EFFECT OF BENEXTRAMINE ON THE INHBITORY ACTIONS OF ADRENALINE AND CLONIDINE ON INSULIN SECRETION IN VITRO

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Insulin secretion is inhibited by adrenaline and noradrenaline, an effect involving an α_2-adrenoceptor mechanism (Morgan and Montague, 1985). There is evidence for heterogeneity among α_2-adrenoceptors and Bond et al (1986) showed an inhibitory effect of noradrenaline in the transmurally stimulated guinea pig ileum resistant to blockade by the irreversible α-adrenoceptor blocking drug, benextramine. Benextramine completely prevented the inhibitory effect of clonidine in this preparation. The present work was undertaken to determine if benextramine can block the inhibitory effects of clonidine and adrenaline on insulin secretion. Rat islets of Langerhans were isolated by collagenase and preincubated in batches of 5 for 30 min in Krebs bicarbonate buffer (37 C; pH 7.4; 95% O_2, 5% CO_2; 3 mg/ml bovine serum albumin; 3.0 mmol/l glucose; with or without benextramine or phenoxybenzamine). Islets were then transferred to incubation media containing 8 mmol/l glucose, with or without drugs and incubated for a further 60 min after which 25 ul medium were removed for insulin assay. Glucose (8 mmol/l) stimulated insulin secretion (insulin secretion, ng $islet^{-1}h^{-1}$ in 3 mmol/l glucose, 0.6±0.1; in 8 mmol/l glucose, 6.0±0.5 $p<0.01$). Clonidine (1×10^{-9}M-7.5×10^{-6}M) or adrenaline (2.7×10^{-9}M - 10.8×10^{-6}M) inhibited insulin secretion concentration dependently. Idazoxan (10^{-7}-10^{-5}M) had no effect on insulin secretion itself and reversed the effect of clonidine (0.2 µmol/l) or adrenaline (0.27 µmol/l) in a concentration dependent manner. Phenoxybenzamine (10^{-6}M) also antagonised clonidine or adrenaline without itself stimulating insulin secretion. Benextramine (10^{-5}M) stimulated insulin secretion itself (control islets, 6.0±0.5 ng $islet^{-1}h^{-1}$; benextramine treated islets, 9.6±0.99 ng $islet^{-1}h^{-1}$, $p<0.05$) and completely prevented the clonidine inhibitory effect. (Two way ANOVA, $p<0.01$ for interaction) However, adrenaline was not significantly antagonised by benextramine. (Two way ANOVA, N.S.)

These data do not allow any conclusions concerning the nature of the antagonism of adrenaline or clonidine by the various agents in isolated islets. However,in view of the ability of benextramine to differentiate between clonidine and adrenaline, a more detailed investigation of the nature of the receptors mediating inhibition of insulin secretion by adrenaline and clonidine is merited. Although benextramine stimulated insulin secretion the present findings confirm our previous view that α-adrenoceptor blockade per se does not necessarily result in enhanced insulin release in vitro (Furman and McMillan 1985).

Maureen Smith is in receipt of an S.E.R.C. studentship.

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FLUORIDE-STIMULATED PHOSPHOINOSITIDE TURNOVER IN HUMAN PLATELETS: DRUG INTERACTIONS

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In several cell types, including the human platelet, sodium fluoride (F) produces mobilisation of intracellular calcium (Poll et al., 1986). We have recently shown that F is also associated with a concentration-dependent increase in phosphoinositide (PI) turnover, which is consistent with current concepts of linkage between these processes. In this study we noted that cyclandelate, a vasodilator and inhibitor of platelet aggregation (Roba et al., 1976), markedly inhibited F-stimulated PI turnover. We have therefore examined a number of other agents in this model system, since the influence of drugs on PI metabolism has not been extensively studied. Three types of compound were used: (i) vasodilators used as hypotensives (diazoxide, hydralazine, minoxidil), (ii) other vasodilators (menadione, nicotinic acid, suloctidil), (iii) other smooth muscle relaxants (menthol, papaverine). Some of these agents affect intracellular cyclic nucleotide levels, but none is thought to interact with a specific neurotransmitter or hormone receptor.

Venous blood from human volunteers was anticoagulated with trisodium citrate and centrifuged (150g, 15 min) to obtain platelet-rich plasma, which was centrifuged at 600g, 10 min. The platelet pellet was resuspended in Ca^{2+}-free Hepes-Tyrode buffer containing 75-100 μCi ^{3}H-inositol (15-20 Ci/mmol). The suspension was incubated for 150 min at 37°, followed by pelleting of the platelets and resuspension in buffer containing 10mM Li Cl. After 30 min incubation this suspension was used in 400 μl aliquots. F concentration was 18mM, and other drugs were added in volumes of 5 μl (total incubation volume 500 μl). Incubation was stopped after 60 min with chloroform/methanol (1:2 v/v) and inositol-1-phosphate (I-1-P) extracted by Dowex-formate resin chromatography, as an index of PI turnover (Berridge et al., 1982).

F (18mM) increased I-1-P levels by 190% compared to control. Among the drugs tested, diazoxide, hydralazine, minoxidil and menthol had no detectable effect on F-stimulated PI turnover, at concentrations up to 100 μM. Menadione (100 μM) inhibited increased PI turnover by 30%, suloctidil (10 μM) by 82% and papaverine (100 μM) by 23%. Nicotinic acid (100 μM) caused a small (25%) but consistent enhancement of PI turnover.

Suloctidil is known to have anti-aggregant activity, though this is greater ex vivo than in vitro (Roba et al., 1976). Vitamin K analogues such as menadione, have also been shown to inhibit platelet function. The effect of nicotinic acid may be related to inhibition of adenyl cyclase, as described in other tissues (Ankories et al., 1980) since the P site inhibitor 2'5'-dideoxyadenosine (100 μM) also increases F-stimulated PI turnover (80%). To develop this study, it would be of interest to examine the effect of these agents, particularly those inactive in the platelet on PI turnover in gut and vascular smooth muscle. The use of F in such experiments may be a useful approach to studying the regulation of PI turnover in different tissues and species, independent of the use of receptor-specific agonists.

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174P

PAF-ACETHER-EVOKED HYPOTENSION IS ENHANCED BY BLOCKADE OF VASOPRESSIN AND RENIN-ANGIOTENSIN SYSTEMS, IN RATS

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The platelet activating factor (PAF) is a phospholipid mediating numerous cardiovascular effects (e.g. hypotension, platelet aggregation, increase in vascular permeability, etc.) through stimulation of specific receptors. Additionally, PAF participates in the functional manifestations of endotoxin and septic shocks (Braquet et al. 1986). Inasmuch as endotoxin was reported to elevate the blood levels of vasopressin in rats (Schaller et al., 1985), this investigation was firstly aimed to assess whether the hypotension evoked by PAF was enhanced by a V-1 vasopressin receptor antagonist (SK&F 100273), and secondly to attempt to determine the possible mechanisms responsible for this effect.

Male Sprague Dawley rats (250-300 g) were anesthetized with pentobarbitone (55 mg/kg, i.p.), and prepared for the measurement of carotid blood pressure and i.v. administrations. PAF-acether (0.035 µg/kg/min i.v.) was infused for 10 min to intact rats, which had been pretreated 10 min earlier with i.v. saline (0.4 ml/kg), SK&F 100273 (15 ug/kg) or enalapril (1 mg/kg). The former two experimental procedures were also performed in rats which underwent bilateral nephrectomy 16-24 hours earlier, or in rats in which the main cardiovascular reflexogenic mechanisms had been abolished by section of vagi and ligation of carotid arteries.

The chosen dose of PAF-acether produced a maximal fall in mean carotid artery blood pressure of 15 ± 3 mmHg from an initial value of 130 ± 5 mmHg (n=6), in intact rats. This effect was not significantly modified by enalapril (8 ± 1 from 115 ± 3 mmHg, n=5), SK&F 100273 (10 ± 4 from 116 ± 5 mmHg, n=6) or bilateral nephrectomy (14 ± 2 from 125 ± 6 mmHg, n=5). However, after SK&F 100273 pretreatment, the latter preparation responded to PAF-acether with a significantly greater hypotension (29 ± 2 from 110 ± 6 mmHg, n=6). Similarly an enhancement of the PAF-induced fall in blood pressure (37 ± 5 from 111 ± 5 mmHg, n=6) was also found in intact rats pretreated with enalapril plus SK&F 100273. In intact or nephrectomized rats in which the vagi had been severed and the carotid arteries ligated, the decrease in blood pressure produced by PAF-acether became 29 ± 3 mmHg (from 139 ± 5, n=5) and 23 ± 2 mmHg (from 150 ± 5, n=7), respectively. Furthermore, when these preparations had been pretreated with SK&F 100273 the hypotensive effects evoked by PAF-acether were 27 ± 6 mmHg (from 124 ± 5, n=5) and 33 ± 2 mmHg (from 120 ± 3, n=10).

These results suggest that the PAF-evoked hypotension induces liberation of vasopressor substances, which appear to be angiotensin II (derived from renal renin) in intact rats and vasopressin in nephrectomized or enalapril-pretreated intact animals. A mechanism of these humoral effects appears to be of a reflexogenic nature. Furthermore, in nephrectomised rats with carotid ligation and the vagi severed, in which vasopressin contributes substantially to maintain base-line blood pressure, PAF-induced hypotension is functionally reduced by vasopressin release through a mechanism to be determined. Finally, the activation ot the renin-angiotensin system by the fall in blood pressure seems to exert a negative feedback control on the vasopressin system.

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SOME PROPERTIES OF AMINE OXIDASES IN HUMAN UMBILICAL ARTERY

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Blood vessels of various species including man are known to contain a semicarbazide-sensitive amine oxidase (SSAO), associated with smooth muscle, and with inhibitor and substrate specificities distinct from those of both the A and B forms of monoamine oxidase (MAO) (Lewinsohn, 1984; Lyles & Singh, 1985). We here describe some properties of these enzymes investigated in the human umbilical artery.

Arteries, dissected from fresh umbilical cords obtained post partum, were stored at -20°C for use within 2 wks. They were homogenized in 1mM potassium phosphate pH 7.8 and used in radiochemical assays for amine oxidase enzymes with [^{3}H]-5-hydroxytryptamine (5-HT), [^{3}H]-tyramine (TYR), [^{14}C]-benzylamine (BZ) or [^{14}C]-2-phenylethylamine (PEA) as substrates. All data below is the mean (± s.e. when shown) of 4-6 homogenates, each from different arteries.

Inhibitor studies: Homogenate aliquots were preincubated with various concentrations of clorgyline (CL) or semicarbazide (SC) before addition of radiolabelled substrate. Inhibition curves were obtained by plotting deaminating activities (as % of uninhibited controls) vs. inhibitor concentration. CL was used to inhibit MAO-A (below 10^{-6}M CL) and MAO-B (between 10^{-6} and 10^{-3}M CL). SSAO activity was defined by its resistance to 10^{-3}M CL, or its sensitivity to inhibition by 10^{-3}M SC. On this basis, 5-HT (1mM) was a substrate for MAO-A alone in this tissue. With BZ (1mM), inhibition by 10^{-3}M CL (13%) and 10^{-3}M SC (89%) indicated a small and a very large contribution by MAO-B and SSAO, respectively, towards BZ metabolism. For TYR (1mM) and PEA (100µM), respectively, the % contributions of MAO-A: MAO-B: SSAO were 63: 22: 15 and 33: 45: 22 from the appropriate CL plots. 10^{-3}M SC also inhibited TYR and PEA metabolism by 27 and 56%, respectively.

Kinetic constants: These were estimated by linear regression analysis of appropriate kinetic plots. Values for SSAO were obtained after preincubation of homogenates with 10^{-3}M CL to inhibit MAO activities.

Substrate/Enzyme	Km (at pH 7.8)	Vmax (nmol/h/mg protein)
5-HT/MAO-A	151 ± 23µM	36 ± 10
BZ/SSAO	172 ± 33µM	269 ± 41
TYR/SSAO	3.34 ± 1.49mM	13.3 ± 6.4
PEA/SSAO	14.9 ± 3.6mM	204 ± 47

Our demonstration of MAO-A and SSAO in umbilical artery is consistent with earlier work (Lewinsohn & Sandler, 1982) although we have found MAO-B to be present also. The very high Km values for TYR and PEA, compared with BZ, as SSAO substrates is in agreement with previous conclusions that these amines are relatively poor substrates for the enzyme in human blood vessels (Hayes et al, 1983; Suzuki & Matsumoto, 1984) but contrast with the rat enzyme, whose Km values towards these three substrates, are considerably lower (e.g. Andree & Clarke, 1982).

We thank the Scottish Home and Health Department for support.

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BALSALAZIDE DOES NOT SHARE SULPHASALAZINE-INDUCED INFERTILITY IN MALE RATS

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Sulphasalazine (SASP), a prodrug which releases 5-aminosalicylic acid (5-ASA) and Sulphapyridine (SP) in the colon, is used to treat inflammatory bowel disease. 5-ASA is the active moiety whereas SP is responsible for infertility in men and rats (Smethurst et al., 1984). Balsalazide (BSZ) is also a prodrug in which SP has been replaced by 4-amino-β-benzoylalanine (Chan et al., 1983). We have investigated the effect of SASP and BSZ on male fertility and plasma hormone profile in rats.

Groups of 10 male Biorex Wistar Rats (400-600g), of proven fertility, received SASP or BSZ via the diet for 7 weeks. Then 4 female rats were introduced nocturnally to each male for 5 days. Vaginal spermatozoa confirmed positive mating. 14 to 18 days after mating, the dams were killed. The number of foetuses and corpora lutea was recorded. Male fertility index (foetuses x 100/corpus luteum), pregnancy rate and live foetus ratio were calculated. After the mating period plasma hormones were assayed from each male rat. At necropsy testes, epididymides, seminal vesicles and ventral prostate were dissected and weighed. Testes were examined histologically for Sertoli-Gonial cell ratio, patterns of tubular depopulation, spermatogenesis and degeneration (or exfoliation).

