FACTORS INFLUENCING CUTANEOUS REACTIONS FOLLOWING ATRACURIUM.

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Atracurium is known to have a histamine releasing potential (Barnes et al, 1984) producing histaminoid cutaneous reactions (Mirakhur et al, 1983) and rarely hypotension and bronchospasm (Siler et al, 1985). Precipitation between alkaline thiopentone (pH = 10.5) and acid atracurium (pH = 3.5) has been suggested as a cause of cutaneous reactions (Fox, 1984) and the recommended method of administration involves either the use of a fast flowing intravenous infusion or flushing of a winged intravenous needle with isotonic saline between the administration of thiopentone and atracurium. It has also been recommended that the atracurium is stored at 4°C until use and injected into a large forearm or antecubital vein over 15-20s.

A randomised clinical trial was designed to investigate some of the factors which might influence the occurrence of cutaneous reactions. Patients in ASA assessment groups 1 or 2, undergoing elective surgery, were allocated at random to one of the groups below. Group 1 received the drugs into a fast-flowing intravenous infusion; Group 2 into a winged intravenous needle with flushing as described above and Group 3 into a winged intravenous needle without flushing. Anaesthesia was induced with thiopentone 5 mg kg^{-1} followed 60 s later by atracurium 0.5 mg kg^{-1} given over 20 s. Cutaneous reactions were noted as was hypotension or bronchospasm if present. The onset and duration of neuromuscular blockade were recorded using a Myotest peripheral nerve stimulator.

The possibility of Hofmann elimination beginning in the ampoule on warming to ambient temperature was investigated by keeping the drug at room temperature for 2-3 weeks before administration (Group 4).

A separate group of patients with a known history of drug allergy was studied by the method in Group 1, as was a further group composed of elderly patients (> 70 yr). Preliminary results are summarised in Table 1.

Table 1. Findings in the various groups of patients receiving thiopentone and atracurium by one of the regimes described in the text.

Group	1	2	3	4	Allergic	Elderly
n	20	20	20	20	13	6
Cutaneous reactions	75%	65%	70%	80%	85%	0%
Hypotension (> 20 mmHg)	25%	5%	35%	30%	31%	83%
Onset of block (Mean±sem)(s)	272.9 ±22.6	266.4 ±13.6	279.1 ±19.9	296.4 ±24.8	285.0 ±26.9	422.0 ±40.3
Duration of block (Mean±sem)(min)	31.2 +1.8	29.6 +1.1	29.4 +1.1	26.7 +1.3	28.2 +0.7	21.4 +1.5

There was a high incidence of cutaneous histaminoid reactions to atracurium which were of short duration and rarely associataed with other signs of histamine release. The reactions do not appear to be related to the method of administration or lack of refrigeration, and they are very uncommon in elderly patients.

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TRAIN-OF-FOUR FADE DURING ONSET OF NEUROMUSCULAR BLOCK WITH ATRACURIUM, VECURONIUM, PANCURONIUM AND TUBOCURARINE.

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Different nondepolarizing muscle relaxants exert different degrees of presynaptic and postsynaptic effects while producing neuromuscular block as shown by different degrees of fade following train-of-four (TOF) or tetanic stimulation (Bowman, 1980; Williams et al, 1980). We report here the relative TOF fade during onset of block with two newer relaxants atracurium and vecuronium and two older relaxants pancuronium and tubocurarine.

Fit adult patients undergoing elective surgery requiring the use of nondepolarizing muscle relaxants were studied after obtaining informed consent and Ethical Committee approval. Anaesthesia was with thiopentone, nitrous oxide-oxygen and fentanyl. Ventilation was adjusted to maintain the end-tidal CO_2 at 4.5-5.0%. The ulnar nerve was stimulated percutaneously at the wrist using supramaximal square wave stimuli at a frequency of 2 Hz in a TOF sequence every 10s. The resultant force of contraction of adductor pollicis was measured and recorded.

Control responses to TOF stimulation were allowed to stabilize for about 10 min following which patients were randomly allocated to receive 1 or 2 x ED_{95} of atracurium, vecuronium, pancuronium or 1 x ED_{95} of tubocurarine. Responses were recorded continuously until maximal block had supervened. TOF ratios were measured at approximately 75, 50 and 25% of control heights of the first twitch (T_1) in the TOF sequence.

TOF ratios diminished (Table 1), indicating increasing fade, as the neuromuscular block increased in intensity (decreasing height of T_1) irrespective of the drug used. At T_1 of 75% the TOF ratios were similar although atracurium 2 x ED_{95} and pancuronium 2 x ED_{95} were associated with significantly lower TOF ratios in comparison to 1 x ED_{95} vecuronium. At T_1 of 50% the TOF ratio was significantly higher (indicating less fade) after vecuronium in comparison to the three other relaxants. Increasing the dosage of the relaxants did not appear to make any difference. The differences between the relaxants were most obvious at deeper degrees of block (T_1 25% of control). TOF ratios after atracurium were not significantly different from that after tubocurarine but both were significantly lower in comparison to vecuronium and pancuronium which were different between each other only at 1 x ED_{95} vecuronium and 2 x ED_{95} pancuronium. At T_1 of 25% vecuronium showed the least and pancuronium, atracurium and tubocurarine increasing degrees of fade in that order.

Table 1. TOF ratios.

Relaxant	ED_{95}	Dosage (μg/kg)	n	TOF ratios at approx. T_1 of 75%	50%	25%
Atracurium	1	226	10	83 ± 6.3	65 ± 7.3	39 ± 11.9
"	2	452	10	81 ± 4.1	68 ± 9.1	41 ± 16.2
Vecuronium	1	40	10	86 ± 4.0	75 ± 5.8	62 ± 6.5
"	2	80	10	85 ± 4.2	74 ± 4.9	55 ± 9.8
Pancuronium	1	60	10	83 ± 6.3	68 ± 9.4	52 ± 16.0
"	2	120	5	79 ± 10.0	69 ± 10.0	53 ± 7.3
Tubocurarine	1	450	10	86 ± 8.0	68 ± 7.4	34 ± 18.6

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EFFECTS OF FLUOXETINE ON NOCTURNAL SLEEP AND DAYTIME ALERTNESS IN MAN.

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Although reduction of rapid eye movement (REM) sleep is a consistent feature of drugs which modify monoaminergic transmission, changes in wakefulness are more specific. Increased wakefulness has been observed with nomifensine, zimelidine and indalpine, but not with maprotiline and mianserin (Nicholson & Pascoe, 1986; Nicholson, Pascoe & Stone, 1986). To investigate further the wakefulness induced by drugs which selectively inhibit the uptake of 5-HT, we have studied the effects of fluoxetine on nocturnal sleep and on daytime sleep latencies.

Nocturnal sleep: 6 males (19-23 years) each ingested 20, 40 & 60 mg fluoxetine and two placebos, and 100 mg nomifensine (active control). There were no effects of 20 and 40 mg fluoxetine on sleep. After 60 mg fluoxetine total sleep time and sleep efficiency were reduced ($p < 0.01$), there was an increase in awake activity and stage 1, and REM sleep was reduced. Nomifensine increased awake activity and stage 1 and reduced REM sleep. The duration (min) of nocturnal sleep stages, means for 6 subjects, are given below (* $p < 0.05$; ** $p < 0.01$):

Sleep stage	Placebo	Nomifensine (mg)	Fluoxetine (mg)		
		100	20	40	60
Awake	2.2	10.3 **	2.9	2.8	10.8 *
Drowsy	42.3	57.1 *	49.3	59.7	78.3 *
REM	103.3	64.4 **	94.0	100.1	57.4 **

Daytime sleep latencies: 5 females (19-29 years) each ingested 20, 40 & 60 mg fluoxetine and two placebos, and 300 mg caffeine (active control). Ingestion was at 1130 h, and latencies and assessments of mood were measured pre-(0900 & 1100 h) and post-ingestion (1300, 1500, 1700 & 1900 h). After each dose of fluoxetine sleep latencies were increased but, paradoxically, subjects reported feeling more sleepy (pooled doses: $p < 0.05$). After caffeine longer sleep latencies were accompanied by greater alertness and anxiety or tension ($p < 0.01$). Daytime sleep latencies (min), means for 6 subjects, are given below (* $p < 0.05$; ** $p < 0.01$):

Time	Placebo	Caffeine (mg)	Fluoxetine (mg)		
		300	20	40	60
Pre-ingestion (mean)	12.8	15.6	12.2	7.0	11.5
Post-ingestion (mean)	8.7	28.3 **	14.9 *	10.8 **	18.9 **

The present studies confirm our previous findings (Nicholson & Pascoe, 1986) that 5-HT uptake inhibition leads to wakefulness during nocturnal sleep, and also increased alertness during the day. 5-HT is believed to be involved in the initiation and maintenance of sleep, and so a possible explanation for the effects of fluoxetine is that uptake inhibition, at least acutely, leads to presynaptic inhibition of transmitter release. The present studies show that 5-HT is concerned with the states of sleep and wakefulness over the 24 h continuum, and in this sense is different from histamine which may be involved in vigilance rather than the underlying state of sleep and wakefulness (Nicholson, Pascoe & Stone, 1985).

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PHARMACOKINETICS OF MIDAZOLAM SEDATION FOLLOWING OPEN HEART SURGERY.

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Midazolam (MDZ) has been shown to be less cumulative than diazepam when used in repeated doses for sedation following open heart surgery (Lowry et al, 1985). However, because of its short $t_{\frac{1}{2}\beta}$ frequent small doses are required to maintain a plasma level adequate for sedation whilst avoiding large swings in plasma levels following each administration. Clearance is adequate following cardiopulmonary bypass and infusions of MDZ should be a safe way of administering sedation for ventilatory support in the postoperative period (Harper et al, 1985).
Twenty adults requiring ventilation following cardiopulmonary bypass were randomly allocated to receive either 2 mg intravenously at hourly intervals or a loading dose of 2 mg followed by an infusion at 2 mg h^{-1}. Arterial blood was sampled prior to the initial dose, at frequent intervals during therapy and for 24 h following discontinuation and plasma MDZ levels estimated using GLC (Howard et al, 1985).
Groups were broadly comparable with respect to age, weight, fitness, operative procedure, duration of bypass and postop ventilation. One patient infused with MDZ showed an atypical response with high plasma levels and a long (16 h) half-life. This patient, excluded from calculations, would appear to be a slow metaboliser of the drug (Dundee et al, 1986).

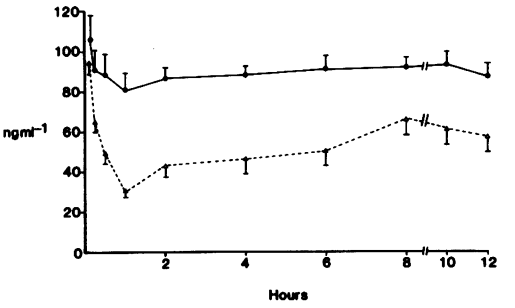

Figure 1. Mean plasma midazolam levels following infusion ●——● and trough levels with intermittent injection Δ------Δ.
A steady state concentration was rapidly attained with the infusion whereas examination of trough levels shows a delay of 6-8 h in attaining a steady state with the intermittent injection. Estimation of near peak plasma levels (10 min after administration) showed a swing between peak and trough levels of 40-50 ng ml^{-1}. Individual clearances in the infusion group, calculated from concentration at steady state, averaged 335 ml $kg^{-1}h^{-1}$. Half-lives averaged 4.5 h in the infusion group and 5.1 h in the intermittent injection group and recovery was prompt in both series..
Midazolam infusion is preferred to intermittent injection for postoperative sedation following cardiopulmonary bypass.

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SINGLE DOSE SEDATIVE AND HAEMODYNAMIC EFFECTS OF MIANSERIN IN YOUNG AND ELDERLY VOLUNTEERS.

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Mianserin is a sedative antidepressant with little anticholinergic activity, widely used in the treatment of elderly patients. Since ageing may modify the pharmacodynamics of some centrally acting drugs, we have carried out a comparative study of the effects of a single 10mg oral dose in two groups of healthy, drug-free, non-smoking volunteers, a young (n=10; age 19 to 37) and an elderly n=9; age 67 to 76).

Each subject took part in three sessions spaced at least one week apart. 'Immediate' (1.5 to 6h post-a.m. drug), 'residual' (11.5 to 16h post-p.m.drug) and placebo effects were measured using a protocol previously described (Swift et al 1985). Pharmacodynamic measures included a continuous attention task (CAT)(Tiplady 1985), critical flicker fusion threshold (CFFT) and choice reaction time (CRT) (Hindmarch, 1975), tapping test (TT), decision making test (DMT) (Tiplady, 1985) and postural sway (PS) (Wright, 1971), together with four visual analogue scales (VAS) and supine and standing BP.

The effects of this dose of mianserin were readily detected. 'Immediate' performance decrements occurred on CAT ($p<0.01$ young; $p<0.05$ elderly), CFFT ($p<0.01$ both groups), CRT ($p<0.05$ elderly only) and PS ($p<0.01$ both groups). 'Residual' impairment was detected on CAT ($p<0.05$) and PS ($p<0.05$) in the young group only and on CFFT in both groups ($p<0.05$ young; $p<0.01$ elderly) (Wilcoxon signed rank test). No effects were seen with TT or DMT and there was no significant difference between groups in the magnitude of impairment (Mann-Whitney 'u' test).

In the young group, immediate impairment was found on three visual analogue scales, alert-drowsy (A-D), steady-dizzy (S-D) and interested-bored (I-B) and residual effects on S-D and I-B scales. ($p<0.05$ each measure). No significant subjective effects were reported by the elderly group. The difference between groups in the 'residual' period was significant for A-D and S-D scales ($p<0.05$). Standing mean BP was significantly reduced in both immediate ($p<0.05$) and residual ($p<0.05$) sessions in the young group only. The immediate reduction differed significantly ($p<0.05$) from the older group, who showed no such effects on mean BP.

These findings confirm the moderate immediate and residual sedation occurring with a small first dose of mianserin. No difference with age was shown with objective measures but subjective appreciation of sedation may be less in the elderly. First-dose postural hypotension occurred in young subjects as previously reported (Elliott et al 1983); its absence in older subjects is surprising in view of their known tendency to reduced baroreceptor sensitivity. Caution is needed on initial mianserin dosage in all age groups, though the compound is usually taken on retiring at night.

