DIFFERENT AFFINITIES OF α_2-ADRENOCEPTOR ANTAGONISTS FOR (^{3}H)-RAUWOLSCINE BINDING SITES IN BRAIN AND SPLEEN MEMBRANES

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Ligand binding studies in brain membranes and platelets have indicated the presence of multiple affinity states of α_2-adrenoceptors (Hoffman et al., 1980). Agonists preferentially label a high affinity state and their use could give misleading data when comparing affinities of antagonists. We have compared the affinity of a series of α_2-receptor antagonists for α_2 binding sites in rat cerebral cortex and rabbit spleen membranes using an α_2-receptor antagonist ligand.

(^{3}H)-rauwolscine (82 Ci/mM New England Nuclear) binding to cortex membranes was determined according to the method of Perry and U'Prichard (1981). Spleen membranes were prepared similarly from the supernatant resulting from initial homogenisation in 0.32M sucrose and centrifugation at 1000g for 15 min. Specific binding (defined by phentolamine 10μM) to cortex and spleen membranes was saturable (65-75% specific binding) with KD's of 3.7nM and 3.2nM respectively in 50mM Na-K-phosphate buffer (pH 7.4) incubated at 4°C for 60 min. Data presented in Table I are means of 3-5 experiments, individual determinations being performed in triplicate.

Table I Ki values (nm) for α-adrenoceptor antagonists

Antagonist - listed in order of potency in pithed rats	(^{3}H)-rauwolscine binding				Ratio $\frac{A}{B}$
	A Brain	Hill No.	B Spleen	Hill No.	
rauwolscine	6.5	1.1	2.6	0.9	2.50
yohimbine	6.2	0.9	2.6	1.1	2.38
phentolamine	33.5	0.9	4.3	0.8	7.79
Rx-781094	2.2	0.9	11.9	1.0	0.18
piperoxan	3.4	1.0	32.6	0.9	0.10
Wyeth 26703	2.7	0.8	42	1.0	0.06
corynanthine	210	0.9	296	1.0	0.71
prazosin	240	0.7	2850	1.1	0.08

Functional post junctional α_2-receptor antagonist activity was determined by ability of compounds to antagonise noradrenaline pressor responses in prazosin treated pithed rats. Pithed rats (Sprague Dawley 280-320g) were pretreated with propranolol (1 mg/kg) and prazosin (1 mg/kg) and the ability of α_2-antagonists (1-3 mg/kg 15 min before challenge) to shift noradrenaline dose response curves was determined in separate groups of rats. The most potent compounds were rauwolscine and yohimbine (dose ratios 7-8 at 1mg/kg) followed by phentolamine > Rx781094 > piperoxan > Wyeth 26703.

The different ranking of affinities of antagonists for rauwolscine binding sites in brain and in spleen (see Table I) provides evidence that α_2-adrenoceptors are not a homogeneous population. The ranking of antagonists in pithed rats correlates better with their affinity for rauwolscine binding sites in spleen membranes than in brain membranes.

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DIFFERENTIAL EFFECTS OF NICOTINE ISOMERS ON (^{3}H)-DOPAMINE RELEASE FROM SLICES OF RAT STRIATUM AND NUCLEUS ACCUMBENS

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A functional, inverse balance is believed to exist between the neurotransmitters acetylcholine (ACh) and dopamine (DA) in certain areas of the basal ganglia (Ladinsky et al, 1975). Indeed, ACh and other cholinergic agonists (both muscarinic and nicotinic) are reported to influence the release of [^{3}H]-DA from slices of rat striatum (de Belleroche & Bradford, 1978; Giorguieff et al, 1977).

The present study investigates the effects of the enantiomers of nicotine tartrate on spontaneous and potassium-stimulated release of [^{3}H]-DA from slices of rat striatum or nucleus accumbens. Female Porton rats were used in all experiments and tissue slices were prepared using a McIlwain tissue chopper and superfused as previously described (Kerwin & Pycock, 1979).

Potassium (20-60mM) stimulated [^{3}H]-DA release from both tissues in a dose-related, calcium-dependent manner. Neither (+)- nor (-)-nicotine (1-100μM) had any effect on the spontaneous release of [^{3}H]-DA from striatal slices. Similarly, (-)-nicotine was without action on accumbens [^{3}H]-DA efflux although (+)-nicotine (1 and 10μM) significantly enhanced the spontaneous overlow of [^{3}H]-DA from this tissue ($p < 0.05$).

The stereoisomers of nicotine displayed opposing actions on the K^+-induced release of [^{3}H]-DA from either tissue. (+)-Nicotine (1-100μM) significantly enhanced K^+-evoked release of [^{3}H]-DA from both striatum and accumbens ($p < 0.05$, $p < 0.01$), whilst (-)-nicotine (10 and 100μM) inhibited such release ($p < 0.01$, $p < 0.001$). In all cases, these effects of 10μM nicotine were reversed by inclusion of the nicotinic receptor antagonist, gallamine (10μM), in the superfusing medium but only partially diminished by hexamethonium (10μM). In each instance, atropine (10μM) failed to influence the actions of either nicotine isomer on K^+-stimulated [^{3}H]-DA release from either tissue.

In comparison to nicotine, the muscarinic agonist, oxotremorine (10μM), potently stimulated both the spontaneous and K^+-induced efflux of [^{3}H]-DA from striatal and accumbens slices ($p < 0.001$). Without exception, these effects were blocked by atropine (10μM) but not by gallamine (10μM).

These data demonstrate that in vitro, the (+)- and (-)-nicotine isomers differentially influence the release of radiolabelled DA from slices of rat forebrain. In view of existing controversy regarding cholinergic-dopaminergic interactions in the basal ganglia (e.g. de Belleroche & Gardiner, 1982; Lehmann & Langer, 1982), such findings suggest a facilitatory action of both (+)-nicotinic and muscarinic receptors on DA transmission in these regions.

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EFFECTS OF PERGOLIDE ON CARDIAC OUTPUT AND TOTAL PERIPHERAL VASCULAR RESISTANCE IN SPONTANEOUSLY HYPERTENSIVE RATS

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In normotensive and hypertensive rats, pergolide produces bradycardia and hypotension. The latter effect is consistent with a reduction of sympathetic drive to the cardiovascular system and is mediated through the stimulation of dopamine receptors on postganglionic sympathetic neuronal varicosities (Cavero, 1982; Hahn, 1981). Since the haemodynamic mechanism of the blood pressure lowering effects of pergolide is not yet known, we studied the effects of this dopamine receptor agonist on the cardiac output in spontaneously hypertensive rats.

Adult (5-6 month old) spontaneously hypertensive rats were anaesthetized with pentobarbitone (55 mg/kg i.p.) and placed under artificial respiration. Catheters were placed in the left femoral artery to measure blood pressure, in the right femoral artery to sample blood at constant withdrawal rate (0.7 ml/min), in the left ventricle *via* the right carotid artery to inject radioactive microspheres and in the jugular vein for drug injections. Cardiac output was measured with radioactive microspheres (^{141}Ce: 15 $\pm$ 3 µM diameter) using the reference blood sample technique (Hales, 1974). The cardiac output was normalized to a kg body weight to obtain the cardiac index (ml/min/kg). Total peripheral vascular resistance (mmHg/ml/min/kg) was calculated by dividing mean femoral artery pressure (mmHg) by cardiac index. Saline and pergolide (30.0 µg/kg) were injected intravenously 10 min before cardiac index measurement. At this moment, the effects of pergolide on blood pressure had reached an apparent steady state.

Ten minutes after administration of saline the value of mean femoral artery pressure, cardiac index and total peripheral vascular resistance were: 147.3 $\pm$ 5.3 mmHg, 242.8 $\pm$ 18.2 ml/min/kg and 0.63 $\pm$ 0.04 mmHg/ml/min/kg (n=8) respectively. They were 113.5 $\pm$ 7.5 mmHg, 237.2 $\pm$ 25.6 ml/min/kg and 0.50 $\pm$ 0.04 mmHg/ml/min/kg (n=7) in pergolide-pretreated rats. The effects of pergolide on blood pressure and peripheral resistance but not on cardiac output were significantly different ($p<0.05$: t-test) from those of saline.

In conclusion, these results indicate that haemodynamically the hypotensive effects of pergolide are due to a fall in total peripheral resistance.

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INHIBITION OF THE PRESSOR COMPONENT OF A DEFENCE REACTION IN CONSCIOUS CATS BY SOME CENTRALLY-ACTING DRUGS

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Electrical stimulation of highly localised areas of the cat brain produces a defence reaction (Abrahams et al, 1960). Stock et al (1976) showed that the benzodiazepine, medazepam, attenuated the cardiovascular changes during stimulation of a defence reaction, being more effective against stimulation of the medial rather than the basal amygdaloid ganglia.The purpose of the present study was to examine the effects of a number of centrally-acting drugs on the pressor component of a defence reaction produced by stimulation of the amygadalo-fugal pathway (Timms, 1981).

SPF cats of either sex were prepared under alphaxalone/alphadolone anaesthesia with an indwelling carotid arterial and jugular venous cannula. A cathodal, monoplar stimulating electrode was implanted into the brain at A11,L6,the precise height being determined by production of a defence reaction on stimulation. A minimum of two days after surgery the freely-moving animal was connected for measurements of blood pressure and heart rate during stimulation via the electrode. Each animal received duplicate stimuli at three different frequencies and that frequency causing a 2mm Hg rise in diastolic blood pressure calculated (F_{20}). Drugs were administered intravenously in saline and the frequency-response curve repeated 10min and 2h after injection. The increase in diastolic pressure occuring at the control F_{20} was calculated and a percentage inhibition figure thus obtained. Three cats were used to study each drug.

Saline injections in a group of seven cats had no effects on the pressor response to stimulation at 10min and 2h. Chlordiazepoxide (1-5mg/kg) caused a dose-related inhibition of the pressor response 10min after injection, but only the high dose produced any effect after 2h.($30\pm2\%$). Clonidine (5-25μg/kg) showed a similar pattern,only 25μg/kg producing $36\pm3\%$ inhibition at 2h. At this time however,the lower doses of clonidine caused a significant reduction in resting mean arterial pressure (5μg/kg, $12\pm6\%$; 10μg/kg, $18\pm3\%$). L-propranolol (1mg/kg) was ineffective at either time after injection. The nicotinic antagonist, pempidine (1mg/kg) caused $52\pm6\%$ and $36\pm15\%$ inhibition at 10min and 2h respectively.

We suggest that this model demonstrates the inhibitory effect of some centrally- and peripherally-acting drugs on the pressor component of a defence reaction. However, the relatively transient attenuation of the centrally evoked pressor responses by chlordiazepoxide and clonidine suggests that the effect is unrelated to the more prolonged anticonvulsant, muscle relaxant and sedative properties usually associated with these drugs. The β-antagonist, propranolol, was also unable to modify the pressor response despite causing an $18\pm4\%$ fall in heart rate.

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ADRENALINE-INDUCED MODULATION OF PRESSOR RESPONSES IN PITHED SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS

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The present study attempts to make an assessment of the role of circulating adrenaline, in the determination of pressor responses to various stimuli, and identify any differential effects in normotensive and spontaneously hypertensive rats.

Male spontaneously hypertensive (SHR) and normotensive Wistar Kyoto (WKY/N) rats weighing 250 - 325g, were anaesthetised with sodium pentobarbitone (60mg/kg i.p.), pithed and respired with air room. Blood pressure was recorded from the right carotid artery and drugs administered by bolus injection and adrenaline by slow infusion via an external jugular vein. Pressor responses, measured as increases in diastolic blood pressure (DBP), to electrical stimulation (0.125 - 4Hz, 30V; 1msec; 15sec) of the entire sympathetic outflow and bolus injections of noradrenaline (0.03 - 10 μg), phenylephrine (0.03 - 10 μg)and oxymetazoline (0.03 - 1 μg) were recorded in intact animals, after acute bilateral adrenal demedullation and during the slow infusion of adrenaline (5 - 500 ng/min in a constant volume of 5 μl/min) in demedullated rats. Plasma adrenaline levels were determined by HPLC.

Diastolic blood pressures in pithed SHR and WKY/N rats were 41 $\pm$ 2mmHg (n =15) and 35 $\pm$ 2mmHg (n = 18) respectively. Noradrenaline, phenylephrine and oxymetazoline induced dose dependent pressor effects in both SHR and WKY/N rats. The responses to each individual agonist were similar in both SHR and WKY/N rats, however, the pressor responses induced by electrical stimulation were significantly ($p < 0.001$) greater in SHR (maximal increase in DBP = 83 $\pm$ 4mmHg, n = 15) than in WKY/N (49 $\pm$ 3mmHg, n = 18).

Bilateral adrenal demedullation reduced the resting DBP in SHR and WKY/N rats to 36 $\pm$ 2mmHg (n = 7) and 29 $\pm$ 2mmHg (n = 9) respectively and caused a 5-fold shift to the right in the pressor response curves to noradrenaline. The maximal stimulation induced increases in DBP in SHR (41 $\pm$ 6mmHg; n = 7) and WKY/N rats (27 $\pm$ 2mmHg; n = 9) were also significantly ($p < 0.001$) reduced. Subsequent infusion of adrenaline induced a dose dependent restoration of the stimulation induced pressor responses, in both SHR and WKY/N rats, but was unable to effect a full restoration of the pressor response to noradrenaline.

The ability of adrenaline infusion to restore fully the stimulation induced pressor responses, in adrenal demedullated rats, but not the noradrenaline induced responses, suggests that adrenaline might facilitate the release of sympathetic transmitter, rather than directly affecting vascular smooth muscle. Adrenaline infusion induced higher levels of plasma adrenaline ($p < 0.001$) in SHR (18.3 $\pm$ 1.9ng/ml @ 500ng/min, n = 6) than in WKY/N rats (4.9 $\pm$ 0.7ng/ml @ 500ng/min, n = 6) and it is possible that the exaggerated effects of adrenaline infusion, upon stimulation induced responses in the SHR rats, may be partly due to a direct effect of the higher circulating levels of adrenaline obtained.

The results indicate that circulating adrenaline is capable of modifying the pressor responses to various stimuli in both SHR and WKY/N rats and might be involved in facilitating sympathetic transmitter release. Furthermore, the removal of adrenaline from the circulation would appear to be less efficient in SHR rats. A combination of these phenomena would contribute considerably to the maintenance and possibly the development of a raised blood pressure in SHR rats.