Table 1 Effects of SASP and BSZ on fertility and plasma hormones of male rats.

Group & daily dose level (mg/kg)		Pregnancy rate (%)	Live foetus per dam (Mean±SEM)	Fertility index (Mean±SEM)	Plasma hormones Mean ± SEM (ng/ml)		
					LH	FSH	Testosterone
Control	0	46	12.0±0.8	96±4	3.3±0.2	8.7±0.4	0.9±0.2
SASP	500	22	4.5±0.8*	34±7*	3.6±0.3	8.5±0.3	1.0±0.3
BSZ	500	34	9.8±1.3	80±10	3.8±0.2	8.2±0.4	0.6±0.1

Results with BSZ were not significantly different from controls, whereas SASP caused a reduction in fertility index and live foetuses per dam (*$p<0.001$). There were no significant differences between the control and treated groups in sex organ weights, testicular function or plasma hormones. These studies confirm those in man that BSZ has no effect on male fertility (McIntyre & Lennard-Jones, 1984). BSZ has shown similar efficacy to SASP in patients with ulcerative colitis and is therefore an important new alternative to SASP (McIntyre et al 1986).

We thank Dr. C.A. Wilson and Dr. R.S. Vine for the hormone radioimmunoassays and testicular histological evaluation respectively.

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MOUSE PLASMA HAPTOGLOBIN: A SENSITIVE PARAMETER FOR THE MEASUREMENT OF NSAID AND NON-NSAID-INDUCED GASTROINTESTINAL TOXICITY.

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We previously demonstrated (Billingham et al 1985) that haptoglobin (HAPT) was a sensitive marker of indomethacin (IND) - induced gastrointestinal tract (GIT) toxicity, and was sensitive to the protective action of sodium salicylate (SAL).

These studies have now been extended to evaluate the protective effects of SAL in more detail. In addition, the known cytoprotective agent 16,16-dimethyl prostaglandin E_2 (DiMPG) (Robert et al 1985) has been investigated along with other GIT damaging agents - hydrochloric acid (HCl) and ethanol (EtOH). Experiments were performed on Alderley Park/CBU female mice fasted for 24 hrs. IND 8 $mgkg^{-1}$ p.o. was used throughout. Increasing doses of SAL (50, 75, 100, 150,and 200 $mgkg^{-1}$ p.o.) were administered 30 min prior to IND to establish a threshold protective dose. SAL 200 $mgkg^{-1}$ p.o. was compared with 200 $mgkg^{-1}$ s.c. 30 min prior to IND to assess the effect of route of administration. To assess the effects of non-NSAID GIT toxicity, EtOH 75% v/v p.o. or HCl 600 mM p.o. were used. SAL 200 $mgkg^{-1}$ p.o. was administered 30 min prior to these agents for assessment of protective activity. Also, varying doses of DiMPG (0.1, 10, 100, 300 and 500 μgkg^{-1} p.o.) were administered 15 min prior to IND. The onset of DiMPG protection was assessed by administering 100 μgkg^{-1} p.o. 15, 30, 60, 90 and 120 mins prior to IND. HAPT was measured post 24 hrs by gel immunodiffusion (Mancini et al 1965) and results were expressed as the mean square of diffusion-ring diameter or as a percentage of IND-induced maximal elevation. Statistical assessment was made using the Student's t-test.

IND produced a significant ($p<0.001$) increase in HAPT levels (314 $\pm$ 20 cf vehicle 34 $\pm$ 6 both n = 50). Prior oral administration of SAL 50-200 $mgkg^{-1}$ caused dose-related reductions ($p<0.05$ and $p<0.001$) in IND-elevated HAPT levels (13% and 45%). When administered s.c. SAL 200 $mgkg^{-1}$, caused no significant change in IND-elevated levels (20% decrease, $p>0.05$ n.s.).

EtOH and HCl produced modest increases in HAPT levels (123 $\pm$ 22 and 126 $\pm$ 16 respectively). Interestingly, prior administration of SAL 200 $mgkg^{-1}$ p.o. caused little change in EtOH-induced levels (154 $\pm$ 24) but caused a marked elevation in HCl-induced levels (239 $\pm$ 17, $p<0.01$).

Prior oral administration of DiMPG 100-500μgkg^{-1} lowered IND-elevated HAPT levels between 23%-62% respectively ($p<0.01$ and $p<0.001$). Lower doses (0.1 and 10μgkg^{-1}) were without effect. Moreover, it was found that 100μgkg^{-1} DiMPG administered 2Hrs prior to IND still produced a lowering of HAPT levels by 35% ($p<0.001$).

These data support the known protective effects of SAL and DiMPG, but indicate different mechanisms may be involved in the generation of NSAID and non-NSAID GIT toxicity. We suggest this model has utility in the evaluation the GIT toxicity of novel agents which interfere with arachidonic-acid metabolism.

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THE EFFECT OF DIGOXIN SPECIFIC FAB FRAGMENTS ON DIGOXIN ELIMINATION IN THE RAT.

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"Digoxin-specific"-Fab fragments (DS-Fab) can be used to treat acute overdosage associated with both digoxin and digitoxin (Wenger et al, 1985; Baud et al, 1983). These antibody fragments attract the drug away from the cardiac receptors and sequester it in the interstitial fluid and plasma. In man, digitoxin, unlike digoxin, undergoes major elimination via the bile and it has been suggested (Ochs and Smith, 1977) that DS-Fab treatment diverts digitoxin elimination away from slow hepatic disposal, towards rapid urinary excretion. Since, in the rat, 40-50% of a dose of digoxin undergoes biliary elimination, we have used a rat/digoxin model to ascertain if DS-Fab have any "diversionary" influence on cardiac glycoside elimination.

In the first experiment, 5 pairs of female Sprague-Dawley rats (200-250g) were anaesthetized with pentobarbitone (60 $mgkg^{-1}$ i.p.), cannulated at the carotid artery, jugular vein and bile duct and given ^{3}H-digoxin (10 μgkg^{-1}, 12.5 $\mu Cikg^{-1}$ i.v.). Bile and blood samples were collected over a 2h period. After 1h, experimental animals were given a dose of DS-Fab (affinity constant $10^{-9}M$) equimolar to that of the digoxin, while control animals received an equal volume of non-immune sheep serum. At 2h the animals were killed, urine produced collected, and tissue samples (brain, heart, lung, liver, kidney, adrenal; ileum, caecum, large intestine, and contents) taken. The second experiment was similar except that DS-Fab were given 2 min after the digoxin. Radioactivity present in biological samples was determined by liquid scintillation counting. ^{3}H-digoxin metabolites in bile and urine were separated thin-layer chromatographically according to Carvalhas and Figueira (1973). Results are means ± s.e. mean. Data were analysed using a non-paired Student's t-test, with a probability of $P<0.05$ being taken as significant.

DS-Fab given at 1h produced a rapid 12-fold increase in plasma digoxin concentration (19.7 ± 5.3 vs 1.6 ± 0.5 $ngml^{-1}$) and concentrations were still elevated at 2h (7.8 ± 0.7 vs 1.1 ± 0.3 $ngml^{-1}$). Cumulative biliary digoxin excretion appeared slightly reduced (7% reduction after 2h) by DS-Fab treatment, although this effect was not statistically significant. There was also no difference in the percentage of digoxin dose directly secreted into the intestines or excreted in the urine. However, the percentage remaining in the kidney was higher for DS-Fab treated rats (5.4 ± 2.0 vs 0.26 ± 0.03).

DS-Fab given at 2 min elevated already high plasma digoxin concentrations 7-fold (77.3 ± 7.6 vs 10.3 ± 1.0 $ngml^{-1}$) and at 2h, they were still three times higher than for controls. Cumulative biliary excretion again appeared reduced (14% reduction at 2h) but this was not significant. As before the amount of drug passing into the intestines was unaffected. However, the percentage of the digoxin excreted in the urine was elevated 3-fold (16.3 ± 1.5 vs 5.0 ± 0.8), whilst that remaining in the kidney was increased 16-fold.

Apart from in the kidney, DS-Fab treatment did not result in any clear differences in tissue digoxin concentration at the end of the experiments. Biliary and urinary radioactivity comprised 80-90% digoxin, this proportion being unaffected by DS-Fab. Although increased renal elimination did not significantly decrease biliary digoxin excretion, the results do suggest that some diversion of digoxin elimination by DS-Fab treatment occurred. However, in the rat model used, the enhanced urinary excretion appeared to have the major influence on the total amount of digoxin eliminated (urinary, biliary excretion and direct intestinal secretion) which was 63 and 53% of the dose for experimental (DS-Fab given at 2 min) and control rats respectively.

The authors wish to thank Mrs M. Andrews for preparing the DS-Fab.

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EFFECTS OF A NOVEL PERFLUOROCARBON EMULSION IN RATS

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We have previously reported the development and preliminary physico-chemical assessment of novel compositions of emulsified perfluorocarbons (PFC) for possible biological uses related to oxygen transport (Davis et al., 1986). The new emulsions were based on perfluorodecalin (FDC) and contained small quantities of perfluorinated high boiling point oils to stabilize against instability caused by Ostwald Ripening. Since previous studies have shown that emulsified PFC are retained in reticuloendothelial and other tissues (Lowe & Bollands, 1985), we now report the effects of a novel FDC formulation on lymphoid tissue and haemagglutination responses to injected sheep red blood cells (SRBC) in rats.

FDC (ISC Chemicals Ltd, Avonmouth) was emulsified by sonication with 4% Pluronic F-68 in an isotonic aqueous phase to give a final 20% preparation. The emulsion contained 1% of a C-16 oil, perfluoroperhydrofluoranthrene, to enhance stability (Davis et al., 1986). Female Wistar rats (body weight (b.w.): 140-160g; n = 8) were given either an intraperitoneal (i.p.) or intravenous (i.v.) injection via a tail vein of 10 $ml.kg^{-1}$ b.w. of this emulsion. Groups of rats were also injected i.p. (n = 5) or i.v. (n = 5) with identical doses of Fluosol-DA 20% (F-DA; Green Cross, Japan); control animals (n = 19) received sterile saline (0.9% NaCl). 24h later, all animals were injected i.p. with 5 x 10^8 double-washed SRBC in 1.0 ml Hank's solution. Blood samples were collected by retro-orbital bleeding at 3 and 5 days later. On day 7, animals were sacrificed and weights of liver, spleen, thymus and mesenteric lymph nodes (MLN) measured. Plasma antibody titres to SRBC were measured by a serial-dilution haemagglutination assay.

Mean liver weight increased by 12-15% ($P<0.001$) following i.p. or i.v. injection of FDC emulsion; in contrast, liver weight was unchanged at 7 days after injection of F-DA. Spleen weight also increased to a maximum of 20% ($P<0.01$) after i.p. injection of novel emulsion but this was less than increases of up to 44% ($P<0.001$) which occurred in F-DA-injected rats. Mean thymus weight showed a small decrease following i.p. injection of FDC emulsion ($P<0.05$) but was similar to control (0.27 ± 0.01% b.w.) in all other cases. MLN weight after injection of FDC emulsion was not significantly different from control (0.08 ± 0.01% b.w.) but increased ($P<0.01$) in response to i.v. injection of F-DA. Mean (± S.E.) $\log_2$ antibody titres to SRBC showed a progressive rise up to day 5 in all animals; titres on day 7 following i.v. injection of either FDC emulsion or F-DA were similar to control (6.0 ± 0.4). However, mean day 7 titres were increased in rats injected i.p. with emulsified FDC (8.9 ± 0.3) or F-DA (7.8 ± 0.4).

These results show that injection of an FDC emulsion containing 1% C-16 oil additive can produce increases in liver and, to a lesser extent, spleen weights in rats. The variation in lymphoid tissue responses to the FDC emulsion and F-DA probably reflects the differences in their composition, since the latter contains 6% (w/v) perfluorotripropylamine (Naito & Yokoyama, 1978). The increase in antibody titre to SRBC following i.p. injection of FDC emulsion supported previous results of similar immunopotentiating effects produced by F-DA in rats and mice (Bollands & Lowe, 1986). However, the mechanism(s) by which emulsified PFC can alter immune system function have not been determined.

This work was supported by I.S.C. Chemicals Ltd. A.D.B. is an M.R.C. Scholar.

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LYMPHIOD TISSUE RESPONSES TO FLUOSOL-DA IN RATS: TIME COURSE EFFECTS RELATIVE TO IMMUNOLOGICAL CHALLENGE

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Perfluorochemical (PFC) emulsion particles can accumulate in cells of the reticuloendothelial system (RES): the extent of this depends upon composition and dose of emulsion administered together with species used and tissue concerned (Bollands & Lowe, 1986a,b,c). In the present study, we have investigated the effects of injection of low doses of the proprietary PFC emulsion, Fluosol-DA 20% (F-DA; Green Cross, Japan), on lymphoid tissue in rats. An objective was to examine the effects of timing and route of administration of F-DA relative to an immunological challenge in the form of sheep red blood cells (SRBC).

Female Wistar rats (body wts: 149 - 155g; n = 62) were used. They were immunized with 5 x 10^8 double-washed SRBC suspended in 1.0 ml Hank's solution and injected either intravenously (i.v.) or intraperitoneally (i.p.); the day of immunization = day 0. Groups of animals also received a single 10 ml. kg^{-1} injection of F-DA via the same route either on day -7, -4, -1, +1, +4 or simultaneously at immunization; controls received identical doses of sterile saline (0.9% w/v NaCl). Animals were sacrificed on day +7 and weights of liver (LIV), spleen (SPL), thymus (THY) and gut mesenteric lymph nodes (MLN) recorded. Plasma antibody (Ab) titre to SRBC was measured by serial-dilution haemagglutination assay.

