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PHARMACEUTICAL INNOVATION IN THE UK (1964-1980)

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The process of research and development in the pharmaceutical industry, leading to the creation of new chemical entities (NCE's), represents the basis from which therapeutic advances can be made. By determining trends in the number of NCE's investigated and whether they progress to the market, it may be possible to identify some of the potential problems in the process of new drug development. Although innovation has been assessed in a variety of ways, the decision of a company to take an NCE into man for the first time for therapeutic purposes is regarded as the most appropriate definition and was therefore used in this study. An NCE was defined as a new chemical or biological compound, but excluded new salts and esters unless they conferred some major therapeutic advantage.

Confidential data were obtained from the research-based pharmaceutical companies in the UK (both foreign and UK owned) on all the NCE's which they had first taken into man during the period 1964 to 1980. The data collected included details of the origin of the compound and its passage through the various stages of research and development eg. biological testing, human pharmacology and clinical evaluation, to the present time, or its withdrawal from further research. This yielded a total database of 690 NCE's and of these 41% were originated by the UK company itself, 37% came from the parent or overseas subsidiary and 12% were licenced from other companies. The UK ranked first on the basis of the number of NCE's innovated (41%), whereas the American companies were responsible for marketing the largest number of NCE's in the UK during the period (42%).

The main therapeutic areas represented by the 690 NCE's were the CNS drugs (26%), anti-infectives (23%) and cardiovascular system drugs (17%). To date 47% of the NCE's have been marketed in the UK (of which 16% were subsequently withdrawn) and research has terminated for 37%. The major reasons for termination of research were inappropriate human pharmacokinetics (26% of those terminated), lack of clinical efficacy (21%), adverse reactions in man (12%) and toxicological effects in animals (11%). At the end of the period under investigation 16% of the NCE's were still in development. These data show an attrition rate of NCE's innovated to those marketed of approximately 2:1.

Further analyses, including data on "drug life", development times, (and their component phases) and changes in the therapeutic areas investigated are under way and will allow an examination of the trends in innovation over a period during which important regulatory changes have taken place.

EFFECT OF ANTIOXIDANTS ON ENDOTOXIN SHOCK IN CONSCIOUS, UNRESTRAINED RATS

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It has been proposed that oxygen centred free radicals (e.g. O_2^-, HO·) can act as toxic agents and may be involved in the pathophysiology of inflammation (Kuehl et al 1979) and of ischaemia (Hess et al.1983). They are formed during the metabolism of arachidonic acid and on activation of phagocytic cells, both of which occur in endotoxin shock. We have studied the effects of two antioxidants, reduced glutathione (GSH) and α-tocopherol, on the metabolic and cardiovascular effects of E.coli endotoxin infusion (41.7 μg kg^{-1} min^{-1} for 4h) in conscious unrestrained rats. The rats were prepared 48h prior to infusion by implantation of catheters in the right external jugular vein (for infusions) and the aortic arch via the right common carotid artery (for blood sampling and arterial pressure recording).

Reduced glutathione (400 mg kg^{-1} i.v.) was ineffective in this model but α-tocopherol (100 mg kg^{-1} day^{-1} for 3 days prior to the experiment and sufficient to elevate blood levels from 1.4±0.2 to 4.0±0.5 μg ml^{-1} ; $P<0.05$) significantly reduced mortality at 72h (mortality in controls = 100%; mortality in α-tocopherol treated rats = 46%; $P<0.05$). The hypotension and tachycardia produced by endotoxin were not significantly modified by α-tocopherol but there was an attenuation of the initial hyperglycaemia, the increase in the plasma lactate concentration and the endotoxin -induced decrease in blood α-tocopherol level (<0.2 μg ml^{-1} in the controls; 2.5±0.7μg ml^{-1}in the treated group).

Kuehl et al (1979) reported the antioxidant MK447 to greatly elevate prostanoid production in vitro and accordingly we studied the effect of α-tocopherol on the plasma concentrations of thromboxane B_2 (TXB_2) and 6-keto $PGF_{1\alpha}$, the stable metabolites of thromboxane A_2 and prostacyclin, respectively. α-tocopherol pretreatment significantly increased the endotoxin-induced elevation (at 4h post-endotoxin) of plasma TXB_2 (control endotoxin 333±87 pg ml^{-1}; endotoxin with α-tocopherol 1410±276 pg ml^{-1} $P<0.05$ and 6-keto $PGF_{1\alpha}$ (control endotoxin, 1506±333 pg ml^{-1} ; endotoxin + α-tocopherol, 3697±438 pg ml^{-1}; $P<0.05$). In contrast, GSH did not modify endotoxin-induced increase in the plasma concentrations of either TXB_2or 6-keto $PGF_{1\alpha}$.

In view of the possible detrimental effects of elevated concentrations of thromboxane and other prostanoids, an attempt was made to improve survival further by additional treatment of α-tocopherol treated rats with BW755C (50 mg kg^{-1} p.o. 1h before endotoxin). Although this treatment suppressed completely the endotoxin-induced elevation in TXB_2 and6-keto $PGF_{1\alpha}$ concentrations, delayed endotoxin-induced hypotension and reduced the tachycardia produced by endotoxin survival was not improved further (endotoxin controls, 100% mortality; endotoxin + α-tocopherol, 46% mortality; endotoxin + α-tocopherol + BW755C 55% mortality). The beneficial effects of α-tocopherol may be related to the removal of oxygen centred free radicals although this remains to be established, as does the explanation for the failure of GSH, another antioxidant, to improve survival.

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THEOPHYLLINE DISPOSITION IN RATS WITH ADJUVANT-INDUCED ARTHRITIS

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High hepatic extraction drugs, for example propranolol, have markedly elevated plasma concentrations in inflammatory disease (Schneider et al, 1981, Bishop et al, 1981). The increase in plasma concentrations has been attributed to increased binding to α_1-acid glycoprotein, rather than inhibition of metabolism (Barber et al, 1983). However, hepatic cytochrome P_{450} and associated enzyme activities are significantly decreased in microsomal preparations from adjuvant-induced rats, a factor which may be important in determining the clearance of low hepatic extraction drugs in adjuvant-induced arthritis. Theophylline was chosen as a model drug for this study, as it is metabolised in the liver by more than one form of cytochrome P_{450}(Grygiel et al, 1980). The aim of this study was to examine the effect of adjuvant-induced arthritis on the pharmacokinetics of a low hepatic extraction drug.

Male Sprague-Dawley rats (160-180g) were injected with 0.1 ml *M. tuberculosis* (heat-killed strains PN, DT & C) suspended in liquid paraffin (3 mg ml^{-1}) into the right hind foot pad. Control rats received vehicle alone. The rats were used approximately 15-20 days later, and were deemed arthritic if their erythrocyte sedimentation rate was greater than 2 mm in the first hour. The rats were anaesthetised with urethane and the carotid artery cannulated for the removal of blood samples at various time intervals. Theophylline (5 mg kg^{-1}) was infused via the jugular vein, or to simulate oral administration, via a cannulated side branch of the hepatic portal vein. Blood samples were analysed for theophylline and its metabolites using a gradient HPLC system with u.v. detection.

When theophylline was administered intravenously, there was no significant difference in the area under the blood concentration time curve $(AUC)_0^{210}$ for the arthritic (581 $\pm$ 115 $\mu g\ ml^{-1}$ min) compared with control (673 $\pm$ 132 $\mu g\ ml^{-1}$ min) rats, nor was there a difference in the blood concentrations of the two metabolites detected (1 methyl uric acid (1MU) and 1,3 dimethyl uric acid (DMU)). After oral administration of theophylline, there was again no significant difference in the AUC_0^{90} of the arthritic rats (261 $\pm$ 62 $\mu g\ ml^{-1}$ min) compared with controls (284 $\pm$ 71 $\mu g\ ml^{-1}$ min) and the concentration of 1MU and DMU was similar for the two groups of rats.

These results show that irrespective of the route of administration, total theophylline blood concentrations are not altered in adjuvant-induced arthritis. Since the plasma protein binding of theophylline is relatively low, changes in binding are unlikely to be important in determining the AUC of theophylline. This suggests that at a dose of 5 mg kg^{-1}, the metabolism of theophylline is not inhibited in adjuvant-induced arthritis.

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EDTA ENTERS ISOLATED HEPATOCYTES AND PROTECTS THEM AGAINST PARACETAMOL INJURY

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In chemical injury to cells there is a time of contact and a time of cell injury and death. These times are often separated by a latent period of several hours during which the mechanisms leading to injury come into action.

Isolated hepatocytes from phenobarbitone treated rats, exposed to 10mM paracetamol for one hour and then resuspended in paracetamol-free medium, show signs of injury and cell death from about two hours onwards. Cell death can be measured by leakage of soluble enzymes, entry of trypan blue or loss of potassium.

Addition of 4mM CaEDTA prevents or greatly reduces development of cell injury when added at the start of incubation or up to two hours later without altering the covalent binding of ^{14}C-paracetamol (Beales et al., 1984). The CaEDTA could be acting at the surface or in the cytoplasmic region of the cells. Isolated cells were incubated with tritiated water (T_2O) and [^{14}C-methoxy] inulin or ^{14}C-labelled EDTA and spun through oil to form a pellet. Total water, extracellular space and EDTA penetration could then be calculated. EDTA does not enter cells in vivo (Klaassen, 1980) nor do we find it to penetrate red blood cells or liver cells in slices. However we find that freshly isolated hepatocytes are readily penetrated by EDTA although they exclude trypan blue and inulin. It seems likely that the protective effect is due to buffering of the cytoplasmic free calcium concentration by EDTA.

PENETRATION OF ISOLATED HEPATOCYTES BY 4mM ^{14}C-CaEDTA

Incubation time (mins)	T-0	T-10	T-30
Concentration of EDTA in intracellular space (mM).	0.82 ± 0.41	1.19 ± 0.19	1.80 ± 0.06

Results are expressed as mean $\pm$ 1SD (n=6 or more results)
T-0 was measured at 5-10 seconds before spinning of sample.

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IN VITRO INHIBITION OF CHONDROGENESIS IN CULTURED RAT EMBRYO LIMB CELLS BY THALIDOMIDE

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The mechanism of thalidomide teratogenicity is still poorly understood. This study examines the mechanism using an in vitro model of chondrogenesis in which rat embryo (13 day; 34-36 somite) limb cells were cultured at various cell densities in the presence or absence of thalidomide. The micromass technique (cells cultured in 20 ul aliquots - Flint, 1983) was employed. An inverse relationship between cell density and chondrogenesis was found. In thalidomide exposed cultures, the threshold cell density necessary for chondrogenesis was much higher than in control cultures (Fig. 1A). Inhibition of chondrogenesis was directly related to the concentration of thalidomide in the culture medium (Fig. 1B). Cytotoxicity was not found below 325 ug thalidomide/ml culture medium. A structurally related non-teratogenic compound, supidimide was inactive. Thalidomide did not inhibit growth or differentiation of neurons in cultures of rat embryo midbrain cells.

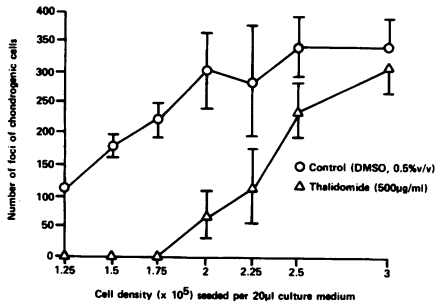

Fig. 1A. Cell density related changes in chondrogenesis in cultures with or without thalidomide.

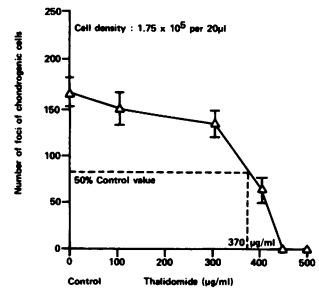

Fig. 1B Chondrogenesis in cultures at fixed cell density exposed to increasing concentrations of thalidomide.

Addition of an in vitro drug metabolising enzyme system during exposure to thalidomide did not influence the inhibition of chondrogenesis. Thalidomide was unstable in culture medium giving in the presence or absence of metabolising enzymes 3 major polar (hydrolysis) products; approximately 10% formation in 2 hours. Thalidomide or a product of its hydrolysis thus appears to be the active inhibitor of chondrogenesis.

Differentiating chondrocytes are uniquely sensitive to thalidomide mediated cytotoxicity because: 1. Cytotoxicity is found at inhibitory concentrations, 2. A reactive metabolite does not appear to be formed, 3. Inhibition of differentiation in another cell type is not found, 4. A structural non teratogenic analogue does not inhibit chondrogenesis. In human embryos, in vivo, thalidomide is most effective as a limb teratogen when exposure occurs as in the cultures, during the early stages of chondrogenesis. The in vitro system described is thus a good model of thalidomide teratogenicity.

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A COMPARISON OF THE ABILITY OF PYRETHROIDS AND THE VERATRUM ALKALOIDS TO INDUCE CUTANEOUS SENSORY EFFECTS

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Pyrethroids are known to induce cutaneous effects in man which are distinct from classical irritation and vascular responses. These effects are characterised by transient facial burning and tingling sensations (Tucker& Flannigan,1983). The aetiology of this cutaneous effect is related to the bursts of repetitive impulses which the pyrethroids can induce in sensory nerves (Vijverberg,et al. 1982). This increased sensory activity is considered to be a function of their ability to prolong the opening of the membrane sodium channel, producing a negative after potential and trains of impulses. The veratrum alkaloids which are structurally disimilar to the pyrethroids are known to affect the sodium channel in a similar manner. Therefore we have compared the ability of two structurally comparable synthetic pyrethroids (cypermethrin and deltamethrin) and a veratrum alkaloid(veratrine) to induce such cutaneous sensations. The guinea-pig flank test which has been developed to study this cutaneous phenomenon (Cagen et al.,1982) was used to quantitatively compare the agents.