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476P

DISTURBANCE OF SERINE METABOLISM AND ENDOGENOUS FORMATION OF β-CARBOLINES IN A GROUP OF EPISODIC PSYCHOTIC PATIENTS.

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Up to now no general biochemical defect has been found to be responsible for the origin of a psychosis. Research in this field is often hampered by the fact that psychiatrists are studying biological correlates in psychotic patients with different diagnoses. Our aim was to find a metabolic abnormality in a rather homogeneous group of neuroleptic-resistent atypical psychotic patients, who fulfill the following conditions: 1) they suffer from acute psychotic episodes characterized by multiple sensory perceptual disturbances; 2) they ameliorate on a carbohydrate-rich, low fat and low protein diet; 3) after oral intake of serine, recovered patients will become psychotic again and 4) recovered patients are biochemically characterized by a low plasma serine and a high plasma taurine level. It was postulated that these patients have a deranged one-carbon metabolism resulting in the formation of β-carbolines and/or isoquinolines which may be responsible for the evoked psychotic symptoms (Pepplinkhuizen *et al.* 1980).

To gain more insight into the pathogenesis of this psychosis, in which a disturbance of serine metabolism seems to be involved, we undertook some biochemical studies using blood and fibroblasts of these patients. The results are the following. No enzymes related to serine metabolism were found to have abnormal activities in fibroblasts obtained from these patients. However, after subculturing fibroblasts in medium supplemented with 10% serum of a psychotic patient, a higher amount of taurine (32%, $2P<0.001$; n=17) and a lower amount of serine (11%, $2P<0.005$; n=17) and methionine (13%, $2P<0.001$; n=17) were formed in these cells compared to supplementation with normal serum. In addition, the concentrations of serine and methionine in the culture media were significantly lower (9%, $2P<0.05$; n=17 and 6%, $2P<0.001$; n=17, respectively) upon subculturing with patient serum. Moreover, the specific activities of serine hydroxymethyltransferase (3.95 vs. 3.05 nmol/min/mg protein) and of cystathionine β-synthase (0.509 vs. 0.370 nmol/min/mg protein) - measured in the fibroblasts without the exogenously added cofactor pyridoxal 5'-phosphate - are significantly higher ($2P<0.01$; n=8 and $2P<0.025$; n=9, respectively) after subculturing with patient serum than with normal serum. So, there is some factor present in the serum of these patients which is responsible for the altered metabolism of serine and taurine.
Analysis of plasma of these patients during acute psychosis by HPLC and mass spectrometry revealed the presence of 0.54 pmol of the β-carboline norharman per ml plasma. This substance was not detectable in plasma of controls as well as of symptom free episodic psychotic patients. Norharman has several pharmacological actions, for instance inhibition of binding to benzodiazepine receptors, inhibition of monoamine oxidase-A and of reuptake of serotonin in rat brain synaptosomes. A combination of these actions may be responsible for the observed psychotic symptoms.

The present results support our hypothesis that these psychotic patients are suffering from an abberation in one-carbon metabolism which will finally result in the endogenous production of β-carbolines.

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ALTERED β-ADRENOCEPTOR CHARACTERISTICS IN AIRWAY SMOOTH MUSCLE OF A PATIENT WITH ATOPIC EXTRINSIC ASTHMA.

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Besides other defects, β-adrenergic receptor dysfunction has been proposed to be a major contribuant to bronchial hyperreactivity in asthmatics.
While investigating muscarinic and β-adrenergic receptor characteristics in human airway smooth muscle, we obtained postmortemly a specimen of trachea from a patient with established bronchial asthma who died from Hodgkin's disease and had positive skin tests for inhaled allergens. The patient did not receive salbutamol at least three weeks before death. Prednison medication was withdrawn gradually in the two weeks preceding death.
Muscarinic and β-adrenergic receptor characteristics were assessed by *in vitro* contraction -relaxation and radioligand binding studies.
Muscarinic receptor function appeared to be normal. Tracheal smooth muscle strips displayed normal sensitivity to methacholine and radioligand binding studies revealed similar muscarinic antagonist and agonist binding profiles as in control subjects.
β-Adrenergic receptor function however was dissimilar. A tenfold decrease in sensitivity for isoprenaline-induced relaxation was observed, in accordance with data presented by Paterson *et al.* (1982).
Receptor binding studies in addition demonstrated a decreased sensitivity of the β-adrenergic receptor high affinity binding site for isoprenaline (39.9 nmol/l versus 9.5 $\pm$ 3.4 nmol/l as in control subjects (N=4)(mean $\pm$ S.D.)).
The high affinity binding site is generally considered to be responsible for the activation of adenylate cyclase.

These findings support the hypothesis of a defective β-adrenergic receptor system in airway smooth muscle of asthmatics.

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POOR METABOLIZER INCIDENCE OF SPARTEINE, MEPHENYTOIN AND NIFEDIPINE IN A DUTCH POPULATION AS ASSESSED BY A "COCKTAIL" APPROACH.

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Oxidative drug metabolism in man is subject to considerable variation and oxidation polymorphism attributes largely to this phenomenon. The cytochrome P-450 system in the liver consists of multiple isozymes and localized deficiencies seem to be the cause for the oxidation polymorphism of sparteine (SP) and mephenytoin (MP). The pattern of inheritance for SP and MP seems to be autosomal recessive which may give rise to 3 distinct phenotypes in a population (1,2). In order to characterize and phenotype an individual subject's drug oxidation status over a broad range, one has to combine model substrates. This study was undertaken to establish the poor metabolizer incidence (PM) of SP, MP and nifedipine (NF) and to investigate any relationships between the oxidation of these substrates.

METHODS:

A "cocktail" of SP (capsule SP-sulphate 25 mg), MP (capsule racemic mixture 100 mg) and NF (capsule 2x10 mg) was administered orally after an overnight fast to 172 healthy volunteers (102 males and 70 females). Urine was collected up to 24 hours (0-8, 8-24 h). SP and metabolites were determined with GC and the metabolite of MP (para-hydroxy-MP: OH-MP) and NF (M1: 2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate-methylester) were analyzed with HPLC.

RESULTS:

PM's were characterized according to the criteria: log SP/metabolites >2, OH-MP <0.5 %, M1 <25 % (0-8 h) (4). The excretion of M1 showed a wide variation (range 15-85 %), the distribution however was not different from a normal distribution (chi*2 test, p=0.54). Based on former criteria (4) the PM incidence would be 9.9 %, but this is further to be confirmed by plasma level data. The PM incidence of MP was 2.3 % and of SP 7.6 %, which is lower for MP and slightly higher for SP than in a comparable Caucasian population (2,3). A computer simulation based on non-parametric statistical evaluation of unimodal and bimodal distributions confirmed the existence of a trimodal distribution of MP and SP with antimodes for MP at 21 % and 2 % and for SP at ratios 1 and 2. No correlation was found between the oxidation of NF, MP and SP.

CONCLUSIONS:

NF polymorphism cannot be characterized by measuring only the metabolite M1 in urine. It is likely that the trimodal distribution of MP and SP represents three distinctive phenotypes for each drug. Different isozymes of the cytochrome P-450 system seem to oxidize SP, MP and NF and a combination of these substrates may be a valuable tool in phenotyping three independent oxidation polymorphisms simultaneously (cocktail approach).

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DISPLACEMENT OF DRUGS FROM SERUM α_1-ACID GLYCOPROTEIN BINDING SITES BY SKF 525 A.

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SKF 525A (2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride) is a well known inhibitor of drug biotransformation. During experiments concerning the disposition of β-adrenoceptor blocking agents, we were surprised to find that in rats pretreated with SKF 525A, the serum binding of oxprenolol, a drug mainly bound to α_1-acid glycoprotein (α_1-AGP), was decreased.

As data on serum binding interactions by SKF 525A are not available, we performed an in vitro study of the influence of SKF 525A on the binding of oxprenolol and propranolol (which are mainly bound to α_1-AGP) and of phenytoin (which is mainly bound to albumin) to serum of rats, dogs and healthy human volunteers, to human serum albumin (HSA, 4 g%) and to human α_1-AGP (80 mg%). In other experiments, instead of SKF 525A, TBEP (tributoxyethyl phosphate), a known inhibitor of α_1-AGP binding, was used. Binding of oxprenolol, propranolol and phenytoin was also determined in serum obtained from male Wistar rats treated with SKF 525A,50 mg/kg intraperitoneally 40 minutes earlier.

Binding was measured by equilibrium dialysis using teflon cells at 25° C. Labeled compounds were added to the phosphate buffer solution (0.15 M, pH 7.4) to achieve concentrations in the protein compartment which are in the therapeutic range; SKF 525A and TBEP were added to the protein solution in a concentration range from 3 to 100 µg/ml.

In vitro, SKF 525A inhibited the binding of oxprenolol and propranolol to serum of the different species studied and to α_1-AGP. Binding of oxprenolol to α_1-AGP e.g. was already affected by SKF 525A 3 µg/ml; with 30 µg/ml, an almost complete inhibition was seen. In the concentration range studied, SKF 525A did not influence the binding of phenytoin to serum of the different species and to HSA. The results obtained with TBEP were similar.

In vivo SKF 525A administration decreased significantly the binding of oxprenolol and propranolol, but had no influence on the binding of phenytoin (Table 1).

Table 1 Percent free drug (+ SD) in serum of rats pretreated with SKF 525A or saline

	Controls	SKF 525A
Oxprenolol	42.3 ± 2.3	55.2 ± 5.9*
Propranolol	10.3 ± 0.6	14.8 ± 2.8*
Phenytoin	13.1 ± 1.5	14.2 ± 1.7

* $P < 0.001$, $n = 6$

Our results show that SKF 525A is a potent inhibitor of the serum binding of drugs mainly bound to α_1-AGP, and it is in that regard about equipotent with TBEP. That SKF 525A does not only affect drug biotransformation but also drug binding must be taken in consideration when studying the influence of SKF 525A on the pharmacokinetics of drugs.

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HAEMODYNAMICS DOSE-RESPONSE EFFECTS OF DILTIAZEM IN CORONARY ARTERY DISEASE.

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The dose-response effects of i.v. diltiazem were evaluated in 12 patients with angiographically documented coronary artery disease. At rest, following a 20 min control saline period with reproducible baseline parameters, the haemodynamic and radionuclide effects of four doses of the drug (0.0625, 0.0625, 0.125 and 0.25 mg/kg at 5 min) were measured in the 3-5 min following i.v. injection. The exercise effects of the cumulative 0.5 mg/kg dosage on haemodynamic and radionuclide parameters during angina were assessed in a control and post drug period of supine bicycle exercise, at the individually titrated anginal workload.
Diltiazem reduced systemic systolic, diastolic and mean arterial blood pressure and vascular resistance index. There was a dose-related reduction in heart rate without change in cardiac index; consequently the cardiac stroke index increased. Over the dose-range, there was no change in left ventricular filling pressure, ejection fraction, or derived cardiac volumes. There was a highly significant reduction in calculated cardiac double-product (Heart rate x Systolic blood pressure).

Variable	Control	X	2X	4X	8X
MAP (mm Hg)	102±3	-2.4	-2.7	-4.7**	-6.7**
HR (beat min-1)	72±4	-1.9	-2.4	-4.0*	-4.7**
PAOP (mm Hg)	10±1	+0.3	+0.1	+1.0	+1.7
SI (ml m-2)	47±1	+1.2	+1.9	+3.4**	+4.6**
EF (%)	51±2	-0.5	-0.1	+0.5	+1.4

Mean±S.E. X=Diltiazem 0.0625 mg/kg. 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 (%)
During supine bicycle exercise, the systemic diastolic blood pressure and heart rate were reduced, without change in cardiac or stroke volume indices, left ventricular ejection fraction or volumes. The exercise double-product was significantly reduced.

These data indicated that diltiazem improved left ventricular function (increased stroke index; reduced double-product) in the resting state. During dynamic exercise, the reduced left ventricular afterload resulted in a probable reduction in left ventricular oxygen requirements (unchanged cardiac index at reduced double-product).

A METHODOLOGICAL APPROACH TO STUDY CELLULAR PHARMACOKINETICS OF DRUGS.

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A method for the isolation of polymorphonuclear and mononuclear leucocytes from both healthy and rheumatoid blood an synovial fluid has been developed. Viable and pure peripheral cell suspensions were obtained after a combination of dextran sedimentation, Ficoll Paque (sp.g. 1.077) and Percoll cushion centrifugation (Raghoebar *et al,* 1986). The density of the Percoll gradient was dependent on the inflammatory conditions of the blood donor (McCarthy *et al,* 1984). Synovial cells were purified from heparinized synovial fluid employing Percoll gradient centrifugation (sp.g. 1.098). Cellular association studies of radiolabeled ligands were performed by the developed silicone oil cushion method (intact cells) and the double centrifugation method (lysed cells) (Raghoebar *et al,* 1984). The combination of these techniques makes it possible to create different in vitro conditions to study cellular pharmacokinetics. Besides variations of the incubation temperature, the incubation time and the ligand and cell concentration, an inward or outward gradient of different cations (Na^+, K^+, H^+, Ca^{2+}, Mg^{2+}) or molecules (D-glucose) can be established. Inflammatory cell conditions can be simulated in vitro by addition of soluble activators such as chemotactic peptide and phorbol myristate acetate to the incubation medium (Simchowitz, 1985) and this enables examination of the influence of cell activation on cell drug association in comparison with the association of drugs to normal cells. The mechanism of association can be studied by adding characteristic pharmacological and biochemical probes to the incubation medium. Furthermore, this methodological approach is also suitable for the determination of intracellular volumes to estimate the intracellular concentration of drugs. The reliability of our experiments has been demonstrated by a lot of studies with different drugs (Table 1).

Table 1 Degree of association of some drugs to leucocytes

DRUG	CONCENTRATION RANGE	% ASSOCIATED	CELL/MEDIUM RATIO*
Sodium salicylate	0 - 20 mM	0.1 - 0.2	1 - 2
Acetylsalicylic acid	0 - 2 mM	0.3 - 0.6	3 - 6
Benzoic acid	0 - 10 mM	0.1 - 0.2	1 - 2
Indomethacin	0 - 20 µM	2 - 4	20 - 40
Ascorbic acid	0 - 150 µM	1 - 3	15 - 30
Dehydroascorbic acid	0 - 100 µM	3 - 4	60 - 80
Chloroquine	0 - 180 µM	5 - 45	60 - 770
FMLP**	0 - 1 µM	0.4 - 1.2	5 - 8
Erythromycin	0 - 159 µM	1 - 2	29 - 33

* cell/medium ratio is expressed as the estimated intracellular concentration divided by the measured extracellular concentration

** N-formyl-L-methionyl-L-leucyl-L-phenylalanine

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PHARMACOKINETICS AND HAEMODYNAMIC EFFECTS OF NISOLDIPINE IN PATIENTS WITH LIVER CIRRHOSIS.