PQ is an SERC CASE student in collaboration with ICI plc.

PROBLEMS ENCOUNTERED IN MEASURING AND INTERPRETING THE EFFECTS OF DRUGS ON BLOOD PRESSURE IN CONSCIOUS DOGS

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The resting conscious dog exhibits a characteristic blood pressure waveform of periodic increases, followed by decreases in both systolic and diastolic blood pressure. The interval between successive pulses decreases as blood pressure rises, and increases as pressure falls again (i.e. sinus arrhythmia). These fluctuations in blood pressure make it difficult to decide on the most appropriate level from which to measure drug induced changes in pressure. Therefore, in an attempt to overcome these difficulties and to better our understanding of the basis of the waveform, we have endeavoured to identify the mechanisms involved.

Cumulative doses of the selective α_1-adrenoceptor antagonist, prazosin (1-100 µg/kg i.v.), or the selective β_1-adrenoceptor antagonist, atenolol (0.1-0.3 mg/kg i.v.), alone, had no effects on the blood pressure waveform, the pulse intervals or mean heart rate.

Cumulative doses of atropine (7-15 µg/kg i.v.) caused a dose-dependent increase in the lowest levels to which systolic and diastolic pressures fell; this effect was accompanied by a dose-dependent shortening of the longest pulse intervals. After 10-15 µg/kg of atropine, the variations in systolic and diastolic pressure, and in pulse interval, were almost abolished. Higher doses of atropine (20-30 µg/kg i.v.) caused a further small increase in diastolic, but not systolic pressure; there was little further decrease in pulse interval. Mean heart rate increased progressively, after each dose of atropine (7-30 µg/kg i.v.), from 59 to 148 beats/min. Pretreatment with prazosin (100 µg/kg i.v.) did not modify the effects of atropine on blood pressure or heart rate. The effect of different doses of atropine, alone, are shown in Figure 1.

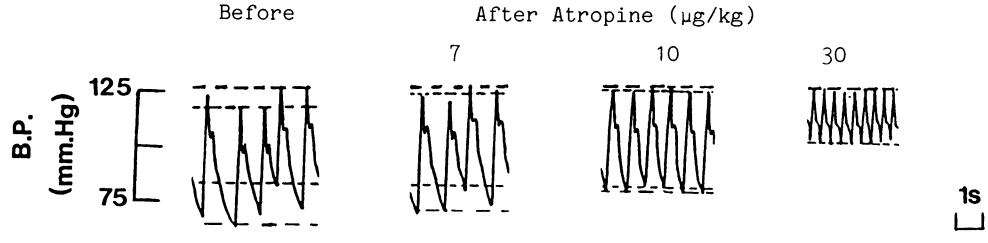

Figure 1. The effects of atropine on blood pressure in a conscious dog at rest. Broken horizontal lines indicate highest and lowest systolic and diastolic pressures.

The failure of prazosin or atenolol to modify the characteristic blood pressure waveform shows that it is not attributable to periodic discharge of the sympathetic nerves supplying vascular or cardiac muscle. In contrast the effects of atropine suggest that the blood pressure waveform merely reflects the variation in pulse interval brought about by changing levels of vagal discharge to the S-A node. A long interval between two successive pulses will allow the diastolic pressure to decline further before the second pulse than would be the case if the interval between pulses is short.

These findings draw attention to the problems involved in interpreting drug effects on the cardiovascular system of conscious dogs because blood pressure changes may reflect changes in pulse interval as well as vascular resistance.

PHENOBARBITONE PRETREATMENT OF RATS LOWERS RESISTANCE TO FLOW IN THE BLOOD PERFUSED SUPERIOR MESENTERIC ARTERIAL BED

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Phenobarbitone pretreatment of rats produces dose-dependent increases both in liver weight and in blood flow to the liver; the change in blood supply is entirely due to a redistribution of cardiac output in favour of the organs draining into the hepatic portal vein. This redistribution is not a consequence of increased hepatic microsomal enzyme activity (Nies et al, 1976), is not shared with all other hepatomegalic agents and does not appear to be the result of differences in the cellular basis of the liver enlargement between phenobarbitone and other drugs causing increases in the liver weight to body weight ratio (Berman et al, 1983; Wilson & Hiley, 1983). Accordingly it is possible that the effects of phenobarbitone on hepatic portal venous return are the result of changes in vascular resistance in the splanchnic rather than the hepatic beds.

Male Wistar rats (Tucks Ltd., Rayleigh, Essex) and male D.A. rats (Bantin & Kingman Ltd., Hull) weighing 250-350g were pretreated for 5 days with 80 $mg.kg^{-1}$ phenobarbitone daily given i.p. in a volume of 4 $ml.kg^{-1}$ saline. Control animals received the vehicle alone. On the 6th day after commencement of treatment the animals were anaesthetized with 40 $mg.kg^{-1}$ pentobarbitone (Sagatal, May & Baker). The animals were prepared for perfusion of the superior mesenteric artery essentially as described by Jackson & Campbell (1980) except that the blood was reheated to 37° before returning it to the animal. Seven flow rates were used ranging from 0.44 to 3.07 $ml.min^{-1}$ and pressure in the perfusion system was determined at least three times in the same animal for each flow rate.

Liver weights were 3.27±0.08g per 100g body wt (n=7) for the control Wistar rats and 4.19±0.02g per 100g body wt (n=10) for the corresponding phenobarbitone pretreated group; this represents a statistically significant increase of 28.1% in the rats receiving barbiturate ($P<0.001$; Student's t-test). The values for the D.A. rats were 2.74±0.06g per 100g body wt (control; n=9) and 4.20±0.12g per 100g body wt (phenobarbitone; n=7) representing an increase in the phenobarbitone pretreated animals of 53% which is statistically significant at the 0.1% level (Student's t-test). In both strains of rat the mean perfusion pressures for each of the seven flow rates in the rats that had been pretreated with barbiturate were lower than those in control rats. Analysis of covariance (Snedecor & Cochran, 1967) showed that pretreatment with barbiturate did not significantly affect the slope of the regression of perfusion pressure on flow rate but that the elevation of the line (i.e. perfusion pressure for any given flow rate) was significantly lower in the animals which had received the drug ($P<0.001$ for both strains).

Hence pretreatment of Wistar or D.A. rats with a dose of phenobarbitone known to increase liver blood flow in at least the Wistar animals (Yates et al, 1978) reduces resistance to flow in the superior mesenteric vascular bed.

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THIOL-DISULPHIDE INTERACTIONS WITH CAPTOPRIL DEPLETE HEPATIC GLUTATHIONE

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Captopril (D-3-mercapto-2-methyl-propanoyl-L-proline; CP) is an orally active angiotensin-converting enzyme inhibitor used in the treatment of hypertension. CP undergoes extensive covalent binding to plasma proteins in man and the rat: probably via thiol-disulphide interchange (Kripalani et al, 1980; Komai et al, 1981; Park et al, 1982). CP covalently bound to plasma protein is cleared rapidly from plasma and appears in urine as a mixed disulphide with cysteine (Park et al, 1982). It was suggested that CP is removed from plasma proteins in a thiol-disulphide reaction prior to renal excretion. To test this hypothesis, we studied the reaction of CP-plasma protein conjugates (CP-PP) with glutathione and cysteine in vitro. In addition, we have determined the effect of CP on hepatic glutathione.

CP-PP prepared as described previously (Park et al, 1982) and incubated with either glutathione (10-500 μM) or cysteine (10-1000 μM) in phosphate buffer (0.1 M; pH 7.4; final vol. 0.5 ml) for 60 min. at 37°. After incubation, CP covalently bound to plasma protein and CP metabolites were determined by equilibrium dialysis and radiochromatography (Park et al. 1982). Hepatic glutatione (Akerboom et al, 1982) was measured 5 hr after i.p. administration of CP (50-300 mg kg^{-1}) to male Wistar rats (250 g) or male DBA mice (35 g).

Gluthatione (20-500 μM) and cysteine (60-1000 μM) significantly ($p < 0.05$) reduced the amount of CP covalently bound to plasma proteins in vitro; the respective EC_{50} values were 204 and 470 μM. In each case there was corresponding increase in the appropriate mixed-disulphide indicating a spontaneous thiol-disulphide interchange. Administration of CP (50-300 mg kg^{-1}) to mice produced a significant ($p < 0.005$) dose-dependent decrease (12-42%) in reduced hepatic glutathione. Administration of CP (300 mg kg^{-1}) produced a time-dependent decrease in glutathione in the rat: after 5 hr hepatic glutatione was 4.25 ± 0.12 μmole g^{-1} liver compared with controls 6.28 ± 0.62 μmole g^{-1}.

In conclusion, we have provided further evidence to support the hypothesis that covalently bound CP is removed from plasma proteins in a thiol-disulphide reaction, which may lead to depletion of hepatic glutathione. It is possible that drug-drug interactions may occur between CP and other drugs which require glutathione for their metabolism.

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(^{125}I)-ANGIOTENSIN II BINDING TO RAT RENAL CORTEX EPITHELIAL MEMBRANES: SENSITIVITY TO DITHIOTHREITOL

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The disulphide bond reducing agent, dithiothreitol (DTT) has been shown to affect the binding of several neurotransmitters and hormones. The situation with angiotensin II (AII) binding to target tissue membranes is confused. Recently Gunther et al. (1980) using rat mesenteric artery membranes, and Chang et al. (1982) with bovine adrenal cortex membranes, have identified a marked inhibition of specific AII binding in the presence of DTT. However, DTT has been frequently incorporated into AII binding assays to prevent peptide degradation with no reported effects on binding (Bennett and Snyder, 1976; Aguilera and Catt, 1981). The present report describes the effects of DTT on ^{125}I-AII binding in rat renal cortex membranes.

Rat kidney cortex crude basolateral and brush-border membranes were prepared as described by Heidrich et al. (1972). Binding assays were performed as described previously for 5 min. at 22 °C, with 0.8-0.9 nM ^{125}I-AII and 1 µM AII to define specific binding. An incubation buffer consisting of 20 mM tris HCl, 120 mM NaCl, 5 mM Na_2 EDTA, 0.1 mM phenylmethylsulphonylflouride (PMSF) and 0.2% bovine serum albumin (BSA), pH 7.4 was used under control conditions. In studies where preincubations with DTT were performed in the absence of NaCl, the NaCl concentration was restored to 120 mM in the final assay.

Specific binding to renal cortex membranes was sensitive to DTT in a concentration dependent manner. Following preincubation of membranes with increasing DTT concentrations for 20 min. at 22 °C, a half maximal inhibition was achieved with 1 mM DTT. Total inhibition of binding was routinely obtained with 5-10 mM DTT. Inactivation of specific ^{125}I-AII binding by DTT was temperature sensitive, the rate at 22 °C being 4-fold faster than the inactivation rate obtained after preincubations on ice.

The ability of unlabelled AII to protect binding sites from DTT was investigated. Addition of varying concentrations of cold AII 15 min. prior to the addition of 10 mM DTT protected specific ^{125}I-AII binding in a dose-dependent manner. Protection of 50% of the binding sites was achieved with 100 nM AII. The rate of DTT inactivation was also affected by the presence or absence of NaCl. Specific ^{125}I-AII binding to renal cortex membranes is partially dependent on NaCl (Cox et al., 1982) and maximal binding was obtained with 120 mM NaCl. The absence of NaCl from preincubations with DTT resulted in a markedly slower rate of inactivation.

This preliminary study has demonstrated sensitivity of specific ^{125}I-AII binding in kidney cortex membranes to DTT. The protection afforded by AII indicated that an essential disulphide bridge is located either within or very close to the ^{125}I-AII binding site. The absence of NaCl appeared to alter the conformation of the binding sites, reducing specific binding and decreasing the rate of DTT inactivation of ^{125}I-AII binding.

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EFFECTS OF CHRONIC ENALAPRIL (MK-421) TREATMENT ON THE RENIN-ANGIOTENSIN SYSTEM IN THE SODIUM DEFICIENT RAT

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Angiotensin converting enzyme (ACE) inhibitors such as Enalapril (MK-421, Merck Sharp and Dohme) show greater antihypertensive effects in states of elevated plasma renin activity (PRA) as caused by Na^+ deficiency (Sweet et al, 1981). We have, therefore, investigated the effects of chronic MK-421 administration on plasma and tissue ACE activity in male Sprague Dawley rats (220-230g) maintained on a Na^+ restricted diet (ICN, Ohio). Preliminary studies showed that 3 weeks or longer on this diet produced significant increases in both PRA and plasma angiotensin I (AI) concentration.

In the present study, rats were maintained on this diet for the duration of the experiment. After 3 weeks Na^+ restriction animals were dosed daily with either MK-421 (10mg/kg/day p.o.) or the methyl cellulose vehicle (5ml/kg/day p.o.) for a further 1 or 3 weeks (N=10/group). ACE activity was assayed in plasma and tissue (aorta, adrenal glands, renal artery, lung and mesenteric bed) before and after the removal of any MK-421 from the sample by a method adapted from Ulm and Vassil (1982). Plasma samples were also assayed for PRA, AI and angiotensin II (A II) by radioimmunoassay.

Treatment for 1 or 3 weeks with MK-421 produced significant falls in blood pressure with no effect on heart rate. PRA was greatly increased by the low Na^+ diet and this was not significantly altered by the MK-421 regimen. Plasma AI was also increased as previously by Na^+ restriction and was further elevated by 1 week dosing of MK-421 (Control, 7.0±0.9: MK-421, 15.4±2.3ng/ml, $p<0.05$) but not by 3 weeks treatment (Control, 8.0±1.6: MK-421, 9.8±2.3ng/ml). Plasma AII was virtually undetectable following 1 or 3 weeks dosing with MK-421.

Plasma ACE activity (units=nmoles substrate hydrolysed per mg protein per unit time) was significantly inhibited after dosing with MK-421 for 1 week (Control, 18.0±6.0: MK-421, 7.3±3.3 units, $p<0.05$) and 3 weeks (Control, 38.7±6.7: MK-421, 3.4±2.1 units, $p<0.001$). Removal of MK-421 from the plasma unmasked an apparent increase in production of ACE at 1 week (Control, 0.14±0.02: MK-421, 0.33±0.04 units, $p<0.005$) and 3 weeks (Control, 0.36±0.04: MK-421, 0.65±0.05 units, $p<0.001$). Significant inhibition of ACE activity in aorta, adrenal glands and lung was also apparent after 3 weeks but not 1 week dosing with MK-421. Removal of MK-421 from the tissue samples revealed small but non-significant increases in ACE production in aorta, adrenal glands and renal artery after 3 weeks MK-421.

