STUDIES ON EXPERIMENTAL IMMUNE COMPLEX (IC)-INDUCED COLITIS: EFFECT OF PREDNISOLONE.

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Prednisolone is a drug, highly effective in the treatment of ulcerative colitis in man. However, prednisolone has been reported to have little effect in some widely studied animal models of colitis. These include dinitrochlorobenzene-induced cell-mediated immune colitis (Norris et al, 1982) and acetic acid-induced colitis (Zeitlin & Norris, 1984), both in guineapigs. While in carrageenan-induced colitis in rats, prednisolone (5mg/kg) is used to enhance the ulcerogenic action of carrageenan (Parke et al, 1986). We now report that experimental IC colitis, induced in mice and rabbits by the method of Hodgson et al (1978), is susceptible to the anti-colitic actions of prednisolone.

The colitis was induced in male BKA mice (20-30 g) or male New Zealand White rabbits (2.0-2.5 kg) by i.v. injection of pre-formed immune complexes of human serum albumin (HSA) and rabbit anti-HSA, concomitantly with intra-rectal formalin (1%) as described by Hodgson et al (1978). Prednisolone (0.67, 4.0, & 30mg/kg) or vehicle alone were given i.p. daily for 3 days prior to induction of colitis and subsequently for a further 5 days. Animals were killed and distal colon was excised. Samples were taken for "blind" histopathological assessment and measurement of oedema. Oedema was determined as increase in tissue water content expressed as a percentage of mean non-inflamed control.

Table 1. Inhibition of oedema determined as change in tissue water content in colon from prednisolone or vehicle-treated colitic mice compared with mean healthy control value. Values given as Mean and SD (bracketed), N=6.

Prednisolone (mg/kg, daily)	Healthy Control Tissue Water (A) (g/g tissue)	Colitic Tissue Water (% diff. from mean A value)	Inhibition (%)	P value (U-test)
0	0.57 (0.05)	32 (4)	0	
0.67	0.57 (0.05)	23 (12)	28	
4.0	0.59 (0.02)	22 (9)	31	< 0.05
30	0.58 (0.04)	10 (8)	69	< 0.05

Dose-related reduction ($P < 0.05$) in colonic mucosal oedema was seen in the prednisolone-treated mice following induction of colitis, although this did not reach statistical significance with the lowest dose (Table 1). Prednisolone-treated animals also showed a reduction in the histopathological features of inflammation assessed blind by a clinical pathologist. A similar suppression ($P < 0.05$) of the colitic oedema response was seen in the rabbits given prednisolone (0.5 mg/kg daily) compared with colitic animals given vehicle alone.

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DECREASE IN β-ADRENOCEPTOR DENSITY DURING LIPID PEROXIDATION.

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The peroxidation of lipids in biological membranes is a destructive phenomenon that can be elicited in various ways. Surface receptor molecules that allow cells to respond to hormones are possibly inactivated during lipid peroxidation. Oxygen radicals may modify the beta-adrenergic response and have also been suggested as a major cause of some heart and lung diseases and aging. We investigated the effect of lipid peroxidation on β-adrenoceptor of lungs and erythrocytes of the rat. The results of this study may be explained by means of the effect of diffusible aldehydes like 4-hydroxy-trans-2,3 nonenal (HNE), which are formed during lipid peroxidation, on sulfhydryl moieties of the β-adrenoceptors, therefore we also investigated the role of HNE.

Membranes of lungs (containing both β_1- and β_2-adrenoceptors)and intact erythrocytes (containing homogeneous β_2-adrenoceptor population) were pretreated with cumene hydroperoxide (lungs with 0.1 mM and erythrocytes with 1 mM) and Fe^{2+} (1×10^{-5} M) for 60 min, which resulted in extensive lipid peroxidation measured as malondialdehyde formation. Lipid peroxidation was shown to reduce beta-adrenoceptor density. The latter was determined with (-)-[^{125}I]-Iodocyano-pindolol and for the estimation of non-specific binding (-) timolol (1×10^{-6} M) was used. In membranes of erythrocytes B_{max} values (fmol /mg protein) and K_d values (pM) change from 947±72 (B_{max} control) and 26±5 (K_d control) to 307±47 and 42±10. Remarkably, the ratio of β_1:β_2 adrenoceptor density in lung membranes did not change and remained 30% : 70%. Pretreatment of lung membranes with 1 mM HNE led to a time dependent inhibition of 50% of specific [^{3}H]-dihydroalprenolol binding. The effect of HNE was also concentration dependent (0.1-2.5 mM).

Summarizing, lipid peroxidation decreased the number of β-adrenoceptors. Not only a change in lipid packing of the membrane but also the effect of HNE may explain our data.

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DECREASE OF α_1-ADRENOCEPTORS AND H_1 HISTAMINE RECEPTORS ON RAT LIVER PLASMA MEMBRANES AFTER CCl_4 PRETREATMENT.

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Maintenance of intracellular calcium ion homeostasis is of utmost importance for the function of the hepatocyte. An extreme rise in cytosolic Ca^{2+} concentration triggers cytotoxicity. During the biotransformation of xenobiotics perturbation of intracellular Ca^{2+} homeostasis may occur. Moreover, changes in intracellular thiol concentrations are accompanied by changes in the Ca^{2+} homeostasis (Jewell *et al.*, 1982). Physiological low concentrations of Ca^{2+} in the cytosol of hepatocytes are maintained by active compartmentation processes and are regulated by α_1-adrenoceptors. Recently, high densities of histaminergic H_1-receptors have been established on rat liver membranes (Imoto *et al.*, 1985). H_1-receptors have also been suggested to play a role in the management of Ca^{2+} homeostasis. In this study we investigated the effect of the toxic agent CCl_4 (Haenen & Bast, 1983), which is known to cause intracellular changes (lipid peroxidation) on α_1- and H_1-receptor densities.

Male Wistar rats were pretreated with CCl_4 via oral administration of a solution (50% v/v in oil, 5 mg/kg). After 18 hrs liver plasma membranes were prepared basicly according to Armstrong & Newman (1985). [^{3}H]-Prazosin (60 Ci/mmol, 1.5×10^{-9} M) in competition experiments with prazosin (2×10^{-11}-10^{-7} M), incubation time 30 min at 25°C was used to determine the amount of α_1-adrenergic receptors. [^{3}H]-Mepyramine (20 Ci/mmol, 5×10^{-10} M) in competition experiments with mepyramine (10^{-10}-10^{-6} M) incubation time 30 min at 25°C was used to quantify the amount of H_1-histaminergic receptors.

The number of H_1-receptor binding sites decreased after CCl_4 pretreatment from (B_{max} fmoles/mg protein) 23639 (control) to 6125 (CCl_4). The K_d value (nM) did change significantly, 6.3 (control) to 5.9 (CCl_4). Preliminary data indicate that the density of α_1-adrenergic receptors decreased after pretreatment with CCl_4 as well. Our data suggest that compounds which perturbate intracellular Ca^{2+} homeostasis may also reduce the density of receptors involved in the regulation of Ca^{2+} mobilization.

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COMPARISON OF THE INTERACTION OF ORPHENADRINE AND NEFOPAM WITH CYTOCHROME P-450 AND THE GLUTATHION-SYSTEM OF RAT LIVER.

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Orphenadrine and its cyclic analogue nefopam are both, in combination with paracetamol, applied as analgesics clinically. In this study we examined the effects of both drugs on several mechanisms, that are involved in the hepatotoxicity of paracetamol.
It is known (Bast *et al.*, 1983) that orphenadrine gives enzyme induction in rat, but that it also inactivates several cytochrome P-450 isoenzymes by the formation of an irreversible metabolic-intermediate (M.I.)-P-450 complex, which can be detected spectrofotometricly. However, nefopam administered i.p. for five days (20 mg/kg on the first day, on the other days 30 mg/kg), does not give an enzym induction and does also not produce a M.I.-complex in vivo. In phenobarbital induced (PB) rat hepatic microsomes however, nefopam produces a M.I.-complex, which is formed after N-demethylation of nefopam, indicated by the observed M.I.-complexation of two of its major metabolites, nefopam-N-oxide and N-desmethylnefopam. The M.I.-complex formation represents the interaction of a nitroxide radical metabolite of nefopam with reduced cytochrome P-450. The M.I.-complex formation was shown to be dosedependent (1-100 μM) both for orphenadrine and for nefopam (optimum resp. at 33 μM and 25 μM). In contrast to the M.I.-complexation of orphenadrine the M.I.-complex formation by nefopam is affected by glutathione (GSH). A concentration of 0.1 mM GSH slightly reduced the M.I.-complexation by nefopam, whereas 0.5 mM GSH reduced the M.I.-complexation for approximately 50%. Also the M.I.-complexation of N-desmethylnefopam was affected by GSH, although to a much smaller extent. These results indicate different metabolic pathways for nefopam and orphenadrine.
After the in vivo administration of nefopam and orphenadrine no changes in the GSH and GSSG (glutathione oxidized) levels in the liver could be detected. There was also no change in the activities of several GSH-dependent enzymes, which might affect the hepatotoxicity of paracetamol. The activities of GSH-transferase (measured with 1-chloro-2.4-dinitrobenzene), Se- and non-Se-dependent GSH-peroxidase (measured with 15 mM H_2O_2 and 30 mM t-butylhydroperoxide) were unchanged after pretreatment with nefopam or orphenadrine.
Finally we studied the effects of orphenadrine and nefopam on the oxidase function of cytochrome P-450. In both control and PB-induced hepatic microsomes both drugs inhibit basal endogeneous hydrogen peroxide formation. The inhibition of hydrogen peroxide formation in control and PB microsomes was respectively 45% and 26% for orphenadrine (50 μM) and 35% and 15% for nefopam (50 μM).

In conclusion, this study indicates that nefopam decreases the hepatotoxicity of paracetamol in the rat, because it lacks any inducing effect on cytochrome P-450, has no effect on the protective GSH-system of the liver and it reduces the overall oxidative stress in the hepatocyte.

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SUPEROXIDE DISMUTASE ACTIVITY OF COPPER(II)-1,10-PHENANANTHROLINE AND COPPER(II)-2,9-DIMETHYL-1,10-PHENANTHROLINE.

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In organisms, molecular oxygen is reduced to water for 98%. Two percent turns into potentially toxic free radicals. These free oxygen radicals play a role in inflammation, toxicity of xenobiotics, radiation damage and ageing. Detoxification of superoxide anion radicals occurs by the enzyme superoxide dismutase (SOD). As copper seems to be directly involved in the dismutase reactions, we decided to test two copper complexes, copper(II)-1,10-phenanthroline ($CU(II)phen_2$) and copper (II)-2,9-dimethyl-1,10 phenanthroline for their SOD activity.

SOD-assays were performed in phosphate buffer (5o mM, pH 7.8, 10^{-4} M EDTA) containing xanthine (5×10^{-5} M) and xanthine oxidase (50 mU/Ml). Reduction of ferric cytochrome c (10^{-5} M) by superoxide anion radicals was monitored at 550nm. Lipid peroxidation (formation of thiobarbituric acid reactive material) experiments were done using hepatic microsomes of male Wistar rats, suspended in phosphate buffer (50 mM, pH 7.4, No EDTA). Lipid peroxidation was initiated by ascorbate (0.2 mM)/Fe^{2+} (10 µM), by cumene hydroperoxide (2 mM and 10 mM) or by H_2O_2 (2 µM - 0.5 mM).

The SOD activity of Cu(II)-1,10-phenanthroline and Cu(II)-2,9-dimethyl-1,10-phenanthroline is concentration dependent. A concentration of 30 µM of the compounds led to 35% and 3% of the maximal activity of SOD for the chelates respectively. The chelates reduced the extent of lipid peroxidation induced by ascorbate Fe^{2+} and stimulated cumene hydroperoxide induced lipid peroxidation. Remarkably, Cu(II)-1,10-phenanthroline stimulated lipid peroxidation produced by H_2O_2 whereas Cu(II)-2,9-dimethyl-1,10-phenanthroline caused an inhibition.

Difference in ultimate molecular structure of the chelates may account for the observed differences. In contrast to the sterically hindered disubstituted phenanthroline (E_o 594 mV) the parent compound (E_o 1.74 mV) forms a planar complex with Cu(II). The favourable aspects of $Cu(II)phen_2$ (SOD activity) are shadowed by its ability to induce lipid peroxidation. It is possible that the stimulation of H_2O_2 induced lipid peroxidation occurs via hydroxyl radicals as follows:

$$Cu(II)phen_2 + H_2O_2 \longrightarrow Cu(I)phen_2 + O_2^{\cdot-} + 2H^+$$
$$Cu(I)phen_2 + H_2O_2 \longrightarrow Cu(II)phen_2 + {}^{\cdot}OH + OH^-$$

BUCCAL DRUG ABSORPTION: AN APPROACH TO PREDICT pH DEPENDENT CHANGES IN BINDING, EXCRETION AND PASSAGE ACROSS MEMBRANES.

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Drugs cross lipid membranes to reach the site of action in the body. Their capacity to do so depends upon their lipid solubility, pKa and pH of body fluids across the membranes. However, the controlling factor influencing the extent of absorption is their innate lipophilicity (Beckett & Triggs, 1967).

Beckett and Triggs (1967) proposed that buccal absorption of drugs at various pH levels particularly pH 5 to 9 can be considered as an in vivo model for the passage of drugs across lipid membranes and that the shape of the buccal absorption-pH curves would give a better indication of the likely passage of drugs through lipid membranes than simple partition or rate of partition experiments between water and organic solvents, and proposed a classification to predict pH dependent renal excretion of basic drugs only qualitatively.

We suggest that the measurement of "area under the curve of buccal absorption versus pH (AUC)" will give an even better indication of the passage of drugs across lipid membranes. The changes in permeability of the buccal membrane can be measured per unit change of pH, giving a "coefficient of buccal absorption". The method can be applied both for acidic and basic drugs and the results can be expressed quantitatively.

$$AUC = \frac{\%BA\ pH5 + \%BA\ pH6}{2} \times (pH6 - pH5) + \frac{\%BA\ pH(n-1) + \%BA\ pHn}{2} \times [pHn - pH(n-1)]$$

The method was applied to 10 basic drugs whose buccal absorption at pH 5 to 9 and renal excretion in acid and alkaline urine have been studied (date obtained from the literature *). A linear relationship ($r=0.954$ $p<.001$) between AUC and log of the ratio of the amount of drug excreted in acid (Ac) and alkaline (Al) urine was observed (Table 1). A similar relationship ($r=0.871$ $p<.001$) was also observed between AUC and % plasma protein binding of some of these drugs (Table 1) because the more highly protein bound drugs tend to be lipophilic (Henry et al, 1974).

The possibility of application of the method for acidic drugs, the passage of drugs across other lipid membranes , i.e. a measure of innate lipid solubility and the toxicolocial significance of the results will be discussed.

Table 1. AUC, urine excretion (ug) & % protein binding of some basic drugs.

Drug	AUC	(n) **	pKa *	24 hr urine excretion (n) **			Ratio	%Protein *
				Ac	Uncon	Al	Ac/Al	binding
Procainamide	12.5	(4)	9.3	160	153	146(4)	1.096	15.5
Practolol	49	-	9.5	150.5	148.7	148(4)	1.017	21
Nicotine (14 hrs)	64.2	(1)	-	454.37	-	53.53(1)	8.47	-
Pindolol	68.5	-	9.3	-	-	-	-	54
Amphetamine	69.5	(1)	9.7	6841	2874.4	700.9(3)	9.76	-
Flecainide	70.4	(7)	9.3	123600	-	19000(6)	6.51	48
Pethidine	71.4	(1)	-	-	-	-	-	64
Mephentermine	73.5	(1)	-	3215.3	919.7	492.5(2)	6.53	-
Norfenfluramine	121.9	(1)	9.22	1731.2	924.4	56.3(1)	30.75	-
Propranolol	131.5	-	9.45	869.5	-	12.25(4)	70.98	91.09
Methadone	137.5	(1)	8.99	1786	867	46.2(1)	38.66	79
Mexiletine	159.2	(4)	4.66	87.3	-	0.77(4)	113.38	70

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* References can be obtained from the authors on request. ** Number of observations.

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ENANTIOSELECTIVE ELECTROPHYSIOLOGICAL PROPERTIES OF TOCAINIDE.

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Tocainide, an orally active antiarrhythmic drug, is used as a racemic mixture of the d- and l- optical antipodes. It has been shown that both chiral forms display a different pharmacokinetic behaviour in man (Conings & Verbeke, 1986). The present work deals with the selective effects of these enantiomers on the electrophysiological characteristics of the guinea pig papillary muscle. The preparation was superfused with a Tyrode solution containing a variable K concentration. Electrical activity was recorded by the intracellular microelectrode technique.

Racemic tocainide decreased maximal rate of depolarization (V_{max}) and shortened the action potential duration (ADP). The effects on V_{max} were frequency dependent, i.e. the dose effect relation was shifted to the left at higher rates of stimulation, and voltage dependent, i.e. more pronounced at elevated extracellular K (K_e) concentrations.

The differential effects of the two isomers (concentration 100 mg/ml) were studied on V_{max} in steady state (60/min) at different membrane potentials (i.e. variable K_e) during recovery from a stimulation. The results can be summarized as follows:

1) The inhibitory effect on V_{max} was greater with l-tocainide (38%; n=6) and less pronounced with d-tocainide (23%; n=7) than with the d,l-mixture (29%; n=5);
2) The shift to the left of the V_{max} / Em (membrane potential) relation was more pronounced with l-tocainide (11.5 mV; n=3) than with d-tocainide (6.6 mV; n=3);
3) The recovery followed a biexponential time course and appeared to be slower in the presence of l-tocainide. The number of experiments up to now are too restricted to allow a quantitative evaluation.

Since the profile of therapeutic activity of class I type antiarrhythmics is dependent upon their interactions with the electrical activity of cardiac cells, one can postulate that differences in electrophysiological and pharmacokinetic properties together should lead to variations of the clinical response in function of the mode of administration, the fluctuations of the steady state blood levels of both steric isomers, etc...

Conings, L. & Verbeke, N. (1986) This meeting.

STEREOSELECTIVE PHARMACOKINETIC BEHAVIOUR OF TOCAINIDE ENANTIOMERS IN MAN.

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Tocainide, an orally active antiarrhytmic drug, has been developed and is used as a racemic mixture of optical antipodes. The present work deals with the selective pharmacokinetic properties of both enantiomers in man, after administration of the racemate, in three experiments.
1) 4 patients received a loading infusion (10 mg/kg) during 30 min immediately followed by a slow (1 mg/min) infusion during 24 hours. Blood samples were taken during the slow infusion and during 36 hours after the end of it.
2) 3 healthy volunteers took 1 Tonocard tablet (400 mg d,l-tocainide) orally. Blood samples were drawn during 11 hours and urines were collected for 96 hours.
3) Blood samples were collected during 1 dosing interval (8 h) from 8 patients on steady state treatment with Tonocard.
Racemic tocainide concentrations were measured by ion paired HPLC (Conings & Verbeke, 1985). Enantiomers were derivatized to form diastereoisomers (Sedman & Gal, 1984) which were separated by HPLC.
Concentration/time data sets were submitted to compartmental and non compartmental analysis.
The results can be summarized as follows :
1) The complex kinetic behaviour of tocainide and its isomers during and after infusions indicated distribution over 3 compartments. The mean t1/2 for d and l isomers was respectively 11.3 and 9.2 h. The mean Vss was similar for both chiral forms, i.e. 2.4 and 2.3 l/kg.
2) Data refering to a single oral administration could be analysed on the basis of a 1+1 compartment system. Absorption was equally quick for both enantiomers, but their elimination rate was very different, i.e. t1/2 = 16.5 h for d-, and 11.7 h for l-tocainide. Varea being similar for both forms, total body clearance of l-tocainide exceeded by 50 % that of d-tocainide. Since their renal clearance, measured from urinary data, was identical, it is concluded that stereoselective metabolism occurs in man.
3) In patients on steady state treatment, the average concentration during one dosing interval was 10 % to 140 % (mean 75 %) higher for d-tocainide than for l-tocainide.

