lung parenchymal strips (HLPS) are frequently used as in vitro models of small airway contractility. However, interpretation of results from HLPS are difficult since these preparations consist of a heterogenous population of contractile elements and the proportion of small airways within the HLPS varies between preparations (cf. Black et al 1983). Furthermore, it is difficult to study bronchodilator effects because of instability of induced tone in HLPS. We have investigated some pharmacological characteristics of an alternative small airway preparation from human lung. The tissue was resected at thoracotomy. The airways were identified by presence of mucus, absence of blood and a subsequent histological examination. Bronchioles with lumen diameters of 0.6 mm-1.5 mm were freed from adjoining tissues and cut into 2 mm long tubular segments. Preparations were placed between two fine prongs, one connected to a force displacement transducer and the other to an adjustable sled. The specimens were bathed in 2.5 ml of Krebs solution (37°C), gassed with carbogen and drugs administered cumulatively. Isometric tension was recorded and a resting tone established at 0.5 g.

Table 1. Responses of human isolated bronchioles to pharmacological stimuli

	n	Range of effective concentrations (M)	EC_{50} (95% conf. lim.) (M)	Mean max.tension mg (s.e. mean)
Constrictors:				
Carbachol	13	$1x10^{-8}$ - $3x10^{-5}$	$4.5x10^{-7}$ (2.7, 7.6)	487 (141)
Histamine	3	$1x10^{-8}$ - $1x10^{-4}$	$1.0x10^{-6}$ (0.15,6.6)	289 (29)
Adenosine	6*	($1x10^{-7}$ - $1x10^{-4}$)	-----	< 70
Dilators:				Mean % relaxation
Theophylline	4	$1x10^{-5}$ - $3x10^{-3}$	$1.5x10^{-4}$ (0.9, 2.4)	127 (20)
Enprofylline	4	$1x10^{-6}$ - $4x10^{-4}$	$2.1x10^{-5}$ (0.8, 5.2)	130 (43)
Terbutaline	2	$3x10^{-9}$ - $1x10^{-5}$	$1.2x10^{-7}$ (0.8, 2.1)	103 (36)

* 3 bronchi had a diameter > 1.5 mm, 1 from a person with bronchial hyperreactivity.

Both carbachol and histamine produced concentration-related, stable contractions of the bronchioles. Relaxations were studied in the presence of $2x10^{-6}$M carbachol (EC_{70}) and calculated as % inhibition of the induced tone (Table 1). Theophylline, an adenosine receptor antagonist, was less potent than the adenosine non-blocking alkylxanthine, enprofylline (Persson 1982). Adenosine by itself induced only weak contractions or relaxations. These observations support the view that antiasthmatic actions of xanthines may not reflect adenosine antagonism. The relaxations induced by terbutaline extend the findings by Persson and Ekman (1976) and show that human small airways are sensitive to β_2-receptor stimulation. Changes in small airway calibre (lumen diameter < 2 mm) are important in obstructive lung disease. The present bronchiolar preparation apparently was 10 times more sensitive to carbachol and histamine than were HLPS (cf. Goldie et al 1982). It is also of interest that the bronchioles relaxed to some degree at concentrations of terbutaline, enprofylline, and theophylline which also are achieved in plasma during treatment of asthma. It is suggested that the present bronchiolar preparations is a relevant model of smooth muscle reactivity in human small airways.

M.J.B. Finney is partly supported by the Lilian Roxon Memorial Asthma Research Travel Grant.

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EFFECTS OF TERBUTALINE, THEOPHYLLINE AND ENPROFYLLINE IN TRACHEAS BRONCHI AND LUNG STRIPS CONTRACTED BY PROPOSED ASTHMA MEDIATORS

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The possibility that relaxant responses to xanthines and β-receptor agonists might vary depending on what type of asthma mediator that has produced bronchoconstriction was examined in guinea-pig airway preparations. The effects of terbutaline (TERB), theophylline (THEO), and enprofylline (ENPRO) were studied in tracheas contracted by traditional and novel mediators. Tracheal relaxatory characteristics of the three bronchodilators were compared also with those obtained in hilus bronchi and lung strips.Open tracheal rings,tubal segments of hilus bronchi and lung strips (1.5 x 1.5 x 20 mm) from guinea-pigs were prepared and mounted in organ baths (see Karlsson & Persson 1981).Tension was increased by mediators from about 0.9 to about 1.5 g, 0.5 to 1.5 g and 1.0 to 1.1 g in tracheas,bronchi and lungstrips,respectively.Isometric tension changes were recorded.The mediators produced sustained contractions from which TERB,THEO and ENPRO produced graded relaxations.The maximum relaxation did not differ significantly($p>0.05$)between these drugs (Table).