Mean spleen weight increased up to 80% and liver weight up to 22% in response to i.v.-injected F-DA; comparable organ weight changes also occurred in some groups of animals injected i.v. with emulsion. Mean thymus weight was reduced to a maximum of 21% when F-DA was injected i.v. on days -7, 0 and +1 and i.p. on day 0; MLN weights were unchanged throughout. Mean (± S.E.) Ab titres were increased in most groups of animals receiving i.v. and i.p. F-DA and reached a maximum value of 9.9 ± 0.7 ($P<0.001$) when the emulsion was injected i.v. at the time of immunization. Changes in mean organ weights and Ab titres with time of F-DA injection relative to SRBC are given below:

	A. I.P. SRBC and I.P. F-DA					B. I.V. SRBC and I.V. F-DA				
Time	LIV	SPL	THY	MLN	Log_2 Ab TITRE	LIV	SPL	THY	MLN	Log_2 Ab TITRE
-7	↑	↑	Unc	Unc	Unc	↑	↑	↓	Unc	Unc
-4	↑	↑	Unc	Unc	↑	↑	↑	Unc	Unc	Unc
-1	Unc	Unc	Unc	Unc	↑	↑	↑	Unc	Unc	↑
0	↑	↑	↓	Unc	↑	↑	↑	↓	Unc	↑
+1	↑	↑	Unc	Unc	Unc	↑	↑	↓	Unc	Unc
+4	Unc	↑	Unc	Unc	↑	Unc	↑	Unc	Unc	↑

↑=increased ($P<0.05$), ↓=decreased ($P<0.05$), Unc.=unchanged compared to controls.

These results show that changes in lymphoid tissue weights and plasma Ab titres in rats immunized with SRBC vary according to the time of a previous or subsequent injection of F-DA via the same route. The increases in spleen and liver weights together with enhancement of Ab production against SRBC were similar to previous findings in rats immunized 24h after injection of F-DA (Bollands & Lowe, 1986a,b). The decreases in thymus weights suggest cytotoxicity or cellular depletion caused by F-DA or its components but this remains to be clarified.

A.D.B. is the recipient of an M.R.C. Research Studentship.

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A PRELIMINARY STUDY OF MILOXICAM PHARMACODYNAMICS AND PHARMACOKINETICS IN THE HORSE

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Miloxicam, a new enolic acid non-steroidal anti-inflammatory drug (NSAID), was administered i.v. to six New Forest ponies at a dosage rate of 0.6 mg/kg in a two-part cross-over study, in each part of which three horses received miloxicam and three were given a placebo preparation. The actions of miloxicam, compared to placebo, were assessed in a carrageenan-sponge model of acute inflammation (Higgins et al., 1984). Concentrations of protein and the enzymes, lactate dehydrogenase (LDH), acid phosphatase (AP) and lysozyme and leucocyte numbers in inflammatory exudates harvested at 4, 8, 12 and 24h were not significantly different in drug and placebo treated animals. The effect of miloxicam on rise in skin temperature at the site of the acute inflammation was also non-significant. However, skin temperature rise and concentrations of protein and LDH in exudate,and exudate leucocyte numbers were significantly reduced ($p<0.05$) in drug-treated horses when data for all sampling times were pooled (n=24). Exudate concentrations of the eicosanoids, 6-keto-$PGF_{1\alpha}$ and TXB_2, were significantly reduced by miloxicam at most sampling times, and the formation of serum TXB_2 was markedly reduced at 4 and 8h but not at 12 and 24h after drug administration, indicating that the action of miloxicam was readily reversible. These pharmacodynamic findings correlate with the pharmacokinetic properties of miloxicam; the plasma concentration-time curve was defined by a three compartment open model in one horse and by a two-compartment open model in five horses. Mean values $\pm$ s.e.mean of pharmacokinetic parameters for the five ponies were C_p^o=9.23 $\pm$ 1.00 ug/ml; $t_{\frac{1}{2}\alpha}$ = 0.40$\pm$0.20h; $t_{\frac{1}{2}\beta}$ = 2.70$\pm$0.44h; Vdarea = 0.16$\pm$0.02 1/kg; Cl_B = 41.8$\pm$2.8 ml/kg/h; AUC_o^{24} = 14.5$\pm$0.8 ug/ml.h.

Table 1. Plasma and exudate concentrations and exudate:plasma concentration ratio of miloxicam (mean $\pm$ s.e.mean for six animals unless stated).

Time (h)	Plasma concentration (ng/ml)	Exudate concentration (ng/ml)	Exudate:plasma ratio
4	896 $\pm$ 117 (n=5)	900 $\pm$ 136	1.04 $\pm$ 0.36(n=5)
8	393 $\pm$ 43	517 $\pm$ 58	1.36 $\pm$ 0.16
12	107 $\pm$ 58	347 $\pm$ 78	2.96 $\pm$ 0.96
24	0	48 $\pm$ 30	-

Exudate concentrations of miloxicam were initially similar to and subsequently greater than concentrations in plasma and this may explain the more prolonged inhibition of eicosanoid synthesis in exudate than in serum. These findings indicate that pharmacodynamic and pharmacokinetic studies in a single investigation in the target species may be useful in selecting dose rate and dosing intervals for NSAID. From the present study it is concluded that doses of miloxicam greater than 0.6 mg/kg should be evaluated experimentally before clinical trials in horses are undertaken.

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PARACETAMOL AS A CAUSE OF DIETARY DEFICIENCY OF SULPHUR AMINO ACIDS

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Paracetamol is metabolised to the glucuronide and sulphate conjugates and also to the glutathione (GSH)/mercapturic acid compounds. In two of these three pathways the sulphur amino acids, methionine and cysteine are utilised, either as a source of active sulphate (PAPS) or for synthesis of GSH. About half of a paracetamol dose ends as S compounds in the urine.

We have observed that male rats of 100 g initial body weight, when fed 1% or 1.5% paracetamol in a stock diet (SDS diet) show an immediate and marked depression of growth. This growth depression is accompanied by a fall in glutathione levels and initially by no evidence of liver cell necrosis (No rise in serum enzymes, no histological evidence of damage). Growth is returned to near normal by addition of 0.5% methionine to the diet as are the glutathione levels in the liver.

Table 1 Effect of 1% paracetamol and methionine in the diet in growth and liver glutathione levels in the young rat.

Diet	C	M	P	P+M
Weight gain g/day	8.5	8.6	2.0	6.0
Glutathione µmols/g liver	6.8	-	3.6	7.7

Rats were fed diets containing 1% paracetamol (P) and 0.5% methionine (M) mixed with SDS powder diet for 8 days.

After a week or two of 1% paracetamol feeding, scattered patches of liver cell injury and necrosis appear in most of the animals but the rats recover and eventually the rats grow slowly to a normal size. We calculate that SDS diet mixture provides a 100 g rat with about 500 µmols S-amino acid/day and 700 µmols paracetamol/day and that metabolism of paracetamol would take up over half of the sulphur amino acid content of standard diets in these circumstances.

The inhibition of growth and depression of GSH levels is not affected by giving phenobarbital (0.1%) with drinking water and is probably due to diversion of S-amino acids from normal pathways such as protein synthesis into paracetamol metabolism, and not to liver injury, at least in the first 10 days.

The onset of patches of liver necrosis is presumably associated with an S-amino acid deficiency and low GSH levels as the amount of paracetamol taken by the rats would not cause liver injury in single dosage and plasma levels of paracetamol are far below those normally found associated with liver injury in rats.

The human diet in the UK contains about 19 mmols of S-amino acids/day and the usual dose of paracetamol is 6.6 mmols (1 g - two tablets) paracetamol. Doses which do not regularly exceed the recommended daily dose (4 g) should not cause problems of S depletion in adults.

The observation of liver tumours in mice fed 1% paracetamol in an unspecified diet has caused some anxiety, but it may well be that these tumours are related to GSH depletion and episodes of liver necrosis.

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Ca^{2+} MODULATORS AS ANTIDOTES TO IMIPRAMINE LETHAL TOXICITY IN THE RAT

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The antagonistic effects of a Ca^{2+} channel entry modulator, nitrendipine, on the functional and morphological toxicity of cocaine intoxication in the rat have been reported. In the present study, the effects of another Ca^{2+} entry modulator, flunarizine, was tested on the toxicity of imipramine, a compound which inhibits synaptic reuptake of noradrenaline, dopamine and of serotonin. Imipramine, the first tricyclic antidepressant, has been used for more than 25 years but there are no specific medication to treat life threatening overdose of this and other tricyclic compounds. Flunarizine was selected among the presently available Ca^{2+} channel modulators because of its antagonistic properties on catecholamines and serotonin. It also exerts a specific action on the cerebral vascular bed and has antihistaminic effects.

Eighteen fasting Sprague Dawley rats weighting 305 ± 21 g are fitted, under ether anesthesia, with a caudal artery catheter connected to a constant micro-infusion pump and to a recorder for on-line recording of arterial blood pressure which is analysed and processed by a microcomputer. Measurements of heart rate and pulse pressure are displayed every 30 seconds. When the animal has awakened, it is placed in a restraining grid.

In a first series of experiments, 10 rats are administered intraperitoneally 100 mg/kg of imipramine; survival time of 5 controls given 10 µl/min saline is 14.6 ± 5.5 min. Five test animals administered flunarizine at the rate of 0.1 mg/kg/min survive 129 ± 89 min ($p < 0.04$). In a second series, 8 rats are administered intraperitoneally 85 mg/kg imipramine. After blood pressure has fallen to 50 mmHg, 4 controls are treated with successive bolus of 0.3 ml of saline; their survival time is 17.0 ± 6 min. Four other rats are treated with successive bolus of saline and 373 µg/kg flunarizine. Total dose of flunarizine given is 2.37 ± 1.21 mg/kg and saline volume 4.9 ± 0.72 ml. All these animals survive and are awake and active 24 hours later.

Table 1

Number of rats	Amounts of I.P. imipramine	Fluid volume administered	Amount of flunarizine (intraarterial)	Survival time (min)
5	100 mg/kg	10 µl/min	none	14.6 ± 5.5
5	100 mg/kg	10 µl/min	0.1 mg/kg/min	129 ± 89 [a]
4	85 mg/kg	1.8 ± 0.3 ml	none	17.0 ± 6.0
4	85 mg/kg	4.92 ± 0.72ml[b]	2.37 ± 1.21 mg/kg[b]	> 1440 [c]

[a]= $p < 0.04$
[b]= In fractionated amounts during the period of treatment (187 ± 86 min).
[c]= Animals conscious and active.

An other Ca^{2+} modulator, nimodipine, also displayed similar protective action againstimipramine toxicity.

These Ca^{2+} modulators might be considered in the treatment of acute poisoning by imipramine and related tricyclic compounds.

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NITRENDIPINE : AN ANTIDOTE TO THE LETHAL TOXICITY OF COCAINE

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In a previous study, it was reported that a Ca^{2+} modulator, nitrendipine, exerted an antagonistic effect on the functionnal and morphological effects of cocaine cardiovascular toxicity. Cocaine is a potent topical vasoconstrictor which inhibits the reuptake of noradrenaline and may be considered as an "indirectly acting sympathomimetic amine". Self-administration of cocaine is associated with dysrhythmia including ventricular fibrillation and myocardial infarction in healthy young adults and also with stroke and intra-cranial hemorrhage. The selection of Ca^{2+} modulators as antidotes to the sympathomimetic effects of cocaine was predicated on their know properties to inhibit the vasoconstrictive effects of norepinephrine. Nitrendipine was selected because its coronary vasodilatator effect in the presence of maintained contractile force of the myocardium. The purpose of thise study was to test nitrendipine as an antidote to a single lethal dose of cocaine.

Ten fasting Sprague Dawley rats weighting 292 ± 31 g are fitted, under ether anesthesia, with catheter in the caudal artery. The catheter is connected to a constant micro-infusion pump and to a recorder for on-line recording of arterial blood pressure which is analysed and processed by a microcomputer. Measurements of heart rate and pulse pressure are displayed every 30 seconds. When the animal has awakened, it is placed in a restraining grid.

Five rats are administered 60 mg/kg of cocaine solution, intraperitoneally, a lethal dose. Five other animals are given the same amount of cocaire, followed by nitrendipine 4 to 5 minutes after cocaine administration. Nitrendipine is given intraarterially first in a loading dose of 7.4 µg, followed by a constant infusion of 1.22 µg/kg/min lasting 85 ± 20 min. The end point of the perfusion was reached when the rat was becoming active and restless. The 5 control animals had a survival time of 8'06" ± 5'20". Death could be attributed to convulsions and respiratory arrest. The 5 animals treated with nitrendipine survived. Total dose of nitrendipine administered was 129 ± 23 µg/kg. The animals were replaced fully conscious in their cage and 24 hours later were active and feeding themselves.

Survival of the animal could not only be attributed to the cardio-protective effect of the Ca^{2+} modulator but also to its stabilizing effect on the central nervous system. Convulsions and respiratory arrest were observed among the control animals which succumbed without presenting arrythmias or cardiac morphological lesions. Nitrendipine appears to have general protective effects against the toxic effects of cocaine on the cardiac as well as the cerebral capillary bed.

Nahas G., Trouvé R. & Maillet M. (1985), Bull.Acad.Nat.Méd., 169:1151-1156.

TISSUE DEPENDENT REGULATION OF ISOZYMES OF CYTOCHROME P-450 CATALYSING THE O-DEETHYLATION OF PHENACETIN IN RAT

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Monoclonal antibodies (MAb's), specific for a single epitope, can provide a valuable means by which to study the expression, regulation, function and substrate specificity of the respective immunoreactive isozymes. We have produced inhibitory and non-inhibitory MAb's to the two major hydrocarbon inducible forms of cytochrome P-450 in the rat, forms c and d, in order to investigate their expression in various tissues before and after induction. Inhibitory MAb's have been used to determine the contribution of forms c and d to the microsomal O-deethylation of phenacetin (POD) to paracetamol.

Treatment of rats with 3-methylcholanthrene (MC) (80 mg/kg in corn oil by i.p. injection 48 h prior to sacrifice) increased hepatic microsomal POD activity by 25-fold and extrahepatic activity (lung, kidney and small intestine) by 3-15-fold. Thus, MC-inducible forms of cytochrome P-450 (c and/or d) contribute to POD activity. Kinetic studies of POD activity with homogeneous preparations of the isozymes showed that both forms are effective in catalysing O-deethylation (c, Km = 5.01 μM; Vmax = 859 pmol/mg/min; d, Km = 2.49 μM; Vmax = 1293 pmol/mg/min).