In view of the enantioselective electrophysiological properties of tocainide isomers (Carmeliet et al., 1986), their different pharmacokinetic behaviour should lead to variations in the clinical response, in function of the mode of administration, the fluctuations of the steady state levels of both isomers, etc... It should also be stressed that the interpretation of TDM results based on racemic drug levels probably needs reevaluation.

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CLINICAL SYSTEMS DYNAMICS PHARMACOKINETICS OF THE METABOLIC CONVERSION OF CAFFEINE INTO PARAXANTHINE.

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Caffeine (1,3,7 trimethyl xanthine) is one of the most widely used drugs in the world. Despite its mass consumption, there are still gaps of knowledge of its metabolism. An incomplete picture of the spectrum of urinary caffeine metabolites exists: as much as 14 metabolites have been reported in urine, together accounting for 30-55 % of an i.v. or oral caffeine dose (Grant *et al.*, 1983). Three of these metabolites have also been reported in blood plasma: the dimethylxanthine phase-1 metabolites, which are generated directly from caffeine by mono-demethylation. The 3-demethylation pathway, which yields 1,7 dimethylxanthine (paraxanthine), outweighs the other two demethylation pathways yielding theobromine and theophylline by an order of magnitude. The aim of the present study was: 1) to determine the fraction of caffeine molecules converted to paraxanthine after administration of an oral or i.v. caffeine dose to human volunteers, 2) to determine the mean (and median) conversion time MCT (MedCT) required for this metabolic stap, and 3) to characterize the pharmacokinetics of paraxanthine. No quantitative data with respect to this metabolic pathway and to the kinetics of paraxanthine have appeared in the literature up to now.

Three healthy male volunteers participated in four consecutive experiments: i.v. and oral administration of both caffeine and paraxanthine. The i.v. infusion plasma concentration profiles of caffeine and paraxanthine were fitted as a series of injections to a sum of exponentials to determine the total body transport functions $\psi_{caf}(t)$ and $\psi_{par}(t)$ of caffeine and parxanthine. Pharmacokinetic parameters of the parent drug and its metabolite were calculated from the dose and the transport function of each respective compound, using statistical moment theory and the theory of systems dynamics pharmacokinetics (Van Rossum *et al.*, 1983).

Numerical deconvolution of $\psi_{caf}(t)$ and the caffeine plasma concentration profile following oral administration of caffeine yielded the absorption-rate-versus-time profile $D_{caf}(t)$ of caffeine. Numerical deconvolution was performed using a Fortran program based on the algorithm of Vaughan & Dennis (Vaughan & Dennis, 1978). The program was written at our department. Mean and median absorption times (MAT and MedAT) and the absorbed fraction of caffeine (F_{caf}) were calculated by integration of $D_{caf}(t)$. The results of an earlier identical kind of study at our laboratory involving six subjects drinking coffee and tea on two separate occasions were as follows: MAT = 27.8 ± 3.5 min, MedAT = 20.4 ± 3.7 min, F_{caf} = 107.2 ± 8.3 %. There were no significant differences between coffee and tea with regard to these caffeine absorption parameters (Teeuwen *et al.*, 1985).

Numerical deconvolution of $\psi_{par}(t)$ and the *paraxanthine* plasma concentration profile following *caffeine administration* yielded the metabolic conversion-rate-versus time profile of caffeine into paraxanthine $M_{par}(t)$. Mean and median conversion times (MCT and MedCT) and the fraction of caffeine converted into paraxanthine (F_m) were calculated from $M_{par}(t)$. At the moment, some of the experiments are still in progress. The results will be presented at the meeting.

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NICOTINE AND COTININE DETECTION IN BIOLOGICAL FLUIDS AND NICOTINE DETECTION IN FILTERS OF SMOKED CIGARETTES.

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Previously published methods for measuring nicotine and its main plasma metabolite cotinine in biological fluids are afflicted with many drawbacks. Sensitivity and specificity of the vast majority of these methods are needlessly reduced by an inadequate choice of work-up procedure, chromatographic column, detector, or internal standards. As a consequence, many procedures require bulky samples (e.g., Stehlik *et al.*, 1982), whereas other procedures are suited to only nicotine analysis (Feyerabend & Russell, 1979; Dow & Hall, 1978) or to only cotinine analysis (Feyerabend & Russell, 1980), or are applicable to only one kind of biological fluid. We developed a procedure for the simultaneous analysis of nicotine and cotinine in concentrations lower than 0.5 ng/ml, applicable to plasma and urine samples. Moreover, a procedure for the analysis of the nicotine content of smoked cigarette filters is outlined.

To 1 ml plasma or 0.4 ml urine samples nicotyrine and nikethamide were added as internal standards for nicotine and cotinine, respectively. Each sample was diluted with an equal volume of 0.05 M borax buffer (pH=9.0) and was flushed through a C-2 solid phase extraction column (100 mg sorbent). The column was washed with 2 ml of water and subsequently with 100 µl 80 % methanol. Then it was dried by air suction. Nicotine, cotinine and their internal standards are eluted by a 500 µl methanol column wash and are caught in a conical vial. 20 µl of propyl acetate were added to the eluate, which then was concentrated to appr. 15 µl by a very gentle stream of dry nitrogen at 45°. Half a µl of the concentrate is splitlessly injected into a gas chromatograph equipped with a 0.2 mm x 12 m dimethyl silicone WCOT column and a nitrogen detector. A two-level three-ramp GC oven temperature program was used, the starting level being 60° and the terminal level being 200°.

Peaks are highly resolved, widths at half-height varying from less than a second (nicotine) to 2-3 seconds (cotinine). Coefficients of variation are 2.7 % for nicotine (5 ng/ml) and 5.0 % for cotinine (25 ng/ml) in plasma. The recoveries are 75-85 % for nicotine and virtually 100 % for cotinine. The chromatogram runtime is 9.0 min including the cooling time of the GC oven (HP 5890). Specificity, sensitivity and rapidness of work-up and chromatography of this method indeed rival with the, in our view, best methods published so far (e.g. Curvall *et al.*, 1982).

To determine the nicotine content of cigarette filters, 10 filters of smoked cigarettes were crushed in a household blender after removal of residual tobacco and ashes. The resulting filter "wool" was quantitatively suspended in 100 ml of methanol, ultrasonically vibrated for 30 min and filtrated through cellulose acetate filters (pore 0.45 µm). Then the *external* standard nicotyrine was added, the filtrate was diluted 25 times with propyl acetate and 0.5 µl were injected into the GC. For this purpose the earlier mentioned GC oven temperature program was cut after appearance of the nicotyrine peak and lasted 5.5 min. Determination of the filter nicotine content allows one to make an estimation of a smokers nicotine intake, if the ratio of the amounts of nicotine in the mainstream of the smoke/ in the cigarette filter is known, as will be shown in another poster contribution.

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LAMOTRIGINE AND CARBAMAZEPINE: A COMPARISON ON PSYCHOMOTOR PERFORMANCE.

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Lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1, 2, 4-triazine] is a novel antiepileptic compound which has a pharmacological profile in animals similar to that of phenytoin (Miller et al., 1984). Lamotrigine has been given to volunteers without important adverse experiences. No impairment of adaptive tracking, eye movements or subjective effects occurred, in contrast to effects after diazepam and phenytoin (Cohen et al., 1985). This study examined lamotrigine (at a higher dose than previously) and carbamazepine, on a range of tests known to be sensitive to effects of sedative drugs, (Nicholson, 1979; Scott et al., 1982; Smith et al., 1981; Tedeschi et al., 1983; Bond & Lader, 1974; Bittencourt et al., 1982) and also subjective effects and autonomic measures.

In a double-blind, placebo-controlled, randomised crossover study, twelve healthy male volunteers received one of the following six treatments at intervals of not less than 7 days: lamotrigine 150 and 300 mg, carbamazepine 200, 400 and 600 mg, and lactose placebo. The carbamazepine was administered as two equally divided doses, one on the study day and one 12h earlier in order to achieve therapeutic plasma concentrations. On other nights placebo capsules were administered to maintain the blinding. Tests were carried out under standardised conditions, 0.5h before, 1.5, 3, 4.5 and 6h after the morning drug administration. Results were analysed by analysis of variance and treatment means compared using Newman Keuls multiple range test, with $p < 0.05$ taken as significant.

Smooth pursuit and saccadic eye movements (mean peak velocity and mean duration of saccade) were impaired after treatment with carbamazepine 600 mg, 400 mg; carbamazepine 600 mg also decreased adaptive tracking score and increased body sway and heart rate compared to placebo (4.6 beats/min). No significant changes occurred after lamotrigine on any variable. There were no effects from any treatment on visual function, salivary secretion, pupil diameter, blood pressure, and subjective ratings, and subjects were unable to distinguish any of the active drugs from placebo.

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A PRELIMINARY EVALUATION OF N-DESMETHYLCLOBAZAM IN EPILEPSY.

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The usefulness of the 1-5 benzodiazepine clobazam as adjunctive therapy for refractory epilepsy is limited by a rapidly developing partial tolerance. While patients who relapse as a result of tolerance will often respond again after a 'holiday' from clobazam, such second or subsequent responses are not invariably as good as the first. Studies in mice (Haigh et al, 1984), baboons (Meldrum and Croucher, 1982) and man (Callaghan and Goggin, 1984; Jawad et al, 1984) suggest that a substantial part, or all, of the anticonvulsant effect of clobazam is due to its principal metabolite, N-desmethylclobazam (NDMC). Furthermore, in an animal model the administration of NDMC produces less tolerance than the administration of clobazam (Feely et al, 1985).

We gave NDMC 30 mgs/day, orally, to 8 adult patients (6F, 2M) who had developed tolerance to clobazam and were having one or more seizures per week. Six had their clobazam withdrawn for at least 3 weeks beforehand and 2 changed directly from clobazam to NDMC. All continued taking other, conventional, anticonvulsants and plasma levels of these were measured before and after the addition of NDMC. Plasma levels of NDMC were measured by reverse-phase HPLC at frequent intervals throughout the study and 24 h profiles were obtained at steady state. We also gave 4 male volunteers NDMC and obtained 24 h plasma level profiles at steady state.

Patients have been followed for between 2 and 16 weeks and all have shown evidence of a favourable response to NDMC. Five of the 6 who had a 'holiday' from clobazam, including one who had no response to a (NDMC) placebo while off clobazam, became seizure free for at least one month. Two have subsequently developed partial tolerance. However, in all 6 patients the response to NDMC has been similar to, or better than, the initial response to clobazam. Neither of the 2 patients who changed directly from clobazam to NDMC became seizure free but one has substantially improved, even though plasma levels of NDMC were similar before and after the change in benzodiazepine ingested. Steady state plasma levels of NDMC were higher (2000-3000 ng/ml) in the male patients than in the male volunteers (700-1900 ng/ml). One patient made a small reduction in carbamazepine dose, to relieve transient diplopia, before plasma levels could be obtained but no changes in carbamazepine levels were seen in 5 others. Two of 3 patients showed substantial (~60%) increases in plasma phenytoin. None of the patients or volunteers found NDMC 30 mgs/day sedative but the only patient who tried a higher dose (30 mgs/60 mgs on alternate days) complained of sedation.

We believe that N-desmethylclobazam merits further investigation as an alternative to clobazam for severe epilepsy.

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THE EFFECTS OF DEXAMPHETAMINE ON ADAPTIVE TRACKING IN HEALTHY VOLUNTEERS.

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Adaptive tracking performance is impaired by sedative antihistamines (Clarke & Nicholson, 1976), and the variability of performance throughout the test is increased (Cohen et al., 1985). Stimulant drugs have not been studied on adaptive tracking. The effects of dexamphetamine are reported here.

12 healthy volunteers (4 M and 8 F) participated in a double-blind, randomised controlled crossover study. Each volunteer received one of the following 4 treatments in identical capsules: lactose dummy (L); dexamphetamine sulphate 2.5 mg (D2.5), 5 mg (D5), and 10 mg (D10), in an order based on a series of Latin squares. Each occasion was separated by at least one week. The volunteers performed a battery of tests before and at 1, 2.5, 4.5 and 6.5 h after treatment administration. An adaptive tracking test was performed as described previously (Cohen et al., 1985). Variability in performance during the test was expressed as the standard deviation of the performance levels assessed at 15 ms intervals during the 10 minutes of the test. A visual reaction time test implemented on a microcomputer obtained an average of 26 reaction times over 5 min. Antero-posterior body sway was recorded by a Wright-Codoc ataxiameter for 3 min. Subjective effects were determined by visual analogue scales (Norris, 1971). In addition to the standard 18 lines the following were included: "Tracking Poor - Good" and "Not at all hungry - Extremely hungry".

Results were analysed by ANOVA and dexamphetamine treatment means were compared with L by the William's test and p values < 0.05 were taken as significant.

The tracking performance was improved by D10 and there was a similar trend after D5. The variability of tracking scores was reduced by D10. Both D10 and D5 reduced visual reaction time. There were no consistent drug related changes in body sway. After D10 subjects were more alert, excited, strong, clear-headed, well coordinated, attentive, sociable and outgoing, and rated themselves better on the tracking test.

It is possible that dexamphetamine reduces or abolishes lapses of concentration that produce periodic transient large deteriorations in performance as was demonstrated by the reduction in variation of performance through the test. The adaptive tracker has been demonstrated repeatedly to be a sensitive instrument for the detection of sedative side effects of drugs. This study has shown that improvement in performance induced by stimulant drugs can also be detected.

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THE EFFECTS OF NEFOPAM ALONE AND IN COMBINATION WITH ETHANOL ON HUMAN PERFORMANCE, VENTILATORY RESPONSE TO HYPERCAPNIA AND MOOD.

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Nefopam is a non narcotic analgesic marketed as Acupan or Ajan (Ger.). The compound has been shown to produce analgesia comparable to that with the oral analgesics aspirin, dextropropoxyphene and pentazocine. Nefopam is structually unrelated to other analgesic drugs and is about 75% protein bound with an elimination half life of 3-8 hours. The present study was designed to mimic a possible combination of nefopam and ethanol in an out patient setting.

12 healthy male volunteers received 4 treatments at weekly intervals. The treatments were of nefopam and ethanol; nefopam and placebo ethanol; placebo nefopam and ethanol; placebo nefopam and placebo ethanol. Each treatment consisted of 2 study days. On the first study day volunteers received nefopam (60 mg) or placebo nefopam at 07.00, 15.00 and 23.00 hours; on day 2 they received a further 2 doses at 07.00 and 15.00 hours. At 13.00 hours on day 2 volunteers were dosed with 0.73 g/kg ethanol (vodka in orange juice) or ethanol placebo. The loading dose was then followed by half hourly 'top-ups' with 0.06 g/kg ethanol (vodka in orange juice) or ethanol placebo as appropriate until 18.00 hours. Saccadic eye movements (Griffiths et al 1985), critical flicker fusion threshold (Hindmarch 1979) and mood (Thayer et al 1978) were measured at the following times before and after the last dose of nefopam (60 mg) or nefopam placebo at 15.00 hours on day 2: -30 mins, 30 mins, 1 h, 1.5 h, 2.0 h, 3.0 h, 4.0 h and 5.0 h. The ventilatory response to hypercapnia (Read 1967) was performed before ethanol/ethanol placebo administration and then 1 h, 2 h, 3 h and 5 h after the last dose of nefopam (60 mg) or nefopam placebo. Blood samples and breath alcohol measurements were taken on day 2 of each treatment. The study was carried out double blind and treatments were administered in a randomised order. Ethanol was prohibited to volunteers for 24 hours preceding each treatment and caffeine was prohibited on day 2. A standard diet was provided on day 2. The study was approved by the Joint Ethics Committee of South Glamorgan Area Health Authority. The data collected was analysed by factorial analysis of variance and then by Student Newman Keuls multiple range test or Tukey's 'a' test. Data from the ventilatory response to hypercapnia was analysed by the method described by Read (1967).

In comparison with placebo, nefopam alone did not reduce peak saccade velocity significantly while both ethanol alone and to a lesser extent nefopam and ethanol reduced velocity significantly ($p<0.001$ and $p<0.005$ respectively). A similar effect was seen with critical flicker fusion threshold (CFFT), where in comparison with placebo, ethanol alone reduced CFFT ($p<0.01$) while nefopam alone and the nefopam/ethanol combination appeared to significantly increase CFFT. No significant effects were seen on any of the mood parameters measured. Nefopam alone marginally impaired ($p<0.048$) respiratory drive 3 hours after the last dose of nefopam, in combination with ethanol this effect was not seen. There were no significant differences between serum nefopam or n-desmethyl nefopam concentrations on either active treatments. Breath alcohol concentrations were not significantly different between the two ethanol treatments.

It is concluded that under these circumstances there is no synergistic interaction between nefopam and ethanol on these measures of drug action.

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FENOLDOPAM VASODILATES FOREARM VASCULATURE BY A DOPAMINERGIC MECHANISM.

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Fenoldopam is a potent and selective vascular dopamine (DA_1) receptor agonist lacking the α and β adrenoceptor agonist activity of dopamine itself (Hahn, 1982). Fenoldopam has been shown to relax isolated human arteries by an action on DA_1 receptors (Hughes et al., 1985). Intra-arterial infusion of fenoldopam has also been reported to increase forearm blood flow in man but the mechanism of this effect is unclear (Redman et al., 1986). We have attempted to antagonise the vasodilator effect of fenoldopam in man using the selective vascular dopamine antagonist R sulpiride (Kohli et al., 1978).

All studies were approved by the Hospital Ethnical Committee. Six healthy subjects (age 23-34) participated. Forearm blood flow (FBF) was measured in both arms by venous occlusion plethysmography, one arm serving as control (Whitney, 1953). Drugs were infused into the study arm via a brachial artery cannula. Infusions were for 5 minutes at each dose level and measurements of FBF were made during the final 3 minutes of each infusion when stable responses had been achieved. Fenoldopam (0.3 - 3 μg/min; i.a.) produced a dose related increase in FBF in the study arm, from resting level of 2.9 ± 0.5 ml/100ml/min to 10.5 ± 1.6 ml/100ml/min (means ± SEM, n = 6) at the highest dose. Control arm FBF did not significantly change during infusion of fenoldopam implying no significant systemic effects over this dose range. (R) sulpiride (0.1, 1 mg/min; i.a.) alone had no significant effect on forearm blood flow. However, these doses of R sulpiride infused for 5 mins prior to and concurrent with fenoldopam antagonised the forearm vasodilatation induced by fenoldopam (0.3 - 10 μg/min; i.a.) in the study arm, antagonism being more marked at the higher dose of (R) sulpiride.

These results demonstrate that the forearm vasodilatation seen following infusion of fenoldopam is mediated by vascular dopamine (DA_1) receptors. The presence of such DA_1 receptors in the human renal and mesenteric circulation is widely accepted. These findings suggest a wider distribution of this receptor in the human cardiovascular system consistent with the reported hypotensive effects of systemic fenoldopam in man (Harvey et al., 1985).

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COVALENT BINDING OF PROPRANOLOL TO HUMAN LIVER MICROSOMES: BIOCHEMICAL AND METABOLIC STUDIES.

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Previous work has shown that propranolol (P) is a potent inhibitor of cytochrome P-450-mediated metabolism in rat liver microsomes and has implicated the involvemnt of a covalently-bound intermediate product of P metabolism (Al-Asady et al, 1985; Schneck and Pritchard, 1982). Furthermore, this binding appears to be associated specifically with the 4'-hydroxylation of P, which in man is known to be under similar control to the polymorphic hydroxylation of debrisoquine (D) (Lennard et al, 1984). We have, therefore, investigated the relationship between the covalent binding and oxidative metabolism of P in human liver microsomes.