Table Relaxatory EC_{50} ($\pm$s.e.mean)values(n=3-8)producing 50% maximum relaxation in airway preparations contracted by carbachol(CARB),histamine(HIST),prostaglandin($PGF_{2\alpha}$),substance P(SP),serotonin(5-HT),leukotriene(LTC4) and ovalbumin(OA,sensitized animals)(molar concentrations in brackets).

	5-Ht	LTC4	OA	SP	$PGF_{2\alpha}$	HIST	CARB	
	(2×10^{-7})	(1×10^{-8})	(0.1µg/ml)	(3.7×10^{-6})	(1.8×10^{-6})	(1×10^{-6})	(6×10^{-8})	
Trachea								
TERB	3.0±0.4	7.0±1.6	8.8±2.6	8.6±1.0	8.7±3.3	4.0±0.7	6.1±0.7	x 10^{-8}M
THEO	4.0±0.8	4.0±0.9	15 ±3.2	15 ±2.0	8.5±1.0	5.7±1.5	9.1±1.4	x 10^{-5}M
ENPRO	0.6±0.04	0.6±0.1	3.7±0.4	2.2±0.3	2.1±0.3	1.5±0.4	2.0±0.4	x 10^{-5}M
Bronchus						(6×10^{-6})	(1×10^{-6})	
TERB						24±6.3	36 ±10	x 10^{-8}M
THEO						15±4.7	33 ±9.3	x 10^{-5}M
ENPRO						3.7±1.0	5.2±0.8	x 10^{-5}M
Lung strip				(1×10^{-4})	(3×10^{-5})	(1×10^{-5})		
TERB				17±4.3	10 ±4.8	48±9.7		x 10^{-8}M
THEO				23±5.4	19 ±5.2	75±16		x 10^{-5}M
ENPRO				16±6.0	6.4 ±0.9	31±6.1		x 10^{-5}M

The three bronchodilators were effective relaxatory agents and each had EC_{50}-values that differed less than sixfold in tracheas contracted by different mediators or by allergen. Thus little selective mediator-antagonism was produced.This result and the findings in bronchi and lung strips agree with the view that β-receptor agonists and xanthines are functional antagonists to mediators in airway smooth muscle.ENPRO,a poor adenosine antagonist(see Persson 1982)was more potent than the adenosine-receptor blocking drug,THEO,suggesting that inhibition by xanthines of mediator- or allergen-induced airway smooth muscle tone is unrelated to adenosine antagonism.(Adenosine, 10^{-6} - 10^{-3}M had dual actions in the trachea but was almost without effects on tone in bronchi and lung strips).A bronchodilator potency ratio between ENPRO and THEO of about 5 seems to be valid in vivo in animals and man (see Persson 1982).The findings in trachea and bronchi confirm this ratio,whereas in lung strips ENPRO was twice as potent as THEO.In conclusion,xanthines and β-receptor agonists exert a functional antagonism and could be expected to be effective relaxants irrespective of type of asthma mediator that has constricted the airway.

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PROSTAGLANDIN RELEASE AND INCREASED VASCULAR PERMEABILITY DUE TO HISTAMINE IN MINI-PIG SKIN ARE MEDIATED VIA H1 RECEPTORS

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An in vivo model system for the study of the interaction between mediators of cutaneous inflammatory states is described. Histamine (H) and prostaglandins (PG) were selected in the present work since levels of these two mediators are elevated in a variety of human skin disorders.
We have measured two responses, namely the Histamine induced release of PG from superfused mini-pig skin and increased vascular permeability. The mini-pig was chosen for these experiments since it had been demonstrated previously (Hensby et al. 1982) to have pharmacological responses to these and other agents similar to those of man.