Thus, although it has been claimed that the antihypertensive effects of ACE inhibitors may not be a result solely of inhibition of plasma ACE activity (Unger et al, 1981) our results suggest that in a state of amplification of the renin-angiotensin system, the blood pressure lowering effects of MK-421 are associated with a decrease in plasma ACE activity and subsequent fall in AII concentration.

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IN VITRO HYDROLYSIS OF THE ANGIOTENSIN CONVERTING ENZYME INHIBITOR ENALAPRIL (MK-421) BY PLASMA AND LIVER

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Lack of antihypertensive activity following oral administration of the angiotensin converting enzyme (ACE) inhibitor N-[1-(S)-carboxy-3-phenylpropyl]-L-alanyl-L-proline (MK-422;Merck, Sharp and Dohme) to man has been attributed to poor absorption. This has been overcome by the preparation of its ethyl ester, MK-421 (Biollaz et al, 1981). The efficacy of esters such as MK-421 is obviously dependent upon hydrolysis rate *in vivo*. The analysis of *in vitro* hydrolysis products by TLC has shown that MK-421 is rapidly de-esterified to its diacid metabolite by rat plasma but that in dog and man, the liver may be more important (Tocco et al, 1982).

In this communication, an *in vitro* method has been used to estimate relative hydrolysis rates of MK-421 at various sites. The metabolic enzyme inhibitor SKF 525A (5×10^{-4}M) was found to inhibit esterase activity completely, without appreciable inhibition of ACE activity. In a typical assay, plasma or tissue homogenate was incubated with MK-421 (1 or 5×10^{-8}M in 50mM NaCl, 1M Na_2SO_4, pH 7.4) at 37°. Hydrolysis was stopped by the addition of SKF 525A. ACE activity was then defined as the ability of the samples to liberate ^{14}C-hippuric acid from ^{14}C-hippuryl-histidyl-leucine over 30 min at 37°.

In the absence of any hydrolysis MK-421 produced a 50% inhibition of ACE activity (IC_{50}) only at a concentration greater than 10^{-6}M. Complete conversion to diacid metabolite however, produced an IC_{50} of 5×10^{-10}M against rat plasma ACE, a value in agreement with that found using synthetic diacid metabolite. Rat plasma (~2mg protein) required 1.1 min incubation with 1×10^{-8}M MK-421, prior to addition of SKF 525A, to result in a 50% inhibition of ACE activity. Cat, dog and human plasma required 74, 1200 and 1200 min respectively ($n \geq 3$ for each) to produce sufficient diacid metabolite for 50% ACE inhibition. Liver homogenates were first purified on a Sephadex G25-40 column to remove an endogenous factor which interfered in the ACE assay. To assay hydrolysis products in samples lacking ACE activity (e.g. liver), a source of ACE (generally monkey plasma) was added following SKF 525A. Expressed per unit protein, the relative hydrolysis rates for dog, monkey, cat and rat liver were 1:2:10:20. The comparative rates for monkey, human, dog, cat and rat plasma were <0.03:0.5:0.6:6:600. Hydrolysis of MK-421 by rat and cat lung, and by cat aorta, mesenteric artery and portal vein was undetectable.

Thus, *in vitro*, only in the rat does MK-421 appear to be hydrolysed faster by plasma than liver. In other species the liver may, therefore, be the more important site of de-esterification. Certainly in the rat and dog (Gross et al 1981) and cat (S.D. Longman, unpublished) the rate of attainment of maximum *in vivo* inhibition of angiotensin I induced pressor responses by MK-421 appears to be related to the rate of hydrolysis of the ester by the liver of the 3 species.

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ESTIMATION OF THE PLASMA MEMBRANE AND MITOCHONDRIAL MEMBRANE POTENTIAL IN HUMAN BLOOD PLATELETS IN SITU

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Blood platelets maintain large transmembrane gradients of Na^+ and K^+, and a negative membrane potential. This membrane potential may be important in initiating the aggregating response and/or stimulus secretion coupling in these cells. The use of lipophylic cations to determine membrane potential is well established, (Rottenberg, 1979) but in eukaryote cells accurate determinations are complicated by problems associated with internal comportmentation of the cation (Lichtshtein etal, 1979). We have attempted to overcome these problems, and to calculate the plasma membrane potential ($\Delta\psi_p$) and the mitochondrial membrane potential ($\Delta\psi_m$) by investigating the simultaneous accumulation of $^{86}Rb^+$ and (^3H) methyltriphenyl phosphonium iodide (^3H-$MTPT^+$). Platelet rich plasma were pre-incubated for 10 minutes with the membrane probes under investigation before the addition of radiolabelled cations, and the incubation continued for a further 90 minutes when steady state accumulation for both Rb^+ and $MTPT^+$ were attained, the accumulation and attainment of steady state equilibrium of $MTPT^+$, but not Rb^+ was enhanced by trace amounts of tetraphenylboron. The partition of cations into the mitochondrial compartment was prevented by either valinomycin or carbonyl cyanide p-trifluromethyxyphenyl hydrazone (FCCP), allowing calculations of the intramitochondrial concentration. The residual accumulation of $MTPT^+$ in the absence of mitochondrial potential is abolished by increasing extracellular K^+ demonstrating that (^3H)-$MTPT^+$ can be used to calculate both $\Delta\psi_p$ and $\Delta\psi_m$. The accumulation of Rb^+ is not influenced by valinomycin or FCCP, indicating that it is excluded from the mitochondrial compartment, but was influenced by raising the external K^+ concentration indicating the Rb^+ accumulation was sensitive to change in $\Delta\psi_p$. From this date it is possible to estimate the membrane potentials (table 1).

Table 1 Accumulation Ratio for ^{86}Rb and (^3H)-$MTPT^+$ and calculate $\Delta\psi_p$ and $\Delta\psi_m$

	$MPTP^+$			Rb^+	
	in/out	$\Delta\psi_p$	$\Delta\psi_m$	in/out	$\Delta\psi_p$
no additions	8.62 ± 2.76			2.71 ± 0.76	
K^+ 130mM	3.88 ± 1.86	-27mV		0.56 ± 0.27	-44mV
Valinomycin 9μM	1.79 ± 0.47		-151mV	2.83 ± 0.94	
Valinomycin + K^+	0.57 ± 0.19	-29mV		0.58 ± 0.39	-42mV
FCCP 50μM	3.14 ± 1.86		-143mV	2.74 ± 0.91	
FCCP + K^+	0.81 ± 0.66	-43mV		0.62 ± 0.18	-40mV

$\Delta\psi = -\frac{RT}{zF}$ Log $\frac{R_1}{R_2}$ where R_1 and R_2 are the mean accumulation ratio in control and high K^+ media respectively.

The usefulness of this technique to the study of platelet responsiveness and the effect of agents that perturb to platelet membrane will be presented.

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METHEMOGLOBIN PRODUCTION BY ρ-AMINOPHENONES

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Administration of ρ-aminopropiophenone (PAPP) gives rise to methemoglobinemia (Vandenbelt et al, 1944). Like other methemoglobin-inducing agents PAPP is effective in cyanide poisoning (Jandorf and Bodansky, 1946), although its onset of action is a little slower than that of DMAP (Bright and Marrs, 1982). Slowness of action is undesirable in the curative treatment of cyanide poisoning, but in prophylaxis where prolonged methemoglobinemia is required, even that produced by PAPP may not be sufficiently long. We have therefore compared methemoglobin profiles produced by ρ-aminohexanoylphenone (PAHP), a longer chain aminophenone known to give rise to relatively prolonged methemoglobin levels (Downey, 1966), with those produced by PAPP. The work was carried out in dogs on account of the similarity of their methemoglobin reductase system to that in man.

After a baseline methemoglobin level had been estimated, PAPP 0.2 mg kg^{-1} or 0.5 mg kg^{-1} and PAHP 0.26 mg kg^{-1} or 0.65 mg kg^{-1} were each administered on separate occasions intravenously to 4 beagle bitches. In subsequent experiments both materials were administered to the bitches by gavage, PAPP at a dose of 1.0 mg kg^{-1} and PAHP at a dose of 1.3 mg kg^{-1}. Methemoglobin levels were estimated every 10 min for 150 min. Except in the case of the lower intravenous doses of the two materials methemoglobin was estimated additionally at 180 min and thereafter hourly until 360 min had elapsed from dosing.

After 0.2 mg kg^{-1} PAPP had been given intravenously, peak mean methemoglobin levels occurred at 1 h (8.6 ± 1.3%; $\bar{x}$ ± SD) whereas after an equimolar dose of PAHP peak levels occurred at 70 min (5.3 ± 1.5%). After the higher intravenous dose of PAPP peak levels again occurred at 1 h (20.5 ± 1.6%), whilst with PAHP they occurred at 80 min (14.1 ± 2.1%). After 1.0 mg kg^{-1} PAPP administered by gavage, peak levels of methemoglobin occurred at 120-140 min during which time they remained constant at 15.3%, whilst after equimolar amounts of PAHP (1.3 mg kg^{-1}) peak levels occurred at 150 min (9.2 ± 2.1%). With both materials the duration of action was slightly longer after oral administration than after the higher intravenous dose: with the former mode of administration levels were at or above 4% at 300 min whereas they were below 3% after intravenous administration. In no case when equimolar doses of the two materials were compared was the rate of onset of action, as exemplified by time taken to reach half peak levels, significantly different.

The results suggested that when equimolar quantities of PAPP and PAHP are administered lower peak levels are obtained with the latter than the former. The action of PAHP is slightly slower than PAPP in that peak levels are attained about 10 min later with PAHP than with PAPP. This difference is not significantly affected by the route of administration which suggests the slower action of long-chain ρ-aminophenones is not due to a slower rate of absorption: it may therefore be due to a slower rate of formation of the N-hydroxylated derivatives, which are postulated to be the active metabolites, (Graffe et al, 1964).

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PERMIXON, A NEW TREATMENT FOR BENIGN PROSTATIC HYPERPLASIA, ACTS DIRECTLY AT THE CYTOSOLIC ANDROGEN RECEPTOR IN RAT PROSTATE

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A large proportion of the aged male population suffers from benign prostatic hyperplasia for which surgical intervention is the ultimate solution. Accumulation of dihydrotestosterone within the gland is considered to be the hormonal mediator of the hyperplasia through its action on the androgenic receptors (Wilson, 1980). The liposterolic extract of the plant, Serenoa repens B, Permixon, has been shown to possess peripheral antiandrogenic activity (Stenger & al., 1982) and, in clinical trials, was found to significantly improve the symptoms of prostatic hyperplasia (Champaut, 1982). As part of the investigation of the mechanism of action, we report here that Permixon inhibits the binding of ^{3}H-methyltrienolone to the cytosolic androgenic receptor of the rat prostate.

Cytosol was obtained by homogenisation (Ultra-turrax) and ultracentrifugation (1h at 100,000 g) of the ventral lobe of the rat prostate. ^{3}H-methyltrienolone (R-1881) (90 Ci/mmol. NEN), a synthetic androgen, was used as radioactive ligand (Asselin & al., 1979). The cytosol was incubated with the radioactive ligand (0.5 to 10 nM) for 2 hours at 0°C. The drugs were dissolved in benzene/ethanol (9/1) and the solvent evaporated under nitrogen and the residue redissolved in the cytosol. Non-specific binding was estimated in the presence of an excess of R-1881 (1 µM). Bound and free radioactivity were separated by filtration through GF/B glassfibre filters, after treatment with dextran-coated charcoal.

^{3}H-R-1881 bound to the cytosolic androgen receptors in a saturable manner giving a linear Scatchard plot with a Kd value of 2.2 $\pm$ 0.1 nM and a maximal binding Bmax of 82.1 $\pm$ 14.1 fmoles/mg protein. The binding of ^{3}H-R-1881 at 5 nM was inhibited by various synthetic drugs known to act at the androgenic receptor (R-1881 IC_{50} : 4.1 nM; Cyproterone Acetate IC_{50} : 13 nM). Permixon, (IC_{50} : 0.33 mg/ml) was 36 times more active than another major antiprostatic drug, Tadenan (IC_{50} : 13 mg/ml). Since Permixon is an oil extract, we investigated the inhibition of the binding of ^{3}H-R-1881 by three other vegetable oils of similar origin. Arachide, Onagre, and Palm oils and a mineral oil, liquid paraffin, all had IC_{50} values greater than 50 mg/ml. The fatty acids from C_8 to C_{20} were also inactive up to 1 mM (final concentration). The inhibition of ^{3}H-R-1881 binding by Permixon was dependent on the concentration of the radioactive ligand (at 5 nM, IC_{50} = 0.33 mg/ml ; at 3 nM, IC_{50} = 0.21 mg/ml ; at 1 nM, IC_{50} = 0.18 mg/ml) in a manner consistent with a competitive inhibition.

Thus the liposterolic extract of Serenoa repens B, Permixon, acts, in vitro, at the cytosolic, androgenic receptor of prostatic tissue in a specific and probably competitive manner suggesting that its antiandrogenic activity (Stenger & al., 1982) results from a direct action at the cytosolic receptor.

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PLASMA CORTICOSTERONE LEVELS AFTER i.c.v. INJECTION OF OPIOIDS IN NORMAL AND ETHER STRESSED MICE

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The relationship between opioids and corticosterone responses to stress is far from clear. Gibson et al (1980) reported that met- and leu-enkephalin administered intracerebroventricularly (i.c.v.) produced opposing effects on plasma corticosterone levels in ether stressed mice. We have extended their studies to include several opioid peptides and clinically used opioid drugs.

Drugs were administered i.c.v. in 0.9% saline into conscious mice (CDI strain) in a volume of 5 µl. Doses of each opioid agonist used were chosen from values in the literature for median antinociceptive activity by the i.c.v. route. The procedure for collection of plasma and fluorimetric measurement of corticosterone was as described by Gibson et al (1980). Corticosterone was measured at 5 and 10 minutes after i.c.v. injection. In addition corticosterone was measured 10 minutes after injection, in mice exposed to ether vapour for 60 seconds at 5 minutes.