Samples of human liver were obtained from 3 renal transplant donors who had been maintained on life support systems until the kidneys were removed. Putative covalently-bound material was measured as radioactivity associated with protein after exhaustive solvent extraction. Labelled proteins were separated by SDS PAGE gel electophoresis. P metabolites were measured by HPLC.

Incubation of microsomes containing 13-24 mg protein with ^{14}C-labelled P (0.5 µC, 100 µM) at 37°C and pH 7.4 gave rise to cofactor-dependent binding which increased almost linearly with time. After 60 min 0.031 $\pm$ 0.004 s.d. nmol. mg protein^{-1} was bound (n = 3 livers) representing 0.18% of the dose. The extent of this binding was decreased considerably by the exclusion of co-factors, boiling, anaerobic conditions and the addition of reduced glutathione (1 mM) and SKF-525A (1 mM). The electrophoresis experiments suggested that ^{3}H-P (44 µCi, 100 µM) modified covalently a large number of microsomal proteins particularly in the 40,000 to 90,000 molecular weight range. Debrisoquine (1mM) decreased total binding by 55 $\pm$ 8 s.d. % but in a apparently non-specific manner. However, debrisoquine at this concentration had little effect on the rate of 4'-hydroxypropranolol formation (91 $\pm$ 13 s.d. % of control values) and even increased the rate of N-dealkylation of P (171% of control values, mean of 2 livers). In contrast, phenacetin (1mM) abolished completely the cofactor-dependent binding as well as the 4'-hydroxylation and N-dealkylation of P.

The findings suggest that the product(s) of the metabolic activation of P bind irreversibly but non-selectively to human liver microsomal proteins. The enzyme system responsible for this activation appears to be related more closely to the cytochrome P-450(s) which metabolise phenacetin than those metabolising debrisoquine.

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STUDY OF THE ACTION OF CICLETANINE IN HYPERURICAEMIC PATIENTS.

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Cicletanine is a new compound reported to have antihypertensive and diuretic properties which may act by stimulation of the chloride-dependent potassium ion flux reducing the chloride gradient responsible for sodium ion membrane transfer thereby inducing prostaglandin release and causing a natriuresis (Braquet et al, 1983; Garay et al, 1984; Braquet and Garay, 1984). During a clinical trial of cicletanine versus indapmide to compare their antihypertensive properties, cicletanine was noted to produce a significant hypouricaemic effect (mean reduction of plasma uric acid 16.6% Gourgon,R data on file, Ipsen International). The aim of this study was to determine the effect of cicletanine on uric acid clearance in a group of patients with stable hyperuricaemia, a group at special risk of diuretic induced gout.

One female and eight male patients with stable hyperuricaemia (plasma uric acid greater than 0.45 mmol/l for males and 0.40 mmol/l for females) and with an age range of 29 to 72 years took part. Eight patients carried a diagnosis of gout, the other had a polyarthritis without crystal proof of gout. Patients whose hyperuricaemia was secondary to other conditions were excluded and concomitant therapy likely to alter sodium or uric acid status was discontinued 2 weeks prior to the study. Two baseline 24 hour urine collections and plasma samples were taken before commencing administration of cicletanine 100 mg orally at 9 a.m. daily for 21 days. 24 hour urine collections and plasma samples were collected on the 1st, 2nd, 20th and 21st days of drug administration. Urinary sodium, potassium, creatinine, uric acid and plasma electrolytes and uric acid were measured along with full blood counts and liver function studies.

One patient developed an acute attack of gout in mid study. This was treated with colchicine and diclofenac. Another had a transient rise in aspartate amino transferase on days 1 and 2 of the study. No significant changes in urinary volume, urinary sodium, uric acid clearance and plasma urate were seen (see Table 1).

Table 1 Results are mean ± SEM (n = 9)

Duration cicletanine therapy	0	0	24hrs	48hrs	20 days	21 days
Plasma uric acid (mmol/l)	0.56 ± 0.022	0.57 ± 0.02	0.54 ± 0.02	0.57 ± 0.02	0.57 ± 0.02	0.57 ± 0.02
24 hour urinary volume (mls)	2223 ± 660	2131 ± 395	1981 ± 200	2080 ± 233	2166 ± 270	1981 ± 247
24 hour urinary sodium (mmol)	124 ± 20	157 ± 22	147 ± 24	147 ± 24	132 ± 11	120 ± 17
Uric acid clearance (mls/min/m^2)	1.77 ± 0.08	1.96 ± 0.12	1.69 ± 0.18	1.95 ± 0.19	2.12 ± 0.23	1.86 ± 0.25

In this group of hyperuricaemic patients, cicletanine did not appear to have diuretic or hypouricaemic activity.

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EFFECT OF AMINOPHYLLINE ON RESPIRATORY STIMULATION AND HEART RATE CHANGES PRODUCED BY INTRAVENOUS ADENOSINE BOLUSES IN MAN.

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Adenosine is an endogenous nucleoside which, when injected intravenously in man, produces transient respiratory stimulation (Watt and Routledge, 1985) and biphasic changes in heart rate (Watt and Routledge, 1986). In laboratory mammals the respiratory stimulant action appears to be exerted in the carotid body (Monteiro & Ribeiro, 1986) via cell surface receptors of the A_2 sub-type (Ribeiro & McQueen, 1986). Little is known of the receptors involved in the respiratory and heart rate changes in man. The bronchodilating drug aminophylline, a competitive antagonist at cell-surface (but not intracellular) adenosine receptors, was used in an attempt to modulate adenosine-induced effects.

Informed written consent was obtained from 10 healthy adult subjects (7 male, aged 26.1 + 6.5 years; mean + SD) according to a protocol approved by the hospital ethics committee. Each subject was studied on two occasions, following single blind intravenous administration over 15 minutes of aminophylline 375 mg or an equivalent volume of saline. At least one hour was left before assessing the dose-response relationship to intravenous adenosine boluses (20 $ug.kg^{-1}$ initially, increasing in steps of 20 $ug.kg^{-1}$, to a possible maximum of 200 $ug.kg^{-1}$). Adenosine-induced respiratory stimulation was assessed using a Lectromed respiration transducer as previously described (Watt and Routledge, 1985). The change in ventilation index was assessed using analysis of covariance over the dose-range 20-120 $ug.kg^{-1}$. The peak adenosine-induced bradycardia and tachycardia were compared to baseline RR interval; the dose-response relationships of the changes in RR interval were compared using analysis of covariance over the dose-range 20-120 $ug.kg^{-1}$. Statistical significance was assumed if $P < 0.05$.

Adenosine produced dose related respiratory stimulation on both the saline ($r = 0.428$, $P < 0.001$) and aminophylline ($r = 0.418$, $P < 0.001$) study days, but the slope ($F = 0.444$, $P > 0.50$) and intercept ($F = 4.180$, $P > 0.05$) of the relationship did not change. Adenosine-induced bradycardia was dose-related on the saline study day ($r = 0.389$, $P < 0.001$) but not on the aminophylline study day ($r = 0.071$, $P > 0.50$). Adenosine-induced increase in heart rate was dose-related on both the saline ($r = 0.591$, $P < 0.001$) and aminophylline ($r = 0.634$, $P < 0.001$) study days, but neither the slope ($F = 0.089$, $P > 0.50$) nor intercept ($F = 2.826$, $P > 0.10$) of the relationships differed.

Our data demonstrate that aminophylline abolishes the dose-response relationship for adenosine-induced bradycardia, which is in agreement with the report of Favale et al. (1985) who used a single dose of adenosine in each subject. The dose-response relationships for ventilatory increase ($0.05 < P < 0.10$) and tachycardia ($P > 0.10$) were not moved significantly to the right, but approached statistical significance. The lack of effect of aminophylline on respiratory effects of adenosine agrees with the findings of Biaggioni et al. (1986). The mechanism(s) underlying the apparent differential modulation by aminophylline of adenosine-induced effects in man remains to be clarified.

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INTRAVENOUS ADENOSINE IN THE ASSESSMENT OF CORONARY PERFUSION RESERVE IN MAN.

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Rapid atrial pacing is established as a test of coronary perfusion reserve but has well recognised limitations. Dipyridamole has proved a useful alternative way of testing coronary reserve and is thought to act by increasing endogenous adenosine levels. The effects of exogenous adenosine on coronary flow in man do not appear to have been previously compared to those of atrial pacing.

We studied 9 patients aged 41 - 65 yrs. (2 men), being investigated for chest pain but shown at arteriography to have normal coronary arteries. Informed written consent was obtained from each patient for a study protocol approved by the local ethics committee. Great cardiac vein flow (by a continuous thermodilution technique), mean systemic arterial pressure, left ventricular (LV) end diastolic pressure (EDP) and indices of LV contractility (peak dP/dt and dP/dt/P) were measured before and for 60 sec following intravenous boluses of adenosine (3.5 mg and 6 mg) and of normal saline as control. Heart rate changes with adenosine were avoided by continuous right ventricular pacing at 100 beats.min^{-1}. Great cardiac vein flow (measured during the last 60 sec of each pacing period) was measured in the same patients during an atrial pacing test (beginning at 100 beats.min^{-1}). All data are given as mean $\pm$ SD and compared by paired t test.

Adenosine 3.5 and 6 mg increased great cardiac vein flow (to a peak within about 20 sec) by 114 $\pm$ 74% from 89 $\pm$ 22 ml.min^{-1} and by 126 $\pm$ 87% from 93 $\pm$ 23 ml.min^{-1}, respectively ($P < 0.001$ in each case). Coronary flow returned to baseline 60 sec after the adenosine injection. Increases in coronary flow were accompanied by a fall in mean systemic arterial pressure by 19 $\pm$ 8% from 100 $\pm$ 16 mm Hg (adenosine 3.5 mg) and by 22 $\pm$ 5% from 97 $\pm$ 10 mm Hg (adenosine 6 mg) ($P < 0.01$ in each case) LVEDP, dP/dt and dP/dt/P did not change. Saline boluses had negligible effect on coronary blood flow (-1% from 97 $\pm$ 21 ml.min^{-1}), mean systemic arterial pressure (-2% from 100 $\pm$ 14 mm Hg), LVEDP and indices of LV contractility. Atrial pacing at 150 beats.min^{-1} increased great cardiac vein flow by 40 $\pm$ 45% from 81 $\pm$ 24 ml.min^{-1} at 100 beats.min^{-1} ($P < 0.05$).

Adenosine increased coronary blood flow three times more than the atrial pacing test did ($P < 0.05$) despite a fall in mean arterial pressure following adenosine. The effect of the atrial pacing test on coronary blood flow is attributable to a metabolic response to increased workload, whereas the effect of adenosine appears to be due to a direct coronary vasodilator action with, by implication, no increase in myocardial energy demands. Adenosine may be useful as an alternative to dipyridamole in the testing of coronary reserve, with the potential advantage in some circumstances of a more rapid onset and brief duration of action.

COMPARISON OF OCULAR AND CARDIOVASCULAR EFFECTS OF ORAL NADOLOL AND TOPICAL TIMOLOL IN PATIENTS AT RISK OF GLAUCOMA.

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Topical beta-blocker therapy is a standard treatment for glaucoma. Oral beta blockade might prove to be a more acceptable form of treatment, if it were shown to be as effective as topical therapy without frequent systemic side effects. We compared the effect of oral nadolol and topical timolol in a randomised, double-blind placebo-controlled, crossover study.

Informed written consent was given by 23 patients (aged 47 - 78 years, 12 female) at risk of glaucoma. The study protocol was approved by the local ethics committee. Active treatment was either nadolol 20mg o.d. orally or timolol 0.25% solution, one drop b.d. in each eye. In the first treatment period each patient received active tablet with placebo drops, or placebo tablet and active drops. Following a four week washout period when placebo tablets and drops were taken, the crossover part of the study was conducted. Intraocular pressure was assessed at baseline, and every 2 weeks for 12 weeks. One patient withdrew while on timolol. Ten of the fittest patients consented to a symptom - limited exercise test. One subsequently withdrew because of an intercurrent back injury. Both nadolol and timolol reduced intraocular pressure (IOP) after 2 weeks and 4 weeks of treatment. At 2 weeks nadolol reduced IOP from 23.7 $\pm$ 4.9 mm Hg (mean $\pm$ S.D) to 18.4 $\pm$ 5.7 mm Hg ($P < 0.001$); timolol reduced IOP from 23.9 $\pm$ 7.1 mm Hg to 16.8 $\pm$ 3.5 mm Hg ($P < 0.001$). At 4 weeks on nadolol IOP fell to 18.3 $\pm$ 5.1 mm Hg ($P < 0.001$ versus baseline) and on timolol to 17.3 $\pm$ 4.1 mm Hg ($P < 0.001$ versus baseline).

Nadolol reduced resting heart rate (79 $\pm$ 12 to 57 $\pm$ 7 beats per minute; $P < 0.001$) and exercise heart rate (110 $\pm$ 27 to 81 $\pm$ 15 beats per minute; $P < 0.001$). On Timolol the fall in heart rates did not reach statistical significance. Nadolol reduced systolic blood pressure on exercise from 181 $\pm$ 20 to 156 $\pm$ 19 mm Hg ($P < 0.001$). Timolol reduced resting (147 $\pm$ 22 to 133 $\pm$ 22 mm Hg) ($P < 0.05$) and exercise (181 $\pm$ 20 to 163 $\pm$ 24 mm Hg, $P < 0.001$) systolic blood pressure. No other effects of drug therapy on blood pressure or heart rate, at rest or on exercise, were detected. The effects of timolol and nadolol on blood pressure did not differ ($P > 0.05$) but nadolol produced a significantly greater fall in heart rate at rest ($P < 0.05$).

Our data suggests that both oral nadolol and topical timolol significantly reduce intraocular pressure. The effects of the drugs in this regard appear similar. Both oral nadolol and topical timolol produced some systemic beta-adrenoceptor blockade, as assessed by the variables described above. Oral nadolol was an effective, well-tolerated treatment; it may prove to be more convenient than topical beta-blocker therapy, and so improve compliance with therapy.

ADENOSINE REPRODUCES EPIGASTRIC PAIN IN PATIENTS WITH RECENTLY SYMPTOMATIC DUODENAL ULCER: EFFECT OF AMINOPHYLLINE.

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Adenosine is an endogenous nucleoside with varied pharmacological effects (Lancet,1985). While studying the respiratory stimulant effect of the nucleoside (Watt and Routledge, 1985) a subject who had a recently symptomatic duodenal ulcer (which had not been disclosed prior to the study) reported transient reproduction of typical epigastric pain for a few seconds after higher dose adenosine bolus injections. Gastric acid appears to produce ulcer pain after an interval of a few seconds (Palmer, 1926). This delay may be attributable to time taken for diffusion or to release of a metabolite . Adenosine appears to be released by acid (Mozsik et al, 1976) and adenosine exerts a negative feedback action on acid production (Gerber et al, 1984). These associations prompted us to prospectively examine the algogenic activity of the nucleoside in patients with recently symptomatic duodenal ulcer, in the absence and presence of aminophylline, an antagonist at cell-surface adenosine receptors.

Informed written consent was obtained from 6 patients with recently symptomatic duodenal ulcer, according to a protocol approved by the local ethics committee. It was made clear to all patients that they were the arbiters of tolerance of any pain which they might experience. Adenosine and placebo were given single-blind by serial rapid boluses, separated by at least 90 seconds, initially in a dose of $20ug.kg^{-1}$, increasing in steps of $20ug.kg^{-1}$, to a possible maximum of $200ug.kg^{-1}$. An electrocardiogram was monitored throughout the study. After each injection patients were asked to record any of their usual epigastric pain on a 10 cm Visual Analogue Scale ("No pain" to "Pain as bad as I can imagine"). Other subjective sensations were also noted. Aminophylline, 250mg, was administered by intravenous injection over 15 minutes, and the adenosine study repeated.

No patient experienced epigastric discomfort with slaine or below a dose of 60 ug kg^{-1} adenosine, but 5 patients reported their typical epigastric discomfort, lasting only a few seconds, at higher doses. The visual analogue pain score for the group was related to adenosine dose both before ($r=0.730$, $P < 0.001$) and after ($r=0.790$, $P < 0.001$) aminophylline. Following aminophylline the relationship was shifted slightly but significantly ($F= 6.516$, $P< 0.05$) to the right, e.g the dose to give a pain score of 25 was 109ug kg^{-1} before aminophylline and 134 ug kg^{-1} after aminophylline. The only other symptom reported was respiratory stimulation.

Adenosine reproduced typical epigastric pain which lasted only a few seconds in 5 of 6 patients with endoscopically proven duodenal ulcer. Whether excess levels of endogenous adenosine stimulated by excess gastric acid play any role in the genesis of spontaneous peptic ulcer pain is speculative. Adenosine exerts many neuromodulatory effects (e.g Stone 1981) in various organ systems. It remains to be determined whether adenosine plays a role in the genesis of pain in peptic ulcer or other painful conditions.

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ORAL SALBUTAMOL DOES NOT AFFECT THEOPHYLLINE KINETICS.

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Reduced serum theophylline levels have been reported in patients taking concurrent oral salbutamol and theophylline when compared to the same patients taking oral theophylline alone (Dawson & Fergusson, 1982- Danziger et al., 1985) but in neither report was the full pharmacokinetic profile of theophylline investigated. We have studied the 24 h pharmacokinetic profile of oral sustained release theophylline (as Nuelin SA, Riker Laboratories) when taken on its own and when taken in combination with oral sustained release salbutamol (Ventolin Spandet, Allen and Hanburys Limited).

Eight healthy male volunteers (age 20-39 years, body weight 60-80 kg) were entered into a randomised cross-over study. Each volunteer was given five doses at 12 h intervals of either theophylline 250 mg alone, or in combination with salbutamol 8 mg, with one week between treatment periods. Immediately before the final dose, 10 ml blood was withdrawn from an indwelling forearm venous cannula for estimation of steady state theophylline concentrations. Further samples were withdrawn at 2, 4, 6, 8, 12 and 24 h after drug administration. Plasma samples were stored at -20°C until measurement of theophylline concentration by H.P.L.C. (Orcutt et al., 1977). From the measured concentrations the elimination half-life ($t_{\frac{1}{2}}$, h) and the total area under the concentration curve (AUC_{0-24} mg $l^{-1}h$) were calculated. Mean ± S.E. mean values are given. As the data were found to be normally distributed, a paired t test was used to compare values from the two patient groups, and a value of $P<0.05$ regarded as significant.

The steady state theophylline concentrations (Cp SS, g l^{-1}), AUC_{0-24} and $t_{\frac{1}{2}}$ in the two treatment groups are shown below in Table 1. No significant differences in any parameter were observed. We conclude that concurrent ingestion of salbutamol and theophylline does not influence plasma theophylline concentrations.

Table 1 Mean Theophylline Pharmacokinetic Parameters (±S.E. Mean)

	CpSS (mg l^{-1})	AUC_{0-24} (mg $l^{-1}h$)	$t_{\frac{1}{2}}$ (h)
Theophylline alone	8.4 ± 0.7	157.4 ± 14.3	10.4 ± 0.6
Theophylline and Salbutamol conbined	8.4 ± 0.8	159.2 ± 10.2	9.9 ± 0.7

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FLUPIRTINE PHARMACOKINETICS IN RENAL IMPAIRMENT.