MAb's specific for cytochrome P-450 form c, 3/4/2 (Boobis et al, 1985), C8 and C9 (Thomas et al, 1984) and a MAb that cross-reacts with forms c and d, 12/2/3/2 (Sesardic et al, 1986), were screened for their ability to inhibit the metabolism of benzo[a]pyrene (AHH) and phenacetin (POD) by reconstituted forms c and d. MAb's C8 and 12/2/3/2 were equally effective at inhibiting AHH and POD activities (> 90%). Additionally, POD activity catalysed by reconstituted forms c and d was inhibited to the same extent by 12/2/3/2. MAb's 3/4/2 and C9 were non-inhibitory, amd were used as negative controls. In hepatic microsomal fractions from control and MC-treated rats, MAb 12/2/3/2 completely inhibited POD activity, whilst MAb C8 was without effect. In marked contrast, POD activity of extrahepatic tissues from MC-treated rats was inhibited by both antibodies, to the same extent.

Immunoquantification of forms c and d with MAb's 12/2/3/2 and 3/4/2 revealed that these two isozymes are co-induced in the liver by MC, but only form c is inducible in extrahepatic tissues. MC induced form c by more than 80-fold in all of the tissues studied.

Two, structurally related (68% sequence homolgy), isozymes of cytochrome P-450, forms c and d, both of which are capable of catalysing POD activity, are both induced by MC in the liver, but are under independent regulation in extrahepatic tissues, where only form c is inducible. Despite the results obtained with the reconstituted isozymes, the major isozyme catalysing POD in the liver is form d, whereas in extrahepatic tissues from MC-treated rats it is form c.

We are very grateful to Drs W Levin and P E Thomas of the Roche Institute, Nutley, NJ for providing MAb's C8 and C9. This work was supported in part by grants from the Medical Research Council and the Wellcome Trust.

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PHARMACOLOGICAL ANALYSIS OF THE Ca^{2+}-DEPENDENCE OF μ-RECEPTOR-MEDIATED AGONISM

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A number of studies in isolated tissues have shown that μ-receptor agonist effects can be augmented or potentiated with reduction in extracellular calcium concentration, $[Ca^{2+}]_o$, (Opmeer and Van Ree, 1979; Hayes and Sheehan, 1986; Johnson et al, 1986). These changes have been ascribed to an enhancement in post-receptor coupling (Hayes and Sheehan, 1986) but it has not been established whether they correspond to a simple alteration in efficacy or whether more complicated post-receptor changes are involved.

In theory (Black and Leff,1983), the events which transduce agonist-receptor occupancy into pharmacological effect can be defined by three parameters: E_m, the maximum effect possible through a particular transducer system; τ, the efficacy of the agonist in that system; n, the slope index of the transducer relation, denoting its sensitivity. The object of the present analysis was to elucidate the effects of $[Ca^{2+}]_o$ variation in terms of these three operational parameters.

Using the isolated, coaxially-stimulated guinea-pig ileum preparation, the μ-receptor-mediated effects of [D-Ala^2,MePhe^4, Gly-ol^5]-enkephalin (DAGO) were studied at 2.5, 1.25 and 0.63mM $[Ca^{2+}]_o$. At each value of $[Ca^{2+}]_o$, the effects of the irreversible ligand, β-chlornaltrexamine (20nM and 100nM, 30 min) were also investigated. The resulting concentration-effect curves were analysed using operational model-fitting procedures (Black et al, 1985; Leff et al, 1985; Barrett et al, 1986) indicating that, with reduction in $[Ca^{2+}]_o$, τ, the efficacy of DAGO in this system was increased and the sensitivity, n, of the transducer relation was also enhanced, the latter indicating a trend towards positive co-operativity. These changes may be rationalised in terms of the known electrophysiological events which transduce μ-receptor occupancy into inhibition of transmitter release from myenteric neurones. No changes in K_A, the agonist dissociation constant, or E_m were detected.

The results of this study confirm previous reports on the Ca^{2+}-dependence of μ-receptor-agonism and they have some important implications for its pharmacological analysis. Manipulation of $[Ca^{2+}]_o$ may provide a useful means of enhancing agonist efficacy, allowing detection of agonism in compounds with low intrinsic efficacies. However, the accompanying change in co-operativity of the transducer relation must be considered when quantifying agonism under these conditions. Neglect of this could, for example, lead to errors in estimation of the relative intrinsic efficacies of agonists at different values of $[Ca^{2+}]_o$.

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FLUFENAMIC ACID MODIFIES NADH-LINKED SUBSTRATE OXIDATION AND CALCIUM-STIMULATED RESPIRATION IN GUINEA-PIG LUNG MITOCHONDRIA

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Previous studies using mitochondrial preparations from either hepatic or pulmonary tissues have shown that low concentrations of the non-steroidal anti-inflammatory drugs (NSAIDS), flufenamic acid and diflunisal have the ability to promote calcium ion (Ca^{2+}) release (McNamee etal, 1985). We now report the ability of flufenamic acid to modify energy metabolism in pulmonary tissue.

Using female Duncan-Hartley guinea-pigs lung mitochondria were prepared by the method of Chappell and Hansford (1969). In the presence of 5mM glutamate plus 5mM malate mitochondrial integrity was confirmed by an ADP:O ratio of 3.0±0.05 (n=45), and a respiratory control index (RCI) of 4.7±0.23 (n=45). Oxygen consumption was measured polarographically using a Clark-type oxygen electrode (Rank Bros. Bottisham, Cambridge, U.K.) based on the method of Sweetman and Weetman (1972). Protein was determined by the method of Gornall etal (1949).

The inclusion of flufenamic acid (0.1-30μM) to tightly-coupled lung mitochondria 1 min prior to the addition of substrate produced a concentration-dependent stimulation of state 4 (ADP absent, substrate and oxygen in excess) respiration, with the rate increasing from 8.8±0.07 to 89.5±5.2 ng atoms O_2 min^{-1} mg of $protein^{-1}$ (both n=5). The EC_{50} value for the stimulatory effect was 7.6±1.6μM flufenamic acid (n=5). Similar results were obtained in the presence of either diflunisal (EC_{50} = 29.7±3.7μM, n=5) or the uncoupling agent 2, 4-dinitrophenol (EC_{50} = 32.4±4.3μM, n=5).

Over the concentration range 2.5 to 80μM flufenamic acid was found to produce a concentration-dependent inhibition of state 3 (ADP present, substrate and oxygen in excess) respiration with the rate being reduced from 114.6±8.4 to 25.7 ±5.7 ng atoms O_2 min^{-1} mg of $protein^{-1}$ (both n=5). The reduction in oxygen consumption was accompanied by a loss in respiratory control, and an IC_{50} value of 41.4±3.7μM (n=5) was obtained. Similar but less significant ($p>0.05$) inhibitory effects were observed with diflunisal (IC_{50} = 86.6±5.8μM, n=5) and 2, 4-dintrophenol. Confirmation of an uncoupling action was obtained when flufenamic acid (2.5μM) released oligomycin (2μg) induced inhibition of state 3 respiration.

Replacement of ADP with Ca^{2+} (1-300μM) produced a concentration-dependent stimulation of state 4 respiration, without loss of respiratory control. The inclusion of flufenamic acid (1-120μM) prior to addition of Ca^{2+} produced a concentration-dependent inhibition of Ca^{2+} - stimulated respiration (IC_{50} value of 9.6±2.4μM, n=5); similar findings were obtained using diflunisal (IC_{50} = 13.8 ±2.1μM, n=5), 2, 4-dinitrophenol (IC_{50} = 66.8±6.7μM, n=5) and the Ca^{2+} influx inhibitor ruthenium red (IC_{50} = 0.12±0.008μM, n=5).

The data presented indicated that flufenamic acid has the ability to prevent ATP synthesis via NADH-linked substrate oxidation and cation transport in pulmonary tissue by uncoupling oxidation from phosphorylation.

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ACUTE DOSING WITH AMINOGLUTETHIMIDE INHIBITS MIXED FUNCTION OXIDATION IN THE MOUSE AND RAT

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Previously it has been shown that chronic dosing with aminoglutethimide (AG) causes induction of mixed function oxidase activity in the mouse and rat (Damanhouri & Nicholls, 1986). As many inducing agents may, on acute dosing, act as inhibitors of hepatic oxidative drug metabolism (Conney, 1967), this possibility has been examined for AG.

In the in vivo experiments with mice (25g, n=10) and male rats (150g, n=8), AG was administered orally 1h before the test drug (i.p.). In female mice, sleeping time to pentobarbitone sodium (45mg/kg) was significantly ($P<0.05$) increased by AG (60mg/kg) administration from 28.7±4.1 min (control) to 171.8±25.5 min (AG). In addition, zoxazolamine (120mg/kg) paralysis in male mice was significantly ($P<0.05$) increased from 30.1±3.6 min (control) to 47.2±3.3 min (AG 60mg/kg). The rate of $^{14}CO_2$ exhalation following administration of (N-dimethyl-^{14}C)antipyrine (Houston et al., 1981) was also significantly ($P<0.05$) altered by AG (60mg/kg) administration, the exhalation $t_{\frac{1}{2}}$ increasing from 22.8±1.0 min to 43.7±3.3 min and from 19.0±0.4 min to 31.4±1.8 min in male and female mice respectively.

In rats, the pentobarbitone sodium (45mg/kg)-induced hypnosis (58.8±20.8 min) was significantly ($P<0.05$) increased in a dose-dependent manner by AG (10 to 40mg/kg; 140 to 310% increase). AG (60mg/kg) significantly ($P<0.05$) elevated the plasma levels of pentobarbitone at 1 and 3h after the barbiturate (45mg/kg) from 27.3± 3.4 and 5.1±1.1µg/ml (control) to 73.1±4.4 and 27.3±3.4µg/ml (AG) respectively. Also in the rat, zoxazolamine (160mg/kg)-induced paralysis (35±5 min) was increased 5-fold by AG (10mg/kg). The O-demethylation of 4-nitroanisole (150µg/ml) by the 10,000xg supernatant of rat liver was inhibited 9 and 21% by the presence of AG 15 and 30µg/ml respectively.

These results are consistent with AG, in acute doses, acting as an inhibitor of mixed function oxidase activity. Although this phenomenon has not yet been shown to occur in man, it would appear prudent to appreciate this potential for drug interaction in the first few days of commencing therapy with AG in breast cancer patients.

PJN thanks CIBA-Geigy, Horsham and the Cancer Research Campaign for financial assistance.

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TAMOXIFEN AND 4-HYDROXYTAMOXIFEN LEVELS IN IMMATURE RAT UTERINE CYTOSOL AND UTERINE KCL-NUCLEAR EXTRACTS.

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The triphenylethylene antioestrogens (e.g. tamoxifen (TAM)) inhibit oestrogen stimulation of target tissues such as the immature rat uterus, most likely by occupying the oestrogen receptor. We have used a gas chromatographic-mass spectrometric (GC-MS) method to measure the relative distribution of TAM and its phenolic metabolite 4-hydroxytamoxifen (4-OHT) in subcellular fractions of immature rat uterus in an effort to assess the potential contribution of 4-OHT to the anti-oestrogenicity of administered TAM; 4-OHT has a very high affinity for the oestrogen receptor.

TAM (10, 40 or 60 µg per rat) was administered i.p. to groups of 25-28 immature female Wistar rats (20-24 days old). After 8 h the rats were killed, the uteri removed and stored in liquid nitrogen until analysed. Cytosolic fractions and 0.5 M KCl-extracts of uterine nuclei were isolated by established techniques. Appropriate radioactive and nonradioactive internal standards were added to each fraction; the fractions were deproteinised and the unconjugated metabolites isolated on a column of QAE-Sephadex A-25 (acetate form). A water:hexane amyl alcohol (5%) extraction removed the KCl in the KCl-extracted nuclear fraction. Sequential processing of the unconjugated metabolites on QAE-Sephadex A-25 (borate form), QAE-Sephadex A-25 (carbonate form), DEAE-Sephadex A-25 (free base form) and SP-Sephadex C-25 (hydrogen form) yielded fractions of catechol, mono-phenol, cationic and noncationic metabolites. Derivatisation and analysis of the individual fractions by GC-MS using selected ion monitoring (SIM) was essentially as previously described (Murphy, Fotsis, Pantzar, Adlercreutz and Martin, 1986).

In 4 experiments TAM levels in the cytosol ranged from 802-2160 pg per uterus and increased with increasing dose administered: in all cases the cytosolic 4-OHT levels were lower giving a 4-OHT : TAM ratio in the range 0.03-0.15 ($\bar{x}$ ± sd: 0.11 ± 0.05). In contrast, TAM levels in the 0.5 M KCl nuclear extracts were lower than in the cytosol (264 - 940 pg per uterus) while 4-OHT levels were of the same order: this yielded 4-OHT : TAM ratios in the range of 0.15 - 0.39 ($\bar{x}$ ± sd: 0.31 ± 0.09). This represents an enrichment of 4-OHT over TAM between the cytosol and KCl-extracted nuclear fraction of 2.5 - 5 fold in favour of the oestrogen receptor rich nuclear extract.

Our results support the suggestion that the high affinity of 4-OHT for the oestrogen receptor results in its selective concentration in rat uterine cell nuclei and implies that it plays a significant part in the antioestrogenic action of administered TAM in this target tissue.

Funded by the Irich Cancer Society. C.M. is in receipt of a scholarship from the Finnish Ministry for Education.

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EFFECTS OF Ca^{2+} CHANNEL ANTAGONISTS ON ACTION POTENTIAL CONDUCTION IN THE GUINEA-PIG HYPOGASTRIC NERVE *IN VIVO*

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Several Ca^{2+} channel antagonists (e.g. verapamil, diltiazem and amlodipine) in very high concentrations (c. 10^{-4}M) inhibit action potential (AP) conduction in and transmitter release from peripheral autonomic nerves *in vitro* (Beattie et al, 1986; Cunnane & Stjärne, 1984). The significance of these actions depends upon whether or not they can be demonstrated *in vivo* at therapeutically relevant doses. The present investigation compared the effects of these three Ca^{2+} channel antagonists on AP conduction *in vivo* with those previously described *in vitro* (Beattie et al, 1986).