Histamine ($1x10^{-8}$ to $1x10^{-5}$ M) caused a dose and time related elevation of PG release from superfused mini-pig skin. This stimulation was rapidly reversed or removed of the H and could be inhibited by Mepyramine (M) but not by Cimetidine (C) suggesting that it is mediated via H_1 receptors.

In the second study PGE_2 (0.3 nmol)synergistically increased the dermal vascular permeability of ^{125}I labelled human serum albumin induced by H. This increased vascular permeability due to H was blocked by M (10nmol) but not C (10nmol) suggesting that it was mediated via H_1 receptors.

TABLE

	PGE_2	H	H + PGE_2	H + PGE_2 + M	H + PGE_2 + C
concentration injected (nmol)	0.3	10	10 + 0.3	10 + 0.3 + 10	10 + 0.3 + 10
plasma exudation (μl)	12±2	30±3	77 ± 15	18 ± 3 ***	68 ± 6 NS

*** $p < 0.001$ relative to H + PGE_2 alone
NS not significant relative to H + PGE_2 alone

Values and means ± SEM N = 12 Samples were injected in 100 μl 0.9 NaCl which gave 10 ± 2 μl exudation.

These results suggest that in cutaneous inflammatory responses the pharmalogical interaction of different groups of mediators may be of more importance than their individual effects.

Hensby et al. Int. J. Immunopharmacol., 4, 352, 1982

LOCALISATION OF BENZO(α)PYRENE HYDROXYLASE AND ALDRIN EPOXIDASE IN NEONATAL RAT SKIN

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Mammalian skin possesses a microsomal drug oxidation system which is highly inducible by the topical administration of polycyclic hydrocarbons (Rettie & Rawlins, 1982). It has been suggested that the epidermis is the major site of drug oxidation in skin (Thompson & Slaga, 1976; Bickers et al, 1982), but this interpretation may have been complicated by inadequate skin separation techniques, and the presence of relatively high specific activities in epidermis compared to those in dermal and whole skin fractions. We have used a reliable skin separation technique (Epstein et al, 1978) to assess the role of epidermal and dermal mixed-function oxidases in the neonatal rat. The suitability of this method was verified by histological examination.

Epidermal, dermal and whole skin microsomes were prepared from neonatal rats following pulverisation in liquid nitrogen and Polytron homogenisation. Microsomal aldrin epoxidase activity was estimated by electron-capture GLC (Williams et al, 1982) and benzo(α)pyrene hydroxylase activity by the fluorimetric method of Nebert and Gelboin (1968). Activities are shown in Table 1 expressed as pmoles 3OH benzo(α)pyrene formed, or pmoles dieldrin formed related to microsomal protein, wet weight of tissue or whole organ activity. Statistical analysis was performed using the Mann-Whitney U-test.

Table 1

	Benzo(α)pyrene hydroxylase			Aldrin epoxidase		
	pmol mg^{-1} min^{-1}	pmol G^{-1} min^{-1}	pmol $organ^{-1}$ min^{-1}	pmol mg^{-1} min^{-1}	pmol G^{-1} min^{-1}	pmol $organ^{-1}$ min^{-1}
Epidermis	0.84±0.08 (n=4)	2.56±0.24	0.51±0.05	0.80±0.18 (n=5)	2.43±0.54	0.48±0.11
Dermis	0.25±0.03* (n=12)	0.86±0.09*	1.04±0.11*	0.23±0.08* (n=5)	0.78±0.26*	0.96±0.32
Whole Skin	0.30±0.04* (n=7)	1.31±0.07	1.86±0.25*	0.29±0.06* (n=5)	1.23±0.32*	1.75±0.46*

*$p < 0.05$ difference from epidermis

Total microsomal protein contents were 1.01±0.15, 2.58±0.15 and 2.77±0.21 $mg.g^{-1}$ for epidermis, dermis and whole skin respectively. The distribution of activity between fractions was similar for aldrin epoxidase and benzo(α)pyrene hydroxylase. Activities of both enzymes, when expressed either on the basis of microsomal protein, or tissue wet weight, were significantly higher in epidermis than in dermis ($p<0.05$). When expressed in relation to whole organ activity, although the sum of epidermis and dermal activity approximated that of whole skin activity, epidermis contributed less than 30% of total activity.