Groups of six guinea-pigs were treated with pyrethroid or veratrine solution on one side of their clipped flanks and the vehicle on the other side. The number of times the animals responded by licking, rubbing or biting the test sites in a 5 minute observation period was recorded. The behavioral response of the guinea-pigs to a single application of a range of concentrations of cypermethrin, deltamethrin and veratrine was assessed at multiple time points during the day.

All three chemicals induced qualitatively similar licking and scratching behaviour. However, the effect of veratrine was less intense, required higher concentrations and was of much shorter duration than that observed with either of the pyrethroids . Veratrine had peak activity30 minutes following application to the flank but this effect had diminished by 2 hours. In contrast both pyrethroids induced peak activity 1 hour following application and the animals were still responding to the test site at 6 hours. Thus two very different types of chemical both of which alter membrane sodium channels also induce similar cutaneous effects and consequent behavioural responses in the guinea-pig flank test.

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TOXICITY OF ORALLY ADMINISTERED TRI-IODOTHRONINE (T_3) IN RATS

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Much is known about the neuropharmacological and endocrinological aspects of T_3 administration using relatively high doses of the hormone (see Atterwill, 1981; Atterwill et al 1983) but little is known of it's general toxicity profile, or the differential sex responses sometimes seen (Heal et al, 1984) e.g. the clonidine-hypoactivity response in neonatal rats. Since we have interests in the toxicological effects of orally administered thyromimetic compounds this information was also required for control purposes. Also since few thyroid hormone studies to date have utilized the oral route of administration the data obtained from the present work should supplement the information presently available following parenteral administration.

Groups of SK&F Wistar rats of each sex were treated orally with L-T_3 in a neutral suspension containing Methocel at 0.25, 2.5 and 25mg/kg B.W. for 14 days. Control groups received vehicle solutions. During treatment the clinical condition, body weight, food and water consumption and body temperature were recorded. Clinical pathology was also performed at mid and end of study. Rats were sacrificed 24 hours following the last dose and a full necropsy procedure carried out.

Treatment with T_3 at the high (H) and mid-dose (M) caused a sex and time-dependent increase in the number of decedents with twice as many males dying as females towards the end of test. All low dose (L) and control (C) animals survived until day 15. In male rats there was a substantial decrease in B.W. from day 4 particularly in the H and M groups with no real effect in food consumption. However, the females responded differently showing no gain in weight but no real loss until Day 11.

Due to the high mortality for H and M groups the end-of-test data refers mainly to the L dose group (0.25mg/kg). The clinical chemistry data reflected the changes expected from a hormone raising metabolic rate. Thus, blood glucose and albumin levels were reduced and the liver-'marker' enzymes: alanine amino-transferase (ALT), alkaline phosphatase (AKP) and aspartate aminotransferase (AST) were elevated. Urinalysis (elevated AKP levels) suggested that possible proximal brush border damage had occurred, particularly in females.

Serum samples taken at necropsy were analysed for free and total T_3 and T_4. Both free and total T_3 levels were markedly elevated as expected whereas T_4 levels were markedly reduced as a consequence of feedback mechanisms. In general, organ weights again highlighted the toxicological sex differences with kidney, heart and adrenal size being elevated (mainly females) and liver weights reduced (mainly males).

These findings provide information on the tolerance of rats to high doses of T_3 and have pointed to important sex differences in response to such treatment. Furthermore, the low dose data add to our knowledge of the wide range of T_3 effects in adult rats tolerating oral administration.

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THE REDISTRIBUTION OF DIGOXIN AFTER TREATMENT WITH DIGOXIN-SPECIFIC FAB FRAGMENTS IN GUINEA PIGS

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Digoxin-specific Fab fragments (DS-Fab) have been successfully used to reverse acute digoxin toxicity (Smith et al, 1982). These antibody fragments are presumed to 'remove' pharmacologically-active digoxin from the heart and other tissues by binding to the hapten and confining it to the plasma and extracellular fluid. In accord with this it has been shown that DS-Fab treatment effects very large increases in plasma digoxin concentration (Smith et al, 1976). However, there have been few attempts to measure the corresponding changes in tissue digoxin concentration following antibody treatment. The present study in guinea-pigs using approximately equimolar amounts of hapten to antibody was undertaken to examine more extensively the effect of DS-Fab on digoxin distribution.

Five pairs of female Dunkin-Hartley guinea-pigs (0.72-1.23 kg), matched for age and weight were anaesthetised with urethane (1.5 gkg^{-1} i.p.) and given ^{3}H-digoxin (22μCi, 10μgkg^{1} i.v.). After 2 h the experimental animals received DS-Fab (1.26$mgkg^{-1}$ i.v.) while controls received an equal volume of saline. The DSFab had a digoxin binding capacity of 8.6μgmg^{-1} and an affinity constant of 10^{9} $lmol^{-1}$. At 3 h the animals were killed and blood and tissue samples taken. Tissue and serum bound digoxin concentrations were determined according to Griffiths et al (1984) and Butler et al (1977) respectively. Results, which are given with percentage changes in parentheses, were analysed using Student's t-test.

Serum digoxin in DS-Fab-treated animals increased 15 fold, 98% of the drug being protein bound compared to 10% in controls. Digoxin concentrations also increased in the kidney (280%), adrenals (140%) and lung (95%), and were unaffected in the heart (ventricle), liver, spleen, brain, skeletal muscle, lymph nodes and fat.

Because of the high relative concentration of digoxin in the serum of antibody treated animals it seemed probable that the presence of serum/blood in the tissues would mask certain tissue distributional changes. To overcome this problem the likely amount of serum digoxin present in particular tissues was subtracted, the tissue serum volumes being assumed to be as reported by Everett et al (1956) for rats.

On applying this correction the antibody administration still appeared to increase kidney digoxin concentration (240%), but decreased it in the heart (50%), brain (85%), liver (90%) adrenals (100%) and lung (100%). The spleen and skeletal muscle digoxin levels were unaffected. No correction factor was available for lymph nodes or fat.

Although DS-Fab do appear to remove digoxin from certain tissues, including the myocardium, the "serum-corrected" results still do not fully relate to the effect of these antibody fragments in reversing digoxin toxicity, since they probably also reduce the effect of the drug by binding to it in the extracellular fluid. The increased kidney digoxin concentration may be due to the presence of large amounts of DS-Fab-bound digoxin in the glomerular filtrate. The reason for the lack of effect on spleen and skeletal muscle digoxin concentrations is unclear.

The authors wish to acknowledge the assistance in these studies of Dr. R. Fraser, Scottish Antibody Production Unit and Dr. M. Smith, Department of Pathology, Ninewells Hospital.

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THE EFFECTS OF DRUGS ON SEXUAL FUNCTION

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Drug induced sexual dysfunction has been sporadically reviewed (Barnes et al, 1979; Millar, 1979; Aldridge, 1982; Buffum, 1982) and is rarely included in teaching. This adverse effect of drugs deserves more attention than it has so far received. Patients are reticent in complaining about it and may even be unaware that any sexual dysfunction they experience may be caused by their medication.

The mechanisms controlling sexual function involve the central nervous system, autonomic nervous system, and local reflexes in the lumbar and sacral regions of the spinal cord. Drugs can affect these mechanisms and so alter:

libido (sexual interest)
genital arousal (erection in man, increased vaginal blood flow and lubrication in the woman)
orgasm in both men and women, or ejaculation in the man.

Many of the effects of drugs on sexual functioning are predictable when the controlling nervous mechanisms are considered. The most frequent pharmacological causes of sexual dysfunction in males are antihypertensive drugs, antidepressants, tranquillizers and ethanol (Comfort, 1983). When men were given the antihypertensive guanethidine 54% were unable to sustain an erection and 60% complained of failure to ejaculate (Bulpitt & Dollery, 1973). The antipsychotic thioridazine is notorious for its interference with ejaculation, the incidence ranging from 30% - 57%; inhibition of erection is less common but has a reported incidence of about 54% (Buffum, 1982).

Female sexual function is less well studied and documented and consequently less is reported on the effect of drugs on female than male sexual function; but there have been several reports of monoamine oxidase inhibitors affecting orgasm in women (Lesko et al, 1982; Moss 1983; Pohl, 1983).

'Adverse effects on sexual functioning are so often dismissed as totally unimportant in relation to the treatment being prescribed, as for example in hypertension' (Wheatley, 1983). But their importance cannot be overemphasized since once a patient has experienced sexual dysfunction and is unaware that it is drug induced, psychological impotence may follow. Furthermore, once a negative sexual response is established it is behaviourally difficult to overcome.

The effects of drugs on sexual function should be monitored in clinical trials. In discussing therapy with patients the sexual effects can be indicated and where the effect is reversible reassurance could be given. If long term therapy is being considered and there are no other alternatives, then ways of helping the patient deal with the sexual problem could be explored.

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COMPARATIVE BINDING STUDIES WITH [^{3}H]-METHYLTRIENOLONE AND [^{3}H]-MIBOLERONE, TWO SYNTHETIC LIGANDS FOR THE ANDROGEN RECEPTOR

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In previous biochemical studies on benign prostatic hyperplasia we have used the androgen receptor ligand, ^{3}H-methyltrienolone (R 1881) (Carilla et al., 1984). ^{3}H-mibolerone, a new synthetic androgen ligand, has recently become available. This ligand is reputed to be more specific than R 1881 for the androgen receptor, with little or no affinity for the progesterone receptor (Asselin et al., 1979 ; Delettre et al., 1980). We report here comparative binding studies with these two ligands on cytosolic androgen receptors of the rat ventral prostate.

Rats were castrated 18 h before sacrifice to reduce testosterone and dihydrotestosterone levels. Cytosol was obtained from the ventral lobe of the prostate by homogenisation and ultracentrifugation (1 h at 100 000 g). ^{3}H-R 1881 (90 Ci/mmol NEN), ^{3}H-mibolerone (80 Ci/mmol Amersham) and other drugs were dissolved in toluene/ethanol (9/1) for distribution into the assay tubes and the solvant evaporated under nitrogen. The residue was then dissolved in the cytosol by sonication. After 2 h incubation at 0°C dextran-coated charcoal was added to complex the free ^{3}H-ligand which was separated by filtration though GF/B glass-fibre filters. Non-specific binding was estimated in the presence of an excess of the non-radioactive ligand (1 μM).

Both ^{3}H-R 1881 and ^{3}H-mibolerone bound to prostate androgen receptors in a saturable manner giving linear Scatchard plots. The apparent dissociation constants (Kd) were respectively 1.6 $\pm$ 0.64 nM and 1.3 $\pm$ 0.62 nM and the Bmax values respectively 76.5 $\pm$ 9.8 fmol/mg protein and 33.3 $\pm$ 10.9 fmol/mg protein. Competition experiments, carried out at 5 nM ^{3}H-ligand, using cyproterone acetate, R 1881, mibolerone, progesterone and Permixon, a plant extract with anti-androgen activity (Carilla et al., 1984), suggest that both ligands label qualitatively similar sites. In the rat prostate which has very few progesterone receptors (Asselin, 1976), progesterone, as expected, inhibited the binding of both ligands with a similar, relatively low, potency.

Ligands	IC_{50}				
	Cyproterone Acetate	R 1881	Mibolerone	Permixon	Progesterone
^{3}H-R 1881 (5 nM)	13 nM	4.1 nM	0.98 nM	330 µg/ml	105 nM
^{3}H-mibolerone (5 nM)	32 nM	5.8 nM	1.1 nM	260 µg/ml	150 nM

Although both ligands appear to bind to qualitatively similar sites ^{3}H-mibolerone labels only half as many sites as ^{3}H-R 1881. Until this anomaly is clarified it is perhaps dangerous to consider the two ligands as equivalent.

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DNA CROSS LINKS IN SENSITIVE LEUKAEMIA CELLS EXPOSED TO CYCLOPHOSPHAMIDE ACTIVATED BY RAT HEPATOCYTES IN VITRO

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Cyclophosphamide (CP) is a widely used antineoplastic agent. It is activated by a P450 dependent oxidation process to form a number of alkylating metabolites of varying cytotoxicity. The clinical utility of the drug is limited by the evolution of resistant cell populations. Because the mechanism of resistance is uncertain, it is difficult to devise chemotherapeutic regimens to counter the development of resistance.

The cytotoxicity of activated CP towards proliferating cells has been demonstrated before by Wiebkin et al (1982). We have used a dual culture system employing isolated hepatocytes and a human chronic myeloid leukemia (CML) cell line (K562) to study the effects of activated CP at the cellular level.

Hepatocytes were isolated from rats preinduced with phenobarbitone and incubated at a density of 10^6 cells/ml. with 0.1 - 1mM CP. Following 1 hour incubation, the supernatant fluid contained less than 1% of the original CP when measured by gas chromatography, but had a high level of NBP detectable alkylating metabolites. Aliquots of this supernatant were added to suspensions cultures of exponentially growing K562 CML cells. Following a 1 hour exposure to the activated drug there was a dose dependent cytotoxic effect as measured by cell proliferation over the next 72 hours and by colony formation in soft agarose.