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Flupirtine is a centrally acting non-opiate analgesic (data on file,Chemiewerk) which has been shown to be effective in post surgical pain (Galasko, 1985). Following on oral dose of 14 C-flupirtine 72% of the label was recovered in the urine and two metabolites have been identified (Hlavica, 1985). We have studied the effect of mild renal impairment on flupirtine pharmacokinetics. With ethics committee approval twelve patients with renal impairment (mean creatinine clearance 59 mls min^{-1} range 44-90) each received 100 mg of flupirtine orally. They were not fasting, and continued to take their usual medication. A 10 ml sample of clotted blood was removed from an indwelling catheter or by venesection at the following times, 0, 0.5, 1, 1.5, 1, 1.5, 3, 4, 5, 6, 8, 10, 24 and 30 hours for serum flupirtine estimation using a specific high performance liquid chromatographic method with fluorimetric detection. No adverse effects were noted.

tmax (h)	1.75(0.5-3)	median & range
Cpmax (ug l^{-1})	788 ± 297	mean ± SD
half life (h)	11.8 ± 3.5	mean ± SD
lag time (min)	21 (0-120)	median & range
mean residence time (h)	14.3 + 4.2	mean ± SD
apparent clearance (mls min^{-1})	263 ± 67	mean ± SD
apparent volume of distribution (L)	262 ± 74	mean ± SD

Data from 13 normal volunteers has shown an elimination half life of 4.7 ± 1.6 hrs (data on file, Chemiewerk), which is significantly different ($p<0.001$) from our own data. Mild renal impairment significantly increases the elimination half life of flupirtine.

We thank Advisory Services for financial assistance and providing flupirtine.

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MEASUREMENT OF SPONTANEOUS MOVEMENT DURING THE NIGHT: A USEFUL OBJECTIVE ASSESSMENT OF PARKINSON'S DISEASE?

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The tremor and rigidity of Parkinson's disease are relatively easy to assess and quantify when compared with the hypokinesia (Marsden & Schachter, 1981). Tests are described for various components of hypokinesia such as reaction time and movement time, but they all require the co-operation of the patient and are hence subject to the variation in performance associated with emotion. In contrast the measurement of spontaneous movement during the night can provide a continuous record of size and frequency of moves and requires no co-operation from the patient. Poverty of movement whilst in bed is of particular importance in the elderly patient since it may lead to functional incontinence and pressure sores. In Parkinson's disease treated with laevodopa, the night-time hypokinesia may be exacerbated by the 'end of dose' effect and consequently be responsive to additional nocturnal doses of laevodopa. We present a double-blind, placebo-controlled, cross-over study of the effect of the laevodopa/carbidopa combination, Sinemet-Plus (Merck Sharp & Dohme Ltd) on spontaneous movement during the night.

Eight patients with idiopathic Parkinson's disease (Hoehn & Yahr (1967) classification: stage III in two patients, stage IV in four and stage V in two), aged 68 to 87 years, consented to take part in the study. All were clinically stable on a regime of Sinemet-Plus which gave optimal control of their Parkinson's disease during the day, with an additional dose of two tablets at 22.00 h. Each patient received three nocturnal treatments in randomised order, the treatments being 2 Sinemet-Plus tablets at 22.00 h and 1 identical placebo tablet at 04.00 h, 1 Sinemet-Plus and 1 placebo at 22.00 h and 1 Sinemet-Plus at 04.00 h, and 2 placebos at 22.00 h and 1 placebo at 04.00 h. Treatments were for 4 nights with a 'wash-out' period of at least three nights inbetween treatments during which the previous regime of 2 tablets at 22.0 h (and none at 04.00 h) was resumed. Daytime medication remained constant throughout. A system involving a load transducer under each bed leg was used to obtain a continuous plot of the lateral position of the patient's centre of gravity. An index of the frequency of moves and the median move size were calculated for the four nights of each treatment (Nicholson et al, 1986).

Six patients completed all three treatments, two completed only two treatments because of intercurrent illness. Neither treatment nor sequence had any significant effect on our index of frequency or on median move size (analysis of variance, $p > 0.05$ in each case). If the end of dose effect is reflected by a reduction in spontaneous movement during the night, this should become increasingly apparent as the night progresses. However, analysis of data on the second half of each night produced results similar to those for the whole night. Although our patients had marked reductions in their median move size as compared with patients of the same age but without Parkinson's disease, this poverty of movement was not reversible by laevodopa. Measurement of spontaneous movement during the night may prove a useful measure of severity of Parkinson's disease, but does not appear to serve as a model of responsiveness to dopaminergic agents.

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DOSE PARACETAMOL MODIFY FRUSEMIDE DIURESIS?

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Indomethacin has been shown to attenuate the natriuretic and diuretic response to frusemide in man (Braker, 1979). Inhibition of frusemide natriuresis by aspirin in normal volunteers has also been demonstrated (Hassan, 1982). We have investigated whether paracetamol, which is thought to have its primary action by inhibiton of central rather than peripheral prostaglandin synthetase, (Goodman, 1985) would affect frusemide diuresis, natriuresis and kaliuresis.

Thirteen healthy volunteers were given a standard breakfast and then received an oral dose of either indomethacin 50 mg (to act as a positive control), paracetamol 1000 mg or placebo on three separate occasions. The experiment was double blind. One hour after analgesic administration, the volunteers emptied their bladders and saved the urine. They then received 20 mg of frusemide orally and measured their hourly urine output for 4 hours. Subjects drank 100 mls of water with each medication (analgesic and diuretic) but no other food or fluid was allowed until the end of the experiment. Urinary sodium and potassium were measured using flame photometry. Data were analysed by multiple regression analysis to identify the independant effect of treatment over the whole study period.

Table 1

	placebo	paracetamol	f value	p
Na mmol	6.3 ± 2.1	2.1 ± 2.1	4.8	p<0.01
K mmol	2.0 ± 0.5	0.2 ± 0.5	0.1	NS
vol mls	46.1 ± 19.6	12.8 ± 19.5	121.8	p<0.001

Table 1 Mean ± SD volume (vol), sodium (Na) and potassium (K) excretion following 20 mg of frusemide orally after pre-treatment with placebo and paracetamol compared with indomethacin. These values represent the excess urinary excretion over the values seen after indomethacin.

As expected, indomethacin inhibited the diuresis and natriuresis of frusemide. We failed to demonstrate a significant effect of indomethacin on urinary potassium, which may have been due to inadequate dietary supervision. Paracetamol significantly decreased frusemide induced natriuresis, and diuresis. This interaction between frusemide and paracetamol may be either pharmacodynamic or pharmacokinetic, but could have therapeutic implications in patients.

We thank students from the School of Pharmacy for their help.

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PLASMA PROTEIN BINDING OF DRUGS IN INSULIN-DEPENDENT DIABETES MELLITUS.

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Alterations in the protein binding of drugs may affect the amount of free drug available for binding to tissue receptors, biotransformation and excretion. Disease states are known to alter drug protein binding but to date few studies have been reported in patients with diabetes mellitus. Reduced binding of acidic drugs (sulfisoxazole and diazepam) has been reported in diabetes (Ruiz-Cabello & Erill, 1984). Warfarin (an acidic drug) and lignocaine (basic) have a low therapeutic ratio and are commonly used in the treatment of cardiovascular diseases, which occur with increased frequency in diabetics. Alterations in the protein binding of these drugs may be of clinical relevance in the therapy of diabetics.

We determined plasma protein binding in 15 well controlled insulin-dependent diabetics (mean age 41 yrs, 13 males, mean glycosylated haemoglobin (HBA_1) 8.6%) and 15 healthy controls (mean age 35 yrs, 13 male, mean HBA_1 6.8%). ^{14}C-radiolabelled drug solutions were added to Na_2HPO_4/KH_2PO_4 buffer to yield concentrations of 2.5 µg/ml Warfarin and 0.9 µg/ml lignocaine. Plasma binding was determined at 37°C by an equilibrium dialysis technique using semi-macrocells and a semipermeable membrane with a molecular cut-off of 10,000. The results are given in the table.

Table Protein binding (mean per cent ± S.E.M.) of lignocaine and warfarin in insulin-dependent diabetics

	Control	Diabetes	P value
Lignocaine	69.2 ± 1.9	57.9 ± 2.1	< 0.02
Warfarin	98.81 ± 0.02	98.57 ± 0.03	< 0.01

These results show a 37% increase in free lignocaine and a 20% increase in free warfarin in the diabetics.

The results of this study suggest that drug binding is altered in diabetes and particular care should be taken when using lignocaine and warfarin in insulin-dependent diabetics.

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720P

CENTRAL EFFECTS OF ASPIRIN AND PARACETAMOL, AND OF THE CENTRALLY ACTING ANALGESICS, MEPTAZINOL AND PENTAZOCINE.

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Previous studies in man have suggested that codeine (Bradley & Nicholson, 1986) and other μ-receptor agonists (Griffiths et al, 1984) may impair neuromuscular activity. However, the possibility arises that such effects may not occur with mixed agonist-antagonist analgesics as their other effects (eg. respiratory depression, hypothermia) appear to be less obvious (Hayes & Tyers, 1983). We have, therefore, studied the effects of meptazinol (M) and pentazocine (PZ) on performance in man, as well as those of the non-opioid analgesics, aspirin (ASP) and paracetamol (PC).

Six females aged between 19 and 30 years took part in two double-blind studies. In the first experiment each subject ingested ASP (325 & 650 mg) and M (100 & 200 mg) and in the second experiment PC (500 & 1000 mg) and PZ (25 & 50 mg). In both studies each subject ingested two placebos and triprolidine (10 mg) as an active control. The order of treatments was randomised, and at least 3 days separated each ingestion. Dynamic visual acuity (DVA), and visuo-motor coordination (VMC) were measured, as well as digit symbol substitution (DSS), critical flicker fusion (CFF), complex reaction time (CRT), and subjective assessments of mood, at 0.75 and 2.0 h after each ingestion.

Reaction time on CRT was shortened by aspirin (650 mg) at 2.0 h (placebo 401 ms, aspirin 374 ms, $p < 0.05$), and the mean of the values at 0.75 and 2.0 h was also less (placebo 398 ms, aspirin 378 ms, $p < 0.05$). However, there were no other effects of aspirin, and similarly, there were no effects with paracetamol. There were also no effects of meptazinol on performance, though with 100 mg the component of subjective assessments related to wakefulness etc. was increased (placebo -0.15, meptazinol 0.31, $p < 0.05$), though this was not observed with the higher dose (200 mg). Pentazocine (25 mg) decreased the number of substitutions on DSS at 0.75 h (placebo 235, pentazocine 225, $p < 0.05$), and the mean of the values at 0.75 and 2.0 h was lower (placebo 233, pentazocine 226, $p < 0.05$), but again the effect was not observed with the higher dose (50 mg).

The effects of triprolidine (10 mg), which was used as an active control, were consistent between the two studies. It impaired VMC, lowered CFF threshold, decreased the number of substitutions on DSS, decreased the number of correct responses on DVA, and reduced the component of subjective assessments related to wakefulness etc. ($p < 0.05$).

Unlike codeine, it would appear that the mixed agonist-antagonists, meptazinol and pentazocine, in equivalent analgesic doses, do not impair neuromuscular activity as indicated by the present studies on dynamic visual acuity and visuo-motor coordination. Indeed, there was no effect of meptazinol except an increased awareness of wakefulness with the lower dose and this could be related to its cholinergic activity. Similarly, the effect with pentazocine on coding was not observed at the higher dose.

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A STUDY OF NEFOPAM KINETICS AFTER SINGLE AND MULTIPLE ORAL DOSES IN YOUNG AND ELDERLY VOLUNTEERS.

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Nefopam (Acupan^{R}, Riker Laboratories) is an established analgesic drug which acts centrally (Irikura et al, 1981). Oral doses of nefopam have been shown to be effective for the relief of pain in a wide range of conditions (Heel et al, 1980). This study was designed to investigate the plasma disposition of nefopam hydrochloride after single and multiple oral doses in young and elderly volunteers.

The protocol was approved by an independent ethics committee and all the volunteers gave written, informed consent to participation. Two groups of healthy volunteers participated; ten young volunteers aged 20-33 years and nine elderly volunteers aged 65-69 years. In the first phase of the study, a single oral dose of 60mg nefopam hydrochloride was given to each volunteer after an overnight fast. Blood and urine samples were collected during the 24h period after each dose. The same volunteers participated in a study of two regimens of multiple oral doses. The first regimen consisted of ten 30mg doses given 8 hourly. Blood samples were taken just before doses 2, 5 and 8 to measure trough levels of nefopam. Further blood samples were taken during the 8h period after the tenth 30mg dose. The second regimen followed straight after the first and consisted of twelve 60mg doses given 8 hourly. Blood samples were taken at intervals up to 26h after the twelfth dose. Concentrations of nefopam and N-desmethylnefopam (NDMN) were measured in plasma and urine by h.p.l.c. with detection by ultraviolet absorbance (Lewis et al., in preparation). The area under each plasma level vs. time curve (AUC) was calculated by the trapezoidal rule.

The results in Table 1 show no significant differences between nefopam kinetics in the young and elderly groups after the single 60mg doses. However, both regimens of multiple doses led to significantly greater accumulation of nefopam in the elderly. Accumulation of NDMN occurred to a similar extent in both young and elderly volunteers after multiple doses. The elderly volunteers may have had a lower capacity for N-demethylation, as has been reported with benzodiazepines (Greenblatt et al, 1982). Nefopam is metabolized by other pathways which may also have been affected by age.

If the concentrations of nefopam found in this study provide safe, effective analgesia in young patients, it might be possible to alter the standard dosage regimen in patients over 65 years of age and retain the same efficacy. Such an alteration must await further research in patients.

Table 1: Pharmacokinetic parameters of nefopam after 3 different dosing regimens of nefopam hydrochloride. Mean (s.e.m.) of each parameter.

	.....Young Volunteers (n=10)....			.....Elderly Volunteers (n=9)...		
Parameter (units)	60mg single dose	30mg 8-hourly	60mg 8-hourly	60mg single dose	30mg 8-hourly	60mg 8-hourly
Peak Level ($ng.ml^{-1}$)	47.1 (5.1)	25.8(2.1)	65.0(7.1)	51.3(9.7)	52.8(10.3)	135.0(24.0)+
Time to peak (h)	2 (1-2)*	2(1-4)	1.5(1-3)	2(1-3)	2(1-4)	2(1-4)
$AUC_{0-\infty}$ ($ng.ml^{-1}.h$)	259.1(33.8)	-	-	479.9(95.2)	-	-
AUC_{0-8} ($ng.ml^{-1}.h$)	-	127.1(10.2)	293.2(25.4)	-	294(60.5)	736.7(128.8)+
Elimination half-life(h)	6.26(0.58)	-	6.63(0.88)	8.58(1.45)	-	10.36(0.67)+
Renal clearance ($l.hr^{-1}$)	0.70(0.08)	-	-	1.27(0.46)	-	-

* Median (range)
\+ significantly greater ($p < 0.05$) than the corresponding value from the young group.

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PRESSURE SORES IN THE ELDERLY: LACK OF INHIBITION OF SPONTANEOUS MOVEMENTS DURING THE NIGHT BY CHLORMETHIAZOLE.

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Most geriatricians acknowledge the dangers associated with use of hypnotics in the elderly, but would resort to their short term trial where 'insomnia' fails to respond to education and reassurance, and where there is no underlying physical disorder. Hypnotics may be valuable in the first few nights after admission to the strange, and often noisy, environment of a hospital ward, and have been used to control nocturnal wandering and disturbed behaviour in demented elderly patients (Magnus, 1978). However, their use may inhibit spontaneous movements during the night and thereby predispose the patient to the development of pressure sores. Meprobamate and certain barbiturates have been shown to have a dose-related effect on these spontaneous movements in non-demented psychiatric patients (Hinton & Marley, 1959; Hinton, 1961). The duration of inhibition of movement was in keeping with the nurses' record of length of sleep. We ask the question whether currently recommended hypnotics for the elderly suppress movements in bed at the recommended doses. Chlormethiazole has been found to be an effective hypnotic in a dose of 384 mg nocté (2 capsules) in the elderly (Briggs et al, 1980). We investigate the effect of chlormethiazole, in doses of 1 or 2 capsules nocté, on spontaneous movement during the night in a placebo-controlled cross-over study in clinically stable elderly in-patients.

Twelve patients, mean age (1SD) 81(5) years, having given their informed consent, each received three treatments in randomised order. The treatments each lasted for 1 week and were as follows: 2 capsules of chlormethiazole, 1 capsule of chlormethia -zole and 1 of an identical placebo, and 2 capsules of the placebo. Capsules were given nightly at 22.00 h. Patients already receiving hypnotics underwent a wash-out period of 1 week prior to the first treatment. A system involving a load transducer under each leg was used to obtain a continuous plot of the lateral position of the patient's centre of gravity (Nicholson et al, 1986). The absolute displacement of the patient's centre of gravity was calculated from body weight and a calibration by a known weight. Movements were classified into eleven size intervals of 4 mm, covering a range, 4 to 48 mm. An exponential curve, $N = Noe^{-\lambda x}$ was fitted to the data on each treatment in each patient, where N is the mean number of moves per hour, in the last four nights of the treatment, in the size interval of mean value x mm. The corresponding regression line ($\log_e N$ on x) had an intercept, $\log_e No$, and a slope, $-\lambda$. $\log_e No$ is a measure of frequency of moves, whilst λ is inversely proportional to the median move size. The effects of chlormethiazole on the values of these two variables were analysed.

Eight patients completed all three treatments, 4 completed only two because of early discharge from hospital. Neither the nature nor the sequence of treatments had any significant effect on either our index of frequency of moves, $\log_e No$, or our index of move size, λ (analysis of variance, $p > 0.05$ in each case). Chlormethiazole, in doses recommended for the elderly, is thus unlikely to accelera -te the development of bed sores. Further work is required to assess whether a reduction in spontaneous movements is a necessary accompaniment of refreshing sleep

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THE EFFECT OF CIMETIDINE ON THE SINGLE DOSE PHARMACOKINETICS OF 1,5 -BENZODIAZEPINES.

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Cimetidine is known to inhibit desmethylation of the 1,4-benzodiazepines diazepam and chlordiazepoxide (Klotz & Reimann, 1980; Desmond et al, 1980) and to facilitate the absorption of diazepam and lorazepam (McGowan & Dundee, 1982). We have investigated the effect of cimetidine on the single dose pharmacokinetics of orally administered clobazam and its active metabolite, N-desmethylclobazam (NDMC), both of which are 1,5-benzodiazepines.

After an overnight fast healthy volunteers were given either clobazam 30mg (n=5) or NDMC 30mg (n=5) orally. Venous blood was sampled after 0, 0.5, 1, 1.5, 2, 3.5, 4. 4.5, 8, 12, 18, 24, 32, 48 hours and thereafter at 24 hour intervals for a further 12 days. After a one week wash out period volunteers were commenced on a 3 week course of cimetidine 400mg b.d. During the 2nd and 3rd weeks of cimetidine the protocol was repeated. Clobazam and NDMC plasma concentrations were measured using reverse phase high performance liquid chromatography. Elimination half-life ($t\frac{1}{2}$) of each substance was calculated using least squares regression analysis and the area under the concentration time curve (AUC o-∞) was assessed using the trapezoid rule. The mean results for the calculated parameters are shown in tables 1 & 2.

Table 1. Calculated parameters means ($\pm$ s.e.m.) - following 30mg clobazam

	Pre-cimetidine		Post-cimetidine	
	Clobazam	NDMC	Clobazam	NDMC
Tmax (h.)	1.7(+0.5)	50(+10)	1.1(+0.3)	77(+14)
Cmax ($ng.ml^{-1}$)	598($\pm$68)	142($\pm$23)	710($\pm$89)	185($\pm$38)
$t\frac{1}{2}$ (h.)	35($\pm$5)	81($\pm$14)	49($\pm$6)	148($\pm$26)
AUC o-∞ ($ng.ml^{-1}h$)	15179($\pm$2411)	31936($\pm$11266)	24424($\pm$2198)	49761($\pm$10216)

Table 2. Calculated parameters - mean ($\pm$ s.e.m.) - following 30mg NDMC

	Pre-cimetidine NDMC	Post-cimetidine NDMC
Tmax (h.)	18(+5)	29(+2)
Cmax ($ng.ml^{-1}$)	269($\pm$22)	279($\pm$21)
$t\frac{1}{2}$ (h.)	45($\pm$3)	56($\pm$4)
AUC o-∞ ($ng.ml^{-1}h$)	25828($\pm$2544)	35358($\pm$4411)

The $t\frac{1}{2}$ for NDMC following both clobazam and NDMC were increased significantly by cimetidine ($p < 0.01$, $p < 0.001$ respectively). Cimetidine also produced significant increases in the AUC o-∞ for clobazam and NDMC following clobazam ($p < 0.01$ & $p < 0.001$ respectively).