Some of the opioids studied showed an inhibition of the elevation of plasma corticosterone levels which are observed in saline treated animals due to the stress of handling and injecting (Table 1). The differences between the effect of opioids on plasma corticosterone could not be correlated with their known selectivity for opioid receptors, and further studies have indicated that these effects do not appear to be dose related. Analysis of variance of all the data shows a significant difference for both comparison of drugs and of time points. There is no significant interaction for drug x times indicating that the pattern of change in corticosterone is the same for each drug.

Table 1 Plasma corticosterone levels in mice after i.c.v. injection of opioids

PRETREATMENT	PLASMA CORTICOSTERONE (µg/100 ml) 5 min	10 min	10 min + ether
Saline	27.1 ± 2.2	36.0 ± 3.0	41.0 ± 5.7
(D-Ala^2,D-Leu^5)enkephalin (50ng)	19.7 ± 2.6**	27.4 ± 0.9*	27.0 ± 2.1*
FK33-824 (5ng)	22.2 ± 1.1	22.6 ± 1.3**	25.1 ± 4.2*
(D-Ala^2,MePhe^{4}Gly(ol)^5 enk. (200ng)	29.3 ± 1.9	37.0 ± 2.2	42.9 ± 3.0
Met enkephalin (50µg)	23.7 ± 2.4	29.9 ± 5.5	40.6 ± 5.5
Leu enkephalin (50µg)	20.5 ± 2.0*	29.4 ± 4.2	36.0 ± 4.3
Morphine (1µg)	38.4 ± 9.4	35.2 ± 5.0	28.2 ± 3.5
Sufentanil (1ng)	19.0 ± 3.6	28.2 ± 6.6	22.1 ± 2.5**
Alfentanil (200ng)	21.0 ± 2.3	30.0 ± 3.4	33.5 ± 5.3

All values are mean ± s.e. mean of at least 5 plasma samples, each from different mice t test vs saline control * $p < 0.05$ ** $p < 0.01$

When morphine (1 µg) and FK 33-824 (2 ng) were given together the corticosterone response to ether stress was markedly potentiated (80.8 ± 16.7 µg/100 ml), an effect which was not observed when morphine was administered with (D-Ala^2,D-Leu^5)-enkephalin. This interaction is of interest in the light of a recent report showing potentiation of analgesia after administration of morphine with FK-33-824 (Lee et al, 1980). In conclusion, the results suggest that there are differences in the effect of opioids on plasma corticosterone, but these effects are not dose related nor can be correlated with selectivity for the µ- or δ-opioid receptor.

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THE BILIARY EXCRETION OF AMARANTH IN GERM-FREE AND CONVENTIONAL RATS

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Germfree (GF) rats are potentially useful in drug metabolic studies where the involvement of the gastro-intestinal microflora is suspected (Goldman, 1982). However, in view of the reported differences in GF/conventional (CV) physiological characteristics (e.g. male GF rats;cardiac output is reduced by 30%, and hepatic and intestinal blood flows are reduced by 50%, Gordon, 1968) which could cloud interpretation of such studies, it was decided to initiate investigations to assess the influence of these differences on drug handling. Since liver blood flow is likely to affect the biliary excretion of xenobiotics, we examined the elimination of amaranth, a polar sulphonazo dye which is normally rapidly excreted by the rat, solely in the bile, as the unchanged compound (Klaassen & Strom, 1978).

Seven littermate pairs of inbred GF and CV BDIX rats (10-12 weeks old) of each sex were compared. Matched pairs (GF versus CV) were anaesthetised (urethane;50%, 1.5gkg^{-1}, i.m.) then bile ducts and left femoral veins cannulated. Bile was collected at 10min intervals, 10min before until 120min after i.v. amaranth (20mgml^{-1}, 20mgkg^{-1}). Bile volumes and amaranth concentrations were determined (Klaassen & Strom, 1978). Whole body, heart, liver, stomach, small and large intestine, and caecal weights were recorded. GF versus CV data were compared using Student's paired t-test (significant difference, $P<0.05$). Results are given as means $\pm$ SEM (GF versus CV respectively).

For male rats bile flow was consistently 50% lower (e.g. 60min values, 22.2 $\pm$ 1.5 and 43.8 $\pm$ 3.7μlmin^{-1}kg^{-1}), amaranth excretion rate was lower during the first 20min (20min values, 423.7 $\pm$ 51.6 and 583.6 $\pm$ 72.3μgmin^{-1}kg^{-1}), and cumulative amaranth excretion was lower during the first 30min (30min values, 9.2 $\pm$ 1.2 and 13.7 $\pm$ 1.7mgkg^{-1}). For female rats, bile flow was only 16% lower (e.g. 60min values, 31.0 $\pm$ 2.2 and 37.0 $\pm$ 1.7μlmin^{-1}kg^{-1}) and no significant difference was seen in the amaranth excretion rate (20min values, 647.2 $\pm$ 49.2 and 588.5 $\pm$ 55.9μgmin^{-1}kg^{-1}, $P>0.5$), or cumulative amaranth excretion (30min values, 14.6$\pm$0.8 and 13.5$\pm$1.2mgkg^{-1}, $P>0.5$). For male rats body weight was 11% lower (0.245$\pm$0.012 and 0.276 $\pm$ 0.006kg), liver weight was 24% lower (36.0$\pm$1.8 and 47.4$\pm$2.6gkg^{-1}), while caecal weight plus contents was 90% greater (32.2 $\pm$ 2.0 and 16.9 $\pm$ 1.0gkg^{-1}). For female rats body weight was similar (0.184 $\pm$ 0.007 and 0.194 $\pm$ 0.008kg, $P>0.1$) liver weight was 28% lower (34.8 $\pm$ 1.4 and 48.0 $\pm$ 2.6gkg^{-1}), while caecal weight plus contents was 290% greater (61.1 $\pm$ 3.4 and 15.6 $\pm$ 0.8gkg^{-1}). With the other organs weighed, the only difference noted was the slightly heavier small intestinal contents of GF rats.

Lower liver weight has not been previously reported, unlike the typical caecal enlargement in GF rats (Gordon, 1968). The former may partly explain reduced bile production in GF rats but not the sex difference in amaranth excretion.

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EFFECTS OF CALCIUM ION REMOVAL, Sr^{2+}, La^{3+} AND VERAPAMIL ON CONTRACTIONS OF GUINEA-PIG AND HUMAN AIRWAY SMOOTH MUSCLE

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Calcium channel blocking drugs eg: verapamil and nifedipine are relatively ineffective in preventing asthmatic attacks (Patel, 1981; Williams et al, 1981). It is conceivable that this is a consequence of airway smooth muscle using an intracellular source of activator calcium ions (Ca^{2+}) such as has been postulated to exist in guinea pig airways (Creese & Denborough, 1981). We report here the results of a study in which we have examined the effects of Ca^{2+} removal, strontium (Sr^{2+}) and lanthanum (La^{3+}) ions and verapamil on contractile responses in airway smooth muscle from guinea pigs and man.

Transverse strips or spirally-cut preparations of guinea pig and human airways were suspended in either Krebs-Henseleit solution (KHS) or Tris buffered KHS at 37°C and bubbled with 5% CO_2 in O_2. The human tissue had been removed during surgery the preceding day and stored overnight in refrigerated KHS. Contractions to equi-effective concentrations (Emax 90) of methacholine, histamine, potassium chloride depolarising solution (KCl) and the calcium ionophore A 23187 were recorded using conventional methods.

The results are summarised in Table 1.

Table 1 Effects of incubation in "Ca-free" KHS, verapamil, La^{3+}, and Sr^{2+} on contractions of guinea pig airway smooth muscle (Mean ± S.E.mean; N > 4)

Drug Emax 90	% Control response: Time in "Ca-free" KHS(min) 60	120	240	Verapamil (1×10^{-6}M)	La^{3+} (1×10^{-3}M)	Sr^{2+} (2.5×10^{-3}M)
Methacholine (1×10^{-4}M)	98 ± 3	42 ± 6	7 ± 1	78 ± 2	54 ± 11	26 ± 1
Histamine (4×10^{-5}M)	86 ± 7	5 ± 3	0	75 ± 7	0	22 ± 5
KCl (9×10^{-2}M)	98 ± 1	41 ± 9	15 ± 3	48 ± 9	101 ± 4	102 ± 5
A 23187 (5×10^{-6}M)	τ	-	-	80 ± 4	ND	ND

τ = Response abolished within 15 min. ND = not done

In both guinea pig and human tissues the rank order for loss of contraction in "Ca-free" KHS was A 23187> histamine> methacholine> KCl. Similarly KCl-induced contractions were most resistant to the effects of both Sr^{2+} and La^{3+}. Verapamil (1×10^{-8}M to 1×10^{-6}M) produced relatively small reductions in developed tension which were not concentration-related.

These results indicate that in airway smooth muscle of guinea pig and man KCl exhibits a novel profile of action. Furthermore, the data are consistent with the existence of both intra- and extracellularly located activator Ca^{2+} stores whose relative contribution to contraction is dependent upon the agonist in question.

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EFFECTS OF CALCIUM DEPRIVATION AND VERAPAMIL ON CONTRACTIONS OF BISECTED RAT VAS DEFERENS

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Triggle et al (1979) and Lin & Swamy (1980) reported that the maximum contractions of rat vas deferens elicited by K^+ and by noradrenaline (NA) depend upon external calcium concentration and are inhibited by calcium antagonists. French & Scott (1981) reported that verapamil potentiates the contractions of the prostatic end to single pulse field stimulation and antagonizes those of the epididymal end. To elucidate the calcium translocation mechanism in the two portions of rat vas deferens, we have studied the effect of calcium deprivation and of verapamil on contractions elicited by NA (10^{-4}M) and KCl (60 mM). Isometric contractions of bisected vas deferens were recorded by conventional methods. After obtaining constant responses (at 20 min intervals) in oxygenated Tyrode solution (37° C, $CaCl_2$ 1.8 mM), the concentration of $CaCl_2$ was stepwise reduced to 0.9, 0.45, 0.22, 0.11 and nominally 0 mM. The effect of stimulants was tested when the tissue had been incubated in the desired calcium concentration for 20 min.

The response to KCl decreased proportional to the decrease of $CaCl_2$ of the medium and at 0.22 mM $CaCl_2$ was 24.1±9.0% (n = 6) and 24.2±4.3 (n = 7) in the prostatic and epididymal portions respectively. On the other hand, there was a significant difference in the responses of the two portions to NA. At 0.22 mM $CaCl_2$, the responses of prostatic and epididymal portions to NA were 65.2±10.0% (n = 7) and 7.6±1.9% (n = 7) of the control contractions respectively.

Verapamil ($2X10^{-8}$M - $2X10^{-5}$M) inhibited the contractions elicited by KCl in both portions dose dependently. With $2X10^{-6}$M verapamil, the responses of prostatic and epididymal portions to KCl were 59.1±8.1% (n = 4) and 24.4±6.1% (n = 4) respectively. On the other hand, verapamil inhibited the responses to NA only in the epididymal portion. With $2X10^{-6}$M verapamil, the responses of prostatic and epididymal portions to NA were 180±20.5% (n = 6) and 57.4±12.5% (n = 6) respectively.

The results suggest that while the contractions to KCl in both portions and to NA in the epididymal end are dependent on external calcium concentration, contractions elicited by NA in the prostatic portion are at least partially dependent on intracellular sources of calcium.

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IN VITRO MYORELAXANT EFFECTS OF SOME DIURETICS ON THE RAT PORTAL VEIN

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It has been suggested that part of the antihypertensive action of the diuretic indapamide may be due to its myorelaxant effect on smooth muscle, as observed in vitro and in vivo (Finch et al., 1977 ; Borkowski et al., 1981). Since long-term treatment with diuretics produces a reduced response to pressor stimuli and lowered blood pressure despite normalized blood and extracellular volumes (e.g. Conway & Lauwers, 1960) we thought it of interest to see if other diuretics had smooth muscle relaxing properties.

In vitro experiments were performed on isolated strips of rat hepatic portal vein maintained in Krebs at 37°C and gassed with 95% O_2, 5% CO_2. The effects of various concentrations of diuretics were studied on the spontaneous contractions of the preparations and the ability of the drugs to inhibit angiotensin II (Ag II, 50nM, administered every 20 min to the organ bath)-induced contractions of the vein.

All diuretics studied dose-dependently reduced spontaneous contractions of the portal vein, total inhibition of activity occuring within 15 min of drug addition with 0.1mM bumetanide, furosemide and piretanide and with 1.0mM amiloride, hydrochlorothiazide and indapamide.

In inhibiting Ag II-induced contractions, the potassium sparing diuretic, amiloride, was the most active compound (IC_{50} 6.8 ± 0.8µM, n=6) followed by the high-ceiling (loop) diuretics bumetanide, furosemide and piretanide (IC_{50} respectively: 82.6 ± 22.9µM, 76.2 ± 15.0µM and 83.8 ± 14.8µM ; n=5-8). The low ceiling diuretic hydrochlorothiazide and indapamide were considerably less active (IC_{50} hydrochlorothiazide, 0.84 ± 0.08mM ;indapamide, 0.28 + 0.02mM ;n=6) and their dose-response curves differed from the other diuretics. This effect was also observed in the $PGF_{2\alpha}$ contracted rat aorta.

The direct myorelaxant effect of amiloride was also observed in vivo using the pithed rat. The animals were binephrectomized 1 hr before pithing so as to exclude any renal action of the drug. In this model, amiloride (10mg/kg, ip) significantly reduced ($p< 0.001$; n=5) the pressor response to i.v. injected noradrenaline.

This work demonstrates that an in vitro myorelaxant action of diuretics is not unique to indapamide. Also observed was a marked difference in the dose-response curves between high and low ceiling diuretics. Results obtained with amiloride reveal a direct myorelaxant effect occurring in vivo. The reason for this myorelaxant action of diuretics is unknown, but it may involve a membrane effect, possibly, though not directly, upon Ca^{++} fluxes, since the drugs concomitantly inhibited spontaneous and Ag II-induced contractions (Hamon & Worcel, 1981).

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COMPARATIVE CALCIUM ENTRY BLOCKING PROPERTIES OF NICARDIPINE, NIFEDIPINE AND PY 108068 ON CARDIAC AND VASCULAR SMOOTH MUSCLE

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Calcium entry blockers (CEBs) inhibit mechanical responses of cardiac muscle and vascular smooth muscle via a blockade of inward calcium currents (Kass & Tsien, 1975; Harder & Sperelakis, 1979). The comparative effects of the dihydropyridine CEBs, nicardipine, nifedipine and PY 108068 (Hof et al, 1981), on these tissues have been investigated. Right ventricular papillary muscle and the superior mesenteric artery were used.