We conclude that both epidermis and dermis may be important in the biotransformation of topical xenobiotics. Dermal activity is likely to be particularly relevant following trans-appendageal adsorption or where the integrity of the epidermis is interrupted.

A.E. Rettie is an MRC student.

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STUDIES ON THE CONTROL OF ARACHIDONIC ACID TURNOVER IN GUINEA-PIG ENDOMETRIUM

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Prostaglandin (PG) $F_{2\alpha}$ output from the guinea-pig uterus increases after Day 11 (Blatchley et al., 1972), and oestradiol (whose output from the ovary increases after Day 10) acting on a progesterone-primed uterus is probably the physiological stimulus. The availability of free arachidonic acid (AA) in the endometrium is the rate limiting step in $PGF_{2\alpha}$ synthesis (Leaver & Seawright, 1982). The uptake of AA into phospholipid (PL), but not into triglyceride, is significantly higher on Day 15 than on Day 7 (Ning et al., 1983). AA could be incorporated into PL either by de novo synthesis or by the addition to lysophospholipid. Since oestradiol stimulates de novo PL synthesis in rat uterus, (Aizawa & Mueller, 1961) an increase in the rate of de novo PL synthesis in guinea-pig endometrium could account for the increased uptake of AA. Consequently, Day 7 and Day 15 endometrium was maintained in tissue culture (Leaver & Seawright, 1982) and incubated with 10 µCi of [^{3}H] choline chloride (sp. act. 58 mCi/mmol) for 6 h. [^{3}H] Phosphatidylcholine (PC) formed was purified by column and thin-layer chromatography (Ning et al., 1983). The rates of PC synthesis (mean ± s.e.m., n = 10) on Days 7 and 15 were 74.4 ± 8.2 and 95.4 ± 27.8 pmole/g tissue/6 h, respectively. These rates did not differ significantly, indicating that the increased uptake of AA into PL is not due to increased de novo synthesis but rather to increased addition to lysophospholipid. Oestradiol may be the stimulus for this latter process.

In the tissue culture system used, oestradiol stimulates and progesterone inhibits $PGF_{2\alpha}$ output from guinea-pig endometrium (Leaver & Seawright, 1982). Therefore, the effects of these steroids on AA release from the various lipids have been measured. Day 7 and Day 15 endometrium was cultured with 20 µCi [^{3}H] AA (sp. act. 120 Ci/mmol) for 24 h. A portion of the endometrium was then analysed immediately, while the rest was incubated for a further 48 h in fresh medium containing no steroids, or containing 10 ng/ml oestradiol, **or 1,000 ng/ml progesterone.** After culture, the various lipids in the endometrium were extracted and purified as described by Ning et al. (1983), and their radioactive content was measured. There were no significant changes in the release of [^{3}H] AA from any lipid class either in the absence or presence of ovarian steroids on Days 7 and 15. A release of 1 to 4% of the total esterified arachidonic acid in the endometrium would account for the amounts of $PGF_{2\alpha}$ synthesised by the endometrium during the culture period. Since the variation in the initial labelling of endometrial lipids among animals and among tissue samples from the same animal is greater than 4%, this technique appears unsuitable for establishing from which lipid pool arachidonic acid is released for conversion into $PGF_{2\alpha}$ by guinea-pig endometrium.

These studies were supported by a grant from The Wellcome Trust.