We conclude that oral cimetidine prolongs the $t\frac{1}{2}$ of NDMC. It also increases the AUC o-∞ for both clobazam and NDMC following an oral dose of clobazan. These latter findings suggest an effect of cimetidine on clobazam absorption and/or elimination.

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TEMELASTINE DOES NOT ALTER THE PLASMA PHARMACOKINETICS OF SIMULTANEOUSLY ADMINISTERED ANTIPYRINE IN MAN.

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Temelastine (SK&F 93944) is a novel histamine H1-receptor antagonist with high fractional protein binding and predominant hepatic clearance.
The present study was designed to assess the effects of single and chronic (1 week bid) 100 mg oral doses of temelastine (TEM) on the plasma pharmacokinetics of simultaneously administered oral antipyrine (AP: 10 mg/kg body weight) in normal subjects.

Eleven normal subjects (6 males, 5 females; mean age: 27y, SD: 4; mean body weight: 69 kg, SD: 8) were enrolled and profiled on 3 occasions. A single oral dose of AP (10 mg/kg BW) was administered on the first study day (R), then one week later, after chronic dosing with placebo bid, simultaneously with a single oral dose of 100 mg TEM (S) and then one week later, after chronic dosing with 100 mg TEM bid, simultaneously with a last 100 mg dose of TEM (L).
On each occasion blood was sampled over 48 h after dosing. Plasma concentrations of AP were assayed according to the method of Danhof et al., 1979. The maximum concentration of AP in plasma (Cmax), the area under the concentration vs time curve extrapolated to infinity, AUC (0-i), the oral plasma clearance (CL/F), the apparent terminal disposition rate constant (K) derived by log-linear regression through the last 4 points beyond 12 h after dosing, and the mean residence time (MRT) were calculated from each individual curve. The log-transformed parameters were analysed for treatment and subject related variance, and the population means for S and L were contrasted vs R by the 95 % confidence interval around the geometric mean of the individual ratios for test:treatment R as a reference.

Table 1 population means for R, geometric means for the ratios for S:R and L:R and the corresponding 95 % CI.

parameter	R	S:R	95 % CI	L:R	95 % CI
Cmax (micromol/l)	95.5	1.05	0.90 to 1.23	1.12	0.96 to 1.32
AUC(0-i)(micromol.h/l)	1441	0.97	0.87 to 1.09	1.04	0.93 to 1.16
CL/F (ml/min per kg)	0.697	1.03	0.92 to 1.15	0.96	0.86 to 1.08
K (1/h)	0.075	0.99	0.92 to 1.08	0.98	0.90 to 1.07
MRT (h)	15.25	0.99	0.91 to 1.08	0.99	0.91 to 1.08

Within all treatments and for the total group, a close correlation could be identified between the CL/F and K.

It is concluded therefore that single and chronic doses of 100 mg TEM did not alter the plasma pharmacokinetics of antipyrine. TEM at the doses used did not appear to inhibit antipyrine-related hepatic oxidative enzyme systems. The inter-subject variability of AUC values related predominantly to differences in disposition rate.

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HERNANDULCIN, A NATURAL SWEETENING AGENT. SYNTHESIS, PURIFICATION AND PHARMACOKINETICS OF THE (±)-HERNANDULCIN RACEMATE.

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(+)-Hernandulcin, (+)-H, is a sweet-tasting triterpene occurring in the leaves and flowers of Lippia Dulcis *Trev.* (Verbenaceae), a plant thriving in Central and Latin America. The Aztec people called the plant *"Tzonpelic Xihuitl"*, "sweet herb". It is likely that they used it for sweetening purposes. Recently Compadre *et al.* traced the plant and identified (+)-H as its sweet principle. A taste panel judged (+)-H to be more than 1000 times sweeter than sucrose on a molar base. This makes (+)-H rank among the most intensely sweetening compounds known today. We synthesized the (±)-H racemate and determined its pharmacokinetic characteristics in the Beagle dog after oral and intravenous administration.

(±)-H was synthesized from 6-methyl-5-heptene-2-one and 3-methyl-2-cyclohexene-1-one in a one-step aldol condensation reaction, which only takes a few minutes. The reaction mixture was subjected to preparative HPLC choosing 60H silica gel as a stationary phase and hexane/ethyl acetate (6/1, v/v) as a mobile phase. (±)-H was satisfactorily purified from its nonsweet diastereomers (+)- and (-)-epihernandulcin, which are minor reaction side products, and from unreacted starting materials and reagents. Attempts to separate (+)- and (-)-H by means of Chiral Stationary Phase HPLC were unsuccessful so far. Consequently, we do not yet know the taste of (-)-H. However, it does not seem to interfere with the sweet taste of (+)-H, regarding the undoubtedly sweet taste of the racemate.

Beagle plasma and urine samples were diluted with an equal volume (1 ml) of 0.5 M phosphate buffer (pH=8.0) and extracted with hexane. Dibutylphthalate was used as an internal standard. After evaporation of hexane, the residue was injected onto a C-18 reverse phase HPLC column (4.5 x 150 mm). The mobile phase consisted of methanol/water (85/15, v/v). The effluent was monitored at 254 nm. Excellent separation and sensitivity and short chromatogram runtimes were thus obtained.

After termination of an i.v. infusion with (±)-H, a multiexponential decline of the Beagle plasma concentration profile was observed, the terminal half-life being 9.2 h. The lipophilicity of the enantiomers was reflected in the high volume of distribution (6 l/kg). After oral administration, a small fraction (6.4 %) reached the systemic circulation quite rapidly (mean absorption time 40 minutes). The complementary fraction was either thermally degraded, destroyed by peptic juices, or, most probably, first-pass eliminated by the liver. A negligible amount of unchanged (±)-H (0.007 %) was recovered in the urine after both oral and i.v. administration, indicating extensive hepatic metabolism of both enantiomers. The nature of the metabolite(s) is not known.

The use of (±)-H as an universal sweetener is prohibited by its thermal lability at temperatures higher than ambient, and by its lipophilicity, which enables it to permeate biological membranes like the gut wall easily but which makes the compound practically insoluble in water.
A more water-soluble and thermo-stable derivative of (±)-H, which shares its enormous sweetening potential, its non-toxicity and non-mutagenity even at massive doses (Compadre *et al.*, 1985) and its cheap and simple synthesis, would be a potent rival to existing non-caloric sweeteners, especially since the most important of them, *aspartame*, suffers from thermal lability as well.

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IN VIVO α_2-ADRENOCEPTOR ANTAGONIST ACTIVITY OF BUSPIRONE AND ITS METABOLITE, 1(-2 PYRIMIDYL) PIPERAZINE (IPP) IN THE RAT.

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Buspirone is a novel anxiolytic, active in animal (anticonflict tests) and in human studies. Its mechanism of action remains obscure, but is unrelated to that of known anxiolytics (Riblet et al., 1983). Its metabolite, IPP is also active in anticonflict tests (Gammans et al., 1983). Studies by Sanghera and German (1983) suggest that buspirone and IPP may have central α_2-adrenoceptor blocking activity. This possibility was investigated by determining the actions of buspirone and IPP, in comparison with known α_2-adrenoceptor antagonists, in blocking a) clonidine -induced mydriasis and b) clonidine-induced hypoactivity, both centrally mediated effects (Berridge et al., 1983; Drew et al., 1979).

Naive, male Sprague-Dawley rats (240-300g) were used. For mydriasis testing, the rats were anaesthetized with phenobarbitone sodium, 60 mg/kg intraperitoneally. The pupillary diameter was measured 30 min after subcutaneous (s.c.) injection of test-drugs. Clonidine, 0.3 mg/kg s.c. was then injected and the pupillary diameter measured 30 min later. The ED_{50} for inhibition of clonidine-induced mydriasis was determined. The effects of single doses of each test-drug and also physostigmine were determined against mydriasis induced by atropine, 0.3 mg/kg s.c.; the test-drugs were injected 30 min before atropine and the pupil size measured 10 min after atropine. For activity testing, rats were injected s.c. with test-drugs 30 min prior to clonidine, 0.03 mg/kg s.c. and placed in plastic cages (26 x 40 x 17 cm high) 30 min later. The number of entries into each quarter of the cage was recorded by observation over a 10 min period and an ED_{50} for reversal of clonidine-induced hypoactivity was determined.

Buspirone, IPP, idazoxan, WY 26392 and yohimbine all antagonised clonidine-induced mydriasis in a dose-dependent manner. The ED_{50} values were respectively 3.7; 0.46; 0.13; 0.44 and 0.42 mg/kg. Buspirone, 8.0 mg/kg, IPP, 1.0 mg/kg, idazoxan, 0.5 mg/kg, WY 26392, 1.0 mg/kg, and yohimbine, 1.0, mg/kg did not antagonise atropine-induced mydriasis, whereas physostigmine, 0.2 mg/kg, inhibited atropine-induced mydriasis by 47%. The test-drugs themselves did not measurably alter pupil size but physostigmine caused miosis. Clonidine-induced hypoactivity was reversed by IPP, idazoxan, WY 26392 and yohimbine with ED_{50} values of 0.18; 0.17; 0.53 and 0.28 mg/kg respectively, but not by buspirone which inhibited locomotor activity per se. The other test-drugs alone had no effect on activity.

These results provide evidence that buspirone and IPP have central α_2-adrenoceptor antagonist actions in vivo. The effective doses of IPP were comparable to those of known α_2-adrenoceptor antagonists and almost 10 x those of buspirone.

The valuable technical assistance of Mr. C. Forler is gratefully acknowledged.

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CHOLINERGIC MEDIATED YAWNING AND ANALGESIA IN RATS: DIFFERENTIAL EFFECTS OF MUSCARINIC AGONISTS AND ANTAGONISTS.

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The existance of muscarinic receptor subtypes in the CNS is now well accepted. Support for this comes mainly from binding studies and depends heavily on the selectivity of the muscarinic antagonist, pirenzepine (Hoss & Ellis, 1985). However, it is important to know whether these receptor subtypes have functional correlates. We investigated a) the ability of cholinergic agonists to elicit 2 centrally mediated responses, yawning (Urba-Holmgren et al. 1977) and analgesia (Karczmar & Dun, 1978) and b) the ability of cholinergic antagonists to inhibit these behaviors elicited by physostigmine, 0.1 mg/kg subcutaneously (s.c.).

Experiments were carried out between 9.00-13.30h using male Sprague-Dawley rats (250-320g). Induction of yawning was assessed by counting the number of yawns elicited in a 30 min period immediately following s.c. injection of the agonists. Effects of antagonists were assessed by injecting them 30 min prior to physostigmine and counting the yawns elicited during 30 min after physostigmine. ED_{50} values for inhibition of yawning were calculated. Analgesia was measured, using an Appelex DS 20 Tail-Flick apparatus, in terms of the tail flick response to a focussed heat stimulus. Effects of agonists were determined 20 min after s.c. injection. Effects of antagonists were determined by injecting them s.c. 30 min before physostigmine and measuring the tail-flick response 20 min after physostigmine. ED_{50} values for inhibition of analgesia were calculated. In view of poor penetration of pirenzepine into the brain, the effects of pirenzepine and atropine were also determined following injection into the lateral ventricle (i.c.v.).

Yawning was induced by physostigmine (0.025-0.4 mg/kg); RS86 (0.05-2.5 mg/kg) and pilocarpine (0.5-4.0 mg/kg) but not by oxotremorine (0.001-0.3 mg/kg) nor arecoline (0.5-2.0 mg/kg). In contrast all 5 agonists produced analgesia over these dose-ranges. Neostigmine (0.05-0.2 mg/kg), bethanecol (0.1-10 mg/kg) and McN-A-343 (5-20 mg/kg) were inactive or marginally active in both tests. Clear differences were obtained in the potencies of drugs inhibiting yawning (Y) and analgesia (A), (ED_{50} values in the table); in particular pirenzepine i.c.v. inhibited yawning but not analgesia.

Antagonist	Route	ED50 Y	ED50 A
Trihexyphenidyl	s.c. mg/kg	0.042	0.73
Atropine SO_4	s.c. mg/kg	0.48	0.065
Atropine Me NO_3	s.c. mg/kg	7.25	1.05
Secoverine	s.c. mg/kg	7.0*	2.6
Pirenzepine	s.c. mg/kg	Inactive (1-10)	
Atropine SO_4	i.c.v. µg/rat	1.0	0.2
Pirenzepine	i.c.v. µg/rat	5.7	inactive (1-100)

* Secoverine 1 and 3 mg/kg potentiated physostigmine-induced yawning.

These results show that it is possible to differentiate muscarinic receptors on the basis of behavioural responses. Yawning was pirenzepine-sensitive and oxotremorine-insensitive whereas the opposite was found for analgesia.

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The technical assistance of C. Forler is gratefully acknowledged.

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VASOPRESSIN-PEPTIDES, PHYSOSTIGMINE AND PRAMIRACETAM ON LATENT LEARNING IN THE RAT.

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Ettenberg et al. (1983) have described a one-trial appetitive learning task (latent learning) in which they demonstrate enhanced effects of vasopressin on retention. During an initial exploration trial, non-deprived rats learn the location of a concealed water source; the rats are then deprived of water. Injection of vasopressin immediately after the acquisition trial results in increased speed of location of the water spout during re-test 48h later. We have used this model to test the effects of several drugs claimed to enhance memory in animal studies, including des-glycine vasopressin and non-peptide drugs.

We used naive Wistar rats weighing 200-220g. The apparatus consisted of a rectangular grey perspex box (60 x 45 x 30 cm high) with a smaller start box (20 x 20 x 15 cm high) attached to one end. A water bottle was concealed, except for 1 cm length of spout, in a grey perspex holder positioned on a long wall, 13 cm from the end wall opposite the start box and 14 cm above the floor. Each rat was first accustomed to handling and familiarized with the test box, without the water bottle being present. On day 2, the water bottle was present and the rat was allowed 5 min exploration of the test box, during which time it was noted whether or not contact was made with the water spout. Rats not making contact were eliminated from the experiment. Immediately after this acquisition trial, the rat was replaced in the home cage for 48h on a water deprivation schedule, with 1h water allowed at 24h. The retention trial was then run in which the time to make contact with a dry water spout was noted (retention latency).

An initial experiment was carried out to validate the method. 4 groups of 10 male rats were treated as follows: Groups A and B: acquisition trial with and without water present respectively, followed by water deprivation (as above) for both groups; Groups C and D: acquisition trial with and without water respectively, followed by ad lib access to water for both groups. In both water deprived and non-deprived rats, retention latencies were shorter for the groups having water present in the test box during acquisition, i.e. A versus B and C versus D. Water deprivation before the retention test shortened latency to contact the dry spout, i.e. A versus C and B versus D. Thus latent learning of the presence of water during acquisition was clearly demonstrated. This result was confirmed using female rats who showed less emotionality and less variability than the males.

For drug testing, groups of 15 rats were used and drugs were injected subcutaneously immediately after the acquisition trial, followed by water deprivation. Vasopressin (5 and 50 µg/kg); des-glycine vasopressin (50 and 150 µg/kg); physostigmine (0.4 mg/kg); pramiracetam (10 and 30 mg/kg) and naltrexone (1.0 mg/kg) significantly shortened retention latencies. Piracetam (500 mg/kg); hydergine (20 mg/kg); d-amphetamine (0.5 and 1.0 mg/kg); naloxone (0.5 and 5.0 mg/kg) and scopolamine (0.5 and 2.5 mg/kg) had no significant effects on retention latencies. The results support the hypothesis that learning is involved in this model and that facilitating effects can be obtained with drugs claimed to enhance memory.

We are grateful to Miss V. Verhoeven for her contribution.

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ELECTROPHYSIOLOGICAL EVIDENCE THAT ISAPIRONE (TVX Q7821) IS A PARTIAL AGONIST AT 5-HT_{1A} RECEPTORS IN THE RAT HIPPOCAMPUS.

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Radioligand binding studies have revealed two serotonin recognition sites in the rat CNS designated 5-HT_1 and 5-HT_2 respectively (Peroutka & Snyder, 1979). Further studies indicate the existence of two 5-HT_1 subtypes, 5-HT_{1A} and 5-HT_{1B}, defined by their high affinity for 8-hydroxy-2-(di-n-propylamino) tetralin (DPAT) and RU 24969 respectively (Marcinkiewicz et al., 1984). The putative anxiolytic isapirone (TVX Q7821) shows a similar regional binding distribution to that found for DPAT at 5-HT_{1A} sites, with particularly high binding in the hippocampus (Glaser et al, 1985) and may act as an antagonist with partial agonist properties at 5-HT_{1A} receptors (Marsden & Martin, 1986). The present study was undertaken to characterise the effects of isapirone on the inhibition produced by iontophoresed 5-HT and DPAT on hippocampal pyramidal neurones (Mason, 1985).

Male Wistar rats were anaesthetised with urethane (1.3-1.5 g/kg, i.p., n=15). Seven-barrel micropipettes were used for extracellular recording and for the iontophoresis of drugs: 5-HT creatinine sulphate (5-HT), acetylcholine HCl (ACh), gamma-aminobutyric acid (GABA), DPAT, isapirone (all at 20 mM, pH 4-5). An outer barrel containing 2% pontamine sky blue in 0.5 M NaCl was used for continuous automatic current balancing and dye marking of recording sites. Iontophoresis of ACh (3-10 nA) was used to activate slowly firing units.

Iontophoresis of 5-HT (0-30 nA) and GABA (1-50 nA) inhibited all hippocampal pyramidal cells studied (n=26) in a current dependent manner. DPAT (1-50 nA) inhibited 11/16 5-HT sensitive neurones. Low ejection currents of isapirone (5-30 nA) were either without effect or occasionally elevated basal discharge rates. Continuous iontophoresis of isapirone at higher ejection currents (20-100 nA) progressively suppressed basal firing rate with a latency of 30-120 s, the magnitude of which increased with ejection current and/or ejection duration; there was no effect on spike amplitude. Isapirone (5-70 nA) antagonised the effects of 5-HT on neuronal firing by 10-75%, proportional to the ejection current. Ejection currents of isapirone >30 nA suppressed firing (20-80%) and blocked 5-HT responses. On ten cells studied isapirone was a 2-4 fold more effective antagonist of responses to 5-HT than to DPAT. Isapirone did not affect GABA-induced suppression of firing (n=7 cells).

These data demonstrate that isapirone (TVX Q7821) is a 5-HT_{1A} receptor antagonist with partial agonist properties on 5-HT sensitive neurones in the hippocampus. The isapirone-induced suppression of neuronal activity might be mediated through a direct agonist action on hippocampal neurones or via antagonism of presynaptic autoreceptors on serotonergic terminals.

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PLASMA AND BRAIN KINETICS OF ZOLPIDEM, A NEW HYPNOTIC, IN THE RAT.

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Zolpidem, N,N-6-trimethyl-2-(4-methylphenyl)imidazo[1,2-a]pyridine-3-acetamide administered as the tartrate salt has been proved to be an effective hypnotic agent in animal and man. This study describes the pharmacokinetic behaviour of zolpidem in plasma and brain of rat following iv and oral administration of 2,63 $mg.kg^{-1}$ of ^{14}C zolpidem (dose expressed as the base). Total radioactivity was measured by liquid scintillation counting while zolpidem was quantified by HPLC using fluorimetric detection. An autoradiographic study was also performed.