Free running papillary muscles and segments of the superior mesenteric artery were removed from 200-300 g Dunkin-Hartley guinea pigs. The mesenteric artery was cut into a spiral strip approximately 20 x 1.5 mm. Both preparations were continuously superfused at 5 $ml.min^{-1}$ with a physiological salt solution (PSS) as described by Patmore & Whiting (1981). The papillary muscle preparations were stimulated through field electrodes at 0.5 Hz, 2-3 V, 0.5 ms and maintained at 30^0C. The muscles were left for 1 h to attain a steady state force of contraction. Contractures of mesenteric artery strips were evoked by superfusion for 4 min in a 15 min cycle with a high potassium concentration PSS where $[K^+]$ was increased from 6 to 120 $mmol.litre^{-1}$ at the expense of $[Na^+]$. The arterial muscle preparations were maintained at 37^0C and left for 2 h to attain a steady state force of contraction. The magnitude of twitch contractures of cardiac muscle and tonic contractures of vascular smooth muscle were measured. Nicardipine, nifedipine and PY 108068 were dissolved in C_2H_5OH and were added to the superfusate to achieve concentrations in the range 10^{-11} to 10^{-5} $mol.litre^{-1}$. In both preparations the effects of these CEBs were monitored over 30 min periods.

The magnitude of contractions in CEB solutions were compared with control values and concentration-response relationships constructed. IC_{50} values were calculated from regression analysis of data from at least 4 separate experiments. Values are shown in Table 1.

Table 1

COMPOUND	IC_{50} ($mol.litre^{-1}$) VASCULAR MUSCLE (1) Mesenteric artery	CARDIAC MUSCLE (2) Papillary muscle	RATIO (2/1)
NICARDIPINE	6.3×10^{-9}	7.0×10^{-8}	11.1
NIFEDIPINE	1.3×10^{-8}	7.1×10^{-8}	5.5
PY 108068	9.0×10^{-9}	2.2×10^{-8}	2.8

The data show that of the dihydropyridines tested, nicardipine is the most potent inhibitor of potassium-induced vascular smooth muscle contractures whilst PY 108068 is the most potent negative inotropic agent. The vascular smooth muscle-cardiac muscle ratios indicate that nicardipine is the most vascular selective CEB.

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ENHANCED RESPONSIVENESS OF VASCULAR SMOOTH MUSCLE TO VASOCONSTRICTOR AGENTS AFTER REMOVAL OF ENDOTHELIAL CELLS

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It has recently been noted that an intact endothelium in vascular smooth muscle preparations is necessary for the expression of vasodilator responses to a number of naturally occurring substances including acetylcholine, bradykinin, adenine nucleotides and substance P. Thus, following mechanical removal of the endothelial cells from such preparations vasodilator responses are abolished (Furchgott and Zawadzki, 1980; De Mey and Vanhoutte, 1981; Cherry et al, 1982). In the present study, we have found that the mechanical removal of endothelial cells from vascular smooth muscle preparations, also enhances the contractile response of these preparations to naturally occurring and other vasoconstrictor substances.

In these studies, pairs of rings (1-2 mm thick) were cut from the thoracic aorta of rats (200-250g Wistar). One ring from this pair was left intact, but the remaining ring was stripped of its endothelial cells by gentle rubbing of the intimal surface with a small piece of tissue held in very fine watchmakers forceps. Selective removal of the endothelial cells was confirmed at the end of each experiment by demonstrating the lack of a relaxation response to acetylcholine (10^{-6} -10^{-5}M), and also by histological examination. Each ring was carefully mounted between stainless steel hooks in an organ bath containing Krebs-solution at 37°C, gassed with 95% O_2/5% CO_2, and placed under a resting tension of 1.5g. The rings were allowed to equilibrate for at least 1 hour, during which time they were washed at regular intervals with fresh, warmed Krebs solution and tension adjusted as necessary. A single dose-response curve to the chosen vasoconstrictor substance was established in pairs of rings by cumulative addition to the Krebs-solution, and measuring isometric tension changes.

Following addition of either noradrenaline, phenylephrine, angiotensin I or angiotensin II both the threshold concentration, and the effective concentration required to produce 50% of the maximum contraction (EC_{50}) were less in the de-endothelialised rings than in the intact rings. (EC_{50} for de-endothelialised : intact: Noradrenaline 0.65 ± 0.14 : 31.8 ± 13.9 nM (n=6), Phenylephrine 7.48 ± 1.62 : 16.6 ± 4.0 nM (n=6), Angiotensin I. 0.96 ± 0.25 : 4.84 ± 0.65 nM (n=6), Angiotensin II 0.43 ± 0.09 : 2.14 ± 0.35 nM (n=6). There was however no difference in the maximum contractile response achieved by phenylephrine and noradrenaline in both preparations whereas contractile responses to angiotensin I and angiotensin II were greatly enhanced in de-endothelialised rings.

Such an enhancement of vascular reactivity to contractile substances may occur in blood vessels when the endothelium is damaged by pathological events. This is of particular importance in view of the recent observations by Ku (1982) demonstrating increased vascular reactivity to thrombin in isolated coronary blood vessels following experimental myocardial ischemia.

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EFFECT OF AMINOPHYLLINE ON AMPHOTERICIN B NEPHRO-TOXICITY IN THE DOG

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The clinical use of amphotericin B is limited because of its ability to cause nephrotoxicity. We have previously suggested that amphotericin initially alters tubular function. The change in renal function may, in situations of sodium depletion, activate tubuloglomerular feedback (TGF) to cause a reduction in renal blood flow (RBF) and glomerular filtration rate (GFR)(Gerkens and Branch 1980).

TGF is an intrarenal mechanism activated by an increase in sodium chloride delivery to the macula densa region (Wright and Briggs, 1977). Osswald et al (1980) have suggested that adenosine may mediate TGF and that adenosine receptor blockade with aminophylline can prevent TGF. The purpose of this study was to examine the effect of aminophylline on the renal response to amphotericin B in the sodium depleted dog.

RBF (measured with electromagnatic flow probes)and GFR (measured by 99 TC DTPA-clearance) were monitored in 3 groups of anesthetized dogs (n = 6 in each group). The first group received amphotericin (0.5 mg/kg i.v. over 20 min), the second group received aminophylline (5 mg/min infused into the renal artery for 140 min) and the third group received both amphotericin B and aminophylline in the same doses and for the same times. Amphotericin B alone caused a sustained reduction in RBF and GFR between 20 and 140 minutes. 140 min after starting the ampho infusion RBF was reduced by 20% (50±13 ml/min) and GFR by 54% (23±2 ml/min). Aminophylline alone increased RBF by 15% (28±14 ml/min) and decreased GFR by 19% (7±4 ml/min). In dogs who received both aminophylline and amphotericin B, RBF was not changed and GFR was decreased by 28% (9±4 ml/min) 140 mins after starting the ampho infusion. This reduction was significantly less than the reduction in GFR in the first group of dogs ($p < 0.05$).

These studies indicate that aminophylline is able to inhibit the acute renovascular response to amphotericin B and are consistent with the hypothesis that the response to ampho is mediated by TGF.

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THE ENCAPSULATION OF THE ANTI-LEUKAEMIC DRUG L-ASPARAGINASE IN INTACT ERYTHROCYTES

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L-Asparaginase is used to treat patients with acute lymphocytic leukaemia but the use of the drug is complicated by side-effects. Liposomes have been employed to avoid allergic and toxic reactions due to the enzyme (Gregoriadis & Allison, 1974). The intravenous use of liposome-entrapped asparaginase in mice reduced blood asparagine levels (Neerunjun & Gregoriadis, 1976). Recently we have encapsulated drugs in intact erythrocytes (Jenner et al 1981) and have shown that the cells survive to their normal lifespan in vivo in the species under test. In this report we have used erythrocytes to encapsulate asparaginase.

Erwinia asparaginase (kindly supplied by Dr. H. E. Wade, Microbiological Research Establishment, Porton, Salisbury, Wilts) was labelled with ^{125}I (Hunter & Greenwood, 1962) and the radioactive material was added to the 'cold' enzyme as a tracer. Erythrocytes were separated from blood obtained by cardiac puncture from Male Wistar strain rats, body weight 150g. Packed cells (1 ml) were swollen in stages by the addition of hypotonic reversed Na^+/K^+ Hanks balanced salt solution (HBSS) to the point of lysis. Asparaginase (2000 Units) dissolved in water (200µl) was added with mixing to the swollen cells which were then resealed and returned to normal tonicity by the addition of hypertonic HBSS. The supernatent was removed and the cells washed 3 x in HBSS. The radioactivity associated with the cells was then determined. In control experiments the erythrocytes were treated with the same amount of enzyme in isotonic HBSS. Calculations showed that 13% w/v of the added enzyme had been taken up by cells in the encapsulation procedure compared with 5% w/v in the controls.

The glutaminase activity of the encapsulated preparation was determined. Packed cells (0.2 ml) containing asparaginase were lysed by freezing and thawing and incubated with 0.633 µmole ^{14}C-glutamine in .075 ml borate buffer (0.1 M) PH 8.5 for 30 min at 37°. After centrifuging the supernatent was subjected to cellulose acetate membrane electrophoresis in pyridine-acetic acid-water buffer (25:1:225 v/v pH 6.0) and the ratio of ^{14}C-glutamine to ^{14}C-glutamic acid determined by radiochromatogram scanning. Approximately 62% of the glutamine had been converted to glutamic acid, equivalent to 40 units of enzyme. One ml of packed cells containing asparaginase was labelled with fluorescein and injected IV into rats. Tail vein blood samples taken periodically showed that the labelled cells survived for up to 50 days in vivo with a half life of 10-12 days. Studies are in progress to test our preparation against tumours in vivo.

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EFFECT OF ACUTE RENAL FAILURE ON THE ELIMINATION OF (^{3}H)-TAUROCHOLIC ACID IN THE RAT

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Renal failure in the rat results in decreased hepatic uptake of dyes, such as indocyanine green, that are commonly used to assess liver function (Bowmer et al, 1982a & b). However, little is known about the effect of uraemia on the liver's ability to remove endogenous substances from plasma. Consequently we have investigated the kinetics of [^{3}H]-taurocholic acid (TCA) in rats with acute renal failure (ARF).

ARF was induced in male Wistar rats (Bowmer et al 1982a) and 48h later the plasma disappearance and biliary excretion of [^{3}H]-TCA (5 mg kg^{-1}; 10 μCi kg^{-1} i.v.) were determined. [^{3}H]-TCA appeared to be eliminated in a bi-exponential manner from plasma in both control and uraemic rats. Kinetic analysis of the results showed that the rate constant for hepatic uptake, k_{12}, was not significantly changed whereas Vc, Vd and the plasma clearance of [^{3}H]-TCA were greater in uraemic rats (Table 1). The rate constant for elimination, k_{el}, was significantly decreased; but in uraemic rats the percentage recovery of [^{3}H]-TCA in bile after 1 h (84.4±6.4%; N=7) was not significantly different than in control rats (90.5±5.2%; N=6).

Table 1 Kinetics of [^{3}H]-taurocholate in control and uraemic rats

Parameter	Control (N=7)	Uraemic (N=8)	
$T_{\frac{1}{2}}$ α (min)	1.2±0.1	1.2±0.2	N.S.
$T_{\frac{1}{2}}$ β (min)	12.5±1.5	18.2±4.0	<0.01
k_{12} (min^{-1})	0.556±0.051	0.533±0.064	N.S.
k_{21} (min^{-1})	0.039±0.010	0.029±0.011	N.S.
k_{el} (min^{-1})	0.061±0.007	0.042±0.009	<0.001
Vc (ml)	41.5±7.3	56.3±13.2	<0.05
Vd (ml)	277±44	510±216	<0.05
Plasma clearance (ml min^{-1} 100g.bw^{-1})	5.2±0.7	7.0±1.5	<0.05
Plasma urea (mg 100ml^{-1})	47±6	269±141	<0.001

Values represent mean ± s.d.

The plasma clearance of [^{3}H]-TCA was increased in the uraemic rats. This change probably results from an increase in Vd and/or increased liver blood flow in uraemic rats (Hiley et al, 1980). In contrast to the results obtained for indocyanine green (Bowmer et al, 1982a), the hepatic uptake of [^{3}H]-TCA was not altered in rats with ARF. Therefore, ARF does not have a universal effect upon the liver's capacity to remove substances from plasma.

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PHARMACOKINETICS OF DIFENACOUM AND WARFARIN IN THE RABBIT

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Difenacoum (3-(3- [1-1^1-biphenyl] -4-yl-1,2,3,4 - tetrahydro-1-naphthyl) - 4-hydroxycoumarin: DF) is a novel 4-hydroxycoumarin anticoagulant which has been developed as a rodenticide. Metabolic studies with radiolabelled vitamin K indicate that DF, like warfarin (W), reduces clotting factor synthesis by inhibition of the enzyme vitamin K epoxide reductase (Park et al, 1979). However DF is a more potent and persistent antagonist of vitamin K in man and the rabbit (Barlow et al, 1982; Park & Leck, 1982). At present, little is known about the disposition of DF. We have therefore made a comparative study of the plasma pharmacokinetics of DF and W in the rabbit.

Male New Zealand White rabbits (2.5 - 3.0 kg) were used in the study. After intravenous administration into the marginal ear vein of either W (6.3 mg kg^{-1} in saline 0.5 ml kg^{-1}) or DF (8.5 mg kg^{-1} in polyethylene glycol 200 0.5 ml kg^{-1}), blood samples were taken from the other marginal ear vein at regular intervals. Plasma was obtained and stored frozen until analysed. W was measured by normal phase chromatography on a spherisorb 5CN column with the solvent system hexane: isopropanol: dichloromethane: acetic acid (85:10:5:1 v:v) (Shearer). DF was measured by reversed-phase chromatography using a Whatman Partisil 10 ODS column, and methanol : water : acetic acid (90:10:1; v/v) as solvent. The interassay coefficients of variation were, respectively, 3.9 and 9.8%.

The plasma concentration of W declined monoexponentially whereas the plasma concentration of DF showed a bi-exponential decay. The data were analysed by least squares regression analysis and the pharmacokinetic parameters obtained are presented in the table.