Following a similar 1 hour exposure to activated drug, cellular DNA was analysed for cross linking (interstrand and DNA-protein) by the alkaline elution technique (Kohn et al 1981). Dose dependent cross linkage is observed in cells exposed to activated cyclophosphamide. This technique enables us to investigate the biochemical basis of CP resistance in individual patients, and to address the question of whether resistance is a property of the activation system, drug entry into cells, DNA repair or some other mechanism.

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THE INTERACTION OF AMINE DEPLETING DRUGS WITH DOPAMINE RECEPTORS IN RAT BRAIN

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Monoamine depleting drugs (reserpine, tetrabenazine, oxypertine) are used to treat movement disorders and psychosis associated with overactivity of brain dopamine function. However, such compounds may also directly interact with brain dopamine receptors (Costall & Naylor, 1973; Nakahara et al, 1980; Login et al, 1982). We now compare the ability of amine depleting drugs and dopamine receptor antagonists (chlorpromazine, trifluoperazine) to interact with behavioural and biochemical indices of cerebral dopamine receptor function.

Stereotyped behaviour was assessed in male Wistar rats (175-250 g) 15 min following administration of apomorphine hydrochloride (1.0 mg/kg sc). Administration of chlorpromazine (0.625 - 20 mg/kg ip 1 h prior to apomorphine), trifluoperazine hydrochloride (0.0625 - 2.0 mg/kg ip 1 h previously) or oxypertine (0.625 - 20 mg/kg ip 1 h previously) inhibited apomorphine-induced stereotyped behaviour (Table 1). Administration of tetrabenazine (0.625 - 20 mg/kg ip 1 h previously) or reserpine (0.1 - 10.0 mg/kg ip 6 h previously) was without effect. The specific binding of ^{3}H-spiperone (0.1 nM; defined using 10^{-5}M (+)-sulpiride to rat striatal membrane preparations was potently displaced by chlorpromazine, trifluoperazine or oxypertine (10^{-9} - 10^{-4}M) (Table 1). Reserpine and tetrabenazine were only weakly effective in displacing specific ^{3}H-spiperone binding to striatal membranes. The dopamine (100 uM)-induced stimulation of adenylate cyclase in rat striatal homogenates was inhibited by the incorporation of chlorpromazine, trifluoperazine and oxypertine (10^{-9} - 10^{-4}M). Reserpine and tetrabenazine (10^{-9} - 10^{-4}M) were without effect on cyclic AMP formation. The specific binding of ^{3}H-piflutixol (0.2 nM; defined using 10^{-6}M cis-flupenthixol in the presence of 3×10^{-3}M sulpiride) to rat striatal membrane preparations was potently displaced by trifluoperazine and chlorpromazine. Oxypertine was less effective but reserpine and tetrabenazine were inactive in the concentrations employed.

Table 1 Effect of drugs on brain dopamine parameters

	ED_{50} (mg/kg)	IC_{50} (M)		
	Stereotyped behaviour	^{3}H-spiperone	^{3}H-piflutixol	Adenylate cyclase
Chlorpromazine	5.3	8.6×10^{-9}	3.0×10^{-8}	1.0×10^{-6}
Trifluoperazine	0.33	2.4×10^{-9}	2.9×10^{-8}	2.2×10^{-7}
Oxypertine	7.8	5.4×10^{-8}	5.3×10^{-6}	7.1×10^{-6}
Tetrabenazine	No inhibition	3.8×10^{-6}	$> 10^{-4}$	$> 10^{-4}$
Reserpine	No inhibition	2.7×10^{-5}	$> 10^{-4}$	$> 10^{-5}$*

The IC_{50} values were determined by log-probit analysis. * The highest drug concentration used in this assay due to solubility problems. n = 5-8 experiments for biochemical determinations; for stereotypy, n = 6 animals for each drug dose.

The results suggest that oxypertine, but not tetrabenazine or reserpine, may have a direct effect on post-synaptic dopamine receptors in brain.

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EFFECTS OF ETHANOL IN VITRO AND IN VIVO ON K^+ STIMULATED PRODUCTION OF INOSITOL PHOSPHATES BY PHOSPHOLIPASE C IN RAT BRAIN SLICES

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The phospholipase C dependent breakdown of phosphatidylinositols to diacylglycerol , phosphatidate and inositol phosphates may play an important role in the release of neurotransmitter from synaptic terminals (Harris et al, 1983). Ethanol at physiologically relevant concentrations (50-100 mM) in vitro reduces the fraction of neurotransmitter released by these terminals to Ca^{2+} dependent stimuli (Lynch & Littleton 1983). Conversely chronic ethanol in vivo results in an increased fraction of neurotransmitter released. Here we report similar effects of acute and chronic exposure to ethanol on the phospholipase C dependent production in inositol phosphates (IP).

Cerebral cortical slices (350 x 350 μm) from chronic ethanol treated or control rats were pre-incubated in a Krebs-Ringer-bicarbonate medium at 37°C containing 1.3 mM Ca^{2+} for 45 min. Slices were then transferred to a similar medium containing Li^+ (5mM) and myo [2-^{3}H]inositol 0.3μM) and incubated for 90 min; this medium was removed and the slices incubated for a further 60 min in an unlabelled inositol (0.3μM) medium prior to stimulation by 40 mM K^+ for 30 min. Where relevant ethanol (100mM) was present throughout the latter 90 minutes of incubation and stimulation. [^{3}H] inositol phosphates were extracted and separated from ^{3}H inositol by Dowex ion exchange chromatography.

Treatment in stimulation period	Control	Chronic ethanol
None	100.0 ± 2.9	100.0 ± 2.4
40 mM K^+	157.0 ± 3.2	185.3 ± 6.5
100 mM Ethanol	83.9 ± 3.0	86.9 ± 4.0
40 mM K^+/100 mM Ethanol	143.6 ± 4.8	162.3 ± 8.9

Values are expressed as percentage of unstimulated [^{3}H]IP dpm in chronic ethanol and control treated animals. (means ± s.e.m of at least 20 determinations).

Results are shown above in the table. Ethanol at 100 mM significantly inhibited both the unstimulated and K^+ stimulated production of [^{3}H]IP in preparations from both control animals and animals treated with ethanol. Chronic ethanol treatment in vivo resulted in a slight but significant increase in the basal production of [^{3}H]IP (108.8 ± 3.3% of control levels), and a much larger increase in the K^+ -stimulated production of [^{3}H]IP (185.3% as opposed to 157.0% of control).

These data suggest that at a physiologically tolerable concentration (100 mM) ethanol has a significant inhibitory effect on the activity of phospholipase C, and that this inhibition is overcome by some adaptive process during chronic exposure to ethanol in vivo such that in the absence of ethanol in vitro these preparations show enhanced stimulated production of [^{3}H]IP. Whilst these findings parallel those of the effects of ethanol on neurotransmitter release and demonstrate a potential biochemical site of action for ethanol in vivo it is not at present possible to determine whether the modulation of phospholipase C activity by ethanol is causal or indeed related to its effects on fractional neurotransmitter release.

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EFFECT OF 8-PHENYLTHEOPHYLLINE ON GLYCEROL-INDUCED ACUTE RENAL FAILURE IN THE RAT

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Aminophylline has been shown to ameliorate the severity of glycerol-induced myohaemoglobinuric acute renal failure (ARF) in the rat (Bidani & Churchill, 1983) and this effect was attributed to adenosine-receptor antagonism. We have investigated the protective effect of 8-phenyltheophylline (8PT) in the glycerol model of ARF since 8PT is a more potent adenosine antagonist than aminophylline (Collis et al 1984).

Rats received i.m. injections of glycerol or saline and were then treated with either 8PT (10 mg kg^{-1}) or vehicle (1.0ml kg^{-1} of 50% v/v polyethylene glycol in 0.1M NaOH) twice daily via the tail vein. A further group of glycerol injected rats received no treatment. Blood samples for determination of plasma urea and creatinine were taken immediately before and at 24 and 48h after i.m. injection of saline or glycerol.

Table 1 Plasma urea and creatinine in rats with glycerol-induced ARF treated with either vehicle or 8PT

	Group 1 no treatment (n=12)	Group 2 vehicle treated (n=12)	Group 3 8PT treated (n=12)
Plasma urea (mg dl^{-1})			
0h	48±1	52±2	49±3
24h	257±19	187±23*	112±11***§
48h	317±37	210±46*	108±10***†
Plasma creatinine (mg dl^{-1})			
0h	0.68±0.01	0.66±0.03	0.69±0.04
24h	3.20±0.31	2.68±0.33	1.92±0.08***
48h	3.61±0.48	2.38±0.49*	1.42±0.10***

Mean ± s.e. mean and number of rats in parenthesis
*$P < 0.05$; ***$P < 0.001$ relative to group 1.
†$P < 0.05$; §$P < 0.01$ relative to group 2.

Treatment with either 8PT or vehicle produced no alteration in levels of urea or creatinine in saline-injected rats. Table 1 shows that the vehicle, an alkaline solution, had a protective effect in ARF. Protection by the vehicle was possibly due to the production of an alkaline urine which has been shown to prevent ARF in patients with crush injuries (Ron et al., 1984). Superimposed upon the protection afforded by the vehicle was an additional effect of 8PT. This effect may be related to adenosine receptor blockade because we have found that 8PT (10 mg kg^{-1}) antagonises adenosine-induced bradycardia in the conscious rat for at least 5h. The degree of amelioration produced by the 8PT solution was similar to that noted with aminophylline (Bidani & Churchill, 1983).

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EFFECT OF ACUTE RENAL FAILURE ON THE PHARMACOKINETICS AND BILIARY EXCRETION OF [^{3}H]-N-ACETYLPROCAINAMIDE ETHOBROMIDE IN THE RAT

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The hepatic uptake and initial biliary excretion of some organic anions are decreased in rats with acute renal failure (ARF) (Bowmer et al., 1982; Bowmer & Yates, 1984). Since separate pathways have been proposed for the hepatic transport of organic anions and cations (Schanker & Solomon, 1963), it was of interest to investigate the effect of ARF on the pharmacokinetics and hepatic handling of the cation N-acetylprocainamide ethobromide (APAEB).

ARF was induced in male Wistar rats by i.m. injection of 50% v/v glycerol (Bowmer et al., 1982) and 48 h later the plasma disappearance, biliary excretion and urinary excretion of [^{3}H]-APAEB (10 mg kg^{-1}; 11.4 µCi kg^{-1}) were determined over 1.5 h. In a separate series of experiments the hepatic levels of [^{3}H]-APAEB were measured at 2.5, 5, 7.5, 10 and 20 min and the cumulative percentage dose in the liver calculated. Both plasma clearance (Clp) and elimination rate constant (β) were significantly reduced in uraemic rats; but the apparent volume of distribution (Vd) remained unchanged (Table 1). There was no significant difference in the cumulative percentage dose of [^{3}H]-APAEB in the liver of either control (5.8 ± 1.9%; N=5) or uraemic rats (5.4 ± 2.1%; N=5) and no significant difference was noted in biliary excretion between control and uraemic rats (Table 1). By contrast, the percentage dose excreted into urine in uraemic rats was significantly lower than in controls (Table 1).

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

Parameter	Control (N=7)	Uraemic (N=7)	
Clp (ml min^{-1})	4.3±0.7	1.9±0.3	<0.05
β (min^{-1})	0.021±0.009	0.0087±0.0037	<0.05
Vd (ml)	268±117	266±129	N.S.
% Dose in bile	12±4	17±5	N.S.
% Dose in urine†	46±3	12±11	<0.001
Plasma urea (mg 100 ml^{-1})	40±9	337±133	<0.001

Values are mean ± s.d. †N=4.

The decreases in Clp and β were probably the result of reduced renal excretion as the hepatic content and biliary excretion of [^{3}H]-APAEB were not significantly altered in uraemic rats. The hepatic handling of [^{3}H]-APAEB appeared to be unchanged suggesting that organic cation transport by the liver is unaltered in ARF. However, biliary excretion failed to compensate for the substantial decrease in the renal excretion of [^{3}H]-APAEB seen in rats with ARF.

We thank the Wellcome Trust for financial support. DJS is supported by a Pharmaceutical Society Scholarship.

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INFLUENCE OF LIPID SOLUBILITY ON THE GLUCURONIDATION AND SULPHATION OF NITROPHENOLS IN RAT ISOLATED HEPATOCYTES

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Conjugation with glucuronic acid and sulphate are major pathways in the metabolism of phenolic compounds, and several factors are known which influence the balance of these two competing pathways. The transferases involved in these reactions are located in different subcellular sites. UDP glucuronosyltransferase is located in the microsomal membrane, whereas aryl sulphotransferase is located in the cytosol, and thus the lipid solubility of a substrate may determine its partitioning between these two locations, hence influencing the pattern of conjugates produced. Previous studies using rat liver microsomes have demonstrated that the rate of glucuronidation of a range of phenols is related to their lipid solubility (Illing, 1980; Illing & House, 1981). This present study was undertaken to investigate if this dependence on lipid solubility also held true if glucuronidation occurred in the presence of the competing sulphation pathway, as occurs in rat isolated hepatocytes.

Rat hepatocytes were isolated as described previously (Paterson & Fry, 1983), diluted to 2 x 10^6 cells/ml medium and incubated for 20 min at 37^0C with 100 µM concentrations of either 4-nitrophenol (4-NP), 2-chloro-4-nitrophenol (2-Cl-4NP), 3-methyl-4-nitrophenol (3-Me-4NP) or 4-methyl-2-nitrophenol (4-Me-2NP). The conjugates produced were hydrolysed by specific enzymes and the aglycones liberated were measured by spectrophotometry. The octanol/0.1M phosphate buffer pH 7.4 partition coefficients (expressed as log P values) were taken from the data of Illing & House, 1981. The results are indicated in Table 1 (values are mean ± S.E.M., n = 6).