The guinea-pig hypogastric nerve-vas deferens preparation was used. Male animals were anaesthetised with urethane (1.7gkg^{-1}, i.p.), the right jugular vein cannulated for the administration of drugs and the left carotid artery for measurement of blood pressure and heart rate. On occasion, drugs were injected into the cannulated external iliac artery. APs were recorded using a suction electrode from a postganglionic branch of the hypogastric nerve at the epididymal end of the vas (Beattie et al, 1986), in response to preganglionic nerve stimulation (0.2 Hz, 0.2-0.5 ms, supramaximal voltage).

Verapamil (0.01-0.3mgkg^{-1}), diltiazem (0.01-1mgkg^{-1}) and amlodipine (0.1-1.6mgkg^{-1}) given i.v., each reduced blood pressure without affecting AP conduction. With the exception of amlodipine (0.1-1.6mgkg^{-1}) which was ineffective, each drug also reduced heart rate significantly. Pentolinium (0.1-0.6mgkg^{-1}) also reduced blood pressure and heart rate but inhibited postganglionic AP conduction, presumably by ganglion blockade.

Verapamil (1-3mgkg^{-1}), on the other hand given i.a., abolished APs and lowered blood pressure and heart rate. Acetylcholine (0.01mg, i.a.) during AP blockade by verapamil (3mgkg^{-1}, i.a.), but not by pentolinium (0.6mgkg^{-1}, i.v.), produced a spontaneous postganglionic AP discharge, presumably by activation of ganglionic nicotinic receptors, indicating that verapamil affects neither these receptors nor postganglionic conduction. Prevention of preganglionic acetylcholine release by verapamil, however, may explain the inhibition of APs, recorded postganglionically.

The results show that prejunctional inhibitory effects of verapamil can be demonstrated *in vivo* only following close i.a. injection. Given i.v., the three Ca^{2+} channel antagonists studied, did not affect AP conduction at doses which significantly affected the cardiovascular system.

Acknowledgements: The support of the SERC and Pfizer Central Research and Glasgow University Medical Research Funds is gratefully acknowledged.

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5-HT_2 RECEPTOR AND α_1-ADRENOCEPTOR INTERACTIONS MAY BE INVOLVED IN HYPERTENSION: IN SPONTANEOUSLY HYPERTENSIVE RATS

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Ketanserin lowers blood pressure in spontaneously hypertensive rats (Van Nueten et al., 1981). It appears that in this species this acute effect is mainly due to its $alpha_1$-adrenergic antagonism (Van Nueten et al., 1985). Since ketanserin is more potent as an S_2-serotonergic antagonist than as an $alpha_1$-adrenergic antagonist (Van Nueten et al., 1981, 1985) the present experiments were designed to determine whether its serotonergic antagonistic properties could also contribute to its blood pressure lowering effect by comparing the effects of ketanserin to those of ritanserin and prazosin. In isolated caudal arteries of Wistar rats ritanserin was 200 times more potent as an antagonist of serotonin-induced contractions than as an antagonist of noradrenaline-induced contractions. In the same preparation ketanserin was 30 times more potent as an S_2-serotonergic antagonist than as an $alpha_1$-adrenergic antagonist. Prazosin was highly selective as an $alpha_1$-adrenergic antagonist. Ketanserin inhibited pressor responses to adrenaline and to serotonin in the conscious spontaneously hypertensive rat (SHR). Ritanserin inhibited pressor responses to serotonin but not to adrenaline and prazosin inhibited pressor responses to adrenaline but not to serotonin in this species. These experiments demonstrate that in vitro and in vivo ritanserin is highly selective as an S_2-serotonergic antagonist, and confirm that ketanserin is a potent S_2-serotonergic antagonist with moderate $alpha_1$ blocking properties whereas prazosin is a highly selective $alpha_1$-adrenergic antagonist. Ritanserin did not affect blood pressure in conscious SHR whereas ketanserin and prazosin effectively lowered blood pressure. These experiments demonstrate that S_2-serotonergic blockade alone cannot explain the blood-pressure lowering effect of ketanserin in the rat. Ritanserin when given in combination increased the blood pressure lowering effect of a low dose of prazosin to the antihypertensive effect of a double dose of prazosin administered alone. In vitro, serotonin amplifies vasconstrictive responses of blood vessels and vascular beds (Van Nueten et al., 1985; Janssens and Van Nueten, in press) and possibly the inhibition by ritanserin of this amplification can explain its potential to enhance the blood pressure lowering effect of prazosin. Therefore the combination of the S_2-serotonergic blockade by ketanserin and its weaker but necessary $alpha_1$-adrenergic antagonism may explain its antihypertensive activity in the rat.

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SENSITIVITY TO α_2-ADRENOCEPTOR AGONISTS IN HYPERTENSIVE RATS

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Spontaneous hypertension in rats is characterized by increased sensitivity to alpha-2 adrenoceptor agonists (Medgett et al. 1984). We have investigated whether the sensitivity of renovascular hypertensive rats to alpha-2 adrenoceptor agonists is likewise increased. The alpha-2 agonist used was UK14304. Phenylephrine was taken as an alpha-1 agonist and angiotensin II as a non-adrenergic agonist.

Three groups of Wistar male rats were used (1) normotensive controls (WKY), (2) renovascular hypertensive rats (WKY:2K1C, clip of 0.2 mm gap on left renal artery for 1 month), and (3) SHR. All rats were 3 months old at the time of the dose-response experiments (1 agonist per rat). The femoral artery and vein were cannulated under pentobarbital anesthesia (50 mg/kg, i.p.). Diastolic arterial pressure (DAP, mmHg) and heart rate (HR, bpm) were recorded. Pentolinium tartrate (5 mg/kg, i.p.) was administered. The absence of reflex bradycardia following pressor responses was taken as an indication of ganglionic blockade.

	DAP		Responses to:		
	after pentobarbital	after pentolinium	UK14304	Phenylephrine	Angiotensin II
WKY	75 ± 4 (52)	45 ± 2			
		maximum	62 ± 6 (26)	85 ± 9 (15)	77 ± 3 (11)
		ED_{50}	2.6 ± 0.3	1.4 ± 0.2	1.3 ± 0.2
WKY:2K1C	113 ± 6 (43)	62 ± 8			
		maximum	53 ± 7 (14)	98 ± 5 (14)	84 ± 4 (15)
		ED_{50}	3.9 ± 0.2	1.8 ± 0.3	19 ± 4
SHR	131 ± 4 (43)	57 ± 4			
		maximum	87 ± 6 (15)	98 ± 8 (17)	92 ± 5 (11)
		ED_{50}	6.9 ± 0.2	1.3 ± 0.4	11 ± 3

Results are means ± SEM. Maximum is maximal increase in DAP (mmHg). ED_{50} values are 10^{-8} moles/kg for alpha adrenoceptor agonists and 10^{-11} moles/kg for angiotensin II.

The alpha-2 agonist, UK14304, had a lower potency in hypertensive rats. The maximal pressor response to UK14304 was greater in SHR (+40% $P<0.01$ compared to WKY) but not in WKY:2K1C. Responses to the alpha-1 agonist phenylephrine were the same in hypertensive and normotensive rats. Angiotensin II showed a lower potency in hypertensive rats. The maximal pressor response to angiotensin II was greater in SHR (+ 20% $P<0.05$ compared to WKY) but not in WKY:2K1C.

In conclusion the increased reactivity to an alpha-2 agonist seen in SHR is not found in WKY:2K1C.

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SENSITIVITY TO SEROTONIN IN TAIL ARTERIES OF HYPERTENSIVE RATS

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The demonstration of increased sensitivity to the vasoconstrictor effect of serotonin in rat models of hypertension appears to depend on the type of hypertension and the vascular bed involved (Vanhoutte, 1985). We have reinvestigated this problem using the isolated perfused/superfused caudal artery of renovascular and spontaneously hypertensive (SHR) rats. Male, 3 month old rats of the following groups were used: (1) outbred normotensive Wistar (WIco), n = 13), (2) inbred normotensive (WKY, n = 11), (3) renovascular hypertensive (clip of 0.2 mm on left renal artery for 1 month, WKY:2K1C, n = 6) and (4) SHR (n = 6). Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and diastolic arterial pressure was measured via a carotid artery cannula. A 2 cm segment of the proximal caudal artery was removed and cannulated at both ends. The segment was perfused (direction of flow: proximal to distal) and superfused with Krebs bicarbonate at 37° and at an initial flow rate of 1 ml/min. Flow rate was increased over the next 30 minutes to 4 ml/min and after 15 mins at this flow rate the basal perfusion pressure was measured. Uptake blockers were not used. Serotonin at concentrations of 10^{-8} to 10^{-4} M was injected (0.1 ml bolus) into the perfusion circuit just before the artery. Vasoconstriction was estimated from the increase in perfusion pressure. Perfusion pressure was allowed to stabilize at baseline values between each injection. ED_{50} values were calculated after the following transformation: logit percentage increase in perfusion pressure versus $\log_{10}$ concentration serotonin.

	WIco	WKY	WKY:2K1C	SHR
n	13	11	6	6
Diastolic pressure (mmHg)	80 ± 6	75 ± 8	120 ± 6	145 ± 3
Tail artery				
Basal perfusion pressure (mmHg)	27 ± 3	47 ± 6	51 ± 6	57 ± 2
Maximal increase in perfusion pressure (mmHg)	219 ± 22	201 ± 9	284 ± 18	287 ± 15
ED_{50} (10^{-7} M)	14.3 ± 3.0	7.3 ± 1.3	5.6 ± 1.2	38.5 ± 2.3
Increase in perfusion pressure (mmHg) at 10^{-7} M	44 ± 6	31 ± 9	22 ± 3	3 ± 1
Slope of the logit/log dose-response curve	1.4 ± 0.2	2.0 ± 0.2	3.5 ± 0.3	2.3 ± 0.3

Results are means ± SEM

Diastolic pressure was higher in SHR than in WKY:2K1C ($P<0.01$). In the tail artery, basal perfusion pressure was higher in inbred rat strains (WKY, WKY:2K1C and SHR) compared to the outbred strain (WIco), independently of whether they were hypertensive or not. The maximal increase in perfusion pressure induced by serotonin was greater in hypertensive (WKY:2K1C and SHR) than in normotensive rats (WICO and WKY). The potency of serotonin was lower in SHR ($P<0.001$ with WKY) but not in WKY:2K1C. The response at a low dose of serotonin (10^{-7} M) was lower in SHR ($P<0.05$ with WKY and $P<0.001$ with WKY:2K1C). The slope of the dose-response curve was steeper in WKY:2K1C ($P<0.001$ with WKY) but not in SHR.

In conclusion, the caudal arteries of both renovascular and spontaneously hypertensive rats show increased reactivity (increased maxima) to serotonin. In the SHR there is a decrease in potency which is not seen in the WKY:2K1C.

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EFFECT OF NICARDIPINE AND REMOVAL OF CALCIUM ON VASOCONSTRICTOR RESPONSES TO PHENYLEPHRINE AND SEROTONIN

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Differences in the degree of attenuation of the vasoconstrictor effects of alpha^{-1} adrenoceptor agonists and serotonin by calcium entry blockers and removal calcium (Fasciolo, 1984 ; van Zwieten & Timmermans, 1985) has given rise to the idea of two independent calcium coupling mechanisms for these two agonists. We have investigated this hypothesis in the ganglion-blocked rat, and the rat caudal artery. Male, three month old, outbred Wistar, normotensive rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The proximal 2 cm portion of the caudal artery was removed (see below). The femoral artery and vein were cannulated and pentolinium tartrate (5 mg/kg i.p.) administered. Diastolic pressure (mmHg) and heart rate (bpm) were recorded. Ganglion blockade was checked by the absence of bradycardia following pressor responses. A dose-response curve for serotonin or phenylephrine (both 10^{-10} to 10^{-6} moles/kg i.v.) was carried out, then repeated under infusion with nicardipine (priming dose 60 nmoles/kg with 6 nmoles/kg per minute infusion). Serotonin produced dose-related increases in diastolic arterial pressure with a maximum of +65±4 mmHg and an ED_{50} of 2.9 ± 0.3 x 10^{-8} moles/kg (n = 12). Nicardipine had no effect on serotonin-induced increases in diastolic arterial pressure. The maximal pressor response to phenylephrine was +73 ± 5 mmHg with an ED_{50} of 1.1 ± 0.1 x 10^{-8} moles/kg (n = 6). Nicardipine did not alter the maximal response but produced a 3 fold (P 0.05) increase in ED_{50} for phenylephrine.

The caudal artery segment was perfused/superfused with Krebs bicarbonate at 4 ml/min and 37°. Dose-response curves were carried out by perfusing various concentrations of either serotonin or phenylephrine (2 minutes/concentration) in the presence of nicardipine (1 uM), prazosin (10^{-8}-10^{-9} M), Krebs without calcium plus 10^{-3}M EGTA.

Maximal increases in perfusion pressure produced by serotonin and phenylephrine were +256±14 and 279±19 mmHg, with ED_{50} values of 3.9 ± 0.4 uM (n = 8) and 15 ± 2 uM (n = 8) respectively. Perfusion with nicardipine produced a slight decrease in the maximal response to serotonin (-17%, P 0.05) and a larger decrease in the response to phenylephrine (-32% P 0.01). There was a 5 fold (P 0.01)increase in ED_{50} for serotonin but no change in the ED_{50} for phenylephrine. Removal of calcium abolished responses to phenylephrine and decreased the maximal response to serotonin by -79% (P 0.001). Prazosin had no effect on serotonin-induced vasoconstriction.

In conclusion our results show that both in vivo and in vitro responses to the alpha-1 adrenoceptor agonist phenylephrine are more sensitive to the calcium entry blocker nicardipine and to removal of calcium than are those to serotonin.