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MULTIEXPONENTIAL PARAMETER ESTIMATION: ELIMINATION OF BIAS

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Linear compartmental models are widely used to describe the pharmacokinetic behaviour of drugs. These models give rise to multiexponential expressions of the type

$$C = \sum_{i=1}^{n} A_i \exp(-a_i . t)$$

where C is plasma drug concentration, n is the number of exponential terms, t is the time measured from the point of drug administration and A_i and a_i are the parameters of the model. Given a set of values for C and t a number of curve fitting techniques are available (Metzler et al, 1974; Provencher, 1976; Sedman & Wagner, 1976) for parameter estimation. One of these is the so-called curve stripping technique also known as feathering, peeling-off or back projection. The principal assumption upon which this technique is based, is that the magnitudes of the exponential rate constants are widely disparate. Violation of this assumption would be expected to result in biased parameter estimates. This effect was demonstrated using computer generated data and the FORTRAN IV curve stripping programme CSTRIP (Sedman & Wagner, 1976).

Bias in the parameter estimates may be eliminated by employing the following iterative procedure. Estimate the parameters by curve-stripping and use these estimates to "correct" the data by substracting the contribution of the fast exponentials from the slow exponentials. Estimate the parameters of the "corrected" data by curve-stripping. Repeat this procedure until the parameter estimates do not alter significantly from step to step. This procedure was applied to noise free computer generated bi-exponential data and the results are tabulated in table 1.

Table 1 Biexponential parameter estimation

Parameter	True Value	Estimated Value		
	Iteration	1	2	3
A_1	33.0	19.1	33.2	33.7
a_1	0.12	0.16	0.13	0.12
A_2	33.0	47.7	33.5	32.3
a_2	0.08	0.082	0.078	0.078

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THE EFFECTS OF BUPRENORPHINE ON OPIOID RECEPTORS IN THE MOUSE PERITONEUM

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The modified abdominal constriction test in which antinociceptive drugs are administered intraperitoneally to mice (Bentley et al. 1981) was used to study the effects of buprenorphine on peripheral opioid receptors, and to compare this drug with three other opioid agonists. It was found that buprenorphine behaved as a full agonist, but was about 20 times less potent than morphine. Buprenorphine had a slower onset of action than other analgesic drugs, and showed several differences from morphine. For example, Naloxone, 3.0×10^{-5} mol/kg S.C. given 23 minutes prior to morphine produced a dose-ratio of 210 for morphine but did not antagonise the other three opioid drugs at all. Pretreatment with a single dose of morphine, 5×10^{-6} mol/kg S.C. given three hours previously caused a 1200-fold desensitization of a subsequent dose of morphine, but caused a less than two-fold desensitization of buprenorphine, while a similar pretreatment with buprenorphine, 5×10^{-6} mol/kg S.C. had not effect on the potency of morphine.

Pretreatment with clonidine, 5×10^{-8} mol/kg S.C. 3 hours previously, caused a cross-desensitization of morphine (480-fold) but desensitized buprenorphine by a factor of only 2.3 times, ketocyclazocine by 4.7 times, and approximately doubled the potency of pentazocine. The α-adrenoceptor agonist piperoxan, 3.0×10^{-5} mol/kg, S.C. given 23 min. previously, antagonised morphine by a factor of 66, while buprenorphine was antagonised only 1.8-fold, and pentazocine 3.3-fold.

It is concluded that the opioid receptors in the mouse peritoneum which respond to buprenorphine are different from μ-receptors. To characterise them more accurately will require the use of a variety of drugs selective for other opioid receptors.

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DELETERIOUS EFFECT OF IBUPROFEN IN A REPERFUSION MODEL OF MYOCARDIAL INFARCTION IN THE ANAESTHETISED DOG

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The non-steroidal anti-inflammatory agent, ibuprofen, has been claimed to reduce the extent of myocardial infarction caused by total occlusion of the left anterior descending coronary artery (LAD), in the anaesthetised dog (Darsee and Kloner, 1981). Reperfusion of a previously occluded coronary artery has also been reported to result in salvage of the ischemic area (Geary et al, 1982). In the present study we have evaluated the effects of ibuprofen treatment in a reperfusion model of myocardial infarction in the anaesthetised dog.