Table 1. Log P values, and rates of glucuronidation and sulphation of four substituted nitrophenols

Substrate	Log P Value	Glucuronidation Rate (nmole/2 x 10^6 cells/min)	Sulphation Rate
2-Cl-4NP	0.73	0.70 ± 0.11	0.32 ± 0.07
4-NP	1.38	1.53 ± 0.14	0.34 ± 0.11
3-Me-4NP	1.84	1.91 ± 0.19	0.47 ± 0.08
4-Me-2NP	2.15	2.37 ± 0.15	0.34 ± 0.11

The rate of glucuronidation exceeded that for sulphation for all four nitrophenols. The rate of glucuronidation was linearly correlated to the lipid solubility, (rate of glucuronidation = 1.14 Log P - 0.10, r^2 = 0.99). The rate of sulphation was, however, similar for all four substrates, and experiments with rat liver slices indicated that this constant level of sulphation was not related to depletion of inorganic sulphate.

It is concluded that the rate of glucuronidation of nitrophenols in rat isolated hepatocytes is related to their lipid solubility, whereas the rate of sulphation is not. It is highly likely that this lipid solubility effect is related to the uptake of compound into the microsomal membrane, the site of glucuronidation in the cell.

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INHIBITION OF PARACETAMOL GLUCURONIDATION IN RAT LIVER MICROSOMES BY PROBENECID

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During the course of a study into the effects of probenecid on paracetamol metabolism in human subjects, it was found that probenecid administration was associated with a significant decrease in the amount of paracetamol glucuronide excreted in the urine, together with a significant increase in plasma paracetamol half-life. It is recognised that probenecid is also metabolised by glucuronidation (Dayton & Perel, 1971) and the possibility occurred that the effects on paracetamol metabolism might, in part, arise through inhibition of glucuronidation. Accordingly, the effects of probenecid on glucuronidation of paracetamol in rat liver microsomes were assessed.

Microsomal fractions were obtained from the livers of untreated rats by the method of Paterson, Fry & Homer,(1984). Incubations were performed for 30 min at 37°C in a total volume of 250µl, which contained phosphate buffer pH 7.4 (final conc. 200mM), microsomes (2mg protein/ml), UDPGA (3mM), Triton X-100 (0.025% w/v), probenecid (5mM) and varying concentrations of paracetamol (0.25 - 5.0mM). The reaction was terminated by the addition of 3N perchloric acid (125µl) and, following centrifugation, the supernatant was stored at -20°C prior to analysis. Paracetamol glucuronide was analysed by the HPLC method of Moldeus (1978). The data were analysed by Lineweaver-Burk plots, the line of best fit being calculated by regression analysis, and statistical analysis was performed by means of a paired sample t-test. The results are indicated in Table 1 (values are expressed as mean ± S.E., n = 6).

Table 1 Effect of probenecid on apparent Km and Vmax values for paracetamol glucuronidation in rat liver microsomes

	Apparent Km value (mM)	Apparent Vmax value (µg glucuronide/mg protein/min)
- Probenecid	19.94 ± 4.23	2.89 ± 0.70
+ Probenecid	1.82 ± 0.36	0.24 ± 0.02
	$P < 0.005$	$P < 0.01$

The presence of probenecid in the incubation significantly decreased both the apparent Km and Vmax values for paracetamol glucuronidation. Furthermore, there was no significant difference in the slopes of lines ($P > 0.10$) drawn according to a Lineweaver-Burk plot. This data suggests that probenecid acts as an uncompetitive inhibitor of paracetamol glucuronidation (Ki value = 0.60 ± 0.14mM), and indicates that the decrease in paracetamol glucuronide excretion and increase in paracetamol half-life observed following concomitant administration of probenecid is, at least in part, attributable to this inhibitory effect of probenecid on paracetamol glucuronidation.

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THE EFFECT OF AGE ON THE ACUTE RESPONSE TO DIAZEPAM

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Open variable dose studies of patients receiving intravenous (i.v.) diazepam for minor procedures have indicated that the dose of diazepam and the plasma total concentration required for sedation decreases with ageing (Reidenberg et al, 1978; Cook et al, 1984). We have examined the effect of a single i.v. dose of diazepam in a double blind study using 11 matched pairs of healthy young and elderly volunteers.

A split unit randomised crossover design was employed using pairs of young and old volunteers who were matched for sex (4 male), weight and alcohol intake. The volunteers all led active lives and none had received any sedation for at least 3 months. The volunteers were studied in the afternoon after fasting for 8 hours, on 2 occasions, 2 weeks apart. Each pair of subjects was given either i.v. diazepam (0.1 mg/Kg) or placebo in one arm. A blood sample was taken from the other arm before and at 3 min after dosing. Psychomotor function was assessed before and at 5 min after dosing using the pair choice reaction time, 2 min letter E deletion test and 3 one-min sway tests. Each subject was given a full practice set of tests to reduce any learning effects. The results were analysed by split plot analysis of variance and the paired t test.

Two young subjects had late blood samples (6.25, 6 min) and the plasma total diazepam concentrations tended to be lower in the young (Y 0.53 $\pm$ 0.26 ug/ml), E 0.76 $\pm$ 0.41 ug/ml; $p > 0.05$). The plasma free fraction of diazepam was higher in the elderly (Y 2.00 SD $\pm$ 0.49%, E 2.24 SD $\pm$ 0.32%; $p < 0.05$). The baseline test values were almost identical on the 2 occasions. The elderly had slower baseline reaction times (Y 464 $\pm$ SD 33, E 591 $\pm$ 66 msec; $p < 0.001$), deleted fewer E's (Y 116 SD $\pm$ 17, E 88 $\pm$ 24, $p < 0.01$) and tended to sway more (Y 5.2 SD $\pm$ 1.7, E 6.17 $\pm$ 1.7°Arc/min; $p > 0.05$). The results are shown in Table 1. All of the changes were statistically significant when expressed as percentage changes. The results confirmed that the acute response to diazepam increases with normal ageing.

Table 1 Change in psychomotor performance (Mean $\pm$ SD).

	Diazepam 0.1 mg/Kg		Placebo		p
	Young	Elderly	Young	Elderly	
Reaction time	+75 ($\pm$ 112)	+376 ($\pm$311)	-38.2 ($\pm$24)	-40.3 ($\pm$27)	<0.005
Sway (°Arc/min)	+4.8 ($\pm$4.1)	+11.8 ($\pm$8.1)	0 ($\pm$0.7)	-0.7 ($\pm$0.8)	<0.025
Letter E Deletion E's/2 min)	-18.9 ($\pm$19)	-31.6 ($\pm$19)	+3.6 ($\pm$10.4)	+5.8 ($\pm$4.6)	<0.1

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STUDIES ON THE METABOLISM OF N-METHYLFORMAMIDE

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N-methylformamide (NMF) is a solvent and potential anticancer drug. It causes centrilobular necrosis in mice (Whitby et al, 1984) and is also hepatotoxic in rats and humans. The mechanism of this hepatotoxicity is unknown. We have investigated the metabolism and hepatotoxicity of NMF in male Sprague Dawley rats. In this strain of rat, NMF in single doses of 200-800 mg kg^{-1} was not hepatotoxic, however, NMF was mildly hepatotoxic at 1000 mg kg^{-1}, as judged both histopathologically and by measurement of the serum transaminases, aspartate and alanine transaminase. The mild hepatic necrosis produced by single doses of NMF was not altered by phenobarbitone pretreatment. High field proton nuclear magnetic resonance spectroscopy was carried out on untreated urine samples and several possible metabolites were identified. These metabolites included methylamine, formate, formamide and some as yet unidentified acetylated compounds.

Rats were dosed with NMF radiolabelled with ^{14}C in the methyl group. After 24 hours, 32% of the dose was excreted in the urine, 1.2% in the faeces and 1% was expired as $^{14}CO_2$. The majority of the compound was still retained in the carcass. At 72 hours, 62% of the dose had been excreted in the urine, 1.9% in the faeces and 6.3% in the breath as $^{14}CO_2$. Phenobarbitone pretreatment had no obvious effect upon the excretion or distribution of NMF. NMF and its metabolites were quantified by GC and NMR in 0-24 hour urine samples of rats dosed with 1000 and 2000 mg kg^{-1} NMF. After a dose of 1000 mg kg^{-1}, 16% of the dose was excreted as unchanged NMF, 1% as formamide, 2% as methylamine and 4% as formate. Increased amounts of formamide and formate were found in urine samples following phenobarbitone pretreatment.

This study has shown that NMF is only mildly toxic to Sprague Dawley rats and also that there is very little metabolism of NMF. Phenobarbitone pretreatment does not alter the toxicity of NMF but does affect metabolism.

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IMPAIRMENT OF LIGNOCAINE CLEARANCE BY PROPRANOLOL - MAJOR CONTRIBUTION OF ENZYME INHIBITION

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Propranolol (P) lowers the systemic clearance (CL) of lignocaine (L) in man by about 40% (Ochs et al, 1980; Conrad et al, 1983), apparently as the result of both haemodynamic and metabolic effects. Thus, using theoretical models of hepatic drug clearance, Tucker et al (1984) have suggested contributions of a maximum 25% decrease in liver blood flow and a 50% decrease in the unbound intrinsic clearance (CLu_{int}) of L due to enzyme inhibition. The haemodynamic effect should lower CL by only about 15%. The "well stirred" model of hepatic drug clearance predicts that the oral clearance of drugs that are eliminated predominantly in the liver is equal to $fu_B.CLu_{int}$, where fu_B is the free fraction of drug in blood. P does not alter the fu_B of L (McNamara et al, 1981). Therefore, a direct 50% inhibition of L metabolism by P is predicted to double the AUC of L after oral administration.

Six healthy male subjects each received $L.HCl.H_2O$ 200mg. orally on two occasions. One dose served as the control (C) and the other (Pr) was preceded by treatment for three days with oral P.HCl, 80mg. bd. The order of C and Pr treatment was randomised. L was given after an overnight fast at 30 min after P dosage. Serial plasma samples were collected up to 8h for L assay by GLC. The subjects remained supine and continued fasting for the first 3.5h of the study. A single intravenous bolus of indocyanine green (ICG) ($0.5mg.kg^{-1}$) was administered 2.5h after dosing with L. Serial plasma samples were taken at 2 min intervals for 20 min and assayed for ICG.

P doubled mean plasma AUC ($\mu g.ml^{-1}.min$) of L (C, 58 ± 19 s.d.; Pr, 117 ± 30 s.d. $p<0.005$), it increased the peak plasma concentration ($ng.ml^{-1}$) of L by 79 ± 50 s.d.% ($p<0.025$) (C, 502 ± 114 s.d.; Pr, 875 ± 250 s.d.) and it prolonged the $t_{\frac{1}{2},z}$ (min) of L by 20 ± 13 s.d.% ($p<0.05$) (C, 103 ± 15 s.d.; Pr, 125 ± 29 s.d.). The time of the peak plasma concentration of L (20-50 min) was not affected by P. P decreased ICG clearance ($ml.min^{-1}.kg^{-1}$) by 11 ± 15 s.d.% (C, 10.1 ± 1.2 s.d.; Pr, 8.9 ± 1.5 s.d.), although this change was not statistically significant.

These experimental findings support the theoretical predictions suggesting that lowering of the CL of L by P is mediated mostly by direct inhibition of its metabolism; any contribution from reduced liver blood flow appears to be small.

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COMPARATIVE ABILITY OF MAN TO ACTIVATE PARACETAMOL TO ITS HEPATOTOXIC METABOLITE

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Paracetamol is widely used as an antipyretic and analgesic. At normal therapeutic doses, up to 4 g per day, it has a wide margin of safety but in overdose it can cause severe, and sometimes fatal, hepatotoxicity. There are marked species differences in sensitivity to the hepatotoxic effect of paracetamol. Thus, hamster and mouse are very susceptible whereas rat and guinea pig are very resistant. There is some question as to how sensitive is man. As little as 10 g of paracetamol may be sufficient in some individuals to cause hepatotoxicity.

Paracetamol itself is not hepatotoxic but rather it is oxidised to a potent electrophile, N-acetyl-p-benzoquinoneimine (NABQI), which is alomost certainly responsible for this toxicity. We have now investigated the ability of man to activate paracetamol to this metabolite and also the explanation for the species differences in sensitivity to paracetamol. Microsomal fractions of human and mouse liver were incubated with [^{14}C]paracetamol and covalent binding of labelled paracetamol to microsomal proteins determined. With human liver binding was 26 ± 2 pmol/mg/min (mean ± SEM, n=28) whereas with mouse liver binding was 190 ± 3 pmol/mg/min (n=3). The active metabolite of paracetamol was also trapped as the glutathione adduct by adding glutathione to the incubation mixture. Mouse liver was 15-20 times more active than human liver in generating the adduct.

The toxicity of paracetamol was investigated in isolated hepatocytes from human, mouse, rat and hamster. Comparative susceptibility of the cells was hamster > mouse >> rat >= human. The maximum concentration of paracetamol tested was 50 mM. At this concentration there was only a 10% decrease in the viability of human hepatocytes (n=4). However, examination of the cells by both light and electron microscopy did reveal morphological changes indicative of early damage.

The putative active metabolite of paracetamol, NABQI, was synthesised and its effects on hepatocytes from the four species investigated. There was a concentration-dependent toxicity to cells from all of the species and the severity of this toxcity was similar in all cases. Thus, the differences observed amongst species in the toxicity of paracetamol are due presumably to differences in the rate of formation of NABQI and not to any difference in sensitivity to this metabolite.