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NICARDIPINE AND α_1-ADRENOCEPTORS IN HYPERTENSIVE RATS

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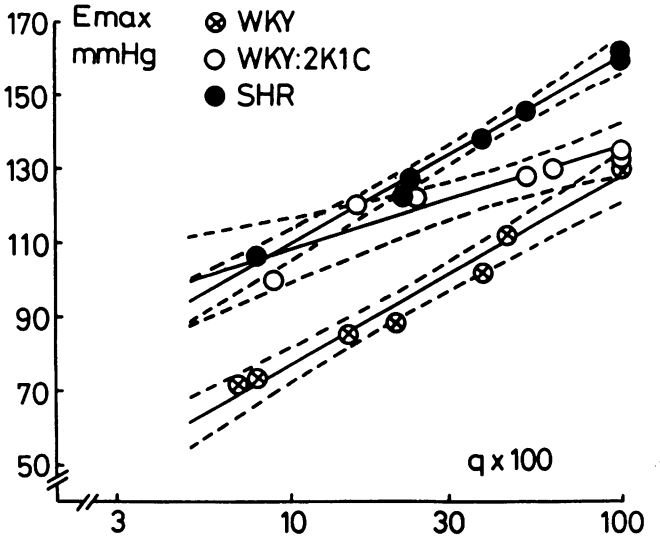
The calcium entry blocker, nicardipine, behaves like an irreversible, competitive antagonist of alpha-1 adrenoceptor-mediated pressor responses. We have determined the relationship between the percentage of receptors not blocked by nicardipine and the maximal increases in diastolic pressure produced by phenylephrine, in normotensive WKY, SHR, and renovascular hypertensive rats (WKY:2K1C, clip 0.2 mm on left renal artery for 1 month). All rats were 3 months old at the time of the experiment. They were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) then pithed and artificially respired at 60 strokes/min and 10 ml/kg. The femoral artery and vein were cannulated. A dose response curve for the alpha-1 adrenoceptor agonist, phenylephrine, was carried out then the nicardipine i.v. infusion started. After 20 minutes, the phenylephrine dose-response curve was repeated. Seven nicardipine doses from 1 to 200 ug/kg per minute were tested. Each rat received one dose of nicardipine; there were 6-8 rats per dose. At each nicardipine infusion rate, the fraction of active receptors remaining (q) was determined as the reciprocal of the slope of the plot of the reciprocals of the concentrations of phenylephrine before receptor blockade, against the reciprocals of the equiactive concentrations of phenylephrine after partial alpha-1 adrenoceptor inactivation by nicardipine (see Furchgott, 1966). The percentage of receptors not blocked (q x 100) was plotted against the maximum increase in diastolic pressure (Emax, mmHg).



Slopes were similar in WKY and SHR over the range of 5 to 100% active receptors. This may mean that the sensitivity to nicardipine is similar in the two strains and that some independent factor like structural adaptation increases responses in SHR. WKY and WKY:2K1C showed similar responses at 100% active receptors but the slope in WKY:2K1C was shallower than in WKY (or SHR). This may mean that WKY:2K1C have a larger number of spare "receptors" than WKY.

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CHRONIC ARTERIAL HYPERTENSION FOLLOWING SINOAORTIC DENERVATION IN CONSCIOUS DOGS: A STUDY OF ADRENOCEPTORS

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The physiological role of carotid and aortic baroreceptors in regulating blood pressure is still discussed. Since arterial baroreceptors are involved in minute to minute regulation of blood pressure, it has been suggested that their dysfunction may lead to the development of hypertension. However, conflicting results were reported : Laubie & Schmitt (1979), Ito & Scher (1981) found a sustained increase in blood pressure (BP) after baroreceptor denervation (BD) in dogs. By contrast, Cowley et al (1973) did not find any evidence for such a change in BP under similar experimental conditions. The present work reinvestigates this problem and examines possible changes in adrenergic receptivity in these baroreceptor denervated animals.

Six dogs of either sex weighing 20-30 kg were trained to stay quiet on a Pavlov table several days before the experiments. After a first measurement of cardiovascular and adrenergic parameters under resting conditions (normal control values in normotensive animals), they were anesthetized with chloralose (80 mg/kg i.v.) and submitted to unilateral (right) BD by section of the aortic and carotid sinus nerves. This procedure was repeated for the left side 2 months later. The effectiveness of BD was checked by the failure of noradrenaline (NA) to induce bradycardia. BP, heart rate (HR), alpha-adrenergic receptivity (assessed by pressor responses to NA (0.5, 1, 2 and 4 µg/kg i.v.) and (^{3}H) yohimbine binding to platelet membranes), beta-adrenergic receptivity (assessed by tachycardic and hypotensive responses to isoprenaline (0.125, 0.25, 0.5 and 1 µg/kg i.v.) and ^{125}I ICYP binding on lymphocyte membranes), plasma levels of catecholamines (measured by HLPC) and plasma renin activity (PRA) were evaluated before and every month after BD.

In trained conscious dogs, BD induced a significant and permanent rise in both BP and HR : the values of systolic, diastolic BP and HR were respectively 161.7 ± 16.2, 63.3 ± 5.4 mm Hg, 85.0 ± 7.9 b/min before BD and 229.2 ± 15.9, 110.8 ± 6.1 mm Hg and 155.0 ± 7.8 b/min 30 days after BD ($p < 0.001$). The number of ^{125}I ICYP binding sites on lymphocytes decreased from 41 ± 8 to 19 ± 4 fmol/mg proteins ($p < 0.05$) (with no change in Kd) whereas isoprenaline-induced tachycardia decreased. Alpha 2-adrenergic receptivity was less altered : the magnitude of the pressor responses to NA increased after 0.5 or 1 µg/kg and remained unchanged after higher doses. The number of (^{3}H) yohimbine binding sites in platelets decreased from 198 ± 12 (before BD) to 133 ± 11 fmol/mg proteins ($p < 0.05$) in hypertensive animals . Catecholamine plasma levels significantly ($p < 0.05$) increased from 395.0 ± 101.9 (before BD) to 1097.2 ± 419.6 pg/ml (day 30) for NA and 458.0 ± 106.9 (before BD) to 925.7 ± 118.3 pg/ml (day 30) for A. PRA levels did not displayed any change.

These results show that chronic sinoaortic denervation in conscious dogs induces a permanent and significant rise in BP with an increase in sympathetic tone. This model of experimental arterial hypertension is associated with a rise in plasma catecholamine levels and a decrease in adrenergic receptivity involving mainly beta- and to a lesser extend alpha$_2$-adrenoceptors.

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ISOPRENALINE-INDUCED IN VITRO DESENSITIZATION OF β-ADRENOCEPTOR MEDIATED RESPONSES OF GUINEA-PIG ATRIA

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The β-adrenoceptor desensitization occurring after prolonged exposure to agonists has been demonstrated in the heart as a reduced sensitivity and receptor density after chronic pretreatment of animals (Hedberg et al., 1984) and after in vitro exposure of isolated atria (Kaumann & Birnbaumer, 1976) and myocytes (Limas & Limas, 1984). The present study examines the conditions for in vitro desensitization of isolated atria by incubation with isoprenaline, measuring tissue responsiveness.

Tension responses of guinea-pig paced left atria (2Hz, 5ms, threshold voltage+50%) and rate responses of spontaneous right atria set up in Krebs-bicarbonate solution containing ascorbic acid (1mM) at 37.5°C gassed with 5% CO_2 in O_2 were recorded. A cumulative concentration-response curve to (-)-isoprenaline was constructed and the maximum concentration (10^{-6}M) left in contact with the tissue for 30, 120, 240 or 480 min. The bath was then washed twice and again at regular intervals for 20, 30 or 60 min. A cumulative curve for isoprenaline was then repeated. Control experiments, performed identically except that isoprenaline was washed out before commencing the sham incubation period, were used to correct the pre-incubation curves of test experiments. Increases in rate or tension were plotted as a percent of the pre-incubation maximum and EC50 or EC30 values calculated. $n \geq 4$ throughout.

After 30 min incubation and 20 min washout, there was no change in rate responses but a small significant ($P<0.05$) depression of the tension maximum response to 85.2±2.7%. Increasing the incubation time to 120 min with a 30 min washout resulted in a significant depression of the rate maximum to 84.9±3.9% and rightwards shift of the curve (EC50 values, 1.9 and 17nM). The tension curve was also displaced. With the same incubation time but extended washout of 60 min, the rate curves were virtually superimposed but the rightward shift of the tension curve persisted. Incubation for 240 min with 30 min washout significantly displaced the rate (EC50, 5.8 and 23nM) and tension (EC30, 5.1 and 79nM) curves to the right with only the tension maximum being depressed to 49.6±1.5%. With a 60 min washout there was still a significant shift of the rate (EC50, 1.1 and 15nM) and tension (EC50, 11 and 46nM) curves to the right, but the depressed tension maximum to 73.7±7.6% was significantly less than for 30 min washout. A reversible phase of desensitization therefore appears to exist, sensitivity being partially restored during washout.

A further increase in incubation time to 8h with 60 min washout resulted in a significant shift of the rate curve (EC50, 5.3 and 32nM) which was not significantly greater than with 4h incubation. The shift of the tension curve (EC30, 11 and 85 nM) was significant, but the depression of the maximum to 56.6±10.3% was significantly greater than for the 4h incubation. Finally, increasing the concentration of isoprenaline to 10^{-5}M (4h incubation, 60 min washout) substantially increased the rightwards shifts and depressions of the rate (58.3±10.2%) and tension (54.7± 9.1%) maxima.

This study demonstrates desensitization of β-adrenoceptor-mediated responses of isolated atria, which depends upon the concentration and time of exposure to isoprenaline and the washout time, and is more pronounced in left atria. Supported in part by the British Heart Foundation.

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CARDIOVASCULAR EFFECTS OF APAMIN AND BRL 34915 IN RATS AND RABBITS

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The recent finding that BRL 34915 (BRL) causes vasodilatation via the activation of vascular smooth muscle K^+ channels has created interest in the role that K^+ channels play in regulating vascular resistance (Weir and Weston, 1986). Apamin, a specific blocker of certain Ca^{2+}-activated K^+ channels, is able to inhibit α-mediated increases in plasma K^+ and potentiate α-pressor responses in guinea pigs with a duration > 2 hours (Coats, 1983) but otherwise little is known of its cardiovascular effects. We have investigated the cardiovascular effects of apamin and BRL in rats and rabbits to further elucidate the role of vascular K^+ channels in blood pressure (BP) regulation.

Normotensive rats were anaesthetized (inactin 150 mg/kg) and the femoral artery cannulated to measure BP and heart rate (HR). Apamin (50 and 150 mg/kg i.v.) caused significant increases in pressor responses to angiotensin II (AII) (duration > 4 hours), but had no significant effect on basal BP or HR. Depressor responses due to isoprenaline were non-significantly reduced after apamin. BRL (0.1 and 0.3 mg/kg i.v.) caused a rapid fall in BP and increase in HR, the latter being abolished by prior β blockade (0.1 mg/kg bopindolol). In rats pretreated with apamin (50 mg/kg), BRL caused essentially the same changes as in untreated animals.

Systemic haemodynamic and regional blood flows (using microspheres) were measured in rabbits as described previously (Hof, 1985). BRL (3,10,30 mg/kg cumulative) dose-dependently lowered BP without changing HR or cardiac output. Marked vasodilation occurred in the stomach, with conductance increases also in the heart, lungs, small intestine and brain. Kidney conductance remained essentially unchanged, and most of these effects had subsided within 30 minutes. Apamin (150 mg/kg) had no significant effects on basal BP, HR or regional blood flows in rabbits and failed to modify the changes caused by the infusion of AII (0.03, 0.1, 0.3 mg/kg/min) as compared to controls.

We conclude that the site at which BRL acts to cause vasodilation in vivo is not sensitive to apamin. Furthermore, the fact that apamin is able to potentiate pressor responses in guinea pigs (Coats, 1983) and rats, but not in rabbits suggests that either metabolic differences (of apamin) or differences in the vascular K^+ channel populations may exist between species.

We wish to thank Dr. P.N. Strong and Beechams Pharmaceuticals for the supply of apamin and BRL 34915 respectively.

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THE VASODILATOR POTENCY OF CALCITONIN GENE RELATED PEPTIDE ON THE HEPATIC ARTERIAL VASCULATURE OF THE DOG

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In many species Calcitonin Gene Related Peptide (CGRP) has been shown to be a potent vasodilator of a wide variety of vascular beds (Brain et al, 1985; Withrington, 1986). The purpose of the present experiments was to assess its potency on the hepatic arterial vasculature of the dog and to compare the results with those previously reported for a wide range of vasoactive substances.

The experiments were performed on 4 dogs (22-26 kg) anaesthetised with a mixture of chloralose (50 mg/kg) and urethane (500 mg/kg). The hepatic artery was cannulated and perfused with blood derived from the femoral circuit. An electromagnetic flowprobe and pressure transducer were incorporated into the perfusion circuit to allow the continuous recording of hepatic arterial mean blood flow (HABF) and hepatic arterial mean perfusion pressure (HAPP) from which data calculations could be made of changes in hepatic arterial vascular resistance (HAVR).

Human CGRP (Bachem) was administered as a bolus dose (100 fmol-200 pmol) directly into the hepatic artery to construct 6 complete dose response curves and to compare these with the responses to isoprenaline (Iso) in the same preparations. In all experiments the hepatic arterial response to CGRP consisted of an increase only in HABF indicating, at constant perfusion pressure, a fall in HAVR and hepatic arterial vasodilatation. This vasodilator response was graded with dose and had a time course similar to the responses to Iso. The threshold dose for CGRP was always less than 1.0 pmol whilst the maximum vasodilator effect was achieved between 50-100 pmol. The mean ED_{50} was 8.1 $\pm$ 2.2 and 757 $\pm$ 74 pmol for CGRP and Iso respectively. The mean maximum increase in HABF was the same for CGRP and Iso (33.6 $\pm$ 1.2, 39.6 $\pm$ 3.7% respectively). The vasodilator responses to CGRP were not antagonised by a dose of the selective B_2 adrenoceptor antagonist ICI 118,551 that shifted the dose-response curve to Iso to the right.