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Nisoldipine is a new dihydropyridine calcium channel blocker which is under investigation for the treatment of hypertension and angina pectoris. The drug is metabolized in the liver and has a high systemic clearance and a low and variable bioavailability on oral administration to healthy subjects. These properties make it relevant to study the pharmacokinetics of nisoldipine in patients with liver cirrhosis.

METHODS. Eight patients with liver cirrhosis (Age 60±6 yrs; Wt. 71±15 kg) and eight healthy control subjects (Age 54±11 yrs; Wt. 74±9 kg) participated. In a randomized cross-over study design they were given nisoldipine intravenously (0.37 mg in 40 min infusion) and orally (patients 5 mg; healthy controls 20 mg tablet). Plasma samples for the determination of nisoldipine and one of its metabolites were obtained at regular intervals and simultaneously supine blood pressure and heart rate were measured. Plasma levels of nisoldipine and metabolite were determined by a selective gas chromatographic technique using electron capture detection. The principal pharmacokinetic parameters were derived by model-independent methods.

RESULTS Pharmacokinetic parameters (Mean±S.D.) of nisoldipine (* $p < 0.05$)

		$t_{\frac{1}{2}}$ (h)	Vss (l/kg)	CLs (l/min)	CLor (l/min)	F (%)	Cmax (ng/ml)
Patients	i.v.	16.6±4.6	6.4±2.3	0.49±0.12			4.7±1.1
	oral	19.0±7.5			4.9±3.6	14.7±10.1	3.5±2.2
Controls	i.v.	9.7±5.4*	4.1±2.1	0.85±0.31*			5.2±1.1
	oral	13.1±6.1			36.6±37.8*	3.7±2.1*	4.5±2.6

After oral administration Cmax of the metabolite was 1.4±0.9 ng/ml with an apparent elimination half-life of 6.7±5.9 h in the patient group. In the control group these data were 6.4±3.6 ng/ml and 5.0±3.0 h. After i.v. administration the metabolite could not always be detected quantitatively.

Haemodynamic effects were (baseline value to maximal effect):

		Blood pressure (mmHg)			Heart rate (bpm)		
Patients	i.v.	113±6/71±9	-->	103±5/61±10	77±20	-->	88±22
	oral	113±9/71±7	-->	103±10/59±4	73±18	-->	83±17
Controls	i.v.	112±18/72±6	-->	102±15/63±8	60±8	-->	71±13
	oral	113±14/75±6	-->	102±12/65±5	60±10	-->	72±14

After i.v. administration a short lasting decrease in blood pressure and increase in heart rate was observed. After oral administration the effects lasted considerably longer.

CONCLUSIONS. In patients with liver cirrhosis oral clearance of nisoldipine is strongly diminished, resulting in a very much increased bioavailability. Bioavailability was highly variable both in patients and in healthy controls. The haemodynamic sensitivity for nisoldipine seems to be unaltered in patients with liver cirrhosis. When these patients are to be treated with nisoldipine dose-reduction might be necessary.

METABOLIC EFFECTS AND EXERCISE TOLERANCE DURING LONG-TERM TREATMENT WITH THREE DIFFERENT β-BLOCKERS.

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Recently, it has been shown that beta blockers reduce submaximal exercise capacity (Lundborg et al, 1980; Kindermann et al, 1984). The effects of beta blockade on the metabolic adjustments to exercise are probably involved in this reduction. However, these studies have been performed in young normotensive subjects after acute or short-term beta blocker administration. With regard to the treatment of physically active hypertensive patients, it is important to know what the effects on exercise capacity are during long-term antihypertensive therapy and whether there are differences between the various types of beta blockers in this respect. Therefore, the effects of long-term (6 months) antihypertensive beta blocker treatment on exercise tolerance and metabolic variables during exercise were studied in 7 subjects with essential hypertension. They exercised on a bicycle ergometer at 70% of estimated maximum work capacity until exhaustion. Maximal duration of the test was set at 90 min. In a single-blind, placebo-controlled, randomized cross-over design, the effects of three beta blockers were compared: propranolol (non-selective, no intrinsic sympathomimetic activity (ISA)), pindolol (non-selective, with ISA) and metoprolol ($beta_1$-selective, no ISA). The dose of the drugs was adjusted to lower standing diastolic blood pressure <90 mm Hg, and was 108±17 mg/day propranolol, 14±2 mg/day pindolol, and 150±31 mg/day metoprolol.

Similar reductions of supine, standing and exercise blood pressures, and exercise heart rate were obtained with all three drugs. During long-term treatment, exercise time was reduced from 69±10 to 52±6 min during propranolol ($p<0.01$), from 62±9 to 38±10 min during pindolol ($p<0.05$; n=6) and from 69±10 to 58±9 during metoprolol ($p<0.01$). Although the reduction tended to be more pronounced during propranolol and pindolol than during metoprolol, the difference was not statistically significant. During exercise, plasma glycerol and non-esterified fatty acid concentrations were reduced during beta blockade ($p<0.05$). The reductions were more pronounced during non-selective than during $beta_1$-selective blockade ($p<0.05$). No consistent effect of the drugs on plasma glucose and lactate concentrations could be demonstrated. Oxygen consumption tended to decrease during non-selective beta blockade ($p<0.05$ during propranolol, $p=0.06$ during pindolol) and respiratory exchange ratio tended to increase with all three beta blockers ($p<0.05$ only during pindolol). Plasma potassium concentrations were significantly increased ($p<0.05$) and a larger increase was found during non-selective than during $beta_1$-selective blockade ($p<0.05$).

The study shows that submaximal exercise performance is impaired in hypertensive subjects during long-term antihypertensive beta blocker therapy. The impairment tends to be more pronounced during non-selective than during $beta_1$-selective blockade and may be related to the effects of beta blockade on fat and/or potassium metabolism during exercise.

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THE COURSE OF BLOOD PRESSURE AND OF PLASMA CATECHOLAMINE LEVELS IN SUBJECTS WITH BORDERLINE HYPERTENSION.

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There are conflicting reports on plasma catecholamine levels in subjects with borderline hypertension (Julius et al, 1980). This could be due to the fact that only part of the group of subjects with borderline hypertension (BHT) develops definitive hypertension in the course of several years, whereas in other borderline hypertensive subjects blood pressure (BP) does not alter or even falls to a normotensive range. We, therefore, studied the course of BP and of plasma catecholamine levels in 26 subjects with BHT (BP after 2 min standing $\geq$140/90 and <160/100 mmHg (mean arterial blood pressure $\geq$107 and <120 mmHg) measured 3 times on 2 different occasions with an interval of 1 week) and in 24 subjects with normotension (NT, standing BP $\leq$125/85 mmHg).

Secondary hypertension had been excluded by appropriate tests in the subjects with BHT. The study started in 1977 and was finished in 1984. In all subjects (male, age 18-30 years) standing BP was measured every 3 months. Subjects with standing mean arterial pressure (MAP, diastolic BP plus one-third of pulse pressure) $\geq$120 mmHg at 2 successive measurements were considered as being definitively hypertensive and were excluded to receive antihypertensive therapy. BP at rest and during exercise was measured with a standard mercury sphygmomanometer. The point of muffling of the Korotkoff sounds (phase IV) was read as diastolic BP. Plasma noradrenaline and adrenaline levels were determined radiometrically (Henquet et al, 1980) at supine rest as well as during bicycle ergometry (75% of the predetermined maximum work load) in 1977, 1979 and 1984.

In 15 subjects with NT, standing MAP remained $\leq$98 mmHg throughout the study (group 1). In 7 subjects with NT, standing MAP increased to levels higher than 98 mmHg but remained <107 mmHg. Two normotensive subjects and one subject with BHT were lost to follow-up. In 10 subjects with BHT, BP remained borderline hypertensive throughout the study (group 2). Between 1979 and 1984, 5 subjects with BHT became definitively hypertensive (group 3). In 10 borderline hypertensive subjects BHT, MAP fell to <107 mmHg. At entrance into the study (1977), plasma catecholamine levels at rest and during exercise did not differ in group 1, 2 and 3. In 1979 and 1984, resting plasma noradrenaline averaged to 0.22$\pm$0.03 ng/ml in group 1 and 0.23$\pm$0.02 ng/ml in group 2. A significantly ($p<0.02$) higher value of 0.42$\pm$0.06 ng/ml was found in group 3. At this time, plasma noradrenaline during exercise was also slightly higher in group 3 than in group 1 and 2 ($p>0.15$). There were no differences between the 3 groups in plasma adrenaline. This study shows that during an observation period of 7 years only 20% of the subjects with BHT become definitively hypertensive and 38% stay borderline hypertensive. Resting plasma noradrenaline is elevated at the time when hypertension develops.

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THE COMBINATION OF NICARDIPINE AND ENALAPRIL IN PATIENTS WITH ESSENTIAL HYPERTENSION: A PLACEBO CONTROLLED STUDY.

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Clinical experience suggests that the addition of a calcium antagonist may be effective in patients receiving treatment with an angiotensin converting enzyme (ACE) inhibitor in whom blood pressure control is unsatisfactory.

Twelve patients with essential hypertension (6M, 6F: 40-64 yrs) had blood pressures greater than 160/95 after a minimum of six weeks treatment with the ACE inhibitor enalapril, 20 mg daily. Nicardipine, a dihydropyridine calcium antagonist, 30 mg three times daily and placebo were each added for two weeks in a randomised, double-blind cross-over study with a two week washout period between treatments. Enalapril 20 mg daily was continued unchanged throughout the study period. Supine and erect blood pressures and heart rate were recorded during four 8-hour study days, with venous blood sampling for measurement of drug levels, plasma ACE activity and plasma catecholamines. The effects of first dose nicardipine, two weeks nicardipine and corresponding placebo were evaluated.

The combination of nicardipine with enalapril was generally well tolerated and no adverse effects were reported. First dose nicardipine was associated with significant reductions in supine and erect blood pressure during the first 4 hours: from a baseline of 165/99 to 128/80 (supine) and 156/101 to 126/82 (erect) at 2 hours after nicardipine compared with 167/101 to 146/90 (supine) and 161/104 to 148/95 (erect) following placebo. A transient increase in supine heart rate occurred at 1 hour (80 bpm compared to 70 bpm).

Two weeks treatment with nicardipine significantly reduced baseline blood pressures 12 hours after the last dose of nicardipine.

Table: Blood Pressure and Heart Rate after 2 weeks treatment with nicardipine and placebo

	NICARDIPINE				PLACEBO			
	Blood Pressure		Heart Rate		Blood Pressure		Heart Rate	
Time (hours)	Supine	Erect	Supine	Erect	Supine	Erect	Supine	Erect
0	158/96 *	150/94 *	74	83	172/106	164/103	75	82
2	124/77 **	120/78 **	72	88	154/96	157/98	70	82
4	129/81 **	122/82 **	70	86	149/95	149/93	69	80
8	135/82	127/83 *	71	87	143/84	144/88	78	85

* indicates significant difference from placebo $p < 0.05$ ** $p < 0.01$

The profile of plasma ACE inhibition and the pharmacokinetics of enalapril were unaffected by the addition of nicardipine.

These results suggest that nicardipine and enalapril are an effective and well tolerated antihypertensive combination.

NICOTINE INTAKE BY SMOKERS OF NON-VENTILATED FILTER CIGARETTES; A SYSTEMS DYNAMICS PHARMACOKINETICS APPROACH.

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An estimate of the intake of a drug inhaled as an aerosol is most frequently made by comparing the area under the plasma concentration curve (AUC) obtained after i.v. administration of a standard quantity of this drug to the AUC observed after inhalation of the aerosol-dispersed formulation of the drug. Either the injection (or infusion) and inhalation of the drug are performed on two occasions separated by a period of abstination of the drug, or they may take place simultaneously if a labeled analogon is available for one of the two dosage routes. The former protocol has been used to estimate the nicotine intake of smokers (Feyerabend *et al.*, 1985); however, the results should be viewed with care, because nicotine disposition kinetics are known to change with the degree of short-term preexposition to the drug, whereas comparison of AUCs presupposes kinetics that are unaffected by an abstination period. The latter protocol meets this objection; however, both protocols require the attendance of a highly skilled medical team at the experiments because of the high toxicity of nicotine.

A safe alternative is to involve the non-toxic long-lived nicotine metabolite cotinine in the estimation of nicotine intake. As cotinine is rapidly and completely absorbed after oral dosage without notable first-pass effect (De Schepper *et al.*, 1986), the cotinine clearance may be computed as: $\dot{V}_{el,cot}$=(oral dose)/AUC. For this purpose, we gave smoking subjects a dose of dideuterated cotinine p.o., the subjects meanwhile kept smoking according to their usual pattern.

For a number of cigarette brands the amount of nicotine retained on the cigarette filter was studied as a function of puff volume and puff duration with the aid of a smoking machine. The relative retention R_{nic} varied only moderately and may be regarded as a constant for each brand. If an experimental subject smokes n filter cigarettes in T hours, and if the amount of nicotine retained on the filters averages q mg, then the gross nicotine input rate $\dot{D}_L$ into the lungs of the subject is: $\dot{D}_L = n.q.(1-R_{nic})/R_{nic}.T$ (mg/h).

The nicotine and cotinine mass balances in the plasma of a steady smoker read as follows: $C_{ss,nic}.\dot{V}_{el,nic}=\dot{D}_L.f_L$ (I) and: $C_{ss,cot}.\dot{V}_{el,cot}=\dot{D}_L.f_L.f_M$ (II) , where C_{ss} and $\dot{V}_{el}$ are the plasma steady-state concentrations and the clearances, respectively, of the compounds indicated in the subscript, f_L is the fraction of inhaled nicotine absorbed in the pulmonary blood, and f_M is the fraction of absorbed nicotine metabolized to cotinine. $\dot{D}_L$ is the formerly discussed nicotine input rate.