Data generated in vivo by other workers (Mitchell et al, 1974; Jollow et al, 1974) were used to calculate the clearance of paracetamol in vivo to the mercapturic acid. This parameter should provide an estimate of the relative rate of production of NABQI. The values obtained were as follows: human, 0.25 ml/min/Kg; mouse, 2.57 ml/min/Kg; rat, 0.40 ml/min/Kg. Thus, data in vitro, in isolated cells and in vivo all indicate that the average ability of man to activate paracetamol to its hepatotoxic metabolite is 10-fold less than that of the mouse and similar to that of the rat, a species relatively resistant to paracetamol hepatotoxicity.

Acknowledgements: ACH was supported by a grant form the Wellcome Trust. Portions of this work were supported by the Medical Research Council.

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EFFECTS OF PROBENECID ON PARACETAMOL METABOLISM AND EXCRETION IN MAN

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A previous study has indicated that the elimination of paracetamol glucuronide (PG) and sulphate (PS) in the kidney of dogs is mediated by an active transport mechanism, as suggested by the inhibitory effects of probenecid, a known inhibitor of this mechanism (Duggin & Mudge, 1975). Probenecid is itself eliminated in part by glucuronidation (Dayton & Perel, 1971) and the possibility was considered that probenecid may inhibit elimination of paracetamol glucuronide partly by inhibition of paracetamol glucuronidation. The present study was initiated to ascertain this.

Ten normal healthy volunteers (5 males; age 20-29 years; weight 56.5-83.5Kg) who had fasted overnight took an oral therapeutic dose of paracetamol (1.5g; 3x500mg B.P. formulation) on two different occasions, once 1h after an oral administration of probenecid (1.0g; 2x500mg Benemid tablets). The two occasions were separated by at least a one week interval. Serial blood samples were collected for up to 6h following paracetamol ingestion and urine samples were collected hourly for these six hours. The levels of paracetamol, PG and PS in the plasma and urine were measured by the method ofHowie et al (1977). Plasma elimination half-lives ($t_{\frac{1}{2}}\beta$) for paracetamol were obtained by the method of least squares regression analysis, statistical analysis being carried out by a paired sample student t-test. The results are shown in Table 1 (values are expressed as mean ± S.E., n = 10).

Table 1. Effect of probenecid on plasma half-lives of paracetamol and urinary excretion of paracetamol, PG and PS.

	$t_{\frac{1}{2}}\beta$ (min)	0-6h excretion of:-		
		paracetamol (mg)	PG (mg)	PS (mg)
- Probenecid	127.2 ± 9.1	33.3 ± 4.2	348.2 ± 33.4	243.4 ± 44.8
+ Probenecid	206.0 ± 32.6	37.9 ± 4.0	74.5 ± 9.9	193.4 ± 29.0
	P < 0.0005	P > 0.20	P < 0.0005	P < 0.05

Serum and urine creatinine concentrations were measured as indices of renal function and were within the normal range for all subjects during the course of the experiments. The results suggest that prior administration of probenecid significantly decreased the urinary excretion of both PG and PS, but that the urinary excretion of paracetamol was unaltered: this agreed with the results of Duggin & Mudge (1975). However, the prior administration of probenecid also significantly prolonged the plasma elimination half-life of paracetamol, this suggesting that probenecid inhibited the metabolism of paracetamol. Preliminary studies indicate that probenecid is an uncompetitive inhibitor of paracetamol glucuronidation in rat liver microsomes. Thus, whilst probenecid may interfere with the elimination of PG and PS by impairment of the renal active transport mechanism, as suggested by Duggin & Mudge (1975), it is also highly possible that it inhibits the metabolism of paracetamol to these conjugates, and thereby also interferes with their elimination.

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140P

AMIODARONE REDUCES PLASMA WARFARIN CLEARANCE IN MAN

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Amiodarone is known to interact with warfarin (Rees et al., 1981) although the mechanism remains unclear. We investigated 8 subjects (4 males) aged 46-72 years who were on warfarin therapy and subsequently required amiodarone for control of cardiac arrhythmias. At the time amiodarone therapy was started the dose of warfarin was reduced by 40-50% as suggested by Hamer et al., 1982, and thereafter adjusted to maintain the BCR within the therapeutic range (2-4).

Blood samples were collected 16-22 hours after the previous warfarin dose, before amiodarone therapy was started and four weeks later. Total (HPLC) and free (equilibrium dialysis) plasma warfarin and one-stage prothrombin time (BCR) were measured. Warfarin dose had been stable for at least seven days before each blood sample. Warfarin clearance was calculated by dividing the daily warfarin dose at steady state by the plasma warfarin concentration.

Total daily warfarin requirement fell from 4.9 $\pm$ 2.3 (SD) to 3.0 $\pm$ 1.4 mg ($p < 0.01$) while the BCR did not change significantly (from 2.6 $\pm$ 0.9 to 3.0 $\pm$ 0.8, $p > 0.05$).

The free fraction of warfarin in plasma did not change (0.013 $\pm$ 0.002 to 0.012 $\pm$ 0.001, $p > 0.05$).

Total plasma warfarin clearance fell significantly from 4.71 $\pm$ 2.34 to 2.65 $\pm$ 0.77 L/day ($p < 0.05$) after four weeks of amiodarone therapy.

We have confirmed that amiodarone significantly reduces warfarin requirements (in our group by 39%). We have shown total plasma warfarin clearance decreases by a similar proportion (in our group by 44%). This indicates that the amiodarone-warfarin interaction is due, at least in part, to inhibition of warfarin metabolism. We have found no evidence that altered protein binding is responsible for the reduction in warfarin requirements.

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THE USE OF FUNCTIONAL ANTAGONISM IN THE DETERMINATION OF FUNDAMENTAL PARAMETERS FOR TACHYKININ-RECEPTOR INTERACTIONS

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Functional interaction occurs when two different receptor types produce synergistic or antagonistic actions in the same cell, and the quantitative properties may, in principle, be used under defined conditions to estimate equilibrium dissociation constants (K_D) or efficacy values for an interactant. A simple additive or subtractive model of interaction of stimulus subeffect in two receptor systems has yielded sets of dose-response curves that correspond quite closely to some experimentally observed interactions (van den Brink, 1973a,b, 1977). In an instance when functional antagonism produces parallel but limited shift of the agonist log dose-response curve, the degree of shift seems to provide an estimate of the efficacy of that functional antagonist relative to others acting on the same receptor.

At a time when reliable substance P antagonists were not available for receptor classification studies we wondered if it were possible to supplement relative potency data for tachykinin agonists with relative efficacy measurements determined through functional interaction experiments, with the possible bonus of some clues as to stimulus-response coupling mechanisms for the tachykinins.

When papaverine is used as a functional antagonist of the tachykinins (or carbachol or histamine) in the guinea-pig ileum the form of interaction seen is depression of the maximum with little lateral shift in the log dose-response curves of the spasmogens. The negative log of the molar concentration of antagonist (papaverine) to depress the maximum to 50% has been termed the pD'_2 (Ariens et al,1964) and may readily be determined from log dose - % depression lines.

A simple subtractive model for subeffect interaction with linear stimulus-subeffect coupling predicts that agonists of lower efficacy would yield higher pD'_2 values with the functional antagonist. However, this did not prove to be the case since pilocarpine, a known low efficacy agonist, gave similar pD'_2 values to acetylcholine and carbachol. Furthermore, the tachykinins substance P, physalaemin and eledoisin-related peptide also gave similar pD'_2 values (about 5.2).

Determination of null equations for simple subtractive interaction of subeffects with a linear stimulus-subeffect relationship showed that, in fact, a more complicated relationship holds.

These results are discussed in relation to null equations derived by Mackay (1981) for more complicated interactions and in relation to the treatment by some authors of the depression of maximum as a quasi receptor occlusion method for the determination of K_D or efficacy values.

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142P

AN EXAMINATION OF THE MECHANISMS OF THE SPASMOGENIC EFFECTS OF TACHYKININS ON SMOOTH MUSCLE

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It has been suggested that substance P (SP), unlike a number of other agents that contract smooth muscle by opening receptor-operated channels (ROC's), instead closes potassium channels so causing depolarisation (Fugisawa & Ito, 1982; Holzer & Petsche, 1983). In support of this mechanism Holzer and Petsche (1983) have shown that the contractile effects of SP in the guinea-pig ileum longitudinal muscle are antagonised by noradrenaline which, they assume, acts by causing an increase in potassium permeability (P_K) in this preparation. However, though this is true of noradrenaline in the case of some intestinal muscles (eg, taenia caeci, Jenkinson & Morton, 1967a) the α-adrenoceptors in the guinea-pig ileum longitudinal muscle seem atypically coupled, and may be inoperative or cause contraction (Bauer, 1982; Halliday & Morton, 1981; Kosterlitz et al, 1970).

In view of this we thought it of interest to determine the adrenoceptor type involved in the inhibition of the actions of tachykinins by the use of selective adrenoceptor antagonists, and at the same time investigate the possibility of multiple tachykinin receptor subtypes in this tissue. Use of phentolamine and propranolol confirmed that noradrenaline was acting not via α-adrenoceptors but by β-adrenoceptors which do not cause increase in P_K (Jenkinson & Morton, 1967b; Morton & Halliday, 1981). The particular form of functional antagonism seen with noradrenaline produced a limited parallel shift of the tachykinin log dose-response line. Interestingly enough, the degree of shift was similar for either eledoisin or physalaemin which are relatively selective for 'E' or 'P' type tachykinin receptors respectively, so no evidence of tachykinin receptor heterogeneity was obtained by this approach.

Nonethless, it was decided that this comparative approach could usefully be extended to other studies and that the inclusion of non-tachykinin agonists would also be of interest. The calcium requirement for contraction in three smooth muscle preparations, the guinea-pig ileum, bladder and rat colon muscularis mucosae, was examined for substance P, kassinin and either carbachol or bradykinin. It was found that, although the tissues differed from each other in calcium sensitivity, within a single preparation all agonists were affected equally by a reduction in the calcium concentration of the bathing medium.

In view of the proposed action of substance P on P_K it was of interest to find what action potassium channel blockers have on the contractile actions of tachykinins. Holzer and Petsche (1983) have shown that tetraethylammonium potentiates the contractile effects of substance P in the guinea-pig ileum, presumably by an action on voltage-sensitive potassium channels, although such an action would be expected to result in non-specific increase in smooth muscle excitability. The peptide apamin which blocks potassium permeability associated with some ROC's (eg, those of α-adrenoceptors and ATP in the taenia caeci) was found to be without action on the contractile effects of substance P and kassinin in this tissue.

It is concluded on the basis of the evidence outlined above that there is little evidence of tachykinin receptor heterogeneity in the guinea-pig ileum, since a variety of experimental procedures failed to distinguish between different tachykinins. The usefulness of studies on calcium dependence is discussed in the light of the inability of such experiments to distinguish between unrelated groups of agonists, and the fact that the time course of effects of changing calcium concentration appear to be more dependent on tissue than on agonist.

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THE D_1 RECEPTOR ANTAGONIST, SCH 23390, AND SULTOPRIDE, HAVE DISTINCTIVE EFFECTS IN A SALT PREFERENCE TEST

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Gustatory factors exert profound effects on the drinking responses of nondeprived and thirsty rats. Considerable overconsumption of fluids can be induced in rehydrating animals given access to palatable sweet or salty solutions (Ernits & Corbit, 1973). Thirsty rats prefer sweet or salty solutions to water in two-bottle choice tests. We have previously reported that the preference for salt solutions was changed following sulpiride, pimozide or clozapine treatments in water-deprived animals (Cooper & Gilbert, 1984), and concluded that dopaminergic mechanisms play an important part in the determination of preference behaviour. The novel compound SCH 23390 has been identified as a specific dopamine D1 receptor antagonist (Iorio et al., 1983), the effects of which differ from those of sulpiride (e.g. Costall et al., 1984). We investigated a possible involvement of D1 receptors in the mediation of salt preference, using this compound.

Forty-eight male hooded rats (General strain, bred in our laboratory) were adapted to daily 22h water-deprivation, and were thoroughly familiarized with a two-choice wooden drinking box. The animals were allocated at random to six groups, which were trained on the choices between 0.064%, 0.16%, 0.4%, 1.0% or 2.5% NaCl concentration versus water, respectively, with the sixth group run under a control water versus water condition. At the end of the deprivation period, a rat was placed in a box for 15 min, and the intakes from the two drinking tubes were measured. Methods are described in detail elsewhere (Cooper & Gilbert, 1985). Each animal served as its own control and received each injection of the compound under investigation according to a balanced design.

SCH 23390 (0.03-1.0 mg/kg, subcutaneous administration, 35-40 min pre-test) produced a marked dose-dependent suppression of total fluid intake. In contrast, sultopride (0.3-10.0 mg/kg, intraperitoneal administration, 2h pre-test) had only modest depressant effects on total fluid consumption. The two compounds exerted different effects on salt preference. In a dose too small to disrupt drinking (0.03 mg/kg), SCH 23390 raised the salt preference curve over the range of salt concentrations. At 0.1 mg/kg, the preferences for the hypertonic solutions (1.0% and 2.5% NaCl) were significantly increased. On the other hand, sultopride (0.3 and 3.0 mg/kg had no effect on the preferences for the more concentrated salt solutions. Instead, preferences for hypotonic solutions were enhanced. It is worth noting that some other drug treatments (e.g. naloxone) decrease salt preference in the same test (Cooper & Gilbert, 1985).