Chloralose anaesthetised beagles of either sex (wt. 10.0 - 15.0 kg) were used. Femoral blood pressure, electrocardiogram (Lead II), left ventricular pressure and its first derivative, dp/dt, were continuously recorded. Following a left thoracotomy an occlusive snare was placed around the LAD distal to its first major diagonal branch. Animals were randomly assigned to two groups, one group (n=9) received ibuprofen (12.5 mg/kg i.v.) 30 minutes before occlusion of the LAD and the other group (n=8) received no treatment. The LAD was occluded for one hour and reperfused for a further two hours. After the reperfusion period the area at risk was delineated by a rapid intravenous infusion of Evans blue dye, whilst the LAD was perfused with saline at mean systemic blood pressure. The heart was then arrested with a saturated solution of KCL, removed and cut into slices (0.5 - 1.0 cm). These sections were counterstained in triphenyltetrazolium chloride, at 37^{o}C for 30 minutes, to differentiate normal and infarcted myocardium. Colour photographs of the serial sections were independently viewed and areas of risk and infarct were measured by computer-assisted planimetry.

Pretreatment with ibuprofen significantly increased the area at risk and the ultimate infarct size (Table 1). Histological examination confirmed that the extension of the infarct was associated with a much greater incidence of microvascular damage and accompanying myocardial haemorrhage. An increased occurrence of death due to ventricular fibrillation (VF) was also apparent in the treatment group. Haemodynamic differences between the control and treatment groups were not significant.

Table 1.
The effect of ibuprofen (12.5 mg/kg i.v.) on area at risk and infarct size in anaesthetised dogs.

	% Risk (R)	% Infarct (I)	I/R %	No. without Infarct	Incidence of haemorrhage	Incidence of VF
Control (n=8)	28.2 (17.2-35.9)	3.1 (0-12.1)	11.5 (0.42-0)	1/8	2/8	0/8
Ibuprofen (n=9)	32.9* (25.0-45.9)	13.3* (1.5-18.5)	37.4** (6.2-46.5)	0/6	6/6	3/9

Median (range) *$p<0.05$, **$p<0.01$ Mann-Whitney 'u' test

These results demonstrate a significant infarct extension due to ibuprofen treatment, and suggest that ibuprofen may impair reperfusion salvage of ischemic myocardium.

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INHIBITION BY OPIATES OF THE EVOKED RELEASE OF (^{3}H)-ACH: DIFFERENCES BETWEEN CORPUS STRIATUM AND TUBERCULUM OLFACTORIUM

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In the striatum (ST), the electrically-evoked release of acetylcholine (Ach) can be modulated through dopamine (DA) receptors (Hertting et al., 1980). The evidence for a modulatory role of opiates on DA release is controversial (Chesselet et al., 1982) and the information for their action on the release of acetylcholine (Ach) in the ST is scarce (Vizi et al., 1977). We report here a comparative study of the effects of morphine (MO) and D-Met-L-Proenkephalinamide (EA, an enkephalinase resistant analog of Met-enkephalin) on the electrically-evoked release of Ach in slices of rat ST and tuberculum olfactorium (TO) labelled with [^{3}H-methyl]-choline and superfused in vitro.

Two periods of electrical stimulation (S_1 and S_2) at 1 Hz, 16 mA, 1 msec, 2 min, were applied with an interval of 44 min. The electrically-evoked release of tritium in S_1, expressed as percent of total tissue radioactivity, was 5.29 $\pm$ 0.28% (n=25) and 2.03 $\pm$ 0.08% (n=61) in the ST and TO respectively.

Morphine produced a concentration dependent inhibition of the electrically-evoked release of ^{3}H-Ach in TO but had no effect on ^{3}H-Ach release in the ST (figure 1A). Exposure to EA inhibited the electrically-evoked release of ^{3}H-Ach in both ST and TO but was at least 10 times more potent in TO when compared with ST (figure 1B). The inhibitory effects of both MO and EA were naloxone-sensitive and sulpiride-insensitive. None of the drugs tested modified the spontaneous outflow of radioactivity.