After iv administration zolpidem plasma disappearance rate fitted a two compartment open model with a rapid phase of 0.2-0.3 hour and a slower phase of 1.3-1.5 hour. About 80 % of the dose was eliminated during the rapid phase. Following oral dosing peak plasma concentrations where attained at 15 minutes (first sampling time).
Independently from the route of administration zolpidem was present in the brain at shorter times at concentrations approaching 30 to 50 % of the plasma values. Up to 1 hour zolpidem accounted for 80-90 % of brain radioactivity. The rate of disappearance from brain paralleled that from plasma.

Autoradiographic studies confirmed the rapid absorption and elimination rate of zolpidem as well as the relatively scarce penetration of metabolites into the brain.

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EXPRESSION OF DES-ARG -BK SENSITIVE (B1) RECEPTORS IN VIVO BY CAPTOPRIL, BRADYKININ AND ENDOTOXIN.

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It has been reported that an intravenous injection of 10 μg of bacterial lipopolysaccharide (LPS) from E.Coli to rabbits induces in 5-20h a hypotensive response to the selective B1-receptor agonist des-Arg^9-BK, an effect not observed in control animals (Regoli et al., 1981). The exact mechanism(s) whereby this effect is brought about is unclear, however it is known that endotoxins can activate the kallikrein-kinin system (Rothschild and Castania, 1968). This study therefore investigates the effect of bradykinin (BK) and the angiotensin converting enzyme inhibitor, captopril (which should theoretically elevate endogenous kinin levels) on the hypotensive response to the selective B1 receptor agonist des-Arg^9-BK.

Male New Zealand White rabbits were used (2-2.5 kg). Groups of conscious rabbits were injected i.v. with 10 μg LPS from E.Coli, captopril, 5 mg kg^{-1} or infused i.v. with BK, 5 μg min^{-1} over a 30 min period. 18-20h later the rabbits were anaesthetised with pentobarbitone, 30 mg kg^{-1} i.v. and blood pressure recorded from a carotid cannula. Dose-response curves were produced to BK, des-Arg^9-BK and acetylcholine or isoprenaline, these being injected i.v. into the jugular vein. A separate group of anaesthetised rabbits were used to investigate the effect of LPS (10 μg i.v.), captopril (5 mg kg^{-1} i.v.) or an infusion of BK (5 μg min^{-1}) for 30 min on BP.

The effect of a single i.v. (10 μg) dose of LPS on BP was variable. Up to 20%Δ in mean arterial BP (MABP) was recorded in some animals (n=3) and no effect was seen in others (n=3). BK infusion produced an immediate drop in BP, the mean maximum Δ MABP being 36.9% (range 28-45.8, n=4). BP gradually recovered almost back to control levels over the 30 min infusion period. Captopril was hypotensive, the mean maximum Δ MABP being 24.2% (range 21.3-30, n=4). Equivalent volumes of saline injected or infused had no effect on BP. The BP's of anaesthetised rabbits pretreated 18h earlier were as follows: saline 65 ± 9.2 mm Hg; LPS 68.4 ± 3.7 mm Hg; captopril 55.1 ± 8.2 mm Hg; BK infusion 70.6 ± 10.6 mm Hg; untreated controls 65.6 ± 3.8 mm Hg (n=3-5). None of these values were significantly different from each other. From dose-response curves, the dose of BK, des-Arg^9-BK, acetylcholine or isoprenaline to produce a 15% Δ in MABP was determined. Doses (moles) of des-Arg^9-BK required were as follows: untreated > 10^{-7}; saline > 10^{-7}; LPS 5 ± 0.2 x 10^{-9}*; captopril 3.1 ± 0.7 x 10^{-8}*; BK infusion 6 ± 2 x 10^{-8}*: *=P<0.05 (n=3-5), value significantly different from untreated or saline groups. The dose of BK producing a 15% Δ in MABP in captopril treated rabbits (1.5 ± 0.7 x 10^{-11} moles) was significantly (P<0.05) different from saline treated animals (12.5 ± 4.2 x 10^{-11} moles). There was no significant difference between the doses of BK, acetylcholine or isoprenaline required in other groups compared to saline controls.

These results confirm the observation that endotoxin can induce a selective increase in the hypotensive response to the selective B1 receptor agonist des-Arg^9-BK in rabbits, an effect also produced by pretreating the animals with an infusion of BK or giving captopril. The present data may indicate that kinins are involved in this process and in addition may provide indirect evidence that captopril can elevate endogenous kinin levels.

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DIFFERENTIAL EFFECT OF TRI-IODOTHYRONINE ENANTIOMERS ON PITUITARY AND PERIPHERAL DEIODINASE FUNCTION IN THE RAT.

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We have previously reported that there are marked sex differences in the toxicity of tri-iodothyronine (T_3) in rats(Atterwill et al, 1985). Since T_3 is metabolised and inactivated mainly by deiodination, we have examined whether these sex differences can be related to differences in the induction of deiodinase activity by T_3 treatment. The effects of D- and L-T_3 on both induction of T_3 metabolism (Experiment A) and on inhibition of pituitary TSH release (Experiment B) were also compared in order to gain information about possible differences in the toxicity of these enantiomers.

Experiment A:- Groups of six male and female SK&F Wistar rats were treated orally with L-T_3 or D-T_3 (both Sigma; >99% optical purity) in aqueous solution at 0.01, 0.1, 1 and 1mg/kg p.o. for 14 days. 24 Hours after the last dose, liver and kidneys were removed and deiodinase activity measured in homogenates by determining production of T_3 from I_4. Deiodinase activity was higher (approximately 2-fold) in male rat control livers than in females but there was little difference in kidney activity. T_3 treatment increased deiodinase activity in a dose-related manner but there were no sex differences. Both D- and L-T_3 increased kidney and liver deiodinase activity in a dose related fashion with very little difference between the two enantiomers. The only significant difference was in the liver at 0.1mg/kg where the activity of the L-form was greater. Very little sex-related difference between the D and L-T_3 effect was apparent.

Experiment B:- In order to study differential effects of D- and L-T_3 on pituitary TSH release an <u>in vitro</u> superfusion technique was used (Jones et al, 1986). Groups of five male SK&F Wistar rats were dosed with either 0.02 or 0.1mg/kg D- and L-T_3 (Sigma, U.K.) s.c. for 14 days. 24 hours after the last dose, basal and TRH-stimulated pituitary TSH release were measured. Basal TSH release in control superfused pituitary glands was 4.81± 0.20ng/ml (n=5). Treatment with both high and low doses of L-T_3 and D-T_3 reduced this basal release approximately 8% and 33 % of controls respectively. TRH increased TSH release to a peak value of 14.5±1.7ng/ml (200% increase) in control animals. Treatment with L-T_3 at either dose, completely abolished the response to TRH whereas TRH increased TSH release to 5.15±0.94ng/ml and 4.70±0.44ng/ml (200% increase) in animals treated with the high and low dose of D-T_3 respectively.

In conclusion there appear to be marked differences in effects of L- and D-T_3 on the pituitary gland with the L-enantiomer being more potent in suppressing both basal and TRH stimulated TSH. In contrast, peripheral deiodinase activity did not show such marked differential responses to treatment with the enantiomers of T_3. Since thyromimetic treatment of both sexes resulted in a similar stimulation of deiodinase activity, it is unlikely that differences in toxicity can be explained in terms of differential effects on T_3 metabolism.

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DIET-RESTRICTION OF RATS DECELERATES AGE RELATED CHANGES IN SERUM PROLACTIN LEVELS AND CNS DOPAMINE RECEPTOR FUNCTION.

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There is a high incidence of tumours, particularly prolactin (PRL) secreting tumours of the pituitary gland as well as ovarian, mammary gland and liver tumours in ageing rats. Diet restriction results in a marked reduction in tumour incidence (1). Since it has been demonstrated that PRL can act as a tumour promoter (2) we have examined the effects of diet restriction on serum PRL levels in rats. In addition we have examined the effect of diet restriction on central dopamine (DA) receptor function since ageing is associated with a progressive increase in circulating PRL (3) as well as a reduction in central DA neuronal function (4).

At weaning, one group of Wistar rats (20 per sex) was fed ad libitum and the other restricted to 6 hours feeding per day. At 6 and 12 months of age blood samples were obtained for estimation of serum PRL by RIA.

In order to assess central DA receptor function the behavioural model, haloperidol-induced catalepsy was employed as previously described (5). Although this model predominantly measures changes in the nigro-striatal DA system it was thought that it may be representative of a generalised change in central DA neuronal function including the pituitary DA receptors controlling prolactin release. Rats were injected intraperitoneally with the DA receptor antagonist haloperidol (2mg/kg) and catalepsy measured at 30, 60 and 90 min after injection by timing the duration of the cataleptic response. At 12 months diet restriction was associated with a significant reduction in serum PRL in female rats (Ad Lib fed: 212 ± 46 ng/ml; Diet restricted: 119 ± 43 ng/ml, $p<0.05$, Student's t-test) but not in male rats (Ad lib fed: 20.7 ± 2.7 ng/ml; Diet restricted 18.7±2.2 ng/ml). There was also a significant decrease in the degree of catalepsy produced by haloperidol in the diet restricted rats ($p<0.001$, chi-squared test) when compared to ad lib fed animals. At 6 months of age although the differences in serum prolaction levels and degree of haloperidol-induced catalepsy were apparent they did not achieve statistical significance indicating that long term diet restriction is required before such changes are fully expressed.

In conclusion, these results demonstrate that diet restriction delays the increase in PRL levels and the reduction in striatal DA receptor function normally associated with ageing. As PRL can act as a tumour promoter the reduced tumour incidence in diet restricted rats may thus be secondary to an inhibition of the progressive reduction in hypothalamic DA neuronal function and subsequent inhibition of the elevated PRL levels normally seen in aged rats.

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FLUFENAMIC ACID PROMOTES CALCIUM RELEASE FROM LUNG MITOCHONDRIA IN NON-SENSITIZED AND SENSITIZED GUINEA-PIGS.

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Previous studies on rat hepatic mitochondria have shown non-steroidal anti-inflammatory drugs (NSAIDs) to be potent inhibitors of ATP synthesis and modifiers of Ca^{2+} transport (McDougall *et al.*, 1983 & McNamee *et al.*, 1985). We have therefore investigated the ability of the NSAID flufenamic acid to modify intracellular Ca^{2+} transport in guinea-pig lung.

Lung mitochondria were prepared from non-sensitized (controls) and sensitized male Duncan-Hartley guinea-pigs (500-800g) by the methods of Chappell & Hansford (1969). Sensitization of the guinea-pigs (200-250g) was produced by intra-peritoneal (50mg) and sub-cutaneous (25mg) administration of ovalbumin dissolved in 0.9%(w/v) NaCl and was allowed to develop for three weeks. Ca^{2+} ion movements were followed using a Ca^{2+}-selective electrode (McNamee et al., 1985).

Lung mitochondria from control guinea-pigs accumulated Ca^{2+} at a rate of 21.4 ± 1.6nmol min^{-1} mg $protein^{-1}$ (n=5) and, following a period of retention, released the cation back into the bulk reaction phase at a rate of 0.9 ± 0.1nmol min^{-1} mg $protein^{-1}$ (n=5). Addition of flufenamic acid (0.1-60μM) prior to the mitochondria produced a concentration-dependent inhibition of Ca^{2+} uptake (IC_{50} value = 39.1 ± 2.6μM; n=5). Conversely, addition of flufenamic acid (0.1-30μM) after the mitochondria had been allowed to accumulate the Ca^{2+} load (125nmol) for 3min resulted in a concentration-dependent stimulation of Ca^{2+} efflux (EC_{50} value = 9.1 ± 2.6μM; n=5). Qualitatively similar results were obtained with the NSAID diflunisal (0.1-80μM) and the uncoupling agent 2,4-dinitrophenol (10-50μM). Statistical analysis confirmed that flufenamic acid produced significantly more potent effects ($p<0.05$) and that each compound was more effective at promoting efflux than inhibiting uptake of Ca^{2+}.

Table 1. The Effect of NSAIDs on Ca^{2+} Ion Movements

NSAIDs	Guinea-Pig Mitochondrial Ca^{2+} Transport.			
	Non-Sensitized		Sensitized	
	Influx IC_{50}(μM)	Efflux EC_{50}(μM)	Influx IC_{50}(μM)	Efflux EC_{50}(μM)
Flufenamic Acid	39.1 ± 2.6	9.1 ± 1.0	36.4 ± 3.4	8.3 ± 1.1
Diflunisal	51.1 ± 2.6	16.2 ± 1.5	56.3 ± 3.2	18.9 ± 3.2

Results are the means of five different experiments ± s.e.mean.

Flufenamic acid (0.1—30μM) and diflunisal (0.1-80μM) also promoted Ca^{2+} release in lung mitochondria prepared from sensitized guinea-pigs. However, the response to these NSAIDs was not significantly altered ($p>0.05$) by sensitization (Table 1). This data indicates that NSAIDs could increase intracellular Ca^{2+} levels by promoting mitochondrial efflux of the cation.

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EFFECTS OF LIPID AMPHIPHILES ON ARRHYTHMOGENESIS IN THE ANAESTHETIZED CAT.

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Lysophophatidylcholine (LPC) and palmitoylcarnitine (PAL) accumulate in the ischaemic myocardium (Shaikh and Downar 1981, Idell-Wenger et al 1980) and may contribute to the development of arrhythmias. LPC produces arrhythmias in isolated hamster (Man et al, 1983) and rabbit hearts (Bergmann et al, 1981).

An anaesthetized cat model was used to study the effect of these amphiphiles *in vivo*. Infusions via the left anterior descending coronary artery were performed by means of a 34 gauge needle. LPC, at a calculated concentration of 500 μM gave reproducible arrhythmias, ventricular fibrillation (VF) and death. PAL had the same effect at a concentration of 50 μM, and also produced ST elevation and a drop in temperature which was reversed by streptokinase, indicative of ischaemia. The time course of the onset of arrhythmias and VF following LPC and PAL was similar to that seen on coronary artery ligation (CAL).

The effects of PAL may be due to the production of ischaemia, thus the effects on platelet aggregation were studied. Neither LPC nor PAL had any aggregatory effects on rat or rabbit platelets. In contrast both attenuated the aggregatory response to ADP (Table 1).

TABLE 1 Effect of LPC and PAL on ADP induced platelet aggregation n=3-5

LPC μM	0	50	200	500					
%response to ADP	100 ±0	94 ±1	67 ±0	54 ±7					
PAL μM	0	10	30	50	100	200	300	500	1000
%response to ADP	100 ±0	93 ±8	85 ±12	87 ±6	76 ±9	64 ±6	66 ±0	64 ±3	16.5 ±16

Therefore, LPC and PAL infusions have been shown to cause arrhythmias in an *in vivo* model, the time course being similar to that seen following CAL. The mechanism of PAL induced arrhythmias may be due to the development of ischaemia although this is unlikely to be initiated by platelet aggregation.

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ROLE OF ENDOTHELIUM IN THE RESPONSE OF IN VITRO RAT AORTA UNDER RESTING AND SUSTAINED TENSION TO BRADYKININ AND ANGIOTENSIN II.

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The obligatory role of endothelial cells in the relaxation of the in vitro rabbit aorta to acetylcholine (ACh) was first demonstrated by Furchgott et al. (1980), and later the dependence of the endothelium for relaxation of a variety of blood vessels to a wide range of agents was shown (Furchgott, 1983). From these and other studies it is clear that the endothelium releases a non-prostaglandin relaxant factor that is not only important in mediating the relaxant response to a wide variety of substances but that it also influences the response seen to a wide range of contractile agents. This study investigates the role of the endothelium in the response of the in vitro rat aorta under resting and sustained tension to bradykinin (BK) and angiotensin II (AII).

The aorta was taken from female Wistar rats (200-300g) and cut into 4mm long rings. The endothelium was removed by turning a narrow wooden cocktail stick in the lumen of the aortic ring. Intact and de-endothelialised preparations were mounted on narrow gauge wire holders in 4ml tissue baths, under a tension of 2g. The tissues were incubated in Krebs-Henseleit solution at 37°C bubbled with 95%-O_2/5%CO_2. The presence of endothelial cells was demonstrated functionally by relaxation of the tissue to Ach, and histologically by a silver staining technique (Poole et al, 1958). After an equilibration period of 1h, concentration-effect curves were constructed to BK and AII on intact and de-endothelialised tissues under resting tension and after contraction with an EC_{50} of phenylephrine.

Under resting tension conditions both BK and AII produced concentration dependent contractions of the tissues with the curves for both agents on de-endothelialised tissues being shifted significantly to the left compared with intact preparations. The maximum contraction to AII was produced with a concentration of 10^{-7}M. Further cumulative addition of higher concentrationss of AII (10^{-6}-10^{-5}M) consistently failed to produce any further increase in contraction. Indeed tissues always relaxed back to resting tension. This relaxant effect of AII was seen on both intact and de-endothelialised tissues. On tissues precontracted with an EC_{50} of phenylephrine both BK and AII produced concentration dependent contractions (AII max, 10^{-7}M). Concentration effect curves to BK and AII on intact and de-endothelialised preparations were superimposable. The combined lipoxygenase/cyclo-oxygenase inhibitor BW 755C (10^{-4}M) failed to inhibit the relaxant response to Ach on intact tissues precontracted with phenylephrine and had no effect on concentration effect curves to BK or AII on tissues under resting tension either intact or de-endothelialised.

In conclusion, the endothelium is clearly modulating the response of the in vitro rat aorta to BK and AII under resting tension but apparently not when the tissues are precontacted with phenylephrine. The relaxant response to AII does not appear to involve the release of endothelial factors. The underlying mechanisms and the role of endothelial factors in the response to BK and AII are being investigated further.

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H.W. is an SERC CASE student with ICI plc.

INFARCT SIZE LIMITATION WITH ALLOPURINOL AND VERAPAMIL ALONE OR IN COMBINATION DURING 48 HOURS OF CORONARY ARTERY OCCLUSION.

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In the canine heart during 24h of permanent coronary artery occlusion allopurinol and verapamil reduce infarct size by up to 40 percent. In the present study we investigated whether combined allopurinol+verapamil would (a) limit infarct size to a greater degree than with allopurinol or verapamil alone and (b) whether the protective effect can be sustained over a 48h period of permanent coronary artery occlusion.

Dogs (25 to 36kg) were pre-medicated with acepromazine (1 mg/ml,i.m.) and anaesthetized with thiopentone sodium (25 mg/kg, i.v.). Coronary artery occlusion was carried out by injecting a 2.0 mm metal bead into the coronary vasculature using a specially designed arterial cannula positioned in the coronary ostium. One minute after occlusion 20 X 10^6 ^{141}Cerium labelled microspheres (15 microns) were injected into the left ventricle to allow subsequent delineation of the anatomic zone of underperfusion (risk zone) and measurement of regional myocardial blood flow. Dogs were divided into 4 groups (n = 6/group): (i) controls given an intravenous infusion of saline, (ii) allopurinol (400 mg; p.o.), 24 and 2h before coronary artery occlusion followed by 10 mg/kg; i.v. 12h and 24h after occlusion, (iii) verapamil (0.2 mg/kg; i.v.) two min after occlusion and thereafter as a continuous infusion (0.005 mg/kg/min; i.v.) during 48h, (iv) allopurinol+verapamil at the same doses given in groups (ii) and (iii). Dogs were sacrificed 48h after coronary artery occlusion and the hearts were sliced into serial 4 mm sections. Infarcted tissue was visualized by triphenyl tetrazolium chloride staining and the risk zone by autoradiography. Transmural biopsies from the risk zone and non-ischemic zone were divided into three fractions (endocardial, mid-myocardial and epicardial), weighed and the radioactivity was measured. Collateral blood flow within the risk zone was expressed as a percent of non-ischemic blood flow.