$C_{ss,nic}$, $C_{ss,cot}$, $\dot{V}_{el,cot}$ and $\dot{D}_L$ are measurable quantities in our protocol. From (I), $\dot{V}_{el,nic}/f_L$, and from (II), $f_L.f_M$ is obtained. For two steady smokers the results were: *subject LdM*: $\dot{V}_{el,nic}/f_L$= 87.9 l/h, $f_L.f_M$=0.73, nicotine intake: 1.78 f_L mg per cigarette (machine yield: 1.2 mg); *subject RV*: $\dot{V}_{el,nic}/f_L$=53.8 l/h, $f_L.f_M$=0.87, nicotine intake: 1.14 f_L mg per cigarette (machine yield: 1.1 mg).

For deeply inhaling smokers like LdM and RV, f_L may well approximate unity. In this case, f_M amounts to 0.73 and 0.87 for these two subjects. f_M has never been published up to now. More experimental results will be presented at the meeting.

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BW A575C, A NOVEL ANTIHYPERTENSIVE AGENT WITH ANGIOTENSIN CONVERTING ENZYME INHIBITION AND β-BLOCKING PROPERTIES.

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Angiotensin converting enyzme (ACE) inhibitors have been shown to be effective in the treatment of several forms of human hypertension. However, such agents do not always offer complete control of hypertension and as a consequence other therapies such as beta-adrenoceptor blocking agents or diuretics, have been given in combination with ACE inhibitors in order to achieve acceptable control of blood pressure (MacGregor et al, 1985).

BW A575C, [N-(1-carboxy-5-[4-(3-isopropylamino-2-hydroxypropoxy)indole-2-carboxamido]pentyl)alanyl proline] was designed as a chemically novel anti-hypertensive agent which exhibits in a single molecule both ACE inhibition and beta-adrenoceptor blocking properties. The present study describes the in vitro and in vivo pharmacological profile of this compound.

The beta-adrenoceptor blocking properties of BW A575C were studied in vitro in isolated, spontaneously beating right atria from male guinea-pigs. Isoprenaline (ISO) concentration response data was obtained as increases in atrial rate and antagonism by BW A575C was analysed using the Schild equation. ACE inhibition in vitro was measured using a partially purified enzyme from rabbit lung by a continuous spectrophotometric assay (Holmquist et al, 1979). ACE inhibition and beta-adrenoceptor blocking properties of BW A575C in vivo were studied using a pithed rat preparation. Angiotensin 1 (AI) or ISO were administered intravenously in an increasing sequence of dose. Only one dose response curve was obtained from each animal, vehicle or drug treatment being randomised to form a balanced design. The anti-hypertensive effect of intravenously administered BW A575C was studied in the conscious renovascular hypertensive dog (24h after left renal artery stenosis, > 70% occlusion).

BW A575C produced a competitive blockade of responses to ISO in the atrial preparation with a pK_b of 7.18 ± 0.05 (cf. pindolol 8.9 ± 0.7). BW A575C inhibited a partially purified preparation of ACE from rabbit lung with an IC_{50} of 10.7 ± 2.1nM (cf. enalaprilat, 4.4 ± 0.8nM). Intravenous administration of BW A575C (1-100 $\mu gkg^{-1}min^{-1}$) to the pithed rat (n=6) inhibited in a dose dependent fashion AI induced pressor responses; comparative studies with enalapril showed it to be approximately 20 times more potent than BW A575C. BW A575C did not significantly inhibit angiotensin II induced pressor responses. Intravenous administration of BW A575C (10-100 $\mu gkg^{-1}min^{-1}$) to the pithed rat (n=6) inhibited in a dose dependent manner ISO induced tachycardia, being approximately 100 times less active than pindolol. Intravenous administration of BW A575C (1 $mgkg^{-1}$) to the conscious renovascular hypertensive dog (n=4) produced a fall in blood pressure (134 ± 11 to 99 ± 4 mmHg) which was sustained for up to 4 hours. The fall in blood pressure was not accompanied by any significant change in heart rate.

In conclusion, BW A575C displays dual activity as an ACE inhibitor/ beta-adrenoceptor blocker both in vitro and in vivo and is a potent anti-hypertensive agent in the renovascular hypertensive dog.

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THE CARDIAC AND RENOVASCULAR EFFECTS OF BW A575C, A NOVEL ANGIOTENSIN CONVERTING ENZYME INHIBITOR AND β-ADRENOCEPTOR ANTAGONIST.

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BW A575C has been shown to possess both angiotensin converting enzyme (ACE) inhibition, and beta-adrenoceptor blocking properties in vitro and in vivo (Allan et al, this meeting). We have therefore investigated the cardiac and renovascular responses to this novel compound in anaesthetised dogs.

In anaesthetised, open-chest dogs (n=5 for each drug) instrumented for the measurement of blood pressure (BP), heart rate (HR), rate of rise of left ventricular pressure (LVdP/dt) and left ventricular internal dimensions (LVID-using sonomicrometry), the effects of BW A575C were compared with the beta-blockers, propranolol and pindolol, at equivalent beta-blocking doses (measured by the inhibition of an isoprenaline tachycardia- response curve). BW A575C (5.0mg.kg^{-1} i.v.) reduced BP (17%), HR (10%), LVdP/dt (16%) with little effect on end-diastolic LVID; Propranolol (0.1mg.kg^{-1} i.v.) reduced HR (19%) LVdP/dt (38%) with no effect on BP but increased end-diastolic LVID (10%). Pindolol (0.01mg.kg^{-1} i.v.) also reduced HR (8%) and LVdP/dt (22%) but had no effect on BP (until higher doses) or end-diastolic LVID.

In anaesthetised dogs (n=4 to 5 for each drug), continuously infused with saline (0.1 ml.kg^{-1} min^{-1} i.v. to promote diuresis), and instrumented for the measurement of BP, HR, LVdP/dt, renal blood flow (RBF) and renal function (UV-urine volume, US-urine sodium), the effects of BW A575C were compared with the ACE-inhibitor, enalapril, and the beta-blocker, pindolol. In animals where the kidneys had intact innervation, BW A575C and enalapril (infused to a total dose of 2.0 mg.kg^{-1} i.v. for equivalent inhibition of an angiotensin I pressor-response curve) increased RBF (34:42%), UV (90:78%) and US (102:226%). Both drugs produced small reductions in BP (7:10%), but only BW A575C reduced HR (13%) and LVdP/dt (28%). In animals where the kidneys were denervated (to obviate any reflex renal responses), the profile of effects of these drugs was unchanged although BW A575C effects on renal function were more marked and better sustained. By contrast, pindolol (infused to a total dose of 2.0 mg.kg^{-1} to elicit its beta-agonist vasodilator actions) in animals with denervated kidneys markedly reduced UV (54%) and US (84%), without changing RBF, but with a significant reduction in BP (35%). In these animals pindolol produced only transient reductions in HR and LVdP/dt.

These studies therefore confirm in vitro findings that BW A575C is a less potent beta-blocker than propranolol and pindolol in vivo, but at equivalent cardiac beta-blocking doses, BW A575C is alone in reducing resting BP. Furthermore, unlike propranolol, BW A575C induces no cardiac dilation, suggesting less cardiodepressant activity. In the kidney, BW A575C, like enalapril, but unlike pindolol, produces renal vasodilation and increases renal function. Unlike enalapril however, BW A575C also reduces HR and LVdP/dt. Thus the combined pharmacological properties of BW A575C produce cardiac and renovascular effects which allow BW A575C to reduce BP without compromising either cardiac performance or renal function.

THE EFFECT OF LOW-DENSITY LIPOPROTEINS ON ENDOTHELIUM-DEPENDENT RELAXATION IN RABBIT AORTA.

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The responses of isolated blood vessels to several vasodilators,including acetylcholine (ACh), ATP and the calcium ionophore, A23187, are dependent on the presence of intact endothelium and are mediated by the release of endothelium-derived relaxing factor,EDRF (Furchgott & Zawadzki,1980). It has been suggested that the generation of EDRF is impaired as a result of perturbation or loss of endothelium in atherosclerosis causing an increase in vascular tone (Vanhoutte, 1983). Furthermore,cholesterol-fed rabbits,which show some characteristics associated with atherosclerosis,exhibit reduced endothelium-dependent relaxation (Coene et al., 1985; Ibengwe et al., 1986). One of the factors implicated in atherogenesis is elevated plasma levels of low density lipoproteins (LDL) (Steinberg, 1983). We have therefore examined the possibility that LDL has a direct and specific effect on endothelium-dependent relaxation.

LDL (density 1.006 to 1.063 g/ml) was prepared from fresh human plasma (Cheung et al.,1980). In some cases the receptor-binding capacity of the LDL was abolished by chemical modification using cyclohexanedione or potassium cyanate (Weisgraber et al., 1978). 2mm wide rings were prepared from the thoracic aorta of Japanese White rabbits (approx. 6 months old),mounted isometrically at 2g resting tension in oxygenated Krebs-Ringer solution at 37°C, and equilibrated for 90 mins. Tissues were contracted with noradrenaline (NA) or 5-hydroxytryptamine (5-HT)at a dose that gave 75% contraction, and relaxed to increasing doses of ACh, ATP or A23187. Wash-out and recovery were followed by incubation with LDL,modified LDL or control buffer. After further wash-out and recovery,tissues were re-challenged with the contracting and relaxing agents. Finally the response to sodium nitroprusside (SNP), an endothelium-independent relaxing agent, was tested. Silver-staining showed that the endothelium was intact before and after treatment.

Rings precontracted with NA or 5-HT relaxed 80 to 100% in response to ACh,ATP or A23187, or to the endothelium-independent agent,SNP. After treatment with LDL the maximal % relaxations to the endothelium-dependent agents were markedly reduced, but the response to SNP was unchanged. Treatment with modified LDL did not inhibit relaxation to any of the agents tested.

These observations show that LDL directly inhibits endothelium-dependent relaxation to a variety of substances,although the smooth muscle retains its ability to relax to SNP. This effect is not due to the removal of the endothelium but requires the interaction of LDL with its receptor.

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NO DIFFERENCE BETWEEN α-ADRENOCEPTOR AGONIST INDUCED CONTRACTION IN ENDOTHELIUM-INTACT AND-DENUDED RING SEGMENTS OF GUINEA-PIG AORTA.

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The spontaneous release of EDRF (Endothelium-Derived Relaxing Factor)(Furchgott and Zawatzki, 1980) depresses α-adrenoceptor agonist-induced contractions of rat aorta. Especially, contraction by partial α-agonists (Lues and Schümann, 1984), which is also very sensitive to calcium entry blockade, is counteracted in the presence of intact endothelium. In the presence of the calcium entry promotor (±)-Bay k 8644 (10^{-6}M), the potency of α-adrenoceptor agonists in endothelium-intact(EI) preparations can be brought to the level of that in endothelium-denuded(ED) preparations (Beckeringh, 1985). In addition, acetylcholine completely relaxes α-adrenoceptor agonist contracted preparations when intact endothelium is present. In contrast to the rat aorta, α-adrenoceptor agonist-induced contractions of guinea-pig isolated aorta are nearly insensitive to calcium entry blockade (Beckeringh et al., 1984). In the present study we have examined the role of the EDRF and the effect of the calcium entry promotor Bay k 8644 on α-adrenoceptor agonist-induced contraction of EI- and ED preparations of guinea-pig isolated aorta. Ring segments of guinea-pig isolated aorta were suspended in Krebs-Henseleit solution containing 1μM dl-propranolol, 10μM cocaine and 20 μM cortisol at 37°C at a resting tension of 2g. Agonists were added cumulatively to the organ bath. Contractions were expressed as a percentage of the maximal response to 100μM (-)-noradrenaline.

	intact				rubbed			
	control		+ Bay k 10^{-6}M		control		+ Bay k 10^{-6}M	
	pD_2	Emax	pD_2	Emax	pD_2	Emax	pD_2	Emax
PE	6.1±0.1	1.02	6.3±0.2	1.06	6.2±0.2	1.03	6.3±0.2	1.05
CIRAZ	7.0±0.2	1.01	7.0±0.1	1.08	7.1±0.1	1.00	7.3±0.2	1.06
CLONI	6.3±0.2	0.61	6.5±0.2	0.63	6.3±0.2	0.64	6.8±0.3	0.71
St 587	5.6±0.2	0.51	5.7±0.3	0.52	5.9±0.2	0.55	6.0±0.3	0.58

(mean ± SEM, n=5-8)

Contractions of EI preparations by (-)-phenylephrine (PE) and cirazoline (CIRAZ), with Bay k 8644 present or absent were not different from those obtained with ED rings. With Bay k 8644 present, contraction by clonidine (CLONI) and St 587 was slightly potentiated but there was no significant difference between EI- and ED rings. By increasing the potassium concentration to 15 mM, control contractions by St 587 were somewhat potentiated, but in the presence of Bay k 8644 and elevated potassium there was a large increase of potency in both EI- and ED rings. The effects of potassium alone and in combination with Bay k 8644 were reversed by the calcium entry blocker nifedipine (10^{-6}M). Acetylcholine (up to 10^{-4}M), in the presence or absence of Bay k 8644,induced only a slight relaxation of EI preparations (±25%). The similarity of the α-adrenoceptor agonist induced contractile effects in the presence and absence of intact endothelium and/or the calcium entry promotor Bay k 8644, as well as the limited relaxation observed in the presence of acetylcholine shows that the EDRF only partly inhibits calcium influx independent contraction. It is suggested that the EDRF interferes with the entry of extracellular calcium following α-adrenoceptor agonist stimulation.

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DEPRESSION OF MAXIMUM RESPONSES OF CIRCULAR PORTAL VEIN BY PRAZOSIN.

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Cohen Wiley & Slater (1979) reported that prazosin, but not phentolamine, reduced the maximum contractile response to noradrenaline (NA), in the rat isolated circular portal vein. The aim of this study was to investigate whether this action of prazosin is related to its selectivity for α_1-adrenoceptors.