Previous reports that dopamine receptor antagonists reduce fluid consumption are extended and qualified by the present data. Dopamine D2 antagonists (sultopride, sulpiride) have little effect on total fluid intake in the preference test. In contrast, SCH 23390, a D1 receptor antagonist, produced marked suppression of drinking. The increases in salt preference brought about by sultopride and SCH 23390 confirm that dopamine receptor-mediated mechanisms may be influential in the control of drinking responses, which are modulated by gustatory sensation.

D.B.G. is supported by an award from the University of Birmingham. Sultopride and SCH 23390 were generously given by Delagrange and Schering Corp., respectively.

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AGE-RELATED CHANGES IN PRE- AND POSTJUNCTIONAL α-ADRENOCEPTORS IN HUMAN SAPHENOUS VEIN

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In the human saphenous vein prejunctional alpha-adrenoceptors are of the $alpha_2$-subtype and postjunctionally the predominant subtype is $alpha_2$, although $alpha_1$-receptors are also present (Docherty & Hyland, 1984). The object of this study was to look for age-related changes in alpha-receptor responsiveness in this tissue, particularly since it has been reported that the responsiveness of prejunctional $alpha_2$-receptors is reduced in the vas deferens of aged rats (Docherty & O'Malley, 1983).

Human saphenous veins were obtained as leftovers from coronary artery bypass grafts of patients aged 38-70 years. Tissues were cut spirally and superfused at 37°C in Krebs-Henseleit solution. In some experiments, tissues were pre-incubated with ^{3}H-noradrenaline.

Field stimulation for 3 min at a frequency of 5 Hz produced an isometric contraction of 1.03 ± 0.11 g, n=29 and an evoked overflow of 1.14 ± 0.15 % of tissue tritium, n=20. The $alpha_2$-adrenoceptor agonist xylazine (10^{-5} uM) significantly reduced the stimulation-evoked tritium overflow to 57.3 ± 3.6 % of control (n=10) but there was no significant correlation between xylazine potency and age.

The $alpha_2$-antagonist yohimbine (0.01-1 uM) produced a concentration-dependent inhibition of stimulation-evoked contractions and potency was calculated in terms of an IC_{30} (concentration producing 30 % inhibition of stimulation-evoked contractions). There was a significant negative correlation between potency of yohimbine and age ($r = 0.70$, $n=11$, $P<0.05$) so that there was a reduced potency of yohimbine with increasing age. The $alpha_1$-antagonist prazosin was less potent than yohimbine at reducing stimulation-evoked contractions, but there was no significant age-related correlation of the prazosin IC_{30}.

Hence, we are able to demonstrate an alteration with age in the responsiveness of postjunctional $alpha_2$, but not of prejunctional $alpha_2$- or postjunctional $alpha_1$-adrenoceptors, in the human saphenous vein. The decreased postjunctional potency of yohimbine may reflect a reduction in the relative proportion of postjunctional $alpha_2$-receptors with age.

Supported by research funds of RCSI and MRC(I). We are grateful to Drs. Neligan and Shaw of the Mater Hospital, Dublin for saphenous vein leftovers.

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INVOLVEMENT OF VASCULAR α_2-ADRENOCEPTORS IN BLOOD PRESSURE CONTROL IN THE ANAESTHETISED SPONTANEOUSLY HYPERTENSIVE RAT

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Alpha$_2$-adrenoceptor agonists produce pressor responses in the pithed rat which are resistant to the alpha$_1$-antagonist prazosin but are inhibited by alpha$_2$-antagonists (Docherty & McGrath, 1980). The objects of this study were to look for an involvement of vascular alpha$_2$-adrenoceptors in the maintenance of blood pressure in the anaesthetised SHR and to determine whether this involved circulating catecholamines or neurally released noradrenaline.

Male SHR (250-300 g) were anaesthetised with pentobarbitone.

The left carotid artery was cannulated for measurement of blood pressure and the left jugular vein for administration of drugs. Some animals underwent adrenal demedullation or sham operation at least two days prior to the experiment.

In untreated SHR, the alpha$_2$-selective antagonist rauwolscine (0.1-10 mg kg^{-1}) produced increasing falls in diastolic blood pressure (DBP), but no maximum effect was reached. In the presence of prazosin (1 mg kg^{-1}), which lowered DBP by 64.4 $\pm$ 7.5 mmHg (n=8), rauwolscine (0.1 mg kg^{-1}) lowered DBP by a further 16.7 $\pm$ 6.0 mmHg (n=3, $P<0.05$ from effects of saline) and rauwolscine (1 mg kg^{-1}) produced a maximum cumulative fall of 23.3 $\pm$ 9.3 mmHg. However, another alpha$_2$-antagonist Wy 26392 (Lattimer et al., 1982) had variable effects on DBP following prazosin, increasing or decreasing DBP, so that Wy 26392 (0.1 mg kg^{-1}) caused an increase of 4.0 $\pm$ 12.0 mmHg (n=5, not significantly different from effects of saline). Wy 26392 (1 mg kg^{-1}) did not raise DBP in pithed rats.

In animals which had undergone adrenal demedullation, resting DBP was significantly less than in sham operated animals (117.8 $\pm$ 11.3 mmHg, n=7 and 161.0 $\pm$ 6.4 mmHg, n=5, respectively; $P<0.05$). There was no significant difference in the fall in DBP produced by prazosin (1mg kg^{-1}) between demedullated (54.3 $\pm$ 12.0 mmHg, n=7) and sham-operated animals (59.0 $\pm$ 6.4 mmHg, n=5). However, rauwolscine (0.1-10 mg kg^{-1}) produced no significant fall in DBP subsequent to prazosin in demedullated animals, whereas in sham-operated animals DBP was reduced 17.0 $\pm$ 2.5 and 24.0 $\pm$ 4.3 mmHg by rauwolscine (0.1 and 1.0 mg kg^{-1}; n=5, in both cases significantly different from effects in demedullated animals, $P<0.05$).

In conclusion, we are able to demonstrate an involvement of alpha$_2$-adrenoceptors in the control of blood pressure in normal SHR but not in SHR following adrenal demedullation, suggesting that circulating catecholamines are responsible for these alpha$_2$-mediated pressor effects. However, there are differences between alpha$_2$-antagonists in their abilities to lower blood pressure in the presence of prazosin: the variable effects of Wy 26392 may be due to a balance between peripheral and central effects.

Supported by research funds of RCSI and by the MRC(I).

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ANTI-ANXIETY ACTIVITY OF SR 41378 A NEW NON-BENZODIAZEPINE HYPNOTIC

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SR 41378, 3-(4'-hydroxy-piperidinyl)-6-(2,4 dichloro phenyl)-pyridazine, is a chemically original compound which exhibits in mice anticonvulsant, sedative and myorelaxant effects with a potency close to that of pentobarbitone. In a rat model of insomnia SR 41378 exhibites a strong hypnotic action with a potency similar to that of pentobarbitone (Bizière et al, 1984). Since such a pharmacological profile is often associated with anti-anxiety activity, we investigated the action of SR 41378 in behavioural experiments specific of anxiolytic compounds.

The methods used were : 1) the approach-avoidance conflict procedure (Davidson & Cook, 1969) in which the desinhibitory (increase in punished responding PR) as well as the neurotoxic effects (decrease in non-punished responding NPR) were measured. Rats (Sprague Dawley, Charles River, France) were dosed i.p., 30 min prior to testing, with SR 41378 suspended in carboxymethylcellulose. In the same test the antagonism of SR 41378 by Ro 15-1788 was investigated. The benzodiazepine antagonist was injected i.p. 30 min before SR 41378 i.e. 1 h before the experiment. 2) The drug discrimination method (standard protocol according to Colpaert et al, 1975). Six Wistar rats were trained with clonazepam (0.25 mg/kg, i.p., 30 min before the experiment). SR 41378 (i.p.) was substituted to clonazepam during generalization tests.

In the conflict paradigm, SR 41378 significantly increased PR (ANOVA $F(4\text{-}30) = 8.0$, $p < 0.001$). The ED50 calculated from the regression curve was 5.2 mg/kg. NPR was also significantly modified by the treatment (Brown-Forsythe's test $F(4\text{-}15) = 8.15$, $p < 0.001$). Comparisons among the means by the Bonferroni's method indicated that the first dose to decrease the emitted responses was 20 mg/kg. Ro 15-1788 (10 and 40 mg/kg) did not antagonize the desinhibitory action of 10 and 20 mg/kg of SR 41378, nor did it modify the 20 mg/kg-induced decrease in NPR. In the drug discrimination paradigm, SR 41378 generalized to the clonazepam cue (ED50 = 6.1 mg/kg) and reduced the total number of lever presses at the highest dose injected (10 mg/kg) (matched pairs t test : $t = 3.2$ $p = 0.02$). Errors were not modified by the treatment (Friedman test statistic = 4.45, df = 3, $p > 0.05$).

Taken together these results suggest that SR 41378 has an anti-anxiety activity with strong sedative effects which are not mediated by a direct action on benzodiazepine receptors. In agreement with the results of the Ro 15-1788 experiments, SR 41378 has been shown not to displace (^{3}H)flunitrazepam from its binding sites in vitro (Bizière et al, 1984).

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THE NATURE OF ARACHIDONIC ACID-INDUCED AGGREGATION OF RAT PLATELETS

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There is some controversy as to the mediators of arachidonic acid (AA) activation of rat platelets. It has been suggested that irreversible aggregation is not entirely dependent on the formation of prostaglandin endoperoxides and thromboxane A_2 (Dutilh et al, 1979; Nishizawa et al, 1983). We have re-examined this situation using the cyclo-oxygenase inhibitor flurbiprofen, the thromboxane synthetase inhibitor dazoxiben and the thromboxane receptor antagonist EP 092 (Armstrong et al, 1984).

In a plasma protein free suspension of rat platelets ($[Ca^{2+}]$ = 0.75 mM) three distinct types of activity, dependent on the AA concentration, were observed.
1. Between 2 and 10 μM AA, shape change and aggregation (usually irreversible) were elicited. These effects were completely inhibited by pre-incubation with either flurbiprofen (10 μM) or EP 092 (2.5 μM) and partially inhibited by dazoxiben (150 μM).
2. Between 20 and 100 μM aggregation responses to AA were markedly suppressed. When ADP was added 2 mins after AA its aggregatory action was inhibited.
3. Between 250 and 1000 μM AA irreversible aggregation insensitive to indomethacin, dazoxiben and EP 092 was obtained.

The washed platelets were sensitive to stable thromboxane mimetics, shape change, reversible and irreversible aggregation being produced. Half-maximal aggregation was produced by 2 μM 11,9-epoxymethano PGH_2 (U44619) and 0.02 μM EP 171 (see Jones et al, this meeting).

In citrated rat PRP the profile of activity is somewhat different. Between 15 and 300 μM AA produced only a shape change response which was blocked by flurbiprofen and EP 092. However, between 500 and 1000 μM AA irreversible aggregation associated with 5-HT release was obtained and this was unaffected by flurbiprofen and EP 092. Although shape change responses could be readily elicited by 11,9-epoxymethano PGH_2 and EP 171 only occasionally were small aggregation waves seen.

These studies show that (a) rat platelets can avidly utilize AA to produce sufficient PG endoperoxide/thromboxane A_2 to elicit irreversible aggregation on their own, (b) platelet inhibitory activity is generated from AA at intermediate concentrations. This is unlikely to be PGD_2 derived from PG endoperoxide since PGD_2 has no inhibitory effect on rat platelets (Smith et al, 1974), (c) only very high concentrations of AA produce a non-cyclooxygenase dependent aggregation. The mechanisms involved in (b) and (c) are under investigation.

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Nishizawa E.E. et al (1983) Thrombosis Res. 30, 289-296
Smith, J.B. et al (1974) Thrombosis Res. 5, 291-299

AN EXTREME OF THROMBOXANE-LIKE ACTIVITY

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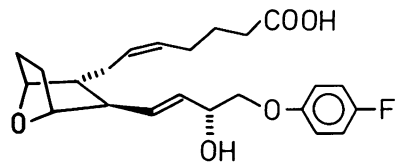
The substitution of a p-fluorophenoxy group for the terminal n-butyl unit of prostanoids which are either very weak agonists (e.g. PGD_2) or partial agonists (9,11-etheno PGH_2) on thromboxane-sensitive preparations generates potent full agonists (Jones et al, 1979). Does this enhancing effect also apply to thromboxane mimetics which already have high activity? We have synthesized and tested the analogue shown below.

EP 171



EP 171 is the most potent thromboxane A_2 mimetic reported to date. EC_{50} values and activities relative to the natural chain compound (Sprague et al, 1983) are as follows: rabbit aorta 0.15 nM, x 60; dog saphenous vein 0.35 nM, x 20; guinea-pig trachea 0.30 nM, x 35; bullock iris sphincter 0.07 nM, x 40. Using washed human platelets shape change responses are elicited between 0.02 and 0.4 nM, and aggregation waves between 0.4 and 2 nM. However, these responses have much slower time courses than those of any other thromboxane mimetic (e.g. 9,11-epoxymethano PGH_2). Shape change typically begins 10 - 15 s after addition and requires 50 - 75 s to reach a maximum. Above 10 nM EP 171 'fast' responses are seen.