Table — Pharmacokinetic parameters for W and DF in the rabbit

	dose (mg kg^{-1})	$t_{\frac{1}{2}}\alpha$ (h)	$t_{\frac{1}{2}}\beta$ (h)	Clp (ml min^{-1})	Vd (l kg^{-1})
W	6.3	-	4.5 ± 0.5	1.3 ± 0.2	0.71 ± 0.1
DF	8.5	3.2 ± 0.2	83.1 ± 20.6*	1.4 ± 0.2	10.4 ± 2.9*

Results are means (n = 4) ± s.d. *$p < 0.001$

DF has a lower rate of elimination from plasma than W because it appears to have a much larger volume of distribution (Vd) than W. These findings partly explain why DF has a much more persistent action than W but do not account for the observation that DF is a more potent antagonist of vitamin K than W *in vivo*.

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PHARMACOKINETICS OF A PHARMACOLOGICAL DOSE OF VITAMIN K IN THE RABBIT

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It has been observed that frequent and repeated administration of vitamin K_1 (K) is necessary to restore clotting factor synthesis after warfarin overdose. However, at present there is no rational regime available for the administration of K as an antedote to anticoagulant poisoning. As part of our studies on the relationship between K metabolism and clotting factor synthesis we investigated the effect of anticoagulants on the pharmacokinetics of K and its 2,3-epoxide metabolite (KO) after administration of a pharmacological dose of the vitamin. The anticoagulants used were chlorovitamin K (Cl-K) and difenacoum (DF) which inhibit the physiologically important K-K epoxide cycle at the epoxidase and epoxide reductase steps respectively (Park et al, 1979).

Male New Zealand White rabbits (2.5 - 3.0kg) were administered K (1 mg kg^{-1}: Konakion®) into the marginal ear vein and blood samples taken at regular intervals from the other marginal ear vein. Plasma was stored at -20° until analysis. Cis and trans K and KO were determined by normal phase h.p.l.c. using 0.2% acetonitrile in hexane solvent with a Partisil PXS 10 (25cm x 4.5mm I.D.) column. DF (0.85 mg kg^{-1}) and Cl-K (10 mg kg^{-1}) were administered as described previously (Park et al, 1979).

The plasma concentration of K declined biexponentially and could be resolved into a fast and slow phase. The α and β elimination rate constants were similar to those obtained with a physiological dose (0.7 $\mu g\ kg^{-1}$) of K (Park et al., 1980) However the plasma concentration ratio of KO:K measured 6h after administration of vitamin K was four fold greater ($P < 0.01$) following the higher pharmacological dose. Cl-K decreased the plasma clearance (Clp) of K and decreased KO formation whereas DF increased KO plasma concentrations without significantly altering K levels (Table 1).

Table 1. Pharmacokinetic parameters for K and KO in the rabbit

	K A.U.C. ($\mu g\ ml^{-1}\ h$)	K Clp ($ml\ min^{-1}\ kg^{-1}$)	KO A.U.C. ($\mu g\ ml^{-1}\ h$)
Control	7.76 ± 1.02	1.92 ± 0.28	2.98 ± 0.41
DF	9.69 ± 2.01	1.55 ± 0.25	6.92 ± 1.69**
Cl-K	19.37 ± 4.72**	0.79 ± 0.19***	1.62 ± 0.47**

Values are expressed as mean (n = 4) ± S.D. **P < 0.01, ***P < 0.001

The results shown above are in agreement with the proposed site of action of the anticoagulants Cl-K and DF. The production of KO is thought to be chemically linked with the formation of γ-carboxyglutamic acid residues in K dependent clotting factors (Bell, 1978). The results suggest, however, these two processes may become dissociated at high pharmacological doses of K.

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ALLERGEN-INDUCED BRONCHOCONSTRICTION IN DOGS EXPERIMENTALLY INFECTED WITH ASCARIS SUUM OVA

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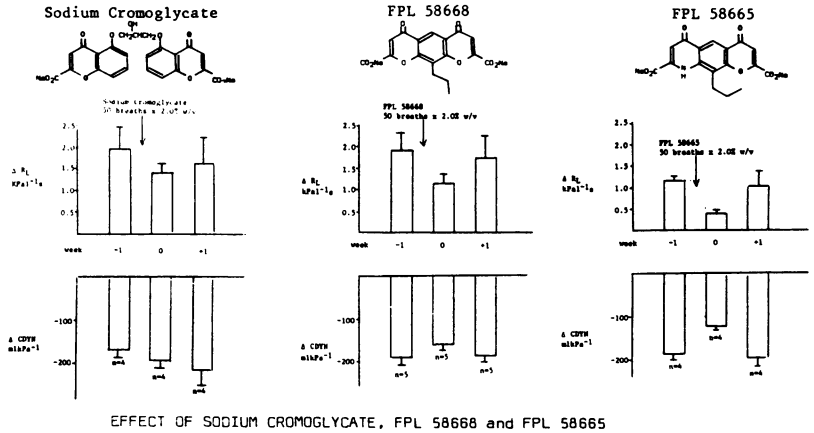
Inhalation of Ascaris suum antigen induces bronchoconstriction in home-bred Macaca arctoides monkeys experimentally infected with Ascaris ova (Richards, et. al.1982). Inhalation of Ascaris in 'naturally' sensitive dogs also induces changes in breathing pattern and bronchoconstriction which have been characterized as IgE-mediated responses, arising from a prior sensitization of the dogs following infestation with cross-reacting antigenically related nematodes (Booth et al.1970). We have now investigated the effects of bronchial provocation with aerosols of Ascaris allergen in dogs which had been bred in a worm-free environment and showed no prior 'natural' sensitivity to Ascaris.

Beagle dogs were anaesthetized and prepared for recording total lung resistance (R_L) and dynamic lung compliance (Cydn). Dogs were selected which did not respond to Ascaris inhalation. The animals were then infected orally with Ascaris eggs, (Richards et.al. 1982). Six weeks after infection inhalation of Ascaris at weekly intervals in anaesthetized dogs produced reproducible increases in R_L and falls in Cdyn. However bronchial reactivity to a standard histamine provocation was unaffected by the infection or by weekly provocation with Ascaris. The changes in lung mechanics were associated with increases in arterial plasma histamine levels. Ascaris-induced bronchoconstriction was reversed by salbutamol indicating that smooth muscle contraction was responsible for the bronchoconstriction, and partially inhibited by atropine suggesting a partial involvement of reflex vagal pathways. Ascaris-induced changes in R_L and Cdyn before and after treatment with sodium cromoglycate and two new anti-asthma drugs, FPL 58668 and FPL 58665 (disodium salts) are shown in fig. 1. FPL 58668 produced a significant inhibition ($P < 0.05$) of changes in R_L but not in Cdyn.' FPL 58665 produced a significant inhibition ($P < 0.05$) of both R_L and Cdyn changes. Sodium cromoglycate did not show a statistically significant inhibition of either R_L or Cdyn changes

Infection of dogs with Ascaris suum ova provides an experimental model which may be useful in predicting the activity of new drugs for the treatment of asthma.



EFFECT OF SODIUM CROMOGLYCATE, FPL 58668 and FPL 58665
BY INHALATION ON ASCARIS INDUCED INCREASES IN R_L AND FALLS IN CDYN

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THE ROLE OF PLATELET ACTIVATING FACTOR (PAF) AND OF PALMITYL-LYSOPHOSPHATIDATE IN SECRETION INDUCED BY THROMBIN AND COLLAGEN

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Platelet-activating factor (PAF) and lyso-phosphatidic acid have been suggested as endogenous mediators involved in the activation of platelets by thrombin or collagen. (Gerrard et al.,1980; Vargaftig et al., 1981). The secretory and aggregatory responses of human platelets induced by thrombin, collagen, PAF and lysophosphatidic acid have been assessed following desensitisation to PAF or lysophosphatidic acid in the presence of an inhibitor of cyclooxygenase.

Human platelet-rich plasma was prepared by centrifugation of citrated whole blood at 220g for 15 minutes at 25°C. After equilibration at 37°C, aggregatory and secretory responses to agonists were continuously monitored using a Payton lumi-aggregometer.

In contrast to some previous reports (e.g. Chesney et al., 1982), aspirin (100μM) or indomethacin (30μM) caused minimal inhibition (10-15%) of the secretion of ATP from human platelets induced by PAF (10-100 ng/ml). PAF (1-5 ng/ml) induced transient aggregatory responses in the presence of aspirin or indomethacin without any detectable secretion of ATP. Subsequent addition of PAF (1-500 ng/ml) failed to induce a further response indicating that the initial dose had caused complete desensitisation of the PAF receptor. Platelets could also be desensitised to lysophosphatidic acid by induction of a small transient aggregatory response which caused complete suppression of further response to this agonist. In such platelets the dose/response curve to PAF was shifted to the right although maximal responsiveness was not decreased.

Desensitation induced by either PAF or lysophosphatidic acid caused a decrease in the maximal secretory responses induced by addition of thrombin. Under conditions where responsiveness either to PAF or to lysophosphatidic acid was completely suppressed, secretion of ATP induced by thrombin (2.0 U/ml) in the presence of indomethacin or aspirin was inhibited by 30% or 25% respectively. If the same preparation was desensitised by exposure to both lysophosphatidic acid and PAF secretion was inhibited by 37%.

In contrast the secretion of ATP from human platelets induced by collagen in the presence of aspirin or indomethacin was not affected by prior desensitisation to PAF or lysophosphatidic acid. These results suggest that although unique receptors exist for PAF and lysophosphatidic acid the mechanism(s) by which these two agonists induce a response share some common features (cf. MacIntyre and Westwick, 1981). PAF and lysophosphatidic acid may be involved in mediation of secretion induced by high concentrations of thrombin in the presence of a cyclooxygenase inhibitor but the action of these mediators does not entirely explain this response. Secretion induced by collagen in the presence of a cyclooxygenase inhibitor does not appear to involve either PAF or lysophosphatidic acid as mediator.

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INCREASED SENSITIVITY OF ARACHIDONIC ACID-INDUCED PLATELET AGGREGATION IN THE PRESENCE OF CARBON DIOXIDE

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While the beneficial effect of acid conditions on the viability of fresh and stored human platelets has been known for some years (Morrison & Baldini, 1967), little is known of the effects of pathological changes in hydrogen ion concentration on platelet activation and aggregation.

The effects of carbon dioxide on human platelet aggregation in vitro, with the concomitant changes in pH, have been studied as a means of imitating the changes in pH and PCO_2 observed in inflammation and tissue fluid stasis (Schade, 1924; Greenwood & Kerry, 1975; Greenwood, personal communication).

A stream of CO_2 (warmed to 37°C) was directed on to the surface of a suspension of platelet-rich citrated plasma (PRP) and the pH monitored. The pH of platelets treated with CO_2 fell to a maximum low of pH 6.2 from a control value of pH 7.4. Adenosine diphosphate (ADP)-induced aggregation was inhibited in CO_2-treated platelets and this effect mimics that seen with PRP taken into acid citrate dextrose (ACD) pH 6.5 (Patscheke, 1981). However, CO_2-treated platelets were rendered up to ten times more sensitive to sodium arachidonate and A23187-induced aggregation. Both these effects were abolished if the CO_2 was allowed to disperse from the PRP on exposure to air, suggesting no permanent alteration in platelet metabolism.

Changes in pH due to dissolved CO_2 greatly altered the sensitivity of platelet aggregation to at least two agonists. The increased sensitivity of arachidonate induced aggregation with lowered pH may be a significant factor in influencing the behaviour of platelets in haemostasis.

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ALTERATION BY PROSTAGLANDINS AND INDOMETHACIN OF THE MOUSE PERITONEAL MACROPHAGE OXIDATIVE BURST

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When exposed to soluble or particulate stimuli macrophages undergo an "oxidative burst" involving rapid enhancement of cellular respiration with the extracellular generation of oxygen radicals (Badwey & Karnovsky, 1980). Under the same conditions, macrophages also produce prostaglandins (PGs) and PGE2 has been shown to inhibit the oxidative burst of different macrophage populations (Weidemann et al., 1978; Smith & Weidemann, 1980; Parnham et al, 1983). We have now further studied the modulation of the mouse macrophage oxidative burst by exogenous and endogenous PGs.

Macrophages (PEM) were obtained from male CBA mice (c. 20 g) by peritoneal lavage after activation 5 days previously with C. parvum (1.4 mg, i.p.), as described previously (Parnham et al., 1983). After washing, 10^6 cells were incubated at 37 °C for 10 min with or without test compound before stimulation with serum opsonized zymosan (OpZ, 0.17 mg/ml) or phorbol myristic acetate (PMA, 50 nM). The production of oxygen radicals was measured after 5 min as luminol-dependent chemiluminescence (CL), in a Lumac Biocounter 2000 or an LKB 1251 luminometer, as described previously (Parnham et al., 1983).
Both PGE2 (10^{-7} - 10^{-5} M) and prostacyclin (PGI2, 10^{-6} - 10^{-4} M) inhibited PEM-CL, induced either by OpZ or PMA in a concentration dependent manner. The extent of inhibition by PGE2 varied markedly, depending on when the experiment was carried out, suggesting seasonal variability in sensitivity of PEM to PGE2. Indomethacin, up to 10^{-5} M, had no effect on either OpZ or PMA-induced CL, though 36 % inhibition was observed at 10^{-4} M, a concentration much higher than that required for cyclo-oxygenase inhibition. However, when administered to mice for 2 days prior to cell harvesting, indomethacin (2 mg/kg/day, p.o.) markedly inhibited (by 70 % vs. control, $p < 0.02$) OpZ-induced PEM-CL ex vivo.

These data indicate that PGE2 and PGI2 produced locally at an inflamed site are capable of inhibiting the oxidative burst of mouse PEM, though responses to PGE2 may be subject to seasonal variation. Amounts of PGE2/PGI2 generated by these cells appear insufficient to exert feedback inhibition. Since mouse resident PEM generate much less CL than activated PEM (Parnham et al. 1983) and since indomethacin has no direct effect on PEM-CL in vitro, the ex-vivo inhibitory effect of indomethacin may reflect inhibition of the recruitment of activated macrophages.

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THERMODYNAMIC CHANGES ACCOMPANYING RECEPTOR-INDUCED LIGAND ORIENTATION

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It can be shown that a charged drug receptor can modify the orientation of an approaching polarised drug molecule. The most probable orientation occurs when the drug molecular dipole is aligned antiparallel to the local electric field line originating from the receptor (Dean, 1981). What has to be determined is whether the receptor-induced ligand orientation is significant in thermodynamic terms. The rotational entropies and associated free energies have been calculated with different dielectric conditions for ethidium and its p-carboxyphenyl derivative in the vicinity of a fully charged portion of B-DNA (12 base pairs of an alternating G-C sequence).