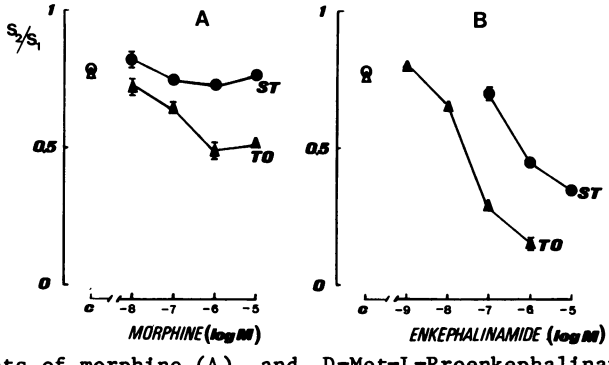

Figure 1 : Effects of morphine (A) and D-Met-L-Proenkephalinamide (B) on the electrically-evoked release of ^{3}H-Ach from striatum (ST) and tuberculum olfactorium (TO).
Abscissa : molar concentrations of drugs added 20 min before S_2. C : controls.
Ordinate : effects of drugs expressed as the ratio of S_2 over S_1.

Our results demonstrate that activation of opiate receptors inhibits the electrically-evoked release of ^{3}H-Ach in the TO. These receptors are mainly of μ and δ subtypes. In the ST the inhibitory effect of EA on ^{3}H-Ach release appears to be mediated by opiate receptors of δ subtype, although rather high concentrations of EA were required.

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CITALOPRAM ANTAGONIZES THE STIMULATION BY 5-METHOXYTRYPTAMINE OF INHIBITORY 5-HT AUTORECEPTORS IN THE RAT HYPOTHALAMUS

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Presynaptic inhibitory autoreceptors modulate the calcium-dependent evoked release of serotonin (5HT) through a negative feed-back mechanism in central serotoninergic neurons (Cerrito and Raiteri, 1979; Göthert and Weinheimer, 1979; Langer and Moret, 1982). It was previously reported that citalopram, a selective inhibitor of 5HT uptake does not affect per se the stimulation-evoked overflow of ^{3}H-5HT but antagonizes the inhibitory effect of LSD on ^{3}H-5HT release (Langer and Moret, 1982). The present experiments were carried out in order to further study this interaction between inhibition of neuronal uptake of 5HT and the effects of agonists on the presynaptic 5HT autoreceptor.

Rat hypothalamic slices were labelled with ^{3}H-5HT (creatinine sulphate) and superfused with Krebs' solution. Two periods (S_1 and S_2) of electrical stimulation were applied with an interval of 44 min and drugs were added 20 min before S_1 or S_2. The parameters of stimulation were 3 Hz, 20 mA, 2 msec for 2 min.

In the absence of inhibition of neuronal uptake, exogenous 5-methoxytryptamine (5 MeOHT) inhibited in a concentration-dependent manner the stimulation-evoked overflow of ^{3}H-5HT, without affecting the spontaneous outflow of radioactivity. Complete inhibition of ^{3}H-5HT overflow was obtained with 3 µM 5-MeOHT, and the IC_{50} was : 0.24 ± 0.06 µM (n=5). This inhibitory effect of 5-MeOHT was competitively blocked by the 5HT receptor antagonist methiothepin (0.1 - 1 µM) indicating that 5-MeOHT inhibits 5HT neurotransmission through the activation of presynaptic 5HT autoreceptors. Citalopram (0.01 - 1 µM) when added 20 min before S_2, did not affect per se the stimulation-evoked overflow of ^{3}H-5HT, while it effectively inhibited neuronal uptake of 5HT in rat hypothalamic slices (90% inhibition at 1 µM citalopram). When citalopram (1 µM) was added to the medium 20 min before S_1 and remained present throughout the experiment, the ratio S_2/S_1 was : 0.93 + 0.06, n=6. In the presence of 1 µM citalopram, the concentration-effect curve for 5-MeOHT on ^{3}H-5HT overflow was significantly shifted to the right (S_2/S_1 : 0.31 ± 0.06, n=6 for 5-MeOHT 1 µM, $p<0.005$ when compared to the value obtained in the presence of 1 µM citalopram, S_2/S_1 : 0.60 ± 0.02, n=4).