The present experiments confirm the high vasodilator potency of CGRP. A comparison with our previous results (Richardson & Withrington, 1981) on the hepatic arterial vascular bed of the dog reveals that, on a molar basis, CGRP is a more potent vasodilator than Vasoactive Intestinal Peptide and prostaglandin E_2 but less potent than bradykinin.

I thank ICI PLC for financial support and the gift of ICI 118,551.

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EFFECT OF ADENOSINE ANALOGUE N^6-CYCLOHEXYLADENOSINE (CHA) ON CEREBRAL BLOOD FLOW IN THE ANAESTHETISED RAT.

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Several factors have been proposed as possible mediators in the mechanism by which the brain regulates its blood flow e.g. hydrogen ions, CO_2, potassium ions and, a more recent addition adenosine (Kuschinsky, 1983). Adenosine has previously been shown to produce increases in CBF (Forrester et al. 1979). Adenosine and its analogues have also been shown to dilate cerebral vessels (Edvinsson and Fredholm, 1983; McBean et al. 1986). In this study the effect of the putative adenosine agonist CHA on CBF was monitored using a laser-Doppler flowmeter. The laser-Doppler method measures the blood cell flux i.e the blood cell flow through the microvasculature from the arterial to the venous side.

The experiments were carried out on halothane anaesthetised male Sprague-Dawley rats (250g - 350g). Polythene catheters (external diameter 0.96 mm) were inserted into both femoral arteries to allow both the measurement of arterial blood pressure, as well as the removal of arterial blood samples to measure blood gas values. PCO_2 and BP values were monitored throughout the course of the experiment, and were found not to differ significantly between saline control and CHA animals.

A 5 mm diameter hole was drilled in the skull of the rat to expose the cortical surface and the laser was positioned over this hole to allow the movement of the red cells to be detected, and thus give a relative measurement of CBF. CHA was administered at a concentration low enough to prevent any hypotensive effects. The CHA was administered via a polythene catheter (external diameter 0.63 mm) inserted in the external carotid artery.

The CHA was administered at a rate of 10^{-10}moles/min (50 ul/min for 15 mins), and the saline was administered at 50 ul/min. The changes in CBF were monitored throughout the course of the experiment. CHA decreases CBF; no transient increases in CBF were noted with CHA as might have been expected (Edvinsson and Fredholm, 1983; McBean et al. 1986). Possible explanations for these changes at low concentrations of CHA are that it acts on another, uncharacterised, sub-type of adenosine receptor or on a receptor distinct from that which mediates dilatation.

Table 1. Effect of CHA on Cerebral Blood Flow.

Time (mins)	0.9% Saline	10^{-10}moles/min CHA
5	4.8 ± 8.9%	-9.7 ± 7.0%*
10	7.0 ± 7.6%	-18.4 ± 14.2%*
15	10.2 ± 7.3%	-19.4 ± 16.0%*

*P<0.01 (Student's t-test, CHA against saline). All values are expressed as percentage change in flow from baseline level (mean ± S.D.). Negative values indicate decreases in flow, positive values indicate increases. n=5 or 6.

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ENDOTHELIAL α_2-ADRENOCEPTORS ON CANINE FEMORAL AND CORONARY ARTERIES DO NOT MODULATE THE CONTRACTILE RESPONSES TO ADRENALINE

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Several investigators have suggested that the presence of α_2-adrenoceptors on the endothelium which when stimulated cause the vessels to relax may modify the constrictor responses to non-selective α-adrenoceptor agonists such as noradrenaline (Cocks & Angus, 1983; Miller & Vanhoutte, 1985). The aim of the present study was therefore to investigate the modulatory role of the endothelium on the constrictor responses to adrenaline in the femoral and coronary artery and to investigate if functional α_2-adrenoceptors are present on these two tissues.

Rings (2-3mm long) of the left circumflex coronary and femoral artery were suspended on two parallel metal pieces, one attached to the bottom of the bath and the top hook being attached to a Devices isometric force transducer by a piece of thread. Isometric contractions of the circular smooth muscle were recorded. The rings were placed in a Krebs-Hensleit solution containing propranolol (1μM) and cocaine (10μM) under a tension of 1 and 5g respectively for the coronary and femoral arteries and allowed to equilibrate for 1 hour. The functional integrity or removal of the endothelium was confirmed in each ring by the presence or absence of relaxation induced by acetylcholine (1μM).

The adrenaline response curves in the dog coronary artery in the presence and absence of the endothelium were compared and the EC_{50} value for the intact (2.3±0.62μM, n=7) and endothelium denuded (1.69±0.23μM, n=7) and the E_{max} values were found not to be significantly different (Paired t-test, $p<0.05$). Similarily in the femoral artery there was no significant difference in the EC_{50} and E_{max} values for the endothelium intact and denuded tissue for the adrenaline dose response curves but there was a significant parallel leftward shift, approximately 200 fold, of the concentration response curve to the α_2-agonist, UK 14,304 although the maximal response was not enhanced.

In order to determine if there was functional α_2 adrenoceptors on the endothelium of these two arteries they were preincubated with prazosin (0.1μM) and then precontracted with U46619 (0.1nM) and 5HT (1μM) in the coronary and femoral vessels respectively. Cumulative additions of UK 14,304 (0.1nM-100μM) in both the femoral and coronary vessels caused dose dependent decreases in tension only in the arteries in which the endothelium was intact. The maximal relaxant responses in the endothelium intact tissues were 50±11% (n=5) of the initial contractile response in the coronary artery and 31±7% (n=7) in the femoral artery.

Therefore, despite the demonstration of functional α_2-adrenoceptors on the endothelium of both the coronary and femoral arteries these did not influence the contractile responses to adrenaline.

A.D. McHarg is supported by an SERC CASE studentship.

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ROLE OF THE ENDOTHELIUM IN AGE-RELATED RESPONSES OF RAT AORTA

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Age can affect the responses of blood vessels to a variety of constrictor agents. These constrictor responses can also be influenced by the endothelial layer (Godfraind et al, 1985). This influence of the endothelial layer may alter with age. Therefore we have studied the effect of the endothelial layer on the responses of the aorta to KCl, noradrenaline (NA), clonidine (CLON) and methoxamine (METH) from rats aged 1, 6 & 12 months.

Pairs of aortic rings (2-3 mm) were cut longitudinally into a flat sheet and set up for isometric recording in a 7 ml organ bath (Krebs' bicarbonate solution, 37°C) under an initial loading tension of 1 g (1 month), 2 g (6 month) and 4 g (12 month). The endothelium was removed from one of the pair by gently rubbing with a cotton bud. A control cummulative dose-response curve to KCl was obtained followed 60 min later by a cummulative dose-response curve to NA, CLON, or METH. The responses were measured as g tension per mg tissue dry weight.

	Endothelium Intact			Endothelium Removed		
	1	6	12	1	6	12 month
KCl						
pD_2	2.08±0.03	1.71±0.02	1.85±0.02	2.39±0.03*	1.95±0.02*	2.07±0.03*
Max. Ten.	0.96±0.05	0.84±0.04	1.28±0.06	0.73±0.05*	0.94±0.06	1.22±0.05
NA						
pD_2	8.67±0.18	8.03±0.20	7.97±0.07	9.04±0.11	8.70±0.20*	8.78±0.09*
Max. Ten.	1.24±0.08	1.08±0.07	1.58±0.15	0.93±0.06*	1.20±0.22	1.51±0.07
METH						
pD_2	6.74±0.13	5.88±0.18	6.13±0.07	6.81±0.10	6.28±0.01	6.54±0.04*
Max. Ten.	1.15±0.07	0.97±0.09	1.41±0.15	0.85±0.11*	1.11±0.07	1.56±0.07
CLON						
pD_2	0.00	7.16±0.11	7.16±0.07	0.00	7.53±0.14	7.49±0.02*
Max. Ten.	0.00	0.47±0.09	0.83±0.19	0.00	0.58±0.08	1.06±0.09

(Max. Ten. = Maximum Tension, *P 0.05 (Intact versus Removed).
Removing the endothelium significantly reduced the maximum tension developed to KCl, NA & METH at 1 month but had no effect at 6 & 12 months. There was no response to CLON at 1 month; at 6 & 12 months maximum tension was not altered by endothelium removal. The pD_2 values increased after the removal of the endothelium at all ages for KCl, at 6 & 12 months for NA and only at 12 months for METH & CLON. One explanation for the decrease in the maximum tension at 1 month could be that at this stage of development a factor is produced which facilitates contraction of smooth muscle (De May & Vanhoutte, 1982). An increase in pD_2 values after the removal of the endothelium is consistent with the idea of the release of endothelium-derived relaxing factor. It is clear that the influence of the endothelium on the responses to constrictor agents at different stages of development is complex and depends on the drug concerned. This might explain contradictory reports by different authors on age-related changes in vascular reactivity to drugs (Duckles & Banner, 1984); changes could have been influenced by the integrity of the endothelial layer in the isolated preparations used.

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Supported by grants from Beecham Research Laboratories and SmithKline (1982) Foundation.

CARDIOVASCULAR PROFILE OF TRANDOLAPRIL, A NEW ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITOR

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The present report describes the ACE inhibitory activity, antihypertensive activity and haemodynamic profile of trandolapril, a new ACE inhibitor.

Administration of i.v. trandolapril to anaesthetized rats attenuated the pressor response to angiotensin I (Ang I, 0.5 ug/kg, i.v.) with an ID_{50} of 13.1±1.3ug/kg (n=6). The corresponding value for enalapril was 83.1±7.1 ug/kg (n=6). Pressor responses to Ang II were unaltered. Oral administration of trandolapril to groups of conscious rats (n=6) attenuated the pressor response to Ang I (0.5ug/kg, i.v.) injected 2 hr later, in a dose-related manner giving 0±5, 18±6, 50±3, 66±4 and 74±4% inhibition at 3, 10, 30, 100 and 300 ug/kg. Enalapril was some 3 fold less potent producing 0±2, 14±6, 54±6 and 72±6% inhibition at 10,30, 100 and 300ug/kg. Pressor responses to Ang II were either unaltered or significantly potentiated (analysis of variance).

Administration of trandolapril i.v. to anaesthetized dogs attenuated the pressor response to Ang I (0.25ug/kg, i.v.) with an ID_{50} of 21.1±2.3ug/kg (n=4). The corresponding value for enalapril was 209.3±43.2ug/kg. Oral administration of trandolapril at 0.1, 0.3 and 1mg/kg to groups of conscious dogs produced dose-related attenuation of Ang I (0.15ug/kg, i.v.)-induced pressor responses, giving respectively 13.1±4.3, 63.0±11.8 and 77.4±9.2% inhibition over 6 hr.Total inhibition over 6 hr induced by the same doses of enalapril were 10.8±6.3, 36.9±3.9 and 61.8±7.9%.

In anesthetized rats, bradykinin (0.25ug/kg, i.v.)-induced depressor responses were potentiated by i.v. trandolapril (ED_{50}, 5.5±0.8ug/kg, i.v., n=6), and enalapril (ED_{50} 13.4±1.5ug/kg, i.v., n=6).

Trandolapril lowered systolic, diastolic and mean BP over 24 hr in conscious SHR after acute oral administration, maximum falls in mean BP being 17±3, 23±4 and 29±3 mmHg for 0.3, 3.0 and 30mg/kg. This antihypertensive effect was significantly enhanced when plasma renin activity (PRA) in SHR was increased by diuretic pretreatment giving maximal falls, seen at 24 hr, of 22±4, 47±3 and 53±4mmHg in mean BP for doses of 0.3, 3.0 and 30mg/kg. Enalapril produced similar results with doses 10 fold higher. There was no antihypertensive effect of trandolapril in binephrectomized SHR suggesting that the kidneys (presumbably renal renin) are a prerequisite for antihypertensive activity. The antihypertensive effect was not modified if SHR were pretreated with indomethacin (5mg/kg, per os) suggesting that prostaglandins are not involved in the antihypertensive response.

In anaesthetized dogs, a single high dose (1mg/kg, i.v.) of trandolapril produced transient and modest (10%) decreases in BP. However, in anaesthetized dogs, pretreated by diuretics to elevate PRA, trandolapril produced dose-related decreases in mean BP of 6.0±2.7, 16.3±6.0, 23.3±5.9 and 28.7±5.8% at 0.03, 0.1, 0.3 and 1mg/kg. Heart rate and cardiac output were not increased so there were dose-related decreases in total systemic resistance, and left ventricular stroke and minute work.

Trandolapril is a potent inhibitor of ACE and has long-lasting antihypertensive activity following oral administration. The drug appears to act by vasodilatation and the degree of response is governed by the state of activation of the renin-angiotensin system.

DIFFERENCES BETWEEN TRANDOLAPRIL AND ENALAPRIL FOR INHIBITION OF TISSUE ACE ACTIVITY IN RATS

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ACE inhibitors have been shown to lower blood pressure (BP) in types of experimental hypertension which are independent on the circulatory renin-angiotensin system (Sweet et al., 1981 ; Unger et al., 1984). Since local generation of angiotensin II (Ang II) occurs in many organs and tissues such as brain, kidney and blood vessels (Deboden et al., 1983) ACE inhibitors could be active at these levels. The present study compared the effects of trandolapril, a new ACE inhibitor, and enalapril on serum and tissue ACE activity in normotensive Sprague Dawley rats.

Following a single oral administration of trandolapril (0.1mg/kg) or enalapril (0.3mg/kg) ACE activity (measured using hippuryl-His-Leu as substrate, Saavedra et al., 1982) was reduced after 30 min and remained significantly inhibited for 6 hr in serum, kidney, aorta, lung, heart ventricle, and adrenal cortex and medulla. There was no inhibition in striatum, suggesting that the compounds did not penetrate the blood-brain barrier. At 24 hr, serum ACE was still significantly inhibited by trandolapril (-63.1±4.7%, n=7), but not by enalapril. Similarly, enzyme activity in the ventricle remained inhibited with trandolapril (-80.5±4.8%) as well as that in the aorta (-91.7±1.3%).