The rotational partition function was computed from the rotation energies determined for 1000 orientations at 28 separate locations round the receptor. These values were then used to derive the rotational entropies and rotational free energies of the two drug compounds (Table 1).

Table 1 Rotational entropies and rotational free energies for ethidium and p-carboxyphenylethidium near a B-DNA receptor

Dielectric condition	ethidium	p-carboxyphenylethidium
	Rotational entropies ($J.K^{-1}.mol^{-1}$)	
in vacuo	34.75 ± 2.4	20.37 ± 3.08
in water (bulk dielectric)	57.38 ± 0.01	54.41 ± 0.36
in water (variable dielectric)	57.17 ± 0.04	52.54 ± 0.68
	Rotational free energies ($kJ.mol^{-1}$)	
in vacuo	-34.64 ± 0.39	-38.68 ± 0.68
in water (bulk dielectric)	-24.20 ± 0.05	-27.99 ± 0.21
in water (variable dielectric)	-25.49 ± 0.19	-29.38 ± 0.36
	(S.E.M. n=28 locations)	

The rotational entropies, calculated from inertial moments by Davidson's method (1962), for free rotation of ethidium and its carboxylated derivative are 146.3 and 151.2 $J.K^{-1}.mol^{-1}$ respectively, with corresponding free energy contributions of -41.5 and -43.0 $kJ.mol^{-1}$ at 310°K. Thus by comparing these values with those in Table 1 it would appear that there are very marked reductions in rotational entropy for both drug molecules in the vicinity of the B-DNA receptor. This reduction is significant in aqueous solvent and occurs where molecular separation between the partners is sufficient to exclude steric collisions.

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KINETIC STUDIES ON RECEPTOR BINDING OF QUINOXALINE ANTIBIOTICS

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Quinoxaline antibiotics, of which the best-known representative is echinomycin, are powerful antitumour agents whose action in vivo is attributable to their binding to DNA (Katagiri et al, 1975; Gale et al, 1981). They are characterised by the presence of two quinoxaline chromophores attached to a cross-bridged cyclic octadepsipeptide; the cross-bridge can be either a thioacetal or a disulphide. Their binding to DNA occurs by a mechanism involving bifunctional intercalation of both chromophores between the base-pairs of the double helix. They display nucleotide sequence-selectivity, the pattern of which varies importantly between different quinoxalines.

Antibiotic-DNA complexes can be dissociated by addition of sodium dodecyl sulphate and the release of the ligand followed spectrophotometrically (Fox et al, 1981; Fox & Waring, 1981). With synthetic DNAs such as poly(dA-dT) and poly(dG-dC) the dissociation reaction follows simple first order kinetics, but with natural DNAs the decay profile is exceedingly complex and, in most instances, requires three successive time-constants for its full description. The proportion of the total decay represented by the slowest time-constant correlates reasonably well with the equilibrium binding constant of a given antibiotic for different species of DNA, and among different antibiotics a correlation between the magnitude of the slowest time-constant and the measured antimicrobial activity can be discerned. We believe that this slowest component of the dissociation profile corresponds to release of antibiotic from its preferred (i.e. tightest-binding) sites in natural DNA. In calf thymus DNA we estimate the frequency of these sites as about 2.4% of the total available sites, corresponding to 3 or 4 of the 136 possible four-base-pair binding sites.

The formation of quinoxaline antibiotic-DNA complexes has been studied by stopped-flow kinetic measurements. The technique is limited by the very low solubility of these antibiotics, but we have been able to measure the rate of the association reactions for both echinomycin and TANDEM by following the absorbance changes at 320 nm associated with the intercalation of the chromophores. The reaction profiles are complex requiring more than one exponential for their complete description. We have attempted to investigate the mechanisms underlying the kinetic processes by measuring the DNA concentration dependence of the various time constants.

For TANDEM, a synthetic analogue of triostin A, the reaction profile with calf thymus DNA is completely described by two exponentials, separated by a factor of about 5, both of which vary with the DNA concentration. This can be interpreted in terms of a two step reaction mechanism in which the initial fast bimolecular reaction (k_2 = 5 x 10^4 M^{-1} s^{-1}) is followed by a slower reversible rearrangement.

For echinomycin and calf thymus DNA that part of the association reaction occurring during the time scale of the stopped flow apparatus (1-2s) is adequately described by a single exponential which varies linearly with the DNA concentration, as predicted for a simple bimolecular association reaction (k_2 = 10^5 M^{-1} s^{-1}). However, this is followed by a much slower reaction which can be observed spectrophotometrically and occurs about 500 times more slowly.

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EFFECTS OF DAZOXIBEN, A THROMBOXANE SYNTHETASE INHIBITOR, ON THE AIRWAYS AND PULMONARY VASCULAR RESPONSE TO E. COLI ENDOTOXIN

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The intravenous administration of E. coli endotoxin to the anaesthetised cat causes an increase in both mean pulmonary artery pressure (MPAP) and airways resistance (R_L) coupled with a fall in dynamic lung compliance (C_L) (Coker et al, 1981). Associated with these changes are increases in plasma concentrations of prostaglandin $F_{2\alpha}$ and the stable metabolites of thromboxane A_2 and prostacyclin (Coker et al, 1983). The thromboxane synthetase inhibitor dazoxiben has recently been reported to modify the E.coli-induced pulmonary hypertension suggesting the involvement of thromboxane A_2 (Ball et al, 1982). The present study was designed to compare the effects of dazoxiben on both the pulmonary vascular and respiratory responses to E.coli endotoxin.

Cats were anaesthetised with sodium pentobarbitone and prepared for measurement of haemodynamic (Parratt, 1973) and lung (Houston & Rodger, 1974) parameters. Dazoxiben (UK 37,248-01; 5 mg/kg or 10 mg/kg i.v.) or saline was administered 15 min prior to E.coli (2 mg/kg i.v.).

The results are summarised in Table 1.

Table 1. Effects of dazoxiben on E.coli-induced changes in MPAP, R_L and C_L (Mean ± S.E. mean; n = 5-7)

	Parameter	Pre-endotoxin	2 min post-endotoxin
Control	MPAP (mmHg)	17 ± 1	36 ± 4 ##
	R_L (% increase)		171 ± 29
	C_L (% decrease)		47 ± 4
After dazoxiben (5 mg/kg)			
	MPAP (mmHg)	15 ± 1	19 ± 1 **
	R_L (% increase)		63 ± 24 *
	C_L (% decrease)		26 ± 8 *
After dazoxiben (10 mg/kg)			
	MPAP (mmHg)	16 ± 3	16 ± 1 **
	R_L (% increase)		149 ± 54
	C_L (% decrease)		34 ± 5 *

* $p < 0.05$) Compared to corresponding 2 min value of control
** $p < 0.01$) group (Mann-Whitney U-test)
\#\# $p < 0.01$ Compared to pre-endotoxin value (Wilcoxon matched pairs test)

Dazoxiben alone did not modify resting MPAP, R_L or C_L. It is evident that while dazoxiben attenuated the pulmonary hypertension and fall in C_L at both doses, it did not modify the increase in R_L at the higher dose. Such an observation illustrates that increases in R_L can occur independently of changes in pulmonary artery pressure. The mechanism by which dazoxiben brings about this effect is not clear. Dazoxiben has been reported to cause a rediversion of endoperoxide metabolism leading to increased concentrations of other prostanoids (Randall et al, 1981). It is possible that after the high dose of dazoxiben it is one, or more, of these products that is responsible for the selective effect on R_L. To help resolve this question, studies involving assay of plasma concentrations of the different prostanoids are being undertaken.

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THE EFFECT OF THROMBOXANE SYNTHETASE INHIBITION UPON COLLAGEN- AND PGH_2-INDUCED PLATELET AGGREGATION IN HUMAN WHOLE BLOOD

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There is some controversy as to whether inhibition of platelet thromboxane (Tx) synthetase can lead to a diversion of prostaglandin (PG) endoperoxides to anti-aggregatory prostanoids or whether the accumulated endoperoxides would produce aggregation themselves (see Bertele et al., 1981; Hornby & Skidmore, 1982). Platelet rich plasma is an unsuitable medium in which to examine these questions since it contains no source of prostacyclin synthetase whereas whole blood does (Blackwell et al., 1978). We have therefore studied platelet aggregation induced by collagen and PGH_2 in the absence and presence of Tx-synthetase inhibition in human whole blood using a recently described method (Lumley & Humphrey, 1981).

In human whole blood collagen-induced aggregation (0.1 - 10.0 µg/ml) was accompanied by the production of TxA_2 (measured as TxB_2, see Hornby & Skidmore, 1982). Aspirin (2×10^{-3} mol/l) completely abolished collagen-induced TxA_2-production but only partially inhibited aggregation (3.1-fold parallel rightward displacement of the collagen aggregation concentration-effect curve) thus revealing the now well reported non-prostanoid mediated component in collagen-induced aggregation (see Lumley & Humphrey, 1981).

The Tx-synthetase inhibitors, N-(1-carboxyheptyl) imidazole (CHI) (Yoshimoto et al, 1978) and 2-isopropyl-3 (1-imidazolyl methyl) indole (UK34787, Cross et al, 1981), in the concentration range 3×10^{-8} - 3×10^{-4} mol/l produced a concentration-related inhibition of TxA_2 production and aggregation to a single concentration of collagen (4.0 µg/ml). Maximum effects against TxA_2 production and aggregation occurred at 3×10^{-4} mol/l with both compounds. Compared with the effect of aspirin (= 100%) (2×10^{-3} mol/l) CHI and UK34787 produced maximally a 97.0 $\pm$ 3.3% and 95.8 $\pm$ 1.8% inhibition of TxA_2 production and a 61.3 $\pm$ 6.6% and 98.5 $\pm$ 20.5% inhibition of aggregation respectively (mean $\pm$ s.e. mean, n = 4). The effect of CHI upon aggregation was significantly less than the effect of aspirin ($P < 0.01$) whereas the effect of UK34787 was not.

In the presence of aspirin (2×10^{-3} mol/l) PGH_2 (0.1 - 1.0 µg/ml) caused platelet aggregation with little production of TxA_2. Under the same conditions CHI (3×10^{-4} mol/l) was without effect on aggregation induced by PGH_2, the TxA_2-mimetic U-46619 or ADP. However, UK34787 (3×10^{-4} mol/l) produced slight inhibition of aggregation to both PGH_2 and U-46619 (2.9 fold and 2.6 fold rightward displacement of the PGH_2 and U-46619 concentration-effect curves respectively). Since UK34787 had no effect on aggregation-induced by ADP it would appear to have some Tx-receptor blocking activity.

The present data suggests that when Tx-synthetase is inhibited, endogenously produced PGH_2 can contribute to the aggregation of human platelets in whole blood. This conclusion was reached using the Tx-synthetase inhibitor CHI; the use of UK34787 is complicated by weak Tx-receptor blocking activity. Furthermore, there was no evidence that Tx-synthetase inhibition resulted in conversion of exogenous PGH_2 to functionally significant amounts of anti-aggregatory prostanoids.

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THE ANTI-INFLAMMATORY ACTION OF DRUGS THAT RAISE ADENOSINE 3',5' CYCLIC MONOPHOSPHATE AND PUTRESCINE LEVELS IN VIVO

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Recently in a search for endogenous anti-inflammatory factors we (Bird, Mohd Hidir and Lewis, 1983) reported that putrescine was a potent anti-inflammatory agent against carrageenan induced oedema and adjuvant arthritis in the rat. Putrescine is produced *in vivo* by the decarboxylation of ornithine by ornithine decarboxylase (ODC). This enzyme is stimulated by adenosine 3',5' cyclic monophosphate (cyclic AMP) which itself is anti-inflammatory (Zurier, Hoffstein & Weissmann, 1973). Theophylline raises cyclic AMP levels by inhibiting phosphodiesterase and also raises liver putrescine levels. It is also anti-inflammatory against the two animal models mentioned above (Bird *et al*, 1983). In this communication we report that isoproterenol and salbutamol raise plasma cyclic AMP levels and putrescine levels in the rat. The drugs were suspended in saline and administered i.p. Cyclic AMP was determined by radioimmunoassay (Amersham International Ltd., Amersham, Bucks.) and putrescine by the dansylation method of Seiler & Asker, (1971). As shown in the table both drugs raised both cyclic AMP and putrescine levels but the cyclic AMP levels with isoproterenol treatment returned to normal after 3.5 h. The drugs were examined for anti-inflammatory activity against carrageenan induced inflammation in that rat. The drugs were administered i.p. 0.5 h before the carrageenan was injected into the foot pad (Winter, Risley & Russ, 1962). The oedema was measured plethysmographically (Bird *et al*, 1983). Isoproterenol (dose 4 mg/kg) suppressed the oedema 3 h after the administration of carrageenan by 52% and at a dose of 8 mg/kg by 89%. At 6 h when the cyclic AMP levels were normal the anti-inflammatory effect of the drug had been abolished. Salbutamol (dose 10 mg/kg) produced a 54% inhibition of oedema at 5 h. In a separate experiment when isoproterenol was administered at 8 mg/kg to rats a 91% inhibition of carrageenan induced oedema was found at 3 h. This was reduced to 79% ($p < 0.05$) at 3 h when 200 mg/kg of difluoromethylornithine (a specific ODC inhibitor) was administered i.p. at the same time as the isoproterenol. It would appear that cyclic AMP and putrescine have a synergistic anti-inflammatory action. Combinations of theophylline with prostaglandin E_1 and aminophylline and salbutamol (Bonta, Parnham & Van Vliet, 1978; Seo & Saeki, 1980) have a synergistic anti-inflammatory action due to their ability to raise cyclic AMP levels by different mechanisms.

Treatment	Plasma cyclic AMP p.mole/ml		Liver putrescine µg/mg dry wt.	
	Time after dosing h.			
	0.5	3.5	5.5	3.5
None (saline)	14.5 ± 1.1			3.9 ± 0.15
isoproterenol (8mg/kg)	25.5 ± 0.3	47.5 ± 0.3	12.0 ± 0.9	14.9 ± 0.5
salbutamol (15mg/kg)	36.0 ± 2.0			19.2 ± 0.7

$P \leq 0.05$ v control. Four animals in each group.