To test the possibility that citalopram antagonizes the inhibitory effect of 5-MeOHT by increasing endogenous 5HT in the synaptic gap, similar experiments were performed in PCPA-treated rats (300 mg/kg PCPA i.p. 48 h before the experiment). Under these conditions, the endogenous level of 5HT in the hypothalamus was decreased by 90% (1077 ± 45 ng/g in control rats ; 109 ± 27 ng/g in PCPA-treated rats ; $p<0.001$). In hypothalamic slices from PCPA-treated rats, exposure to citalopram, 20 min before S_2, increased in a concentration-dependent manner the stimulation-evoked release of ^{3}H-5HT (maximum effect S_2/S_1 : 2.05 ± 0.14, n=6 at 10 µM citalopram, EC_{50} : 0.3 µM). When citalopram 1 µM was added 20 min before S_1 and maintained in the superfusion medium throughout the experiment, the control ratio was S_2/S_1 : 0.90 ± 0.02, n=5. After PCPA pretreatment, citalopram still significantly shifted to the right the concentration effect curve for the inhibitory effects of 5-MeOHT on ^{3}H-5HT release (S_2/S_1 : 0.19 ± 0.03, n=5 for 5-MeOHT 1 µM, $p<0.001$ when compared to the value in the presence of 1 µM citalopram, S_2/S_1 : 0.50 ± 0.03, n=5). These results suggest that the interaction between citalopram and the 5HT autoreceptors is not due to changes in the concentration of endogenous 5HT in the synaptic gap but that a direct interaction between the neuronal uptake mechanism and presynaptic 5HT autoreceptors may be involved.

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COMPONENTS OF CIRCLING BEHAVIOUR PRODUCED BY INTRANIGRAL INJECTION OF AMPHETAMINE

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Intranigral application of amphetamine causes dendritic release of dopamine (Leviel et al, 1979). Injection of dopamine into the zona reticulata of substantia nigra of rats produces contraversive circling (Jenner et al, 1983). We now investigate the ability of focal injections of amphetamine into substantia nigra to cause rotation.

Unilateral injection of amphetamine (12.5 - 50 μg in 0.5 μl 0.9% saline) into the zona reticulata of rats produced dose-dependent contraversive rotation. Nigral GABAergic mechanisms appear involved since prior intranigral injection of picrotoxin (0.25 μg in 0.5 μl 0.9% saline 15 min previously) reduced contraversive circling to intranigral amphetamine (50 μg) (Table 1). Prior intranigral injection of sulpiride (10 μg in 0.5 μl 0.9% saline 15 min previously) or a unilateral 6-hydroxydopamine lesion of the medial forebrain bundle 21 days previously enhanced contraversive rotation caused by injection of amphetamine (50 μg) into zona reticulata. So, the contraversive rotation observed following intranigral administration of amphetamine does not involve release of dopamine alone. Amphetamine (10_3- 500 μM) released ^{3}H-dopamine, ^{3}H-5HT and ^{3}H-noradrenaline, but not ^{3}H-GABA, from nigral slices. So, another transmitter might be involved in amphetamine actions in substantia nigra. Injection of cinanserin (10 μg in 0.5 μl 0.9% saline 15 min previously) into zona reticulata increased contraversive rotation to intranigral amphetamine (50 μg) in intact animals. So, release of 5HT itself cannot account for the effect of amphetamine in the intact animal. However, in 6-hydroxydopamine lesioned animals cinanserin reduced contraversive rotation suggesting a role for 5HT.

Table 1 Drug manipulation of amphetamine-induced circling

Treatment	Dose (μg)	Number of contraversive rotations in 15 min	
		Intact	6-OHDA Lesioned
Saline	-	98 ± 12	177 ± 7*
Picrotoxin	0.25	39 ± 18*	54 ± 21$^+$
Sulpiride	10	153 ± 14*	166 ± 25
Cinanserin	10	196 ± 12*	68 ± 28$^+$

* $p < 0.05$ compared to amphetamine alone; $^+$ $p < 0.05$ compared to 6-OHDA + amphetamine.

Amphetamine's action in substantia nigra does not appear only dependent on dendritic dopamine release. 5HT is not involved in the intact animal but appears important following removal of dopamine neurones. Amphetamine-induced rotation from substantia nigra is a complex phenomenon involving more than one transmitter substance.

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