Circular preparations of the portal vein were removed from male Wistar rats (230-280g) and mounted as described by Cohen et al (1979). Non-cumulative concentration-response curves (CRC) to NA or phenylephrine (PHE) were constructed following an initial contraction to 1.0μM NA. A second CRC was then elicited 60 min later in either the absence of antagonist or in the presence of either prazosin or corynanthine. All responses have been expresssed as a percentage of the maximum control response and the dose ratio values have been determined from the EC_{50} of the first CRC. In the absence of antagonist, the second CRC for both agonists was shifted slightly to the left of the first CRC with a significant increase in the maximum response (Table 1). The selective α_1-adrenoceptor antagonists prazosin (5nM) and corynanthine (1μM) (McGrath, 1982) produced similar dextral shifts in the CRC for the non-selective agonist NA and the selective α_1-adrenoceptor agonist phenylephrine (results in table 1 have been corrected for the shift in the control CRC.) In contrast to corynanthine, however, prazosin significantly reduced the maximum response to both agonists (see Table 1).

Table 1: Effect of prazosin and corynanthine upon CRC to NA and PHE.

Antagonist	NORADRENALINE		PHENYLEPHRINE	
	log dose ratio	% reduction max. response	log dose ratio	% reduction max. response
control	-	-10.6±3.0	-	-28.8±3.2
prazosin	1.14±0.9	15.3±3.4*	1.17±0.08	2.6±5.1*
corynanthine	1.00±0.1	-10.8±3.5	1.13±0.05	-28.5±7.6

*: significantly different from control (unpaired Students' t-test)

A 25-fold increase in the concentrations of both antagonists caused a further dextral shift in the NA CRC and prazosin produced a greater depression of the maximum. In only 2 out of 6 preparations studied did the selective α_2-adrenoceptor agonist UK-14304 elicit a response and in both cases the maximum response was less than 10% of the maximum response to NA.

In conclusion, we have confirmed the observation of Cohen et al (1979). However, based upon the reported selectivity of the agents used in this study (see McGrath, 1982), the reduction of the maximum responses by prazosin in the circular portal vein is unlikely to be related to its ability to selectively block α_1-adrenoceptors.

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CHARACTERIZATION OF VASCULAR NEURONAL DOPAMINE RECEPTORS IN THE RAT.

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We have shown in vivo that apomorphine administered locally into the isolated autoperfused hindquarters, renal and mesenteric vascular beds inhibits neurogenic vasoconstriction through stimulation of neuronal dopamine receptors (Dupont et al, 1985; 1986). To further characterize the dopamine receptors involved, we studied the influence of the selective DA_1-receptor antagonist SCH 23390 [(R)-(+)8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol] and the selective DA_2-receptor antagonist domperidone (Hilditch & Drew, 1985) on the inhibitory effect of apomorphine in these 3 vascular beds.

The experiments were done in Wistar rats weighing 270-440 g, anesthetized with pentobarbital. Autoperfusion of either the hindquarters or the renal or the mesenteric vascular beds was performed using extracorporeal flow circuits as described previously (Dupont et al, 1985; 1986). Flow was adjusted at the start of the experiment, so that perfusion pressure was equal to systemic blood pressure, and was kept constant during the experiment. The lumbar sympathetic chains, the periarterial renal nerves or the periarterial mesenteric nerves were stimulated (supramaximal voltage, 1msec, 4Hz) with a bipolar electrode. The animals were pretreated with atropine (1 mg/kg IV). Electrical stimulation was performed 4 times at intervals of 5 min. Domperidone (10 µg/kg, n = 6 in each vascular bed) or SCH 23390 (50 µg/kg, n = 6 in each vascular bed) was administered locally, in bolus, immediately after the 1st stimulation. Apomorphine (1 µg/kg/min) was infused locally for 5 min, starting immediately after the 2nd stimulation and stopping after the 3rd. In the absence of antagonists, this dose depresses the pressure response to electrical stimulation at 4Hz by approximately 50 % in each vascular bed (Dupont et al, 1985; 1986).

In neither vascular bed, the administration of domperidone and of SCH 23390 had an effect on perfusion pressure per se or on the pressure response to stimulation at 4Hz. The local infusion of apomorphine had no effect on perfusion pressure per se. After pretreatment with SCH 23390 50 µg/kg, a dose shown to antagonize the blood pressure lowering effect of the selective DA_1-receptor agonist fenoldopam (Sengupta & Lokhandwala, 1985), apomorphine reduced the responses to stimulation in the hindquarters, renal and mesenteric vascular beds to 53.3 $\pm$ 2.0 % (mean $\pm$ sem, n = 6, $p < 0.05$, Wilcoxon test), 51.6 $\pm$ 2.2 % (n = 6, $p < 0.05$) and 53.1 $\pm$ 5.1 % (n = 6 , $p < 0.05$) of the responses to the 1st stimulation respectively. After pretreatment with domperidone, the responses to electrical stimulation during apomorphine infusion were respectively 99.1 $\pm$ 1.6 % (n = 6), 102.1 $\pm$ 1.8 % (n = 6) and 97.3 $\pm$ 2.1 % (n = 6) of the responses to the 1st stimulation.

Our results show that the inhibition of neurogenic vasoconstriction by apomorphine in the isolated autoperfused hindquarters, renal and mesenteric vascular beds is antagonized by the selective DA_2-receptor antagonist domperidone, but not by the selective DA_1-receptor antagonist SCH 23390. This suggests that the neuronal dopamine receptors present in these vascular beds belong to the DA_2-type.

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POSTJUNCTIONAL α_1- AND α_2-LIKE ADRENOCEPTORS IN HUMAN ISOLATED DEEP DORSAL VEIN OF THE PENIS.

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Erection is a vascular phenomenon depending on arterial and venous constriction (Toduriu and Boumer, 1983).

Dynamic cavernography allows to study the venous component and the impotent patients with an erectile flow superior to 120 ml/min are considered to have a venous leakage (Wespes et al, 1984). The real mechanism of venous constriction is not yet understood but ligation of the deep dorsal vein seems to restore their erection (Wespes and Schulman, 1985).

In the present study, we have made a pharmacological analysis of the postjunctional alpha adrenoceptors in the isolated deep dorsal vein. Rings of those veins were mounted for isometric tension in organ chambers filled with Krebs solution (13 patients, aged from 23 to 67 years old, mean 48; 24 rings).
Noradrenaline (NA), phenylephrine (Phe) and too a lesser extent Clonidine (Clo) induced a dose dependent contraction and the order of activity was NA > Phe > Clo (ED 50 for NA and Phe : 3,57 μM $\pm$ 1,09 (s.e. mean), 5,89 μM $\pm$ 0,88 (s.e. mean) respectively. These responses were not modified after preincubation with cocaine (30 μM) or β oestradiol (30 μM).

Various antagonists were added to elucidate the nature of these responses. Propranolol (1 μM) did not modify them while prazosin competitively antagonized the responses to NA and Phe.
However prazosin (10 nM, 100 nM and 1 μM) reduced the Phe (10 μM) induced contractions (% of inhibition : 46%, 85% and 93% respectively) more than those induced by NA (10 μM) (% of inhibition : 28%, 50% and 64% respectively). Yohimbine (10 nM, 100 nM and 1 μM) slightly reduced the NA (10 μM) induced responses (% of inhibition : 20%, 30% and 40% respectively) and had no effect on Phe induced contractions.

In conclusion those results indicate the presence of both alpha 1 and alpha 2 like adrenoceptors on venous smooth muscles cells of the human deep dorsal vein of the penis with a preponderance of alpha 1 adrenoceptors.

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AT WHICH α-ADRENOCEPTOR SUBTYPES DOES UK14304 ACT?

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UK14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) has been reported to be a selective alpha$_2$ adrenoceptor agonist (1). We have re-examined the selectivity of UK14304 for alpha$_2$ adrenoceptors in three studies in the rabbit; in vitro ligand binding studies, in vitro platelet aggregation studies and in vivo studies in the conscious rabbit. In the first study the ability of UK14304 to displace the alpha$_1$ adrenoceptor ligand [^{3}H] prazosin (P) and the alpha$_2$ adrenoceptor ligands [^{3}H] idazoxan (I) and [^{3}H] yohimbine (Y) from specific binding sites on kidney and brain membranes and whole platelets was examained. In the second study the effect of UK14304 on the aggregatory response of rabbit platelets to ADP and the effect of antagonists on this response was examined and compared to that of other alpha$_2$ adrenoceptor agonists. In the third study, in conscious rabbits, pressor dose response curves to IV UK14304 were constructed, the immediate pressor responses measured and the effects of a range of alpha$_1$ and alpha$_2$ adrenoceptor antagonists on these responses studied.

In the ligand binding studies IC_{50} values for the displacement of [^{3}H] I from brain and kidney membranes were 1×10^{-7} M and 6×10^{-8} M for the displacement of [^{3}H] Y from brain, kidney and platelet 1×10^{-8}, 6×10^{-8} and 5×10^{-8} M respectively but for [^{3}H] P displacement was 10^{-4} M or greater consistent with the previously reported alpha$_2$ adrenoceptor selectivity of UK14304. In the platelet aggregation studies UK14304 enhanced the pro-aggregatory response to ADP, maximum response 3.3 ± 1.2, OD, C_{50} 2.2 ± 2.4 x 10^{-8} M compared to a maximum of 5.1 ± 1.4 and C_{50} 1.6 ± 0.4 x 10^{-8} M for adrenaline. The aggregatory response to UK14304 was not attenuated by P 10^{-5} M but was abolished by I and Y 10^{-5} M which again is consistent with alpha$_2$ adrenoceptor agonist activity. In contrast, in vivo Y and I failed to significantly attenuate pressor responses to UK14304, although responses were attenuated by P and doxazosin (D) . These results would be more consistent with alpha$_1$ adrenoceptor agonist activity.

Table: Effects of P and I on pressor responses to alpha adrenoceptor agonists

Drug	Dose (ug/kg)	Pressor Response (mmHg) Control	I (0.5 mg/kg)	Control	P (0.5 mg/kg)
BHT 920	35	29 ± 8	20 ± 5**	26 ± 2	19 ± 4
methylnoradrenaline	3	33 ± 4	23 ± 9**	32 ± 4	23 ± 4*
Noradrenaline	1.5	42 ± 9	37 ± 8*	47 ± 5	15 ± 3**
Phenylephrine	8	39 ± 3	37 ± 3	42 ± 8	4 ± 2**
UK14304	250	40 ± 8	37 ± 7	38 ± 11	19 ± 4**

n = 6-8. *p < 0.05 **p < 0.01

However whereas D exhibits its maximum antagonist effect on phenylephrine responses 4 hours after administration (2) attenuation of UK14304 responses was greatest after 15 minutes.

Although most studies have shown UK14304 to be an alpha$_2$ adrenoceptor agonist it has been reported to behave more like an alpha$_1$ adrenoceptor agonist in some isolated smooth muscle preparations from rabbit, guinea pig and rat (3,4). Our studies are not entirely consistent with a classification of UK14304 as a selective alpha$_1$ or alpha$_2$ or mixed alpha$_1$/alpha$_2$ agonist. Anomolous results have also been observed with other agonists showing alpha$_2$ adrenoceptor activity (4,); it is possible that further subclassification of alpha adrenoceptors may need to be considered.

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PHARMALOGICAL STUDY OF THE ADRENOCEPTORS OF ISOLATED HUMAN CORONARY ARTERIES.

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Alpha - adrenergic tone playing a role in chronic stable angina (Mudge et al , 1976, Berkenboom et al, 1986) we have studied the reactivity of adrenoceptors of isolated human coronary arteries.

Rings of proximal coronary arteries from children (group A, mean age 5 ± 3 years, n = 4, 11 segments) and from adults (group B, mean age 48 ± 4 years, n = 9, 21 segments) were suspended in aerated Krebs solution under 4g and 7g tension respectively.

On preparations contracted with KCl (20 mM), concentration response curves were assessed for isoproterenol, and the ED_{50} was determined in group A (0.43 ± 0.07 μM)(mean ± s.e.mean).In group B, segments of 7 patients failed to relax to isoproterenol, while in the remaining 2 patients (without heart disease) the ED_{50} for isoproterenol was 0.48 μM.
A competitive antagonism by atenolol 1 μM and propranolol 1 μM was demonstrated and the shift in ED_{50} was 10 ± 3 and 101 ± 7, respectively.

In group A, norepinephrine 10 μM induced a relaxation of 19 % (percent of maximal contraction induced by KCl 30 mM : 2.1 ± 0.2g).
After preincubation with atenolol 1 μM, norepinephrine 10 μM induced a contraction of 26 ± 6 % , while after propranolol 1 μM the contraction was 84 ± 7 % ($p < 0.05$ versus atenolol).
In group B, norepinephrine 10 μM induced a large contraction 99 ± 11 % (KCl 30 mM : 2.7 ± 0.4g) which was not modified after preincubation with atenolol 1 μM or propranolol 1 μM.In both groups, prazosin 1 μM partially antagonized norepinephrine (10 μM)-induced contraction (45 % of inhibition in group A and 51 % in group B), while a mixture of prazosin 1 μM and yohimbine 1 μM completely abolished the response.

In conclusion,the population of adrenoceptors of isolated human coronary arteries seems to vary according to age and/or to pathological status.
In preparations from children, the presence of α-and β-adrenoceptors can be demonstrated, while in adults, α-receptors prevail in most preparations.These α - receptors appear to be of α_1 and α_2 subtypes.

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CARDIOVASCULAR EFFECTS OF THE NEW ANTIHYPERTENSIVE COMPOUND DU 29373.

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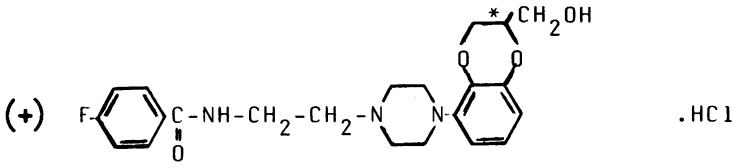
DU 29373, flesinoxan.HCl*, was found to represent a potent, longacting antihypertensive agent with a new, probably central mode of action.
The structural formula is shown in Figure 1



The effects of DU 29373 on blood pressure (Pm) and heart rate (HR) were studied in several species. Blood pressure was measured via arterial cannulas and heart rate derived from the pulse pressure. In conscious Spontaneously Hypertensive (SH) rats oral DU 29373 produced a rapid, long lasting (> 8h) decrease in Pm and a 20% reduction (ED_{80}) was found at 1.5 mg/kg. A comparable result was obtained in renal hypertensive rabbits. HR decreased only moderately in these species. In anaesthetised cats and dogs, DU 29373 potently lowered Pm, reaching ED_{80} values of 0.014 and 0.06 mg/kg i.v. respectively. In cats, but less in dogs, HR clearly decreased at higher doses.
The decrease in Pm (25%) was well maintained in SH rats applying DU 29373 (20 mg/kg/day) during 10 days via osmotic minipumps, and rebound effects on HR or Pm were not observed upon removal. Instead, the parameters gradually returned to base-line.
The compound is active both after oral and i.v. administration and metabolism studies indicate that no active metabolites are produced in the species tested.