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THE EFFECT OF THE NONSTEROIDAL ANTI-INFLAMMATORY AGENTS, DIFLUNISAL AND FLUFENAMIC ACID ON MICROSOMAL NADPH OXIDATION

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As part of an investigation into the effect of nonsteroidal anti-inflammatory agents on organelle function, we have shown that diflunisal affects the oxidation of respiratory substrates in isolated mitochondria (McDougall et al, 1981). The present communication describes the results of a study designed to determine the action of diflunisal and flufenamic acid on oxidation reactions in the microsomal fraction of rat liver. The reaction chosen for the study was the flavoprotein, NADPH cytochrome c reductase (E.C. 1.6.2.3.). This enzyme is a key component of the mixed function oxidase system, and mediates the transfer of electrons from NADPH to the terminal oxidase, cytochrome P-450.

The activity of NADPH cytochrome c reductase was determined by following the reduction of cytochrome c at 550 nm, using a Pye Unicam SP1800 spectrophotometer (Masters et al, 1967). The experimental cuvette contained 0.1 M phosphate buffer, pH 7.4, 53.3 μM cytochrome c and rat liver microsomes (2 mg protein) at 37°C. The reaction was initiated by the addition of NADPH (16.7 μM) to the cuvette, to give a final volume of 3 ml. The anti-inflammatory agents were added 2 min before the NADPH.

Under control conditions, NADPH cytochrome c reductase activity was found to be 110 ± 21 nmol cytochrome c reduced/min/mg microsomal protein (n = 10). This activity was reduced to 16 ± 4 nmol/min/mg protein (n =5) when diflunisal (0.5 mM) was included in the reaction medium. Full inhibition curves for diflunisal (0.1 - 1 mM) gave an IC50 value of 0.3 ± 0.02 mM (n = 5). Dixon plots revealed that the inhibition was competitive in nature (Ki = 0.095 ± 0.015 mM; n = 5).

Similar results were obtained with flufenamic acid (IC50 = 0.2 ± 0.04 mM; n = 5), which was also found to be a competitive inhibitor of cytochrome c reduction (Ki = 0.185 ± 0.035 mM; n = 5).

Acetylsalicylic acid failed to affect the activity of NADPH cytochrome c reductase, even at concentrations up to 2 mM.

Rat liver microsomal NADPH-linked oxidations are susceptible to attack by the nonsteroidal anti-inflammatory agents, diflunisal and flufenamic acid. It should be pointed out, however, that the concentrations required to inhibit the reaction are probably too high to contribute to the anti-inflammatory action of the compounds.

P. McD. was an S.E.R.C. student.

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MODULATION OF CELL ACCUMULATION AND INFLAMMATORY ENZYME RELEASE IN IMPLANTED SPONGES

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In various inflammatory lesions, lysosomal enzyme release has been reported to be inhibited (Boyle & Mangan, 1982), potentiated (Blackham & Woods, 1979), or unaffected (Ammendola et al., 1975) by administration of anti-inflammatory agents. In the present study the effect of various agents was investigated on cell accumulation and lysosomal enzyme release in a rat model. Male CFHB rats (220-250g.) were implanted s.c. with saline-soaked polyester sponges, under pentobarbitone anaesthesia. After 24h. rats were killed in ether and sponge exudates were assayed for inflammatory cells and β-glucuronidase activity. Rats were dosed p.o. immediately prior to sponge implantation, except for cyclophosphamide which was administered to deplete circulating leukocytes based on the method of Van Arman, 1974. Results are shown in Table 1.

Table 1 Modulation of cell accumulation and β-glucuronidase release

Treatment		Percentage Change	
		Cells/ml. exudate	β-glucuronidase/ml. exudate
Indomethacin	1mg/kg	-26	+33*
	10	-56*	+49*
Tiaprofenic Acid	10	-7	+45*
	50	-49*	+63*
Phenylbutazone	20	+17	+2
	100	-38*	+32*
Prednisolone	1	-16	-1
	10	-41*	+7
Dexamethasone	0.01	-22	-2
	0.1	-61*	+1
Cyclophosphamide	10x4	-66*	-16

*$p < 0.05$ (students 't' test)

All agents inhibited cellular accumulation in agreement with published data (Walker et al., 1976; Higgs et al., 1980; Boyle & Mangan 1982). However, β-glucuronidase activity was potentiated by all non-steroidal agents, whereas the steroids had no effect. Leukocyte depletion with cyclophosphamide had little effect on β-glucuronidase activity suggesting that the inflamed tissue is the major source at this time.

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ELEVATION OF CYTOPLASMIC CALCIUM CONCENTRATION IN HUMAN PLATELETS BY PLATELET-ACTIVATING FACTOR

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Responses to platelet-activating factor (PAF) were examined in human platelets loaded with the fluorescent Ca^{2+} indicator, quin2 (Rink et al, 1982). Platelets were separated from acid citrate dextrose PRP by centrifugation at 350 g for 15 minutes and resuspended in Hepes-buffered saline. With 1 mM external Ca^{2+}, 20 ng/ml PAF (a near-maximal effective dose) evoked a rapid approximately 10-fold rise in $[Ca^{2+}]_i$ (cytoplasmic free Ca^{2+}) to between 0.6 and 1.0 μM. $[Ca^{2+}]_i$ then declined towards resting levels with a half-time of about 1 minute. Further additions of PAF were without effect consistent with rapid receptor desensitisation (Henson, 1976). Parallel studies showed that in 1 mM external Ca^{2+}, 20 ng/ml PAF produced shape change, substantial aggregation and 50% ATP secretion (cf. maximal thrombin-induced release).

In Ca^{2+}-free medium, with 0.5 mM EGTA, PAF could elicit only a small transient rise in $[Ca^{2+}]_i$ (to 150-250 nM) presumably by partial discharge of an internal pool since subsequent addition of ionomycin produced a larger rise in $[Ca^{2+}]_i$. These results show that the major source of Ca^{2+} under normal conditions is by PAF-triggered influx across the plasma membrane. Although, in Ca^{2+}-free medium PAF could not nearly raise $[Ca^{2+}]_i$ to the Ca^{2+} threshold for cell activation, as previously established with Ca^{2+} ionophores, PAF could still produce shape-change, 15% secretion and a hint of aggregation. It appears therefore, that PAF can activate the putative Ca^{2+}-independent route, previously postulated to explain the response to thrombin in Ca^{2+}-free media (Rink et al, 1982).

Large doses of acetyl salicylic acid (100 μM for > 10 minutes) or indomethacin (10 μM) had little effect on the $[Ca^{2+}]_i$ response and only partly inhibited secretion and aggregation. Shape change was not affected. These results suggest that endoperoxides do not play a major role in mediating the effects of PAF. The alleged Ca^{2+} antagonist, verapamil (40 ng/ml), was without effect on the $[Ca^{2+}]_i$ transient, though it somewhat reduced the secretion and aggregation in Ca^{2+}-free conditions. These data may imply that however verapamil acts in platelets has little to do with Ca^{2+} flux.

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CONVERSION OF PROSTACYCLIN TO 6-KETO PROSTAGLANDIN E_1 BY RAT AND RABBIT PLATELETS

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6 keto prostaglandin E_1 (6 keto PGE_1) is an enzymatically formed metabolite of prostacyclin (PGI_2) which retains considerable biological activity (Quilley et al, 1980). Synthesis of 6 keto PGE_1 catalysed by the enzyme prostaglandin 9 hydroxy dehydrogenase (PG 9HDH) occurs in rabbit liver, rabbit, pig and human kidney, and human platelets. A recent study from this laboratory has also demonstrated the spontaneous release of 6 keto PGE_1 from human platelets in the absence of added PGI_2 (Lofts & Moore, 1983). The purpose of the present investigation was to study conversion of PGI_2 to 6 keto PGE_1 and spontaneous release of 6 keto PGE_1 from rat and rabbit platelets.

Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared by differential centrifugation of citrated blood. PRP and PPP were incubated with and without PGI_2 (0.1 - 1.0µg/ml) at 37°C and aliquots removed at timed intervals for bioassay on the rat stomach strip (RSS) and against ADP induced human platelet aggregation as described previously (Hoult et al, 1981). In other experiments 0.2 ml aliquots of PRP were incubated and extracted after acidification to pH 3.4 with 2 vol. ethyl acetate and subsequently bioassayed against ADP induced human platelet aggregation. In some cases the extracts were subjected to thin layer chromatography in solvent F6 (ethyl acetate:acetone:acetic acid, 90:10:1 v/v). After development for 40 min the chromatogram was cut into 1 cm strips from the origin, eluted with 2ml methanol, and bioassayed against ADP induced human platelet aggregation.

Incubation of PGI_2 (1µg/ml) with rat or rabbit PRP resulted in increased spasmogenic activity on the RSS (max. 2.9± 0.4µg/ml PGI_2 equivs., n=6 and 2.5±0.5 µg/ml PGI_2 equivs., n=6 at 7.5 min for rat and rabbit respectively). In PPP spasmogenic activity declined rapidly (0.35± 0.07µg/ml PGI_2 equivs., n=6 and 0.28±0.05µg/ml PGI_2 equivs., n=6 at 30 min for rat and rabbit respectively). Anti aggregatory activity of PGI_2 incubated with PRP or PPP was prolonged compared with its stability in phosphate buffer (pH 7.4). In rat PPP the decline in spasmogenic and anti-aggregatory activity was similar wheras in rabbit PPP the anti-aggregatory activity persisted for longer than the spasmogenic activity.

These results are consistent with formation of 6 keto PGE_1 by platelets. It is unlikely that 6 keto PGE_1 formed by platelets contributes significantly to the prolonged anti-aggregatory activity since this was similar in PRP and PPP and especially since 6 keto PGE_1 is some 10-20 times less potent than PGI_2 as an inhibitor of human platelet aggregation.

After extraction of PRP anti-aggregatory activity was detected and reached a peak after 15-30 min. After t.l.c. anti-aggregatory activity co-chromatographed with authentic 6 keto PGE_1. After 30 min incubations contained 48.9± 10.9ng/ml 6 keto PGE_1 equivs, n=12 and 45.1± 17.9ng/ml 6 keto PGE_1 equivs., n=8 for rat and rabbit respectively. Small amounts of anti-aggregatory activity were present in the 6 keto PGE_1 zone after t.l.c. of incubations containing rabbit (but not rat) PRP without added PGI_2.

These results suggest that rat and rabbit platelets convert PGI_2 to 6 keto PGE_1.

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EFFECTS OF INDOMETHACIN, SALICYLIC ACID OR BW775C ON 6-KETO-$PGF_{1\alpha}$ PRODUCTION FROM RAT GASTRIC TISSUE EX VIVO AND IN VITRO

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The dual lipoxygenase/cyclo-oxygenase inhibitor BW755C in doses which inhibit prostaglandin production in inflammatory exudates was reported not to inhibit prostacyclin generation or to induce damage in the rat gastric mucosa (Whittle et al, 1980). Moreover, BW755C (0.5-80μM) increased mucosal prostacyclin production in vitro (Boughton-Smith & Whittle, 1981). Using a specific radioimmunoassay for 6-keto-$PGF_{1\alpha}$, the stable breakdown product of prostacyclin, we have investigated the effects of BW755C, salicylic acid (sal.) and indomethacin (ind.) on rat gastric corpus tissue both ex vivo and in vitro. The experimental protocol is described below.

Male Wistar rats (200-250g), starved for 18h, were dosed orally with one of the following drugs: ind. (1-5mg Kg^{-1}), sal. (150mg Kg^{-1}), BW755C (1-100mg Kg^{-1}) or vehicle (0.5% methyl cellulose). After 2h the animals were sacrificed and their stomachs removed, opened along the greater curvature, washed in buffer (Tris 50mM, pH 7.4) and pinned out. Gastric corpus sections (area - $8mm^2$) were removed and placed in 200μl of buffer, vortexed (5s) and incubated (5min at 37°C). Aliquots were assessed for the presence of 6-keto-$PGF_{1\alpha}$ using radioimmunoassay (New England Nuclear). The above drugs were also tested in vitro using the same protocol except that sections were incubated in either 1ml buffer or buffer plus drug. In preliminary experiments drugs were tested on the mucosa alone in order to eliminate any effects on smooth muscle cyclo-oxygenase. Results, expressed as ng 6-keto-$PGF_{1\alpha}$/section, were analysed using Student's paired or unpaired 't' tests.

BW755C (1-100mg Kg^{-1}) reduced the amount of 6-keto-$PGF_{1\alpha}$ ex vivo, the maximum effect (from 14.8 ± 1.0 to 9.4 ± 0.4ng, $p<0.01$) being observed with 5mg Kg^{-1}. Ind., 5mg Kg, also reduced the amount significantly (from 12.2 ± 1.5 to 2.2 ± 0.2ng, $p<0.001$) whereas sal. 150mg Kg^{-1} failed to alter it. Results in vitro showed that sal. (362μM) or BW755C (2μM) increased the amount of 6-keto-$PGF_{1\alpha}$ (from 26.0 ± 2.0 to 36.5 ± 3.5ng and 41.5 ± 7.0ng respectively; both $p<0.05$) whereas BW755C (40μM) decreased it (from 26.0 ± 2.0 to 18.0 ± 2.0ng; $p<0.02$). When the mucosa alone was subjected to ind. (2.8μM) or BW755C (40μM) both compounds reduced the amount (from 4.0 ± 0.5 to 1.3 ± 0.3ng and 2.10 ± 0.3ng respectively, both $p<0.02$). In contrast, BW755C (2μM) increased it (from 4.0 ± 0.5 to 8.1 ± 1.9ng, $p<0.05$).

The present results demonstrate that gastric corpus tissue can generate prostacyclin ex vivo and in vitro, the amounts of which are significantly reduced by ind. Sal. failed to reduce the amount ex vivo whereas BW755C inhibited it at doses reported not to affect mucosal prostacyclin levels (Whittle et al, 1980; Peskar et al, 1982). In vitro, BW755C (2μM) and sal. (362μM) increased the amount of 6-keto-$PGF_{1\alpha}$; however BW755C (40μM) inhibited it which contrasts with previous findings (Boughton-Smith & Whittle, 1981). In experiments using mucosa alone the results clearly show that, as with combined tissue, BW755C produced qualitatively similar effects.

In conclusion, BW755C (2μM) stimulated prostacyclin production as previously reported (Boughton-Smith & Whittle, 1981) whereas the higher concentration (40μM) was inhibitory in the present experimental system.

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