Infarct sizes (expressed as a percent of risk zone size) were: (i) controls = 75.0 $\pm$ 20.7%, (ii) allopurinol = 49.5 $\pm$ 17.2%, (iii) verapamil = 51.2 $\pm$ 15.4%, (iv) allopurinol+verapamil = 47.0 $\pm$ 8.7% (mean $\pm$ SD). Risk zone size for all of the groups was identical (p:NS); however, the size of the infarcts in all drug-treated groups was lower ($p<0.05$) than controls. In control dogs there was a negative correlation ($r = -0.81$) between infarct size and collateral blood flow in the subepicardial fractions such that, given collateral blood flow, infarct size could be reliably predicted. Any drug which achieves true salvage (without altering collateral blood flow) would be expected to produce a smaller mean infarct size than that predicted by the collateral blood flow. In the present study this was found to be the case. The ratio of the actual infarct size to the predicted infarct size we term the 'salvage index'. Tissue salvage is indicated when this index is less than 1.0. The salvage index in the allopurinol+verapamil group, the allopurinol alone group and the verapamil alone group was 0.6 $\pm$ 0.1, 0.6 $\pm$ 0.2 and 0.8 $\pm$ 0.2 (mean $\pm$ SD), respectively.

In conclusion, allopurinol, verapamil, or both drugs in combination, can limit infarct size for up to 48h of sustained coronary artery occlusion.

MEPTAZINOL-INDUCED DIURESIS IN THE WATER-LOADED RAT IS ANTAGONISED BY ATROPINE.

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Recently, we reported that meptazinol produced diuresis in the water-loaded rat and that this effect was antagonised by the opiate antagonists naloxone and MR2266 (Algate, Brammer & Lawrence, 1986). However, in addition to opioid actions meptazinol is also reported to have cholinomimetic properties (Green, 1983, Cowlrick and Shepperson, 1985). In this study the effect of the muscarinic antagonist atropine on meptazinol-induced diuresis was examined.

Female Wistar rats (200 - 250 g) were treated subcutaneously with atropine 15 minutes before administration of meptazinol (30 mg/kg s.c.). The animals were water-loaded at a dose volume of 25 ml/kg and placed individually in metabolism cages. The cumulative urine output was then measured hourly over a period of 5 hours. There was a minimum of 5 animals per group and animals were tested not more than once weekly.

Meptazinol produced a dose-related diuresis in the rat. A volume of between 2.5 and 5.3 ml above control urine output was achieved 5 hours post-dose following subcutaneous administration of meptazinol at 30 mg/kg. In comparison, the kappa agonist cyclazocine at 3 mg/kg (s.c.) produced an increase of 4.5 - 5.1 ml above control urine output levels.

Pretreatment with atropine sulphate at 0.3 and 3 mg/kg reduced the meptazinol (30 mg/kg)-induced diuresis by 29.4% and 41.2% respectively at 5 hours post-dose. Atropine sulphate pretreatment was without effect on cyclazocine (3 mg/kg)-induced diuresis. A slight antidiuretic effect was observed following administration of atropine alone, but was not sufficient to account for the antagonism of meptazinol-induced diuresis. Atropine methylnitrate pretreatment at 3 mg/kg also reduced the meptazinol (30 mg/kg)-induced diuresis by 41.2%.

The results of this study indicate that meptazinol exerts its diuretic effect in the water-loaded rat through a peripheral cholinergic action, in addition to the opioid action previously reported.

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CLONIDINE BLOCKS THE PRESSOR EFFECT OF ELECTRICAL STIMULATION OF THE C1 AREA IN NORMOTENSIVE RATS.

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Biochemical evidence suggests that the blood pressure lowering effect of clonidine may be linked to an inhibition of the release of adrenaline in the hindbrain (Atkinson et al, 1984) possibly in the A1/C1 area (Atkinson et al, 1986). Stimulation of the C1 area electrically increases blood pressure (Reis et al, 1984). We have investigated the effect of clonidine on the pressor response to electrical stimulation of the C1 area.

Three month old, male, normotensive Wistar rats were anesthetized with sodium pentobarbitone (50 mg/kg i.p.). Anesthesia was later supplemented as required with Dial (R) (a mixture of 4 parts urethane plus 1 part allobarbitone w/w). The femoral artery and vein were cannulated and tracheoctomy performed. Rats (n=14) were artificially respired with room air (10 ml/kg, 60 strokes/min) and received tubocurarine (1.5 mg/kg i.v). The back of the skull was removed and a glass electrode was inserted 1.5 mm rostral and 2 mm lateral to the obex, and 2.5 mm below the surface of the brain. The exact location of the electrode was checked histologically at the end of the experiment. The electrode was made from a glass capillary and had a 4 mm tip with parallel walls of 30 micron external diameter. It was filled with 0.15 M NaCl. Electrical stimulation was carried out for 10 sec at 100 Hz, 0.2 msec duration and 40 V. Changes in diastolic arterial pressure were recorded. A series of 3 stimulations were carried out over 10 min. Clonidine (0.5 mg/kg s.c. n = 5) or solvent (n = 9) was injected and stimulations repeated every 10 minutes for the next 30 min.

	Diastolic arterial pressure (mmHg)	
	Controls	Clonidine (0.5 mg/kg, s.c.)
n	9	5
Before stimulation	72 ± 10	61 ± 7
Change following stimulation before injection	+69 ± 7	+68 ± 7
Change following stimulation 30 min after injection	+66 ± 8	-5 ± 8

Our results are consistent with the hypothesis that electrical stimulation liberates a neurotransmitter in the C1 pressor area and that clonidine inhibits this liberation. Adrenaline may be the transmitter liberated or may be liberated together with another transmitter.

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THE BLOOD PRESSURE LOWERING EFFECT OF CAPTOPRIL IS RELATED PRIMARILY TO THE PLASMA RENIN LEVEL.

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The fact that converting enzyme inhibitors lower blood pressure in low, as well as high plasma renin, states (Sweet and Blaine, 1984 and Antonaccio et al, 1981), has raised the question as to whether (1) blockade of the circulating renin angiotensin system is the universal mechanism for the blood pressure lowering effect and (2) some other mechanism, such as blockade of a vascular renin-angiotensin system may be involved, especially in low renin situations. In order to gain further information on this point we have studied the blood pressure lowering effect of captopril in rats with widely varying renin status. Differences in renin status were produced by various combinations of unilateral renal artery clipping (gap 0.2 mm, for 1 month) and 24 h nephrectomy. Captopril was infused (3 mg/kg i.v. per minute for 10 minutes) under urethane anesthesia. Plasma and aortic renin levels (PRL and ARL, ng AI . mg protein^{-1}. h^{-1}) were determined by incubation with rat renin substrate and radioimmunoassay of ANG I formed. Results were as follows:

Group	n	Clipped kidney	Nephrectomy	Mean arterial pressure Basal (mmHg)	Mean arterial pressure %fall after captopril	PRL	ARL
A	36	sham	sham	92 ± 12	33.3 ± 2.2	11.4 ± 1.0	0.93 ± 0.01
B	18	sham	right	90 ± 17	33.0 ± 2.1	6.8 ± 0.9	-----
C	24	sham	bilateral	81 ± 15	8.9 ± 0.4	0.006 ± 0.002	ND
D	41	left	sham	123 ± 22	38.0 ± 2.8	30.1 ± 4.5	2.20 ± 0.29
E	49	left	left	81 ± 17	23.0 ± 1.4	1.5 ± 0.3	0.35 ± 0.06
F	14	left	right	132 ± 41	37.4 ± 3.6	46.0 ± 10.0	-----
G	18	left	bilateral	81 ± 45	17.0 ± 0.8	0.06 ± 0.02	0.46 ± 0.12

ND = not detectable. Results are means ± SEM.

Renin values were not normally distributed. Correlation coefficients were calculated using $\log_{10}$ transformation of renin values.

Within each group there was no significant correlation between the fall in blood pressure produced by captopril and either PRL and ARL. Correlations between the fall in blood pressure and either PRL or ARL across the groups, were highly significant:
($r = 0.99$ $n = 5$ $p<0.001$ for PRL and $r = 0.94$ $n = 3$ $p<0.05$ for ARL).

In conclusion (1) the blood pressure lowering effect of captopril appears to be correlated to plasma renin status and (2) it does not appear necessary to invoke an action on the vascular renin angiotensin system in order to explain the effect of captopril.

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COMPARISON OF THE ACUTE HAEMODYNAMIC EFFECTS OF PINDOLOL AND LABETALOL IN HEALTHY VOLUNTEERS.

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Pindolol and labetalol are non-selective ß-blockers to which additional vasodilating properties are ascribed, caused by stimulation of vascular $ß_2$-adrenoceptors (ISA) and by α_1-antagonist activity respectively. The purpose of the present study was to compare the ß-blocking and vasodilating activities of both drugs in healthy volunteers. Six subjects with a mean age of 23 years (range 20-28 years) and a mean weight of 71 kg (range 63-82 kg) participated. Informed consent was obtained from all subjects. All subjects received an oral dose of pindolol (10 mg) or labetalol (200 mg), in a random order with at least 1 week in between. Before and 1, 2 and 3 hours after drug administration, the following measurements were made with the subjects in the supine position: systolic and diastolic blood pressure (SBP, DBP, by an automatic device), heart rate (HR, via ECG), forearm blood flow (FBF, by plethysmography). At the beginning of the study (control) and 3 hours after administration of each drug, bicycle ergometry was done at 3 levels (25, 50 and 100 Watt), each level lasting for 5 min. Results are expressed as means ± SEM. During the 3 hours after administration of either pindolol or labetalol, no significant changes were found in supine BP, HR or FBF (table 1).

Table 1: Haemodynamic effects before and during 3 hours after drug administration

	Pindolol (10 mg)				Labetalol (200 mg)			
Hours	0	1	2	3	0	1	2	3
SBP (mmHg)	115±2	112±2	113±2	113±2	111±2	107±2	110±2	113±2
DBP (mmHg)	54±2	52±2	58±3	55±1	55±2	55±2	58±2	57±3
HR(beats/min)	60±2	56±2	57±4	57±3	62±3	59±4	61±3	59±2
FBF(ml/100ml/min)	3.5±0.4	3.2±0.4	2.8±0.3	3.0±0.5	3.3±0.5	2.6±0.3	2.8±0.2	2.6±0.2

Changes in BP and HR during bicycle exercise in the sitting position, before and 3 hours after drug administration are given in table 2.

Table 2: Bicycle ergometry before and 3 hours after drug administration

	Control		Pindolol		Labetalol	
Watts	0	100	0	100	0	100
SBP (mmHg)	119±3	148±4	105±4*	118±4**	100±2**	126±4**
DBP (mmHg)	71±4	46±3	67±4	59±5**	65±2	47±4[+]
HR (beats/min)	88±6	123±5	69±3**	104±4**	78±5	113±4[+]

Statistical significance (Student's t-test for paired observations):
versus control : *$p<0.05$, **$p<0.01$, versus pindolol: [+]$p<0.02$

SBP was significantly reduced by pindolol and labetalol before and during exercise. DBP was not reduced before exercise by either of the ß-blockers. However during 100 W exercise DBP was higher after pindolol when compared with control or labetalol. The reduction in HR before and during exercise was greater for pindolol when compared with labetalol.

Conclusions: After oral administration of pindolol and labetalol no peripheral vasodilation could be shown. The different haemodynamic changes during exercise between pindolol and labetalol can be explained by the α_1-antagonist activity of labetalol.

PAF-INDUCED BRONCHIAL HYPER-ACTIVITY: EFFECTS ON β-ADRENOCEPTOR FUNCTION.

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Exposure of experimental animals and normal volunteers to PAF results in an increase in non-specific bronchial reactivity (Mazzoni et al., 1985a; Cuss et al., in press) which is poorly reversed by beta-adrenoceptor agonists (Mazzoni et al., in press) and PAF has been shown to cause beta-adrenoceptor desensitisation (Braquet et al., 1986). In the present study we have investigated the possible mechanisms underlying this poor reversibility.

Anaesthetised guinea pigs were monitored for changes in intra-thoracic pressure (ITP) using the Konzett-Rossler technique. Responses to histamine (H) (10 μg/kg) or bombesin (0.5-10 μg/kg) injected i.v., were measured before and after an i.v. infusion of PAF (600 ng/kg/hr) or bovine serum albumin (BSA). Bombesin was administered to induce a comparable level of prolonged airways obstruction in both BSA and PAF treated animals and the effects of cumulative doses of isoprenaline (ISO) (0.1-10 μg/kg) on reversal of airways obstruction was determined. Infusion of PAF increased the mean increase in ITP to H from 2.1 ± 0.4 to 12.6 ± 1.0 mm Hg (n=6) compared to BSA treated controls (4.1 ± 0.7 before; 7.1 ± 1.0 mm Hg after, n=7). ISO (0.1, 1 and 10 μg/kg) reversed the increase in ITP in bombesin treated control animals from 10.3 ± 1.6 mm Hg (n=12) to 8.1 ± 1.9 (n=8), 4.8 ± 0.8 (n=11) and 3.5 ± 0.1 (n=8) mm Hg respectively. However, the effect of bombesin in PAF treated animals eliciting a comparable increase in ITP (9.8 ± 2.1 mm Hg, n=6) was not significantly reversed by ISO (0.1-10 μg/kg). These animals were sacrificed and tracheal transverse strips mounted in organ baths for isometric recording and the ability of cumulative doses of ISO to reverse a H-induced contraction ($5x10^{-6}$M) was studied. This produced equal contractions in both PAF and BSA treated tissues and the EC_{50} values for ISO in PAF and BSA treated animals were not significantly different ($-\log_{10} EC_{50}$=7.44 ± 0.2, n=5, in PAF treated animals and 7.02 ± 0.28, n=6, in control animals). Similarly, there was no difference in maximal relaxation to ISO between PAF treated and control animals. Aliquots of lung membrane preparations from the above animals were incubated with ^{125}I-cyanopindolol (CYP) (12-200 pM) at 37°C for 60 min. Non-specific binding was determined by adding 200 μM ISO. Scatchard analysis of these data yielded the affinity constant of binding (K_d) and the maximum number of receptors (B_{max}). There was no significant difference between the values of K_d (46.5 ± 12.3 pM v 32.7 ± 4.0 pM) and B_{max} (306 ± 50 fmol/mg protein v 293 ± 50 fmol/mg protein) for control and PAF treated animals respectively. Chopped peripheral lung from untreated animals was incubated for 45 min with or without PAF (10^{-7}M) and subsequently used for membrane preparations on which ^{125}I-CYP binding was examined. No significant differences were found between the K_d and B_{max} values (K_d 31.0 ± 4.0 pM v 30.6 ± 7.0 pM; B_{max} 244 ± 48 fmol/mg protein v 230 ± 54 fmol/mg protein) of control and PAF treated lungs.

These results suggest that PAF-induced hyperreactivity is not associated with the loss of beta-adrenoceptor function and that the inability of isoprenaline to reverse airways obstruction in hyperreactive animals probably reflects the contributions of non-spasmogenic elements.

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A NOVEL ASSAY FOR ASSESSING THE BINDING OF PHOSPHOLIPASE A_2 TO SUBSTRATE.

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Phospholipase A_2 activity is often estimated using pre-labelled E. coli organisms as a substrate. The action of phospholipase A_2 (PLA_2) is regulated by its ability to attach to its substrate. The enzyme has a well-characterised Interface Recognition Site which binds to organisational defects in phospholipid bilayers (Verheij et al, 1981). Binding of the enzyme to pure phospholipids has previously been measured using spectroscopic methods, by equilibrium gel filtration and by isothermal calorimetry. We describe here an adaptation of the E. Coli hydrolysis assay to measure the binding of PLA_2 to a biological membrane.

[^{125}I]-labelling of commercially available pure pig pancreatic PLA_2 was performed using the Chloramine T method (Hunter & Greenwood, 1962). Appropriate aliquots of the labelled enzyme (50 - 150 ng, 50 - 200,000 dpm) were incubated routinely in 10 mM MOPS buffer pH 7.4 containing 10 mM Ca^{2+} and 1 mg/ml bovine serum albumin with autoclaved E. coli (KRAB 1157 strain, 10^8-10^9 cells/ml) for 15 min at 37°C. 'Bound' enzyme was removed by centrifugation at 10,000 xg for 3 min to sediment the E. coli, and aliquots of the supernatant removed and counted. Binding of [^{125}I]-PLA_2 to E. coli occurred rapidly (<5 min) at 4°C and at 37°C, was related to the number of E. coli added, and was inhibited by unlabelled enzyme. The binding was Ca^{2+}-dependent (max. at 5 - 10 mM) and Mg^{2+} did not substitute. Maximum binding occurred at pH7-pH8. The binding was inhibited by chlorpromazine (IC_{50}~30 μM) and enhanced by 4mM hexanol. Neither procaine (0.1 - 10 mM) nor mepacrine (500 μM) had any effect. The detergents Triton X-100 and Tween-20 (0.01%) inhibited binding whereas deoxycholate (0.5 mM) enhanced binding. In contrast, hydrolysis of sonicated egg phosphatidylcholine by pig pancreatic PLA_2 was increased by Triton X-100. The differences may be related to the physicochemical structure of the substrate in each case.

Human recombinant lipocortin (LC; Wallner et al, 1986) inhibited binding of [^{125}I]-PLA_2 to E. coli with an IC_{50} of approx. 10^{-8} M. A similar IC_{50} was found in the E. coli hydrolysis assay. The effect of LC appears to be specific, since several other proteins had no consistent effect upon the assay at concentrations up to 1000-fold higher. Furthermore in preliminary experiments, the antibody RM23 raised against macrocortin from rat peritoneal fluid (Flower et al, 1984) reversed the inhibitory effect of LC.

We suggest that the binding of [^{125}I]-PLA_2 to E. coli may prove a rapid and sensitive screening procedure in the investigation of anti-PLA_2 proteins and other substances, and may help to elucidate their mechanisms of action.

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THE EFFECTS OF Zn^{++} ON PIG PANCREATIC PHOSPHOLIPASE A_2 ACTIVITY AND CARAGEENIN-INDUCED PLEURISY.

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Hydrolysis of the 2^1 fatty acid ester bonds in diacyl phosphoglycerides by mammalian pancreatic phospholipase A2 (PLA_2), shows an obligatory requirement for calcium ions (Pieterson et al, 1974). Binding of Ca^{2+} to PLA_2 induces distinct changes in the ultra violet (UV) difference spectrum of the protein caused by the conformational change necessary for the binding and hydrolysis of substrate. Other divalent cations will not substitute for Ca^{2+} but the related Group II metal ions Ba^{2+} and Sr^{2+} induce qualitatively similar UV changes to Ca^{2+} and permit binding of enzyme to substrate, although the resulting complex is catalytically inactive. Ba^{2+} and Sr^{2+} therefore act as competitive inhibitors of PLA_2 in the presence of Ca^{2+} (Pieterson et al, 1974). We have investigated the effect of Zn^{2+} on PLA_2 activity and the binding of pig pancreatic PLA_2 to its substrate.