Several observations suggest that the basic mechanism of action of EP 171 on the human platelet is similar to that of other thromboxane mimetics.
(a) Using the lumi-aggregatometer the release reaction induced by EP 171 is delayed, but its onset relative to the magnitude of primary aggregation is the same as for the fast-acting agonists.
(b) In platelets labelled with quin 2 ($[Ca^{2+}]_o$ = 1 mM), EP 171 (1 - 25 nM) produced concentration-dependent rises in internal calcium. [^{32}P]-Phosphatidate is correspondingly increased up to a maximum of 7-fold over basal. Relevant data for 9,11-epoxymethano PGH_2 can be found in Pollock et al (1984).
(c) Shape change and aggregation induced by EP 171 are blocked by the thromboxane receptor antagonists EP 045 (1 μM) and EP 092 (0.1 μM) (Jones et al, 1983), whereas indomethacin (10 μM) has little effect.

It is possible that the singularly slow activation of platelets by EP 171 reflects a slow approach to equilibrium occupation of thromboxane receptors. This might be expected from a high affinity agonist present in the biophase at very low concentration.

Jones et al (1979) Chemistry, Biochemistry and Pharmacological Activity of Prostanoids pp 210-220, Pergamon Press
Jones et al (1983) Adv.Prostaglandin, Thromboxane & Leukotriene Res. 11,345-350
Pollock et al (1984) Biochem.J. 219, 833-842
Sprague et al (1983) Adv.Prostaglandin, Thromboxane & Leukotriene Res. 11, 337-343

EFFECTS OF SERUM AND THYROID HORMONE ON THE VOLTAGE-DEPENDENT Na^+ UPTAKE OF CULTURED CEREBELLAR NEURAL CELLS

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Thyroid hormone affects the maturational increase in Na^+,K^+-ATPase activity in the cerebellum (Valcana & Timiras, 1969). Na^+,K^+-ATPase-related Na^+ fluxes, voltage-dependent Na^+-channels and 'passive' Na^+-permeability are involved in the maintenance of the bioelectrical activity of excitable cells (see Catterall, 1982). Cerebellar interneurons in culture show developmental increases in all these parameters (Atterwill et al, 1983; Beale et al, 1980) but hitherto only the effect of thyroid hormone on the Na^+,K^+-ATPase activity has been investigated. We now sought to examine the effect of T_3 on the voltage-dependent and veratridine-insensitive Na^+ uptake using the ^{22}Na flux assay developed by Catterall.

Cultures were obtained from 6-8 day old rat cerebella as previously described (see Atterwill et al, 1983). Cells were dissociated and plated on 35mm plastic dishes precoated with 5µg/ml poly-L-lysine (cerebellar granule neurones), or directly on plastic (cerebellar astrocytes) at a density of 2.5×10^6 cells/dish. Neurones were cultured in either a serum supplemented medium (SSM) containing 25mM KCl and 10% foetal calf serum (FCS), or a serum-free, chemically-defined medium (CDM) as described previously (Atterwill et al, 1983). 10µM Cytosine arabinoside was added to the SSM, but not the CDM cultures after 18h. For the studies of hormone action CDM cultures were treated with 2nM T_3 every 48 hours. The ^{22}Na flux assay was performed as described by Beale et al (1981). Veratridine and ouabain were used at a concentration of 100µM and 5mM respectively. The final concentration of $^{22}NaCl$ was 5mM.

In all culture conditions studied, the veratridine-sensitive component accounted for between 83 and 87% of the total ^{22}Na uptake at 7 DIV in the granule neurones. Comparing cells cultivated in the presence or in the absence of serum, there was no difference in either the rate of voltage-dependent or 'passive' veratridine-insensitive Na^+ uptake. However, whereas thyroid hormone failed to alter the veratridine-sensitive component in CDM cultures, it caused a significant 33% increase in the 'passive' ^{22}Na flux. Na^+ fluxes were also investigated in cultured astrocytes first grown in the SSM medium and then transferred to the serum-free, CDM medium (and treated with T_3). No veratridine-sensitive ^{22}Na-flux was detectable in the astrocyte cultures grown in the serum-free medium. Furthermore, T_3 had no effect on ^{22}Na uptake in these cultures.

In conclusion, we have shown that T_3 treatment of serum-free cultures of cerebellar granule cells does not alter veratridine-sensitive Na^+-uptake but does affect the 'passive' Na^+ permeability of the cell membrane. Furthermore, the much reduced manifestation of stimulation-coupled glutamate release from granule neurones in serum-free medium compared with those cultured in the presence of serum (Gallo, V unpublished) does not correspond to alterations in the expression of veratridine-sensitive Na^+ channels.

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Valcana, T. & Timiras, P.S. (1969) J. Neurochem., 16, 935-943.

This work was carried out at the MRC Developmental Neurobiology Unit, Institute of Neurology, London.

150P

SIMILAR EFFECTS OF ACETYLCHOLINE AND ADENOSINE ON CYCLIC GMP LEVELS IN GUINEA PIG ATRIA

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George et al (1970) showed an inverse relationship between force of contraction and intracellular cyclic GMP (cGMP) levels after administration of acetylcholine (ACh) to the perfused rat heart. Since then, however, further studies in various myocardial preparations have yielded conflicting results (eg, Linden & Brooker, 1979). The present investigation sought to confirm the effect of ACh on cGMP levels in paced guinea-pig atrial preparations and also to compare the effect with that produced by adenosine, another endogenous agent with very similar negative inotropic actions.

Guinea-pigs (300-600g) were stunned and exsanguinated, and both atria removed. These were placed in Krebs solution gassed with 95% O_2, 5% CO_2 at 32°C and electrically paced by adjacent platinum electrodes with a 3 mm separation at a frequency of 3.3 Hz, voltage twice threshold and pulse width 0.5 ms, developed tension being measured isometrically. Initially dose-response curves were constructed for ACh and adenosine in order to determine the concentration of each drug giving a 75% reduction in force of contraction. This concentration was used subsequently in the cGMP studies.

Atria were set up and paced for a 90 min pretreatment equilibration period, the drug under test was then added and after 2 min when the negative inotropic effect was established the atria were rapidly removed, frozen in liquid nitrogen and freeze dried for 2h. A number of atria thus prepared were pooled (n = 2 - 4), homogenised in 0.47 M perchloric acid and the supernatant assayed for cGMP using a radioimmunoassay kit (Amersham International). Samples were also assayed for protein content (Bradford, 1976).

As can be seen from Table I, ACh and adenosine, at equi-active concentrations with respect to effects on contractility, elevated tissue cGMP levels more than twofold. With respect to this action on cGMP levels ACh and adenosine did not differ significantly ($p < 0.05$) in their effects.

Table I Effects of ACh and adenosine on cGMP levels [pmol/mg protein : means ± s.e.m.] in paced guinea-pig atria.

	Control	ACh (0.8 μM)	Adenosine (112 μM)
cGMP	0.188 ± 0.007 (11)	0.395 ± 0.028 (11)	0.389 ± 0.026 (11)

It appears that although ACh and adenosine act via different membrane receptors, namely muscarinic and P_1 purinoceptors respectively, these agents initiate common actions both on atrial contractility and on intracellular cGMP levels. It is of interest that both are also known to activate increased potassium conductance; however, the link between these various events is not clear. There is certainly good evidence that cGMP per se is not the direct mediator of the actions of ACh on either potassium conductance (Trautwein et al, 1982) or contractility (Diamond & Chu, 1984).

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EFFECTS OF CALCIUM REMOVAL ON RESPONSE OF LUNG PARENCHYMAL STRIPS TO KCl, HISTAMINE, CARBACHOL OR ANTIGEN

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The isolated guinea-pig lung parenchymal strip has been extensively used in the assessment of the effects of drugs on the peripheral airways. We have examined the effect of reducing calcium on the contractile response of the parenchymal strip to KCL, carbachol, histamine and antigen challenge under isotonic conditions. Cummulative dose-response curves are difficult to repeat on the same tissue (Brink et al, 1981). Hence the experimental conditions were designed with this in view.

Male guinea-pigs (Dunkin Hartley, 200g), sensitised to egg albumin (1mg i.p.) for 21 days, were killed by dislocation of the neck and exsanguinated. The lungs were perfused via the pulmonary artery with Krebs bicarbonate solution at room temperature to remove excess blood. Four strips were cut from the lower lobes. Each strip was approximately 20mm long and 3mm wide. Each strip was blotted dry and trimmed until the wet weight was 80-90mg and was set up in a 7ml organ bath containing Krebs bicarbonate solution at 37°C gassed with 95% CO_2 / 5% O_2, and attached to a Hugo Basile isotonic transducer under 400mg tension. Initially, all strips were subjected to an extra tension of 600mg for 30 minutes after which the extra weight was removed and the tissue was allowed to equilibrate for a further 30 minutes. During this period the tissue was washed every 15 minutes. After this, the tissue was dosed cummulatively with either KCL, histamine or carbachol; upon reaching the maximum response the tissue was washed and the above equilibration procedure repeated before recording a second cummulative dose response curve. On any tissue, only 2 dose response curves were determined. Using sensitised parenchymal strips, it has been shown that:

reproducible dose-response curves can be obtained on separate tissues to carbachol, histamine and KCL if expressed in terms of the maximum response to KCL,
if tissues were washed in calcium free Krebs bicarbonate , the response of the tissue to histamine and antigen were unaltered. However the responses to carbachol and KCL were reduced to 47% and 64% of their respective control responses,
if EGTA (2.5mM) was added to calcium free Krebs bicarbonate fluid the response to antigen was abolished: a response to KCL and carbachol could only be obtained with the highest concentrations of these substances; the dose-response curve to histamine was displaced to the right and the maximum response was reduced by 58%.

In conclusion, a) simply washing the tissue in calcium free Krebs bicarbonate fluid is insufficient to remove all extracellular calcium, b) histamine appears to utilise both sources of calcium whereas with carbachol and KCL mainly extracellular calcium is important.

Brink et al., 1981 J. Pharmac. Exp. Ther., 291, 1.

THE RELEASE OF ENDOTHELIUM DERIVED RELAXANT FACTOR IS CALCIUM DEPENDENT

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The discovery by Furchgott (1980) that acetylcholine (ACh) induced relaxation in blood vessels is entirely dependent upon an intact endothelium has led to a considerable effort to discern the nature of the signal to the underlying smooth muscle cells. The relaxation of vascular preparations by many other drugs has also been shown to be dependent on the endothelium (for review see Furchgott, 1984). A series of experiments by Griffith et al. (1984) has shown that the signal is capable of expression in cascade systems and that the relaxation signal is a humorally released factor. The endothelium derived relaxant factor (EDRF) has been shown to have a very short half life (6 secs) in cascade systems (Griffith et al., 1984) thus making difficult any investigation into the nature of EDRF, its identity and its release mechanism.

In this investigation we utilised a cascade system in which a de-endothelialised rat aortic strip was mounted isometrically to act as a bioassay for EDRF. The bioassay strip was superfused with Krebs solution which had passed through an intact (endothelium bearing) aorta. The bioassay was also simultaneously perfused with a Krebs solution containing 2μM noradrenaline (NA). The two perfusion systems mixed prior to the bioassay and were arranged such that the time delay between endothelium and bioassay was 2 seconds. The bioassay strip was de-endothelialised by gentle abrasion with filter paper and the effectiveness of this technique was tested by the lack of a relaxant response to 1μM ACh after pre-constriction with 1μM NA. Additionally, the status of the endothelium in both the bioassay strip and the intact aorta were determined histologically after staining with the method of Griffith et al. (1984). We found that the de-endothelialisation technique was effective in removing practically all of the endothelium whilst the endothelium of the intact aorta covered virtually the entire luminal surface of the vessel.

100μM ACh perfused through the intact aorta relaxed the bioassay by 23.6±7.9% (n = 4) of its precontraction tension indicating that EDRF had been released from the intact aorta. If the perfusion through the intact aorta was changed to be essentially calcium free Krebs (calcium omitted) while the other perfusate contained 5mM calcium (maintaining $[Ca^{2+}]$ at the bioassay of 2.5mM) there was no relaxation in response to ACh. When the $[Ca^{2+}]$ through the entire system was restored to 2.5mM whilst maintaining the ACh perfusion, a relaxation of 52.0±17.2% (n = 4), indicating a release of EDRF, occurred immediately.

We therefore conclude that the release of EDRF is calcium dependent.

We thank the British Heart Foundation for support.

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EFFECTS OF ETHANOL AND MEPACRINE ON ARACHIDONATE MOBILISATION IN THROMBIN AND A23187-STIMULATED PLATELET PHOSPHOLIPIDS

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Ethanol inhibits platelet aggregation and the release reaction in vitro. This may be due to inhibition of phospholipase A_2 (PLA_2) because in unstirred platelets ethanol reduces the release of arachidonic acid (AA) from phosphatidylcholine (PC) but not from phosphatidylinositol (PI) in response to thrombin (Fenn & Littleton, 1984). We extend these findings to stirred platelets and to A23187 stimulation, and compare ethanol with mepacrine.

Platelets from human volunteers were washed in the presence of 300 ng/ml prostacyclin and incubated at 37°C for 60 min with 0.08 µCi/ml ^{14}-C AA (50 ng/10^8 platelets) before the final wash. Stirred platelets were allowed to aggregate in a Payton aggregometer after 2 min drug preincubation, and phospholipids were extracted and analysed by radio-t.l.c.

		Aggreg^n	PC (45.8 ± 2.6%)*	PI (22.1 ± 1.3%)*
2	Thrombin (0.1-0.5 U/ml)	++	-11.5 ± 3.5	-28.0 ± 4.6
3	+ ethanol	+	-3.3 ± 1.9	-27.5 ± 3.0
4	+ mepacrine	0	+5.8 ± 2.1	-4.5 ± 4.1
5	A23187 (5-10 µM)	++	-12.6 ± 5.4	-31.2 ± 1.0
6	+ ethanol	+	+4.0 ± 10.2	-29.3 ± 8.9
7	+ mepacrine	0	-3.0 ± 5.4	-21.6 ± 3.1

++ = full aggregation, + = partial aggregation. Values in PC and PI columns indicate percent change in proportion of AA found in that phospholipid zone. * = proportion of AA taken up into that phospholipid class after lipid labelling.