In search for its mode of action, DU 29373 was administered simultaneously via both vertebral arteries in anaesthetised cats according to Porsius (1980). Via this route, the potency of DU 29373 increased 35 times compared to i.v. administration. A nearly similar potency increase was seen with cisterna magna administration.

These data indicate that DU 29373 is a potent antihypertensive compound and that the central nervous system is involved in its mode of action. Receptor binding showed that DU 29373 has no α_2-affinity (pKi=4.2) but represents a selective 5-HT_{1A} ligand (pKi=8.7) (Bevan et al., 1986). It is concluded that DU 29373 represents a potent longacting antihypertensive which differs from the currently used antihypertensive principles. At present, DU 29373 is undergoing clinical evaluation.

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*proposed INN

α_2-ADRENOCEPTOR INHIBITORY CONTROL OF TRANSMITTER RELEASE IN THE RABBIT MESENTERIC ARTERY; AN ELECTROPHYSIOLOGICAL STUDY.

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There exists some controversy whether the release of the neuroeffector transmitter in the rabbit mesenteric artery is controlled by an inhibitory α_2-adrenergic mechanism (Kuriyama & Makita, 1984; Mishima et al., 1984). In the present experiments the periarterial nerves of isolated jejunal branches of the rabbit mesenteric artery (<0.5 mm diameter) were stimulated with trains of 15 pulses at 1 Hz; the amplitude of intracellularly recorded excitatory junction potentials (e.j.ps) was used as a measure of transmitter release. The stimulation voltage was adjusted so that the amplitude of the first e.j.p. was before drug-application about 7 mV. The train of pulses caused facilitation and subsequent depression of e.j.ps. The α_2-agonist clonidine reduced the e.j.p. amplitudes in a concentration-dependent manner. Noradrenaline (1 µM), which is non-selective at α-adrenoceptor subtypes had a similar effect to that of clonidine 0.1 µM. The α_1-agonist phenylephrine was inactive at 0.1 µM and produced a moderate depression at 1 µM. The percent inhibition by all agonists tested was inversely related to the length of the train. The α_2-antagonist yohimbine did not influence the first e.j.p. at 0.3 µM, but depressed it at a higher concentration of 3 µM. Yohimbine concentration-dependently enhanced the later e.j.ps in the train. The α_2-selective stereoisomer of yohimbine, rauwolscine, and the non-selective α-antagonist tolazoline acted in a manner similar to yohimbine. The α_1-selective corynanthine was much less potent than its stereoisomer yohimbine; another α_1-antagonist, prazosin, was also less potent than yohimbine. The α_2-selective antagonists at a concentration of 3 µM reduced the effect of clonidine 0.1 µM to a larger extent than the α_1-selective antagonists. Yohimbine 3 µM reduced the effect of phenylephrine 1 µM to a greater degree than corynanthine 3 µM. None of the ligands tested changed the membrane potential of the smooth muscle cells. We conclude that in the mesenteric artery, activation of presynaptic α_2-adrenoceptors inhibits the release of the motor transmitter. The enhancement of transmitter release by α_2-antagonists indicates that these receptors are physiological targets of endogenous noradrenaline.

Table 1 Effect of drugs with affinity for α-adrenoceptors on the 1st and 15th e.j.p. elicited by trains of 15 pulses at 1 Hz

Treatment (µM)		n	Inhibition (%; mean±S.E.) 1st e.j.p. amplitude	15th e.j.p. amplitude
None		9	3.7±4.3	-1.7±2.7
Noradrenaline	1.0	5	74.2±3.6*	61.3±4.4*
Phenylephrine	0.1	6	-4.6±5.9	-6.9±7.9
Clonidine	0.01	7	30.7±5.8*	4.6±3.6
	0.1	7	87.2±3.0*	64.0±8.2*
Yohimbine	3.0	7	32.9±5.2*	-60.2±7.0*
+ Clonidine[a]	0.1	7	29.7±4.1*	18.9±4.0*
Prazosin	3.0	7	6.6±8.8	-20.3±8.0*
+ Clonidine[a]	0.1	7	58.7±7.5*	58.0±3.6*

* P<0.05-0.001; significant differences from zero. [a] Inhibition with respect to the preceding train recorded in the presence of antagonist alone.

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A NON α-OR β-ADRENOCEPTOR MEDIATED EFFECT OF SOME CATECHOLAMINES IN RAT GASTRIC FUNDUS .

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It has previously been reported that the rat gastric fundus contains inhibitory postjunctional α_1-adrenoceptors (Dettmar et al, 1984, Verplanken et al, 1984) and inhibitory postjunctional β-adrenoceptors (Lefebvre et al, 1984). Catecholamines can relax this preparation by an action mediated via both populations of inhibitory α_1- and β-adrenoceptors. The present study involved an investigation of an α- or β-adrenoceptor - independent response in the rat gastric fundus, using a number of catecholamine and non-catecholamine α_1-adrenoceptor agonists in the presence of the mixed β_1- and β_2-adrenoceptor antagonist propranolol.

Strips of rat gastric fundus were suspended in Kreb's solution containing propranolol (2 μM), atropine (2 μM) and guanethidine (5 μM). In experiments involving catecholamines, cocaine (3 μM), hydrocortisone (30 μM), EDTA (30 μM) and L-ascorbic acid (30 μM) were also present. Tone was induced by the addition of barium chloride (0.5-2 mM) to the bath.

Noradrenaline, adrenaline and cirazoline were approximately equipotent at inducing relaxations (IC50 0.07 - 0.08 μM). Alpha-methyl noradrenaline (IC50 0.3μM), phenylephrine (IC50 0.5μM) and dopamine (IC50 5μM) were less potent in inducing relaxations. Prazosin (0.01-1 μM) shifted the dose-response curve of the agonists to the right. The antagonism of noradrenaline, adrenaline and α-methyl noradrenaline was incomplete and Schild plots gave slopes significantly less than unity. Further addition of propranolol (30 μM), idazoxan (0.1 μM) or haloperidol (30 μM) did not improve the antagonism. In contrast, cirazoline, phenylephrine and dopamine were completely antagonized by prazosin and Schild plots gave slopes of unity. 3,4-dihydroxyphenylacetic acid (DOPAC), had no relaxant effect (1-100 μM).

In conclusion all the α_1-adrenoceptor agonists studied produced dose-related relaxations of the rat gastric fundus in the presence of propranolol. The effects of cirazoline, phenylephrine and dopamine were completely antagonized by prazosin, indicating an action mediated via postjunctional α_1-adrenoceptors. The relaxations induced by noradrenaline, α-methyl noradrenaline and adrenaline were only partially antagonised by prazosin and propranolol and were unaffected by idazoxan, indicating an effect not mediated via α-adrenoceptors. Relaxant responses to noradrenaline resistant to α- and β-adrenoceptor blockade have previously been reported in the rabbit intestine (Wikberg, 1977) and dog colon (Grivegnee et al, 1984). In the former case the effect was attributed to a non-specific effect of the catecholamine nucleus, but this cannot explain the present results since DOPAC, containing the catecholamine nucleus, was without effect and since dopamine was completely blocked by prazosin. Further investigation into this non α- or β-adrenoceptor-mediated effect of catecholamines is required.

JK holds an SERC CASE award in collaboration with Reckitt & Colman plc

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DIETARY EFFECTS ON β-ADRENOCEPTOR REACTIVITY IN GUINEA PIG TRACHEA.

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Old case studies suggested a beneficial role for poly unsaturated fatty acids (PUFA's) in asthmatic disease (1). Using semi-synthetic diets (35 en%) we demonstrated that very moderate variations in guinea pig dietary linoleic acid (18:2ω6) exerted profound attenuated effects on tracheal reactivity to histamine (2).

In the present study we investigated if diets that differ in PUFA content influenced relaxing properties of tracheas after β-adrenergic receptor stimulation. Inmediately after weaning, forty male guinea pigs were put on different diets during a six week period. The diets varied in the amount of linoleic acid (6, 12 and 22% fat respectively); a diet with low linoleic acid (3%) and additional (6%) linolenic acid (18:3ω3) was also present.

Respiratory β-adrenergic receptor function was investigated in carbachol precontracted tracheal spirals (in vitro). The isoprenaline dose response curves of the tracheas showed a maximum relaxation in the dietary group receiving 12% linoleic acid. The relaxations of the other linoleic acid groups were significantly ($P< 0.01$) decreased (20%). In the linolenic acid group maximal relaxation was decreased (13%) though not significantly.

These results suggested that an optimal dietary poly-unsaturated fatty acid content exist for β-adrenergic receptor function, and that slight changes in that will cause a marked negative effect in response.

To analyse whether the observed differences in β-adrenergic receptor responsiveness should be explained in terms of a specific linoleic acid effect, four alternative diets (35 en%) were tested. These diets also differed in the amount of linoleic acid (3, 6, 12% fat respectively), while to two groups that received 3% linoleic acid 3% linolenic acid or 3% fish oil was added additionally.

Isoprenaline induced maximal relaxation in the different dietary groups was calculated relatively to the group receiving 12% linoleic acid. Maximal relaxation in the dietary group receiving 3% linoleic acid was significantly ($P< 0.01$) diminished (24% decrease). Addition of 3% extra linoleic acid had a similar effect (25% decreased maximal relaxation). In contrast, addition of 3% linolenic acid or 3% fish oil, i.e. addition of fatty acids with more double bonds than linoleic acid, resulted in an almost complete restoration of the maximal response as was found in the 12% linoleic acid group.

These results strongly suggest that the amount of PUFA and the grade of unsaturation of the PUFA's present in the diets affect the guinea pig tracheal response to isoprenaline, possibly by affecting membrane fluidity and thereby the β-adrenergic-adenylate cyclase collision coupling.

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500P

EVIDENCE FOR BLOOD VESSEL SELECTIVITY OF BRL 34915.

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BRL 34915 (Ashwood et al. 1984; Buckingham et al. 1984) is an antihypertensive agent which increases K^+-conductance in blood vessels (Hamilton et al., 1985; Buckingham et al., 1985). We compare the effects of BRL on rat portal vein, guinea-pig atria and on intracellular recordings in guinea-pig papillary muscle.

Portal veins removed from male rats (Sprague-Dawley: 250-300 g) were incubated in Krebs' bicarbonate under 0.5 g tension. Spontaneous myogenic activity was quantified using a Grass integrator (7P10B). Left and right atria were obtained from male Duncan Hartley guinea-pigs and incubated in Krebs' bicarbonate at 32°C. Spontaneously beating right atria triggered a Grass tachograph (7P44A) to measure heart rate. Left atria were field stimulated (2.5Hz, 30V, 1ms) to measure contractile force. Conventional glass microelectrodes were used to record intracellular membrane potentials (MP) from right papillary muscle paced at 0.5 or 2.0 Hz in standard PSS at 30°C. These preparations were also partially depolarised using $BaCl_2$ (2 mM) which induces rythmic slow action potentials (AP).
BRL (0.01 - 1.0 µM) was a potent inhibitor of the myogenic activity of the rat portal vein ($-\log IC_{50}$ = 7.32 ± 0.1, n=6; cf. diltiazem = 6.8 ± 0.1). In contrast, BRL failed even at 30 µM to modify the rate of spontaneously beating atria. The decrease in force of contraction of electrically driven left atria observed at 10 - 100 µM, was associated with a solvent (ethanol) action.
Neither BRL (10 or 30 µM) nor solvent significantly modified the resting MP of the papillary muscle. BRL (30 µM) slightly increased (2.5 ± 0.9%) the maximal rate of rise (V_{max}) of the AP at 2.0 Hz (Control: 217.7 ± 16.1 V/s), however a pronounced shortening of the AP duration (APD) was observed at lower frequencies. The effects of BRL on APD_{50} (msec; effect at 50% repolarisation) are shown in the following table:

Hz	n	Control	+10.0 µM	+30.0 µM	Wash
0.5	3	317.4 ± 3.8	306.7 ± 4.6	211.6 ± 28.3*	318.6 ± 1.8
2.0	7	210.6 ± 5.7	204.3 ± 5.4	176.7 ± 7.2*	205.8 ± 7.3

* P<0.05, paired t test

BRL did not inhibit the maximal amplitude of $BaCl_2$ evoked AP's in contrast to diltiazem (1 µM) which decreased this parameter (-28 %). However, BRL (30 µM) caused a slight variation in the diastolic depolarisation (<-5 mV) which resulted in either an increased cycle length (Control: 981 ± 31 msec; BRL: 1222 ± 68 msec, P<0.05) or a blockade of the spontaneous firing of the slow AP's.

In conclusion, BRL is a potent myorelaxant in the rat portal vein with no effect on either rate or force of atrial contraction, therefore BRL has high blood vessel/heart selectivity. The effects of this compound on (a) the repolarisation phase of the stimulated AP and (b) the diastolic depolarisation seen during $BaCl_2$ induced automaticity in papillary muscle are consistent with a K^+-channel stimulant activity of BRL with no pronounced effects on the cardiac Na^+ or Ca^{2+} channels in accordance with Cain and Metzler (1985).

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ANTIHYPERTENSIVE EFFECTS OF RS-93427-007.

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Recently, several prostaglandin-like compounds have been found to possess activities which might be useful in cardiovascular diseases. One of these compounds, RS-93427, 4-Z-((3'S,1S,2S,3R,6S)-2-(3'-cyclohexyl-3'-hydroxy-prop-1-ynyl)-3-hydroxybicyclo[4.2.0]oct-7-ylidene)butyric acid, (Kluge, 1986) has been reported to prevent platelet aggregation in several species (Willis et al., 1986). This paper describes the antihypertensive activity of RS-93427 in comparison to dl-15-deoxy-16-hydroxy-16 vinyl prostaglandin E_2 methyl ester (CL-115347) (Chan et al., 1983).