PLA_2 was measured by three methods: (i). a conventional radiochemical method; (ii). a continuous direct spectrophotometric method; (iii). a continuous direct turbidimetric method. Enzyme activity in all assays was characterised by an initial lag phase of 2-3 minutes after which a linear reaction rate was observed for at least 5 minutes. In all assays Zn^{2+} as the Cl^- salt had an IC_{50} of approximately 30 µM. Other divalent cations Mg^{2+}, Fe^{2+}, Cu^{2+}, Co^{2+}, Pb^{2+}, Cd^{2+}, Au^{2+}, Mn^{2+} and Ni^{2+} had no effect on enzyme activity at concentrations up to 200 µM. Ba^{2+} and Sr^{2+} at a concentration of 250 µ M inhibited enzyme activity by 35% and 47% respectively. The inhibitory effect of Zn^{2+} was dependent on the Ca^{2+} concentration with the IC 50 at 10 mM, 20 mM, 30 mM, 40 mM and 50 mM, Ca^{2+} being 4 µM, 12 µM, 26 µM, 30 µM and 38 µM respectively. Chelation of zinc by the addition of CaEDTA or 1,10 phenanthroline relieved the inhibition of PLA_2. Furthermore addition of these chelating agents to samples in which hydrolysis had been totally inhibited by 200 µM. Zn^{2+} resulted in the initiation of reaction without the lag phase, indicating that in the presence of Zn^{2+} an inactive enzyme-substrate complex had been formed. UV difference spectra of PLA_2 in the presence of Zn^{2+} showed qualitatively similar absorbance changes to those induced by Ca^{2+}, suggesting that Zn^{2+} binds at or near the Ca^{2+} binding site of PLA_2. In addition, Zn^{2+} and Ca^{2+} induced qualitatively identical fluorescence emission spectra of PLA_2 in the presence of the fluorescent probe ANS. Zn^{2+} and Ca^{2+} were also effective in protecting PLA_2 against inactivation by incubation with p-bromophenacyl bromide (BPB). Incubation of PLA_2 alone with 1 mM BPB for 3 hours resulted in an 85% loss of enzyme activity whereas in the presence of 100 µM Zn^{2+} or 5 mM Ca^{2+} inhibition was only 17% and 22% respectively. Direct binding studies of 125I PLA_2 to E. coli phospholipid membranes showed that like Ca^{2+} Zn^{2+} (10 - 100 µM) enables PLA_2 to bind to phospholipid.

On the basis of its PLA_2 inhibitory effects Zn^{2+} was tested as an anti-inflammatory agent. Using the carageenin pleurisy model 50 µg of Zn^{2+} given by i.v. injection simultaneously with carageenin was found to inhibit cell infiltration by 56% while having no effect on exudate volume.

The present results show that Zn^{2+} ions can compete at the Ca^{2+} binding site, or a closely related site, on PLA_2 and permit binding of PLA_2 to its substrate, although the resulting Zn^{2+}-enzyme-substrate complex is catalytically inactive. This mechanism of inhibition of PLA_2 may have useful implications in the control of inflammation.

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HAEMODYNAMICS OF BUMETANIDE IN CHRONIC AND EXERCISE-INDUCED LEFT HEART FAILURE.

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The haemodynamic and radionuclide (nuclear stethescope) effects of bumetanide were determined in 24 patients with impaired left ventricular function due to angiographically documented coronary artery disease. Two groups of patients with raised (>18 mmHg; Group I) or normal (<18 mmHg; Group II) resting pulmonary artery occluded pressure (PAOP) were prospectively studied. Both groups had impaired exercise cardiac function (PAOP > 25 mm Hg). Following a stable 20 min control period, resting haemodynamics were determined 3, 7, 15 and 30 min following bumetanide 25 ug/kg i.v. The exercise effects (30 min) compared haemodynamics during 4 min constant-load supine bicycle exercise with a control period at the same workload. At rest systemic arterial blood pressure and vascular resistance index were increased in both groups. The heart rate was unchanged, and there were significant reductions in cardiac index and PAOP. The left ventricular ejection fraction was unchanged in Group I, but reduced in Group II patients. Cardiac volumes were unchanged. There were directionally similar effects during dynamic bicycle exercise. However the cardiac index was maintained at a reduced PAOP, and there was a smaller increase in systemic vascular resistance index.

		REST		EXERCISE	
Variable	Gr	Control	Drug	Control	Drug
MAP	I	98±5	102±5**	108±6	113±6**
	II	97±4	103±5**	117±5	126±5**
HR	I	85±5	84±6	110±5	108±5
	II	76±3	74±3	100±4	100±4
PAOP	I	24±2	21±2	35±1	29±2**
	II	11±1	9±1	26±2	17±1**
SVI	I	35±3	33±2	38±3	37±3
	II	45±3	39±2**	53±4	51±3
EF	I	35±4	33±2	26±3	28±3
	II	54±6	49±5**	39±4	39±3

Mean±S.E. Drug effects at 30 min. Statistics control vs drug ** $p<0.01$. Variables - Mean Arterial Pressure (mm Hg), Heart Rate (beat min-1), Pulmonary Artery Occluded pressure (mm Hg), Stroke Volume Index (ml m-2), Ejection Fraction (%)

The immediate effects of bumetanide were on systemic arterioles (pressor response) and capacitance vessels (reduced PAOP); left heart volumes were unchanged.

A SAFER METHOD TO OBTAIN INTRAVENOUS PHARMACOKINETIC DATA.

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To obtain fundamental pharmacokinetic data of half-lives, clearance, volume of distribution, absolute bioavailability and first pass metabolism, for the prediction and adjustment of dosage regimens, it is essential to administer the drug by the intravenous route. The most common method of intravenous dosing is by bolus injection, but this is not without risk because of possible side effects with high initial plasma levels. Also, once all the drug is in the body, the clinician's options become severely restricted should adverse effects occur, particularly when dealing with new drugs.

Constant rate intravenous infusions which mimic oral doses in terms of maximum plasma levels (Cpmax) and time they are reached (tmax) offer a suitable alternative without these disadvantages. A simple method has, therefore, been devised for the calculation of infusion rate which can best be considered in two phases: (a) the infusion rate to obtain the desired level (Cpmax) at steady-state and (b) the adjustment of the infusion rate so that Cpmax is also achieved at the desired time (tmax).

(a) At steady-state, the rate of drug entering the body must equal that eliminated or:

infusion rate = clearance x steady-state plasma levels (Cpss) ...1

An infusion rate can be calculated by substituting Cpmax for Cpss and using an estimate of clearance obtained from previous data in animals and/or man.

(b) Theoretically, under these conditions Cpmax will not be achieved until approx 4 half-lives after infusion started. To increase the infusion rate to achieve Cpmax at tmax, the plasma levels (Cp) at tmax must first be back calculated, assuming the elimination approximates to a monoexponential decline:

$Cp = Cpss\ (1 - e^{-\lambda t})$ where λ is the exponented constant ...2

The final infusion rate can then be obtained by correcting the initial infusion rate, obtained from equation 1, by the ratio Cpmax to Cp, obtained from equation 2.

This technique has been used routinely and successfully for non-radiolabelled and radiolabelled studies. Twelve subjects received d-fenfluramine at a calculated infusion rate of $6.5 mg.h^{-1}$, resulting in mean maximum plasma levels of $30.3 \pm 6.3 ng.ml^{-1}$, which was in good agreement with a predicted value of $30 ng.ml^{-1}$. Furthermore, all the pharmacokinetic parameters of interest, including half-life (15.5 ± 6.6h), clearance ($745 \pm 232 ml.min^{-1}$), volume of distribution (913 ± 379 litres) and absolute bioavailability (83 ± 17%) were calculated. Similarly, for tertatolol, an infusion rate of $3.5 mg.h^{-1}$ was calculated to give maximum plasma levels of radioactivity from [^{14}C]-tertatolol of $90 ng.equiv.ml^{-1}$ at 1h and, experimentally, a value of $91 \pm 27 ng\ equiv.ml^{-1}$ was obtained. Most importantly, no adverse side effects have been observed using this procedure.

PLASMA CONCENTRATIONS OF ALFENTANIL IN CHINESE, NEPALESE AND CAUCASIAN SUBJECTS AFTER INTRAVENOUS INFUSION.

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Alfentanil is a more predictable narcotic for use as an intravenous anaesthetic induction agent than fentanyl. Its kinetics in the Caucasian population is well documented (Hull, 1983). Very little information is available on the disposition of alfentanil in other ethnic groups. The study presents preliminary data on plasma concentrations of alfentanil after intravenous infusion in 4 Chinese (CHI, male, age 37-59), 4 Nepalese (NEP, male, age 23-29) and 5 Caucasian (CAU, 3 female & 2 male, age 30-39) patients. None of these patients had hepatic disease nor renal dysfunction. This study was approved by the Local Ethical Committee.

These patients underwent surgery when general anaesthesia was standardised and alfentanil was used as narcotic supplement during induction (loading dose: 5 $\mu g\ kg^{-1}\ min^{-1}$, maintenance dose 0.5 $\mu g\ kg^{-1}\ min^{-1}$). Infusion was stopped 5 min before the anticipated completion of surgery. Blood samples (5 ml) were collected at various intervals during induction and half-hourly for 3.5 h after the cessation of infusion. Alfentanil in plasma was assayed by a gas-liquid chromatographic technique (Aun & Chan, 1986).

Mean ($\pm$ S.D.) doses of alfentanil used were 5.0 $\pm$ 0.5, 5.5 $\pm$ 1.3 and 6.0 $\pm$ 1.7 mg respectively for CHI, NEP & CAU patients. The plasma concentrations at which unaided respiration commenced during recovery period were not significantly different (mean $\pm$ S.D. were 111.1 $\pm$ 35.4, 104.2 $\pm$ 48.4 & 120.3 $\pm$ 28.4 for CHI, NEP & CAU). None of these patients experienced respiratory depression during recovery after surgical operation. The mean elimination $t_{\frac{1}{2}}$ calculated after cessation of infusion was shorter in the Asian groups (mean $\pm$ S.D. were 56.7 $\pm$ 14.7, 59.4 $\pm$ 10.5 and 90.4 $\pm$ 25.9 min respectively for CHI, NEP and CAU patients). Among other interpretions one possible explanation for this difference may be that in the Chinese group shorter periods of infusion duration, though not significant (mean $\pm$ S.D. were 52.8 $\pm$ 21.9, 72 $\pm$ 34.7 and 65.6 $\pm$ 15.6 min for CHI, NEP and CAU patients), were encountered during surgery.

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PERTUSSIS TOXIN INDUCED HYPOTENSION IN RATS: A NON-ENDOTOXIC SEPTIC SHOCK.

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Although Bordetella pertussis vaccines are accepted to be effective, adverse reactions including circulatory shock are still a matter of concern. Recently it was demonstrated that pertussis toxin from B.pertussis causes a strong hypotension accompanied with vascular β_2-adrenoceptor hyporesponsiveness and a severe cardiac anticholinergic effect 4 days after administration to rats. The present study was undertaken to get more insight in the underlying mechanism of the pertussis toxin induced hypotension.

In all experiments pertussis toxin was given 4 days prior to the final measurements. After 4 days the rats were anaesthetized with urethane and blood pressure was measured directly. Autonomic receptor function was measured by establishing dose-response curves for salbutamol and arecoline with respect to changes in diastolic BP and heart rate, respectively. Experimentally there are indications that endogenous opiates might contribute to the hypotension during circulatory shock. Naloxone appears to restore BP partially during shock. Therefore pertussis toxin and saline treated rats were chronically pretreated with naloxone (10, 20 and 50 $mg.kg^{-1}$. 24 h^{-1}, i.v.) during 4 days by means of mini-osmotic pumps. A dose of 20 $mg.kg^{-1}.24\ h^{-1}$ prevented partially but significantly the pertussis toxin induced hypotension: diastolic BP values amounted to 95±5 mm Hg, 64±4 mm Hg and 80±4 mm Hg ($\bar{x}$±s.e.) for the controlgroup, pertussis toxine treated group and the naloxone pretreated pertussis toxin rats, respectively. The highest dose of naloxone did not result in a preventive action because this dose reduced BP intrinsically. In contrast, in another series of experiments it was demonstrated that an established hypotension 4 days after administration of pertussis toxin was not counteracted by acute doses of naloxone (0.3-10 $mg.kg^{-1}$, i.v.). Neither the β_2-adrenolytic nor the cholinolytic effect of pertussis toxin was influenced by naloxone pretreatment. In order to investigate the role of the adrenergic system in the hypotensive action pertussis toxin was given to reserpinized and adrenalectomized rats. In these animals pertussis toxin induced a further reduction in BP (from 70±3 mm Hg down to 49±5 mm Hg). This additional hypotension might be explained by either direct vasodilatory actions of the toxin or by other mechanisms e.g. stimulation of the release of endogenous opioids from the pro-opiomelanocortine molecule due to low amounts of circulating corticosteroids. Pretreatment with dexamethasone (5-10 mg. kg^{-1}, i.p.) during 4 days did not affect the hypotensive effect of the toxin. Since suppression of the release of opioids from the pro-opiomelanocortine by dexamethasone could not prevent the hypotension it can be speculated that also neuronal mediated release of opioids from this complex must contribute to the development of the overall hypotensive action of the toxin. Both dexamethasone and adrenalectomy/reserpinization could restore the pertussis induced β_2-adrenoceptor hyporesponsiveness. The anticholinergic effect was not influenced.

From the present findings and recent histological studies it must be concluded that pertussis toxin can induce circulatory shock. Opioid rather than adrenergic mechanisms seems to be partially involved in the development of the hypotension.

THE MUSCARINIC RECEPTORS OF THE HEART AND OF THE SALIVARY GLANDS MAY BELONG TO TWO DIFFERENT SUBTYPES.

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The differentiation between M_1 and M_2 receptor subtypes is well documented. It is mainly based upon experiments with the anticholinergic compound pirenzepine and the muscarinic agonist McN-A-343 (M-chlorophenylcarbamoyloxy-2-butynyltrimethylammonium). However, studies in vitro revealed differences between the M_2 receptor type in the heart (Fuder et al., 1985; Kilbinger et al., 1984) and that in the ileum.

It was the aim of the present investigation to study with the aid of five muscarinic agonists in vivo and in vitro the possibility of the existence of different muscarinic receptor subtypes in the heart and in the salivary glands. In the in vivo study we used the pithed rat model for M_2 present in the heart and the anaesthetized rat was used for the study of the effect of the muscarinic agonists on the salivary glands. The radioligand binding experiments were performed using ^{3}H--N-methylscopolamine (0.4 nmol/L: spec.act. 81 Ci/mmol) on both tissues. The following muscarinic agonists were used: pilocarpine (P), bethanechol (B), oxotremorine (O), aceclidine (A) and AH-6405 (1,4,5,6-tetrahydro-5-phenoxy-pyrimidine)(AH). The following parameters were assessed: decrease in heart rate (HR) and the production of salivation (SAL). The results are shown in table 1.

Table 1: Influence of muscarinic receptor agonists on heart rate, production of saliva and the results of radioligand binding studies. For details see text.

	In vivo		In vitro	
	*$HR_{ED_{40}}$	*$SAL_{ED_{15}}$	Heart (PKi)	Salivary gland (PKi)
O	6.97	6.91	6.30	5.78
B	5.88	6.00	4.32	3.89
A	5.36	5.72	5.16	4.72
P	4.75	6.66	5.17	5.29
AH	5.17	4.41	4.25	4.39

*mol/kg.

Pilocarpine is a more potent agonist in vivo for the muscarinic receptor type activating the salivary glands, and less so for the cardiac muscarinic receptor. Conversely, AH shows in vivo the opposite preference since it is more potent in activating the cardiac muscarinic receptor than that in the salivary glands. However, radioligand binding studies show that both agonists possess equal affinity for the cardiac muscarinic receptor type and for that in the salivary glands. Since both agonists possess the same affinity in radioligand binding studies the differences in potencies in vivo can only be explained by differences in efficacy, because of the opposite selectivity, this difference can not be explained by different receptor reserve for both agonists and therefore may indicate a heterogeneity of M_2 receptor subtypes.

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INOTROPIC AND PHOSPHODIESTERASE INHIBITING EFFECTS OF PIMOBENDAN ON HUMAN ISOLATED CARDIAC PREPARATIONS.

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Pimobendan is an orally effective cardiotonic agent with vasodilator properties. The positive inotropic effect of the drug may be attributed to a partial inhibition of cAMP phosphodiesterase (Berger et al., 1985), to an increase in calcium sensitivity of the contractile proteins (Rüegg et al., 1984), to a prolongation of the action potential (Honerjäger et al., 1984), or to a combination of these effects. The aim of the present study was to investigate whether or not pimobendan produces cardiotonic and phosphodiesterase inhibiting effects in human isolated cardiac preparations. Therefore, the effects of pimobendan on force of contraction (FC) and crude phosphodiesterase (PDE) activity were investigated in left ventricular papillary muscle strips (diameter 1 mm or less) isolated from NYHA grade III patients undergoing mitral valve replacement operations. The methods used have been described earlier (Berger et al., 1985, Böhm et al., 1985).

300 µmol l^{-1} pimobendan increased FC by about 90 % in electrically driven (0.5 Hz) papillary muscle strips. The PIE began after about 2 min and was maximal after about 30 min. Upon cumulative application of 1-300 µmol/l, pimobendan was less effective. Pimobendan (1-300 µmol l^{-1}) diminished PDE activity of these preparations concentration-dependently, with a maximal inhibition of only about 40 %. These values were comparable to those obtained in normal guinea-pig myocardium (Berger et al., 1985).

The results indicate that pimobendan has a positive inotropic effect not only in the normal guinea-pig heart but also in preparations isolated from the failing human heart. As in the former, the partial inhibition of PDE may contribute to the increase in force of contraction also in the latter.

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EFFECT OF PIMOBENDAN ON CALCIUM SENSITIVITY OF SKINNED FIBRES ISOLATED FROM HUMAN PAPILLARY MUSCLES.

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Pimobendan (=UD-CG 115 BS) is a positive inotropic agent with vasodilatory properties. Different mechanisms have been proposed to participate in the positive inotropic effects, e.g. a (partial) cAMP-phosphodiesterase inhibition (Berger et al., 1985) and an increase in calcium sensitivity of myofibrils in the presence of pimobendan (Rüegg et al., 1984). The latter effect was demonstrated in preparations isolated from porcine trabecula septomarginalis (Rüegg et al., 1984). The aim of this study was to investigate whether or not pimobendan was able to influence the calcium sensitivity of chemically skinned fibers isolated from human papillary muscles. The papillary muscles were obtained from patients undergoing mitral valve replacement operations. Myocardial protection was performed with Bretschneider HTH solution.

Fibers of fresh left ventricular human papillary muscles were extracted for 12-18 h in a solution containing 50 % glycerol and 50 % of a buffer (20 mmol/l HEPES, 10 mmol/l sodium azide, 2 mmol/l dithioerythriol and 0.5 % Lubrol PX, pH=7.0 at 4°C). Subsequently the preparations were stored in the same solution without Lubrol at -20°C for several days. After mounting, the fibers were relaxed by immersion in a solution containing 10 mmol/l ATP, 12.5 mmol/l $MgCl_2$, 5 mmol/l EGTA, 25 U/ml CPK, 20 mmol/l HEPES, 5 mmol/l NaN_3 and 10 mmol/l CP resulting in a free calcium concentration of pCa<8. Contraction was induced by immersion into an analogues solution in which EGTA was replaced by EGTA-calcium buffer. The effect of pimobendan (0.1 mmol/l) was measured at different free calcium concentrations. Data are presented as % tension development of maximal tension (obtained at pCa=4.33). Results are expressed as means ± SD (n=11).

-log (Ca^{++})*	% tension	% tension in the presence of 0.1 mmol/l pimobendan	
6.90	6.4 ± 4.0	8.8 ± 5.1	p < 0.01**
6.18	21.3 ± 7.9	26.6 ± 9.6	p < 0.01
5.78	45.2 ± 16.6	51.3 ± 15.6	p < 0.01
5.46	73.1 ± 13.1	79.7 ± 12.4	p < 0.01
4.98	83.5 ± 8.4	87.6 ± 10.4	p < 0.01

*Free calcium concentrations were calculated as described by Fabiato and Fabiato (1979). ** Paired t-test.

The results demonstrate that pimobendan also increases the calcium sensitivity of myocardial fibers isolated from human papillary muscles.

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