Results are shown in the Table. Most AA was incorporated into PC and PI (see line 1), with less (about 10-12% of the total) in phosphatidyl-serine and -ethanolamine. Non-esterified AA was < 5% of extracted radiolabel. Thrombin and A23187 caused mobilisation of AA from PC and PI (lines 2 & 5); this is presumed to reflect PLA_2 and PLC (phospholipase C) activity, respectively. PC but not PI hydrolysis was inhibited by 100-200 mM ethanol (lines 3 & 6) and 50 µM mepacrine (lines 4 & 7), although PI and thrombin/A23187 was an exception (line 4). Thus there is no clearcut correlation between phospholipase inhibition and aggregation.

In conclusion, both ethanol and mepacrine inhibit PLA_2, but PLA_2 activation is not a prerequisite for platelet aggregation (lines 3 & 6). Similarly, activation of PLC and aggregation are not inseparable since mepacrine fully inhibited A23187 aggregation without substantially blunting release of AA from PI (line 7). This may reflect the different mechanisms of platelet activation by thrombin and A23187.

Fenn, C.G. & Littleton, J.M. Br. J. Pharmacol. 81 175P (1984).

Supported by an MRC studentship to S.H.P.

CENTRAL α_2-ADRENOCEPTOR FUNCTION FOLLOWING ACUTE AND CHRONIC DOSING WITH IMILOXAN

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Imiloxan (RS-21361) is a selective α_2-adrenoceptor antagonist as determined in both the pithed rat (Michel et al, 1981) and in the isolated transversely bisected, rat vas deferens preparation (Michel & Whiting, 1981).

Brown et al (1984) have demonstrated a significant 'down regulation' of β-adrenoceptors in rat cerebral cortex following chronic twice daily administration of imiloxan over a period of 10 days. This study examines the central α_2-adrenoceptor function in conscious rats following acute and chronic administration of imiloxan as demonstrated by a reversal of the effect of clonidine on locomotor activity.

Male Sprague Dawley rats (100-150 g bodyweight) were orally dosed twice (9pm/9am) or twice a day (9pm/9am) for 14 days with either imiloxan (100 $mg.kg^{-1}$) or distilled water (10 $ml.kg^{-1}$). Activity was measured over a 10 min period using an Ormed Actisystem in which both test and control animals could be tested at the same time. Activity was measured at 30, 60 and 90 min following the 9am dose. Clonidine (0.3 $mg.kg^{-1}$ p.o.) was administered to all the animals 30 min before their activity was measured. Brain samples were taken following the activity measurements and analysed for imiloxan levels, by HPLC with U.V. detection following solvent extraction.

Table 1 shows the results of the acute and chronic dosing. In the acute study there was a significant reversal of the clonidine-induced decrease in locomotor activity only at 30 min post-dose, whereas following the chronic dosing there was a significant reversal at all three time points. The brain imiloxan levels in the chronically and acutely treated animals at 60 and 90 min were significantly lower ($p < 0.001$) than the 30 min levels.

This study shows that chronic administration of imiloxan prolongs the duration of the reversal of clonidine-induced decrease in locomotor activity. Further, this is possibly due to an increase in the catecholamine sensitivity or receptor 'down regulation' rather than a prolonged α_2-adrenoceptor blockade due to compound accumulation in the CNS as the levels are not maintained after 30 min.

Table 1 Activity measurements following acute and chronic dosing of imiloxan

	ACUTE DOSING		CHRONIC DOSING	
POST DOSE	IMILOXAN ($100\ mg.kg^{-1}$ x 2)	CONTROL	IMILOXAN ($100\ mg.kg^{-1}$ x 28)	CONTROL
30 min	463 ± 59* (n = 6)	219 ± 65 (n = 6)	638 ± 44** (n = 12)	325 ± 41 (n = 12)
60 min	292 ± 56 (n = 6)	194 ± 37 (n = 6)	570 ± 50* (n = 12)	352 ± 35 (n = 12)
90 min	165 ± 33 (n = 6)	228 ± 31 (n = 6)	616 ± 37* (n = 11)	421 ± 28 (n = 12)

Significant difference from controls using paired 't' test:
*$P < 0.01$ ** $P < 0.001$

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COMPARISON OF CHOLINERGIC ACTIVITY OF SUBERYLDICHOLINE WITH THAT OF CARBACHOL IN VITRO

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Suberyldicholine has been reported to exhibit full agonist activity at ileal muscarinic receptors (mACHRs) and no activity at mACHRs mediating aortic vasodilation (Eglen & Whiting, 1984). This apparent selectivity may either reflect different efficacies of the compunds or receptor heterogeneity of tissues. This study has further investigated the agonist activity of suberyldicholine and carbachol at mACHRs in a wider range of tissues.

Agonist potency (pD_2) of suberyldicholine and carbachol was determined in guinea-pig uterus, bladder, trachea and paired atria (paced and unpaced). The antagonist affinity (pK_B) of atropine (1×10^{-8} mol.litre^{-1}) at ileal mACHRs against both suberyldicholine and carbachol was also determined (Clague et al, 1984). The action of either carbachol or suberyldicholine at ganglionic nicotinic receptors was studied before and after hexamethonium (1×10^{-4} mol.litre^{-1}) incubation. The efficacy of the agonists was measured at ileal mACHRs using the irreversible muscarinic antagonist, propylbenzlylcholine mustard (pr-BCM), after the method of Furchgott and Bursztyn (1967).

Carbachol, exhibited similar agonist potencies (pD_2 range was 6.4 - 6.7) in all preparations used. Conversely, suberyldicholine was a full agonist only at ileal mACHRs and a partial agonist at mACHRs mediating negative inotropic effects. NO activity was observed in the other tissues.

The affinity of atropine at ileal mACHRs was similar when either suberyldicholine (pK_B = 8.60 $\pm$ 0.12, mean $\pm$ sem, n = 4) or carbachol (pK_B = 8.59 $\pm$ 0.10, mean $\pm$ sem, n = 4) was used. This indicates that both agonists act on mACHRs

However, preincubation of ileal tissue with hexamethonium, although not affecting the potency of carbachol, did abolish the actions of suberyldicholine. This indicates that suberyldicholine, in contrast to carbachol, possesses activity at nicotinic receptors in this preparation.

Finally, although both agonists were full agonists at ileal mACHRs, their respective potencies differed(carbachol pD_2 = 6.40 $\pm$ 0.10, suberyldicholine pD_2 =5.58 $\pm$0.05 mean $\pm$ sem, n = 4). Estimation of their dissociation constants using pr-BCM showed that carbachol possessed a lower affinity than suberyldicholine at ileal mACHRs (Carbachol pK_A = 3.99 $\pm$ 0.04, suberyldicholine pK_A = 5.70 $\pm$ 0.05, mean $\pm$ sem, n=4.) Consequently, the efficacies of these agonists were also very different (carbachol = 256, suberyldicholine = 1.8).

In summary, suberyldicholine is inactive at mACHRs present in a number of tissues, in addition to the aorta. The compound produces a response at mACHRs present in the ileum, and to a lesser extent in the atria. We conclude that this apparent selectivity is ascribable to the low efficacy of the compound and not to any selectivity for the ileal mACHR subtype.

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156P

TISSUE SIZE IS A MAJOR FACTOR INFLUENCING TISSUE SELECTIVITY STUDIES WITH NIFEDIPINE

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Apparent tissue selectivity for calcium antagonists has been assessed by comparing EC50 values for relaxant effects in several tissues (Flaim, 1982). However, binding studies for dihydropyridines have revealed only small differences between K_D values in different tissues (Janis & Scriabine, 1983). This report shows that functional studies can be biased by tissue size.

Longitudinal strips of taenia coli, portal vein and bladder muscle, helical strips of aorta, mesenteric and renal artery and single tracheal rings were dissected from albino male rabbits (1.5 - 2.5 kg) and mounted in 10 ml organ baths filled with Tyrode solution at 35°C, gassed with 95%: 5% CO_2. The responses to taenia, trachea and bladder preparations were recorded isotonically (1 g load) whereas the responses to the other preparations were recorded isometrically (1 g resting tension). After a 30 min incubation period the tissues were contracted with a K^+-depolarizing solution (mM: NaCl 97, KCl 40, $CaCl_2$ 1.8, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5) and when the contractile response had stabilized nifedipine (1-1000 nM) was added cumulatively. Nifedipine responses were expressed as percentages of the response to papaverine (30 µM). The tissues were blotted and weighed at the end of the experiment. The sensivities of the preparation to nifedipine and their weights are shown in Table 1.

Table 1. Comparison of nifedipine EC50s (nM) in rabbit tissues with the weight of the tissues. The values are the mean ± s.e. mean.

Tissue	EC50, nM	Weight, mg	n
trachea	2.3 ± 0.6	19.7 ± 4.3	10
portal vein	3.3 ± 1.2	36.2 ± 11.0	6
mesenteric artery	4.2 ± 0.9	16.0 ± 3.1	8
renal artery	5.7 ± 2.1	6.2 ± 1.1	8
taenia	8.8 ± 1.8	17.6 ± 3.6	14
aorta	25.4 ± 4.1	31.4 ± 4.5	19
bladder	125.5 ± 32.4	267.9 ± 40.8	26

Although the trachea and portal vein preparations were clearly the most sensitive, there was a significant ($p < 0.05$) correlation between the mean EC 50s and tissue weight. Within tissues, the bladder, with the greatest weight range, showed the highest correlation ($r = + 0.594$, $p = 0.001$) which was further increased by correlating ln weight against ln EC50 ($r = + 0.879$, $p < 0.0001$). Adding additional bladder tissue to the bath also reduced sensitivity to nifedipine. Small bladder preparations were quite sensitive to nifedipine (EC50 7.1 ± 1.2 nM; weight 61.8 ± 7.0 mg, n=5) indicating that the amount of tissue is a crucial factor. The correlation (ln weight against ln EC50) was also marked in the taenia ($r = + 0.579$, $p = 0.029$) and in the aorta ($r = + 0.494$, $p = 0.032$) but was not significant in the smaller tissues perhaps due to the small weight ranges. Thus the amount of tissue influences sensivity to nifedipine, which may reflect a site of loss in the muscle reducing the concentration at the Ca^{++} channels.

I thank Mrs C. Berg for technical assistance.

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EFFECTS OF INTRAVENOUS AND INTRASPLENIC INFUSIONS OF GLUCAGON ON CARDIAC OUTPUT AND ITS DISTRIBUTION IN THE RAT

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Glucagon has been reported to increase liver blood flow in man (Feruglio et al, 1966) and dog (Hulstaert et al, 1974). Ohnhaus (1972) showed that bolus intravenous (i.v.)injections of glucagon increased renal blood flow and also blood flow to some organs in the gastrointestinal tract in the rat. He used a rubidium clearance technique but did not carry out simultaneous determination of cardiac output (CO). Accordingly it was not possible to ascertain to what extent the increases in tissue blood flow were the result of the increased CO produced by the hormone (Glick et al, 1968) rather than changes in its distribution. Here we report the effects of a single rate of glucagon infusion given to rats either i.v. or into the spleen (i.sp.). The latter was an attempt to correspond to its physiological delivery into the hepatic portal vein.

Cardiac output and its distribution were determined using 15.7±0.3μm diameter plastic microspheres labelled with ^{113}Sn (NEN) as described by Hiley et al (1980) in pentobarbitone anesthetised (60mg/kg) male Wistar rats (220-240g; Bantin & Kingman, Hull) after they had received a 10 min. infusion of glucagon (2μg/kg/min) or saline (20μl/min) either i.v. or i.sp.

The i.v. infusion of glucagon increased heart rate to a greater extent than i.v. saline and CO was 23% greater ($P<0.05$; Student's t-test) in the rats receiving glucagon (20.6±1.6ml/min/100g body wt, n=6) relative to the controls (16.7±1.0ml/min/100g body wt, n=6). The percentages of the CO passing to the heart (control, 7.7±0.7%; glucagon, 10.2±0.9%)and kidneys (control, 12.8±1.6%; glucagon, 16.7±1.0%) were also significantly increased ($P<0.05$). The increased CO resulted in greater blood flow per unit mass of tissue in pectoral skeletal muscle, testes, spleen, stomach, small intestine and liver although these organs received no greater share of the CO.

CO was not significantly changed by the i.sp. infusion of the same dose of glucagon relative to that in rats given i.sp. saline (26.6±1.6 and 29.4±1.6ml/min/100g body wt respectively for the control, n=7, and glucagon, n=6, groups) nor were there changes in the fractions of the CO received by the heart and kidneys. However, the percentages of the CO passing to the stomach (control, 0.74±0.07%; glucagon, 1.16±0.16%) and small intestine (11.7±1.2%, control; 16.1±1.5%, glucagon) were significantly greater ($P<0.05$; Student's t-test) in the animals given the hormone. Blood flow per unit mass of tissue was significantly increased by i.sp. glucagon only in the stomach, small intestine and liver ($P<0.05$; Student's t-test).

These observations show that the single infusion rate used for glucagon had different effects when given directly into the hepatosplanchnic vascular region rather than i.v. and that, although given "downstream" to the gastric and superior mesenteric arterial beds by infusion into an organ draining into the hepatic portal vein, it is capable of a selective increase in their blood flow.

AJN is an MRC research student.

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