Male spontaneously hypertensive rats (Tac:N(SHR)fBR) weighing 300-400 g were prepared for blood pressure recording under ether anesthesia. After surgery the ether was removed and wound edges were infiltrated with lidocaine. Compounds were administered cumulatively at 30 to 60 min intervals to groups of 3-4 rats. Doses of compound to reduce mean femoral arterial blood pressure by 20% (ED_{20}) were estimated graphically. The potential for orthostatic hypotension was evaluated by the technique of Lee et al., (1982) using the spontaneously hypertensive rats. Mongrel male and female normotensive cats weighing 2 to 5 kg were anesthetized with pentobarbital and prepared for monitoring blood pressure, heart rate and myocardial force. RS-93427 was administered in cumulative dose increments at 30 min (i.v.) or 60 min (i.d.) intervals.

Epoprostenol (PGI_2) had an ED_{20} of 1.5 µg/kg, i.v., in the rats. CL-115347 produced a comparable antihypertensive effect at 12 µg/kg while the ED_{20} for RS-93427 was 42 µg/kg. Orally, CL-115347 and RS-93427 had ED_{20}s of 300 and 470 µg/kg, respectively. Unlike CL-115347, when applied topically at 600 µg/kg, RS-93427 did not induce a reduction in blood pressure. At high antihypertensive doses, RS-93427 induced signs of peripheral vasodilation. When evaluated for orthostatic hypotension, RS-93427 at 1-100 µg/kg was inactive. Repeated administration of vehicle or RS-93427 at 80 µg/kg day for 4 days gave ED_{20}s of 34 and 20 µg/kg, i.v., respectively, for the two groups suggesting a lack of tolerance.

The hypotensive ED_{20} of RS-93427 administered to 3 cats was 1.5 µg/kg, i.v. and 30 µg/kg, i.d. Heart rate was elevated following 10-30 µg/kg, i.v. or 100-1000 µg/kg, i.d. The ED_{20} of CL-115347 was 21 µg/kg, i.d.

In summary, RS-93427 appears to be a potent antihypertensive by the intravenous and oral routes in both rats and cats. As published elsewhere, it also possesses potent platelet aggregation inhibiting and antiatherosclerotic properties which should be useful in obstructive coronary artery disease.

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RU38486 ANTAGONISES GLUCOCORTICOID INHIBITION OF PROSTACYCLIN AND PGE_2 SYNTHESIS BY CULTURED RAT GASTRIC AND DUODENAL EXPLANTS.

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Administration of corsticosteroids is known to sometimes result in the genesis of peptic and duodenal ulcers (Messer et al., 1983). As corticosteroids are known to inhibit eicosanoid synthesis in other tissues, and as prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) protect the gastric and duodenal mucosa against ulcerogenesis, (Robert, 1979), it is possible that corticosteroid-mediated ulcerogenesis is a result of PGI_2 and PGE_2 inhibition in these tissues.

We therefore studied the effect of hydrocortisone (HC) and betamethasone (BM) on PGI_2 and PGE_2 synthesis by cultured explants of rat stomach and duodenum. The effect of the antiglucocorticoid RU38486 (Chobert et al., 1983) on corticosteroid action was also studied. Culture conditions were modified from the methods of Jeremy et al. (1986a, 1986b). Gastric and duodenal explants were cut out with a 5 mm diameter ophthalmologist's trephine. Twelve discs of stomach or duodenum were placed in sterile culture flasks, containing 100 ml medium 199 (containing 100 $mg.l^{-1}$ streptomycin and penicillin G and designated steroid treatments) and incubated at 37 °C for designated times. Following incubation, the explants were washed and placed in polypropylene tubes containing 1 ml medium 199. Eicosanoid synthesis was stimulated by the addition of calcium ionophore A23187 (final concentration: $3x10^{-5}$ $mol.l^{-1}$), and the tubes incubated for 1 h at 37 °C in a shaking water bath. Tubes were then centrifuged and aliquots taken for estimation of PGE_2 and 6-oxo-$PGF_{1\alpha}$ synthesis by radioimmunoassay (Jeremy et al., 1985). HC and BM effects were studied over a ceoncentration range of $1x10^{-10}$ to $1x10^{-5}$ $mol.l^{-1}$, and at 4, 8, 12 and 18 h culture. HC and BM inhibited PGI_2 and PGE_2 synthesis in a time- and concentration-dependent manner in both duodenal and gastric explants. Since adequate inhibition of eicosanoid synthesis was achieved at 18 h, all subsequent experiments were carried out following 18 h incubations. Aldosterone, progesterone, 17β estradiol and testosterone were all without effect on explant eicosanoid synthesis. Since corticosteroids have been shown to inhibit eicosanoid synthesis through stimulation of inhibitory proteins (Flower & Blackwell, 1979; Hirata et al., 1979), the effect of protein synthesis inhibitors, actinomycin D (AcD) and cycloheximide (Chex), on HC and BM ($1x10^{-6}$ $mol.l^{-1}$)-inhibited PGI_2 and PGE_2 synthesis was investigated. Both AcD ($3x10^{-5}$ $mol.l^{-1}$) and Chex ($3x10^{-5}mol.l^{-1}$) were without effect on the inhibitory action of HC and BM. RU38486 antagonised the inhibitory action of HC and BM in a competitive manner.

These data indicate that the cytoprotective eicosanoids, PGI_2 and PGE_2, are inhibited in cultured gastric and duodenal explants by corticosteroids which do not depend on de novo protein synthesis, and that corticosteroid inhibitory action was competitively blocked by RU38486. RU38486 may prove to be beneficial in an antiulcerogenic role in stressed and steroid-treated patients.

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PROSTACYCLIN PROLONGS BLEEDING FROM THE MESENTERIC ARTERY BUT NOT THE GASTRIC MUCOSA OF THE RAT.

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Platelet adhesion, aggregation and subsequent plug formation play a major role in the control of skin and vessel wall haemostasis (Sixma and Wester, 1977). Although bleeding of the gastric mucosa from ulcer sites and following damage by aspirin-like drugs and topical irritants is common, little is known about the haemostatic process in this tissue. In the present study, bleeding from a standard incision in the rat gastric mucosa has therefore been investigated. The actions of prostacyclin, which prevents platelet aggregation, (Moncada et al, 1976) and of the thromboxane synthase inhibitor, 1-benzyl imidazole (BZI) (Whittle et al., 1981) as well as those of low doses of heparin which interfer with blood coagulation, have been compared on bleeding time in the gastric mucosa and in the mesenteric artery.

Male Wistar rats (200g) were anaesthetised with pentobarbitone, the stomach was opened along the greater curvature and mounted in a plexiglass chamber with its vascular supply intact. Ileal mesentery was placed over a similar chamber. Gastric mucosal bleeding was produced by a 2 mm blade cut through a rugal fold, while mesenteric bleeding was induced by puncturing a mesenteric artery close to the ileal wall with a 25 gauge needle (Zawilska et al, 1982). Isotonic saline (37°C) was superfused over the surface (3.0 ml/min gastric; 6.0 ml/min mesenteric), collected at timed intervals, and the haemoglobin (Hb) output determined using spectrophotometric techniques (absorbance measured at 540 nm). The bleeding time was determined as the time from initial bleeding to the first sample in which Hb output was <0.1 mg/min.

The initial rate of bleeding from the standard incision (5.1 ± 0.8 mg/min Hb, mean ± s.e. mean, n=13) was comparable during the superfusion of isotonic saline or acid-saline (pH 2). Neither the rate of Hb output, nor the bleeding time (3.4 ± 0.2 min, n=13) was significantly prolonged (4 ± 0.4 min, n=6) by intravenous infusion of prostacyclin (0.5µg/kg/min) in a dose which inhibits platelet aggregation ex vivo. Likewise, intravenous administration of BZI (50 mg kg), 10 min prior to the incision, in a dose reducing thromboxane B_2 formation in clotting blood by $90\pm2\%$ (n=5), did not significantly prolong bleeding time (4.1 ± 0.4min, n=8). However, intravenous injection of heparin (100 U/kg), prolonged the bleeding time ($P<0.001$) beyond 15 min. In contrast to the gastric mucosa, in the mesenteric artery, the bleeding time (3.0 ± 0.2 min n=7) was significantly ($P<0.05$) prolonged by administration of prostacyclin (6.4 ± 0.9 min, n=6) or BZI (6.1 ± 0.7 min, n=6), but not by heparin (3.3 ± 0.3 min, n=6).

These findings confirm the importance of platelet aggregation in the haemostatic processes that terminate primary bleeding from arterial vasculature. In contrast in the gastric mucosa, neither prostacyclin nor BZI had any significant action on bleeding, yet heparin substantially prolonged the bleeding time. These findings therefore suggest that haemostasis in the gastric mucosa is more dependent on the coagulation system than platelet aggregation, which thus has therapeutic implications for the use of drugs which affect platelet aggregation or coagulation.

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CARDIOPULMONARY AND GASTRIC ACTIONS OF INTRAVENOUS PAF IN ANAESTHETIZED CATS.

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PAF (platelet-activating factor; 1-O-alkyl-2-acetyl-sn-glycerophosphorylcholine) has many properties consistent with a role in inflammation and may be an important mediator of anaphylaxis (Morley et al., 1985). It has been found to provoke marked bronchoconstriction and systemic hypotension in a variety of animals (see for example Vargaftig et al., 1980, Halonen et al., 1985). In addition a potent ulcerogenic action of PAF in rats has recently been described (Rosam et al., 1986). In the present study we have conducted a preliminary investigation of the cardiopulmonary and gastric effects of bolus intravenous injections of PAF in anaesthetised cats.

Male, mongrel cats (3-4 kg) were anaesthetised initially with halothane (5%) and thereafter with chloralose (60-80 mg kg^{-1} i.v.). Each animal was placed on its back and ventilated mechanically with approximately 50 strokes of 7 ml of laboratory air per kg body weight through a cervical tracheostomy. The pleural cavity was opened and intrapulmonary pressure measured with a Statham PM131TC differential pressure transducer. Airflow was measured with a heated Fleisch size 00 pneumotachograph and a Validyne MP45 differential pressure transducer. Pulmonary airways resistance (R_A) and dynamic lung compliance (C_D) were calculated on-line on a breath-to-breath basis by a pulmonary mechanics analyser (Buxco Electronics Model 6). Arterial blood pressure and heart rate were recorded from a catheter placed in the left femoral artery.

PAF (1-300 ng kg^{-1} i.v.) provoked rapid, and in some cases sustained, bronchoconstriction evidenced by a cumulative rise in R_A of up to 303 ± 77% and a synchronous cumulative fall in C_D of up to 59 ± 6% (mean ± s.e. mean; n=5). In contrast, vehicle alone (bovine serum albumin 0.25%) or the same doses of lyso-PAF were without effect. PAF-induced bronchoconstriction was accompanied by a more transient dose-related fall in arterial blood pressure (max. Δ -83/69 ± 11/9 mm Hg). A progressive rise in haematocrit from 31 ± 2% to 39 ± 3% was also evident, consistent with increased intravascular permeability. Following completion of the initial dose-response curve, a further dose of PAF (300 ng kg^{-1} i.v.) was administered making a cumulative total of approximately 750 ng kg^{-1} in all and causing a further rise in haematocrit to 47 ± 6%. One hour later a segment of the stomach wall (corpus) was removed for morphological investigation. Macroscopically the stomach appeared blanched with patches of hyperaemia. Subsequent histological examination provided evidence of extensive vasocongestion of mucosal and submucosal vessels, marked subepithelial oedema and focal regions in which extravasation of erythrocytes was evident. In a few places, the epithelium was broken and this was associated with haemorrhage and fibrin deposition. These changes were not seen following corresponding doses of lyso-PAF.

The results of the present study indicate that the previously described cardiopulmonary and gastric actions of PAF in other mammals can be extended to the cat. The mechanism by which PAF exerts these actions in this species, which may involve the release of secondary mediators, remains to be established.

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PLATELET ACTIVATING FACTOR MODULATES INTERLEUKIN-2-INDUCED PROLIFERATION OF HUMAN T-LYMPHOBLASTS.

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Platelet activating factor (PAF, 1-O-octadecyl-2-O-acetyl-sn-glycero-3-phosphoryl-choline) is produced by and activates a range of inflammatory cells. However, to date, its effects on lymphocyte function have not been characterised. We have examined the role of PAF in lymphocyte mitogenesis by determining the effect of a) PAF and two non-hydrolysable PAF agonists, PR1501 and PR1502 (Rasmussen, personal communication), b) three selective PAF receptor antagonists, namely L-652,731 (Hwang et al 1985), BN52021 (Bourgain et al 1985) and CV3988 (Terashita et al 1983) and c) a selective PAF synthesis inhibitor, L-648,611 (Robbins et al 1985) on IL-2 induced proliferation of human T-lymphoblasts.

Human T-lymphoblasts were produced essentially according to the method of Smith and Cantrell (1985). Aliquots (160μl) of cell suspensions in RPMI 1640 + 10% heat-inactivated foetal calf serum containing $1.6x10^5$ viable lymphoblasts were added to microtitre wells. Quintuplicate aliquots were then treated with 20μl drug vehicle, PAF agonists (0.03-300nM), PAF receptor antagonists or the synthesis inhibitor (1-30μM) followed 10 min later by the addition of 20μl recombinant IL-2 (0.1-30ng/ml). The cells were then incubated, pulsed with ^{3}H-thymidine (^{3}H-TdR) and harvested (Gordon et al, 1979).

IL-2 produced a dose-related ^{3}H-TdR incorporation, (ED_{50} 2.2±0.18 ng/ml, n=6 donors). L-652,731 and CV3988 produced dose-related inhibition of sub-optimal IL-2 induced ^{3}H-TdR incorporation with IC_{50}'s of 11±2.5 and 13.4±4μM respectively (n=3 donors). L-648,611 was also an effective inhibitor (IC_{50} = 15μM). However, BN52021 at 30μM was ineffective at modulating IL-2 induced ^{3}H-TdR incorporation (n=3).

Conversely the PAF agonists produced a dose-related (0.3-300nM) and marked potentiation of sub-optimal IL-2 induced ^{3}H-TdR incorporation, with maximal potentiation ranging from 1.2 to 5.0 fold enhancement of control values (n=4). None of the above drugs in the concentration examined exhibited cytotoxicity as determined by trypan blue exclusion. Furthermore the inhibitory effects of 10μM L-652,731 could be prevented by the prior addition of PAF or PR1501 (0.3-300nM). However the inhibitory effect of 10μM L-648,611 could not be reversed by similar treatment.

In conclusion, PAF can exert a modulatory role in T-lymphoblast proliferation and the PAF receptor present on lymphoblasts is apparently distinct from that on platelets, since BN52021 is inactive in the above studies.

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INVESTIGATION OF THE EFFECTS OF DU 29373 ON THE CARDIOVASCULAR SYSTEM OF THE ANAESTHETISED CAT.

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DU 29373, (+)-N-[2-[4-(2, 3-dihydro-2-hydroxymethyl-1, 4-benzodioxin-5-yl)-1-piperazinyl]ethyl]-4-fluorobenzamide hydrochloride, was found to lower blood pressure and heart rate effectively in several species. On administration via the vertebral arteries in cats, the potency of DU 29373 increased 35 times compared to i.v. which strongly suggests an involvement of the central nervous system (Calis et al., this meeting). Therefore, the effects of DU 29373 on sympathetic outflow (PSNA) have been studied. Further, DU 29373 is studied on carotid sinus nerve activity (CSNA) and in vagotomised cats.

Cats were anaesthetised with α-chloralose (70 mg/kg, i.v.) and pentobarbitone sodium (12 mg) and paralysed with decamethonium (0.25 mg/kg). Simultaneous recording of branchial arterial pressure (BP), heart rate (HR), femoral arterial conductance (FAC) and thoracic preganglionic sympathetic nerve activity (PSNA) were made as previously described (Ramage, 1984). Carotid sinus nerve activity (CSNA) was recorded in separate cats (Ramage, 1986). A cumulative dose response curve (3-300 μg/kg) was produced for DU 29373 with injections given into the jugular vein at 5-15 min intervals; control animals received the equivalent injections of saline.

DU 29373 (n=5) caused a significant ($p<0.05$), dose dependent fall in mean BP which reached a maximum of 53±7 mmHg (mean ±s.e.). At the lowest dose, the decrease in BP was associated with a small but significant increase in PSNA, 10±1% but, as the dose was increased, PSNA declined with respect to vehicle treated animals along with BP. However, this decline in PSNA was not significant. Higher doses induced bradycardia reaching a maximum of 86±7 beats/min. The decrease in BP was associated with no significant change in FAC. Injection of atropine methonitrate (0.1 mg/kg) caused a 77% reversal in the bradycardia caused by DU 29373.

In preliminary experiments (n=2) DU 29373 caused a dose related fall in CSNA along with a fall in BP. (In these experiments the bradycardia was unaffected by MDL 72222 (0.5 mg/kg)). In vagotimised animals (n=3) DU 29373 caused a dose dependent fall in BP and PSNA associated with a dose related increase in HR reaching a maximum of 27 beats/min.

The results indicate that DU 29373 lowers blood pressure and heart rate in intact cats, without causing a reflex rise in central sympathetic tone. The bradycardia is largely vagally mediated. DU 29373 binds selectively to 5-HT_{1A} receptors (pK_i=8.7, Bevan et al., this meeting) and its cardiovascular profile seems to resemble that of 8-OH-DPAT (Fozard and Ramage, 1984), suggesting that central 5-HT_{1A} receptors may play an important role in the cardiovascular effects of DU 29373.

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IDENTIFICATION OF Ca CHANNELS IN MICROVESSELS ISOLATED FROM RAT BRAIN.

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Cerebral microvasculature presents unique morphological, physiological and biochemical properties. It constitutes a barrier between general circulation and the central nervous system. Furthermore, regulation of blood flow through cerebral microvessels plays a fundamental role in maintaining the integrity of cerebral functions. We developed a procedure by which intact and functional microvessels can be isolated from the brain, so that Ca channels in cerebral microvessels can be identified and characterized.

Cerebral microvessels were isolated from the gray matter of rat brain by mild disruption of the tissue and trapping of the vessels on nylon sieve. The preparation typically consisted of multibranching capillaries with segments of small arteries. Enzymatic and microscopic examination allowed to ascertain the purity of the preparations. For instance, compared to the whole cerebrum homogenate, microvessels fraction was enriched in alkaline phosphatase (14:1) and in gama-glutamyltranspeptidase (36:1). A first indication for the existence of functional Ca channels was given by the observation that K-depolarization increased the rate of ^{45}Ca influx and that this increase was blocked by nifedipine (Morel and Godfraind, 1985).

Ca channels were further identified by the specific binding of a 1,4-dihydropyridine Ca entry blocker, $^{3}H(+)PN$ 200 110 (Lee, Roeske and Yamamura, 1984). Saturation studies revealed that microvessels contained a high affinity specific binding site for $^{3}H(+)PN$ 200 110 (K_D: 57$\pm$7 pM - B_{max}: 30-60 fmol/mg protein). $^{3}H(+)PN$ 200 110 specific binding was completely inhibited by other dihydropyridine antagonists and by flunarizine. Verapamil only displaced 40% of the $^{3}H(+)PN$ 200 110 specific binding; d-cis diltiazem enhanced and l-cis diltiazem poorly inhibited the specific binding.

In conclusion, it appears that $^{3}H(+)PN$ 200 110 specific binding in microvessels presents characteristics similar to those described in other tissues.

Nicole Morel is I.R.S.I.A. fellow.

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THE PHARMACOLOGICAL CONSEQUENCES OF MIXING SODIUM NITROPRUSSIDE WITH HYDROXOCOBALAMIN OR THIOSULPHATE .

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Sodium nitroprusside (SNP) is a clinically-used short-acting vasodilator. It is generally claimed that the cyanide ligand of the nitroprusside ion is released in the blood and accounts for the acute toxicity of SNP (Cole, 1978). To retain its pharmacological benefits but avoid cyanide toxicity, a "mixed infusion" technique has been advocated (Schulz, 1984) in which sodium thiosulphate (ST) and SNP are mixed (12:1 molar ratio) prior to injection. Hydroxocobalamin (HOCb) has also been suggested for use in SNP-associated cyanide toxicity (Cottrell et al, 1978), the cyanide combining with the HOCb to form the relatively non-toxic and readily-excreted cyanocobalamin. Since it was conceivable that a "mixed infusion" of HOCb and SNP could be used, and since ^{13}C-n.m.r. spectroscopy has demonstrated an interaction between HOCb and SNP (Butler et al, 1986) we initiated studies to examine the pharmacological consequences of such an interaction. It was also decided to ascertain if there were any consequences of mixing SNP and ST.

Male Sprague-Dawley rats (450-650g) were anaesthetised with urethane (1.5g kg^{-1} i.p.) and cannulae inserted in a carotid artery (measurement of blood pressure or withdrawal of blood) and a jugular vein (injection of drugs). Comparisons were made between SNP and a SNP/HOCb mixture, or SNP and a SNP/ST mixture. For each comparison a high (A) and low (B) dose of SNP were used along with a high (C) and low (D) dose of mixture; the low dose was 25-50% of the high dose. Each dose was given four times in a randomised order (ABCD, ACBD, CABD, BCDA) to allow for any sensitivity changes during the course of the experiment. Maximum BP lowering, "onset" and "offset" times were measured. In separate experiments, ^{14}C-NaNP (5μmol kg^{-1}, 0.3μCi kg^{-1}) was injected into anaesthetised rats either alone or with HOCb or ST. Blood samples (0.4ml) were withdrawn at 1,2,4,6, 10,20,40 min and plasma radioactivity determined by scintillation counting. Means ± s.e. mean are given. Significant differences ($P<0.05$) were determined using Student's t-test.

HOCb added to SNP in a 10:1 molar ratio, caused prolongations in both the onset (30-50%) and offset (20-40%) of the depressor response to maximal doses (600 or 200 nmol) of SNP but, as would be expected, had little effect on the degree of BP lowering achieved. However, a similar pattern was obtained with submaximal doses of SNP (20 and 5 nmol) using a 10:1 molar ratio HOCb:SNP mixture. For example, the higher submaximal dose (SNP alone or mixture respectively) in a typical experiment, gave BP lowering, onset and offset time values of 20 ± 0.3, 18 ± 1 mmHg; 26 ± 2, 50 ± 7 sec; and 71 ± 4, 85 ± 4 sec. HOCb added to SNP (600 or 200 nmol) in a 0.5:1 molar ratio had no effect on the depressor response. Addition of HOCb to the injection solution in a 10:1 HOCb:SNP molar ratio, resulted in an elevation of plasma ^{14}C-SNP-derived radioactivity. During the first 10 min the plasma elimination profiles for the SNP alone and the mixture were roughly parallel, with the concentrations of radioactivity associated with the latter being 2-3 times greater.

ST added to SNP had no effect on the depressor response to SNP using submaximal doses (12 and 6 nmol) of SNP either alone or as an ST:SNP mixture (12:1 molar ratio). This mixing of ST and SNP had a less marked influence on the plasma ^{14}C-SNP-derived radioactivity than occurred with HOCb. The main effect was an elevation of 50-60% for plasma radioactivity at 4,6 and 10 min.

Prolongation of the depressor response associated with mixing HOCb with SNP may be linked with complex formation as 10 and 1:1 molar ratios of HOCb:SNP result in 2 and 1 molecules of HOCb respectively combining with each SNP molecule (Butler et al, 1986).

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THE INFLUENCE OF SODIUM SALICYLATE ON THE RECTAL ABSORPTION OF CEFOXITIN SODIUM ADMINISTERED AS BOLUS OR AS INFUSION TO RATS.

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Cefoxitin sodium is a second generation cephalosporin antibiotic with marked resistance to the action of beta-lactamases. Cefoxitin is given by i.v. or i.m. injection because of its poor absorption after oral and rectal administration. Enhancement of the absorption of cefoxitin could enable the administration of this antibiotic in particular by the rectal route.

Several studies concerning the enhancement of the rectal and intestinal absorption of cefoxitin in animals and humans have been published. As absorption enhancers have been used phenothiazines, sodium salicylate, sodium 5-methoxysalicylate, epinephrine metabolites, concanavaline A, phosphate derivatives, disodium EDTA, Brij 35 and medium chain glyceride. Among these sodium salicylate could be an interesting absorption enhancer which can be used relatively safely in humans because of its extensive use in pharmacotherapy and well known side effects.

We wanted to study the enhancing effect of salicylate on the rectal absorption of cefoxitin in unanesthetized rats with respect to the influence of rate of administration, site of delivery and presence of phosphate buffer.

Solutions with or without phosphate buffer pH 7.4, containing 3 mg cefoxitin sodium with or without 12 mg sodium salicylate, were used for rectal administration in unanesthetized female Wistar rats. Solutions were administered in the rectum at a distance of 2 mm or 1 cm from the anus. The solutions were delivered as a bolus injection or as a linear infusion for 32 min. Arterial blood concentrations of cefoxitin were measured using a RP-HPLC method. Minimal bioavailability was determined by comparing the minimal AUC(-inf) after rectal administration with the AUC(-inf) obtained after i.v. infusion of cefoxitin.

Rectal infusion of cefoxitin resulted in low mean bioavailabilities (17 to 32 %), which could not be enhanced by salicylate. Rectal bolus administration of cefoxitin resulted in low mean bioavailabilities (8 to 17 %), which could be significantly enhanced by salicylate in the presence of phosphate buffer. The absorption after bolus administration with enhancer at a distance of 1 cm from the anus was complete (101 %) and was significantly higher than at a distance of 2 mm (46 %). In general the variances in bioavailability were larger after administration without phosphate buffer.

The absorption enhancing effect of salicylate proved to be dependent of the rate of administration. After rectal infusion absorption enhancement could not be achieved, possibly because of rapid absorption of salicylate or because of development of oedema at the site of delivery. Lower bioavailabilities after bolus administration at 2 mm from the anus could have been a consequence of ligating the infusion device in the rectum. Use of buffered solutions seemed to reduce variability by creating and maintaining in different rats a comparable environment for absorption. Further studies are currently undertaken to elucidate influence of rate and site of delivery on absorption enhancement.

CONSTRUCTING IN VITRO AREA UNDER CURVES FOR TESTING ANTITUMOUR AGENTS WITH THE MEAN RESIDENCE TIME AS STARTING-POINT.

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In vitro sensitivity testing of antitumour agents has attracted much attention during the last two decades. An important problem with any in vitro system remains simulation of in vivo drug behaviour, as characterized by pharmacokinetic parameters. Usually a concentration of 0.1·peak plasma level as determined after systemic administration to patients and a one hour exposure time is chosen. Sometimes 0.03·peak plasma level and continuous exposure is preferred. In several studies exposure times have varied extensively but at large intervals and usually not in relation to in vivo behaviour of antitumour agents.
We studied effects of variation of exposure time (texp) and exposure concentration (cexp) of antitumour agents on cultured human ovarian cancer cells. Colony survival was determined using the human tumour colony forming assay. In Figure 1 a representative colony survival versus in vitro area under the concentration-time curve(AUC) of mitomycin C(MMC) is given for two human ovarian cancer cell lines.

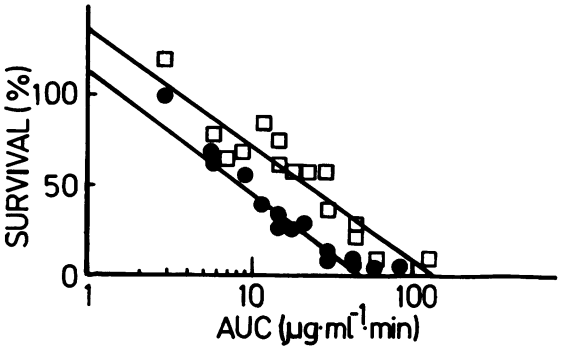

Figure 1. Relationship between colony survival in the human tumour colony forming assay and in vitro AUC of MMC for two different human ovarian cancer cell lines.

The AUC's were constructed by variing the texp between 30 and 180 min and the cexp between 100 and 500 ng/ml simultaneously. The texp range was based on data of mean residence times(MRT) of MMC following different ways of administration to cancer patients(de Bruijn et al., 1986). In this way in vitro AUC's were created which were actually determined in patients. MMC appeared to be stable in culture media during conditions of exposure as determined by high-performance liquid chromatography. From the data it could be concluded that small changes of texp result in clear changes of effects as determined by colony survival. This was confirmed by data of G_2-content of the same cells exposed to MMC as mentioned before and determined by DNA flowcytometry. The MRT can be suggested to be taken as a starting-point for the choise of texp while construction of in vitro AUC's as illustrated above is recommended for in vitro testing of antitumour agents.

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