The effects of different doses of trandolapril, enalapril or solvent (water) were investigated 2 hr after oral administration. Trandolapril inhibited (compared to solvent-treated animals) ACE activity in a dose-related manner in serum and in all tissues studied giving an ID_{50} of 4.0±0.5, 20.5±3.5, 19.7±2.8, 6.7±0.8, 7.7±0.9, 10.8±0.9 and 21.0±2.1ug/kg (n=7) in serum, ventricle, aorta, kidney, lung, adrenal medulla and adrenal cortex respectively. Enalapril was some 6-10 fold less potent producing ID_{50} of 47.5±6.3, 137.8±33.8, 57.8±9.3 and 53.1±8.4ug/kg (n=7) in serum, aorta, kidney and lung. In ventricle and adrenal medulla and cortex enalapril produced a plateau inhibitory effect of 40%, there being no further inhibition with increasing doses.

The doses of trandolapril and enalapril administered did not lower B.P. However, significant reductions in mean BP were obtained after 3.0 mg/kg trandolapril (-11.9±1.7%, n=10) and after 30.0 mg/kg enalapril (-10.7±1.2%, n=10). This suggests that plasma and tissue generation of Ang II are not responsible for maintaining BP in conscious normotensive Sprague Dawley rats.

The results confirm the potent oral ACE inhibitory activity of trandolapril. The differences in enzyme inhibition observed in ventricle and adrenal tissue between enalapril and trandolapril is difficult to explain, but could be due to the presence of more than one type of enzyme in these tissues, as has been proposed in the brain (Zubenko & Nixon, 1985).

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AUTORADIOGRAPHIC LOCALIZATION AND CHARACTERIZATION OF HUMAN CARDIAC β2-ADRENOCEPTORS

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Radioligand binding studies in homogenate preparations of human heart have demonstrated the existence of β_2-adrenoceptors in variable proportions (Stiles et al, 1984). Although there is evidence for β_2-adrenoceptor mediated positive inotropic and chronotropic responses (Gille et al, 1985; Zerkowski et al, 1986), the interpretation of receptor binding experiments in homogenates is limited by cellular heterogeneity, with contributions from β_2-adrenoceptors associated with nerves, blood vessels, and fibroblasts. The aim of this study was to characterize β_2-adrenoceptors in the human heart and investigate their distribution by autoradiography.

Tissue samples were obtained from right atrial appendage (n = 4), left atria (n = 1), pericardium (n = 1) and papillary muscle (n = 4) from patients undergoing cardiac surgery. No patient had received β-adrenoceptor or calcium antagonists. Samples were frozen within 20 min of removal and stored at -70°C until 10μm sections were cut, mounted onto microscope slides and labelled with (-) [^{125}I]-cyanopindolol ([^{125}I]-CYP) (50 pM) for 150 min at 25°C (Lew & Summers, 1985). After washing, the sections were wiped and counted in biochemical studies, or in autoradiographic studies apposed to either nuclear emulsion coated coverslips, (Kodak NTB3) or X-ray film and exposed dessicated at 4°C.

Specific binding of (-) [^{125}I]-CYP was stereoselective, (pK_D (-) propranolol, 8.97±0.02; pK_D (+) propranolol, 6.88±0.06; n=3) and saturable. A significant proportion of β-adrenoceptors present in atria and papillary muscle were β_2-adrenoceptors. Computer-assisted iterative curve fitting of antagonist competition curves to CGP 20712A (β_1-selective) and ICI 118,551 (β_2-selective) revealed 37-45% β_2-adrenoceptors in atria and 34-36% β_2-adrenoceptors in papillary muscle. In atria the pK_D values for CGP 20712A were 9.20±0.03 and 5.56±0.14 (n=4) and for ICI 118,551 9.17±0.44 and 6.41±0.14 (n=4). These values were used as a guide in choosing concentrations of CGP 20712A (100nM) and ICI 118,551 (70nM) required to delineate β_1- or β_2-adrenoceptors. Non-specific binding was defined by propranolol (1μM). X-ray film images showed a homogeneous distribution of β_1- and β_2-adrenoceptors in atria and papillary muscle. Higher resolution studies confirmed the homogeneous distribution of β_2-adrenoceptors localized to the cardiac myocytes. A low density of β_2-adrenoceptors was also found overlying the intimal surface of the intramyocardial blood vessels and in the pericardium.

In conclusion, β_2-adrenoceptors have been localized to cardiac myocytes, the intimal surface of intramyocardial blood vessels and to the pericardium. The distribution of β_2-adrenoceptors in the human myocardium supports a role for these receptors in control of the rate and force of the human heart and suggests other roles in the coronary vasculature and in the pericardium.

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STIMULATION OF CARDIAC LIPOLYSIS BY ISOPRENALINE: A STUDY USING THE PERFUSED RAT HEART

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Activation of cardiac lipolysis has been suggested to be responsible for a significant component of the increase in myocardial oxygen consumption which follows the administration of adrenoceptor agonists in both experimental animals (Vik-Mo et al, 1979) and man (Simonsen and Kjekshus, 1978). Despite this, the effects of pharmacological agents on lipolytic activity in this tissue have been little studied. The objective of this study, therefore, was to characterise the lipolytic activity of isoprenaline in isolated, working rat hearts, with particular reference to other metabolic and mechanical effects.

Hearts from male AH/A strain rats were perfused using the methodology of Strong et al (1979), except that acetate (1 mM) was the sole exogenous substrate. Once cannulated, the atrial perfusion pressure of the working heart was increased to 20 cm water. Hearts which could not develop peak left ventricular pressures of greater than 160 mmHg for 1 min were rejected. Working hearts were then equilibrated for 20 min, at a mean aortic pressure of 40 mmHg, before being exposed to isoprenaline for 4 minutes. Lactate and glycerol concentrations were determined in coronary effluent, collected both before and during exposure to isoprenaline. An index of ventricular contractile activity, dP/dt_{max}, was derived from the left intraventricular pressure signal. In these experiments isoprenaline enhanced glycerol efflux in a concentration-dependent manner, (Table 1). Interestingly, effects on both glycerol and lactate efflux, and ventricular contractile activity (dP/dt_{max}) occurred over the identical concentration range.

Table 1. Isoprenaline stimulated glycerol efflux in the working rat heart

Isoprenaline Concentration (M)	Mean glycerol efflux rate (± SEM; nmol/min) Before isoprenaline	After isoprenaline
10^{-9}	2.5 ± 0.4 (5)	2.4 ± 0.2 (5)
10^{-8}	2.3 ± 0.3 (6)	5.4 ± 0.8 (6)
10^{-7}	2.4 ± 0.2 (6)	8.5 ± 0.8 (6)
10^{-6}	2.3 ± 0.2 (6)	7.7 ± 0.2 (6)

In a subsequent study, pre-perfusion of the hearts with $3x10^{-8}$M propranolol (for 20 min) completely abolished the increased lipolytic activity following 10^{-8}M isoprenaline, whilst the increase in the rate of lactate efflux was inhibited by 98%.

In summary, isoprenaline stimulates lipolysis in a dose-dependent manner in the isolated, perfused rat heart, and this is probably consequent upon activation of ß-adrenoceptors. Thus, the isolated, working rat heart may be used to study the lipolytic potential of phamacological agents, and to simultaneously assess their effects on other aspects of cardiac metabolism and contractile function.

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Ca^{2+} AND OXYGEN DEPENDENCE OF NORADRENALINE-INDUCED CONTRACTION OF THE ISOLATED RAT PORTAL VEIN

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Contraction of the rat portal vein to noradrenaline (NA) is highly dependent on $[Ca^{2+}]_o$ (Sigurddson et al, 1975; Ebeigbe 1982) and on PO_2 (Fasehun et al 1986) ($[Ca^{2+}]_o$ = [free Ca ions]). In 95%O_2 (PO_2 = 580±12mmHg), responses to agonists are monophasic, and bigger than at a lower, more physiological PO_2 (16% O_2: 112±10mmHg), in which responses are biphasic.

We have now examined the $[Ca^{2+}]_o$ dependence of contraction in different O_2 tensions to determine whether $[Ca^{2+}]_o$ dependence and the effects of drugs which alter this are altered by PO_2. Isometric contractions of longitudinal strips of portal vein were recorded (male Wistar rats 245-255g; Krebs' bicarbonate saline, Ca^{++} 1.25mM, 16%O_2: 79%N_2: 5%CO_2. $[Ca^{2+}]_o$ sensitivities of spontaneous activity and contraction to NA 0.3µM were determined and repeated in 95%O_2 and 5%CO_2. Responses were obtained (1) as discrete responses (5 min) at each $[Ca^{2+}]_o$ level (non-cumulative), illustrating effects on the time course of contraction, or (2) NA was added when $[Ca^{2+}]_o$ was low and $[Ca^{2+}]_o$ was increased in steps (cumulative), allowing more rapid estimation of $[Ca^{2+}]_o$ sensitivity. These protocols were repeated (i) with $[Ca^{2+}]_o$ unbuffered, i.e. total Ca = $[Ca^{2+}]_o$ = 44µM to 5mM, (ii) buffered with NTA (nitrilotriacetic acid) and EGTA (2.5mM of each), wherein total Ca = 2.5mM to 10mM but $[Ca^{2+}]_o$ = 1µM to 5mM. The buffers allow accurate determination of $[Ca^{2+}]_o$ (otherwise impossible <0.1mM. Buffering is particularly necessary when normal sensitivity to $[Ca^{2+}]_o$ is increased. Sensitivity to $[Ca^{2+}]_o$ is expressed as $-\log(EC_{50})$ where EC_{50} is the $[Ca^{2+}]_o$ producing a response 50% of the maximum obtained within the range of $[Ca^{2+}]_o$ tested. The main points are summarised in the table. Mean values±s.e.m.

Table 1 Characteristics of $[Ca^{2+}]_o$/response relationship (cumulative Ca^{2+})

		Unbuffered		Buffered	
	O_2	16%	95%	16%	95%
$-\log EC_{50}$	spontaneous	2.92±.02	2.84±.06	2.70±.20	2.65±.6
	NA control	3.38±.06	3.35±.10	3.70±.03	3.50±.10
	NA Bay K 8644			4.10±.05	3.90±.05
Max.tension	NA control	0.83±.08	1.37±.07	0.59±.05	0.87±.06

O_2 did not influence spontaneous activity or the $[Ca^{2+}]_o$-sensitivity of NA-induced contraction with any experimental protocol. The increase in response at high PO_2 applied only at high levels of $[Ca^{2+}]_o$ (>2.5mM). The buffers caused some depression of maximum response to NA. However, the absolute responses at low $[Ca^{2+}]_o$ were not affected by the buffers, justifying their use in estimating $[Ca^{2+}]_o$ sensitivity. Judged by $-\log EC_{50}$, sensitivity was slightly higher in the buffers but this can be accounted for by the changed maximum. More interestingly, a clear increase in sensitivity was produced by Bay K 8644 (0.3uM) in buffers. This is difficult to demonstrate without buffers since the $-\log EC_{50}$ is approximately 4.

The early, transient component of the biphasic response, which is obvious only at the lower O_2 tension, appeared only at $[Ca^{2+}]_o \geq 0.63$mM. The very high $[Ca^{2+}]_o$ sensitivity of this component contrasts with those in several other blood vessels which survive in very low $[Ca^{2+}]_o$ and have been attributed to the release of intracellular Ca^{++} stores (van Breemen, 1977).

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ELECTROCARDIOGRAPHIC ANALYSIS OF THE ANTI-ANGINAL EFFECTS OF NICARDIPINE AND NITROGLYCERINE.

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In the majority of cases of human angina pectoris, sclerotic narrowing of one or more of the major coronary arteries results in a disequilibrium of O_2 supply and demand resulting in regional myocardial ischaemia. We have developed a protocol of transient myocardial ischaemia (TMI) in the dog (based on the original model described by Szekeres et al, 1976) which provides reversible and reproducible ECG responses to myocardial ischaemia.

Pentobarbitone-anaesthetised beagles were respired with room air and thoracotomised via the left 5th intercostal space. The cradled heart was prepared to receive a reversible ligature applied to the the left anterior descending coronary artery (LAD). Unipolar epicardial recording electrodes were sutured onto the predicted ischaemic zone of the left ventricle to provide 8 epicardiograms. An intravascular pacing catheter inserted into the right atrium was used to pace the heart at 50-80 beats. min^{-1} above resting HR for 1 min followed by a further 2 min during which the LAD was occluded. Ten min were allowed between challenges, thus establishing a 13 min repeat protocol. Control episodes were repeated until two consecutive challenges produced the same degree of S-T segment elevation in the epicardial ECGs. The S-T segment rises during control and post-drug challenges were calculated for two distinct phases:-a) during the rising peak insult from measurements made at 45, 60, 75, 90 and 105 s in the LAD occlusion period (5 time values) b) early during the recovery phase at 2 s intervals for the first 20 s (10 time values) following switching off pacing and releasing occlusion. The average of the final two control values was taken as 100% against which subsequent values were assessed. The time values were summated for each lead during each phase.

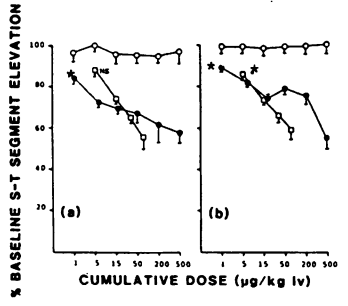

Figure 1 The cumulative i.v. effects of nicardipine(●,n=7) and GTN (□ n=5) on S-T segment elevation during the insult (a) and recovery (b) phases of TMI in the dog. Controls (o,n=4) represent an increasing no. of challenges. Each value is a mean ± SE bar. All points are significantly lower than controls at $P<0.001$ except where shown *$P<0.01$ and NS, Student's t-test.

Nicardipine and GTN dose-dependently inhibited S-T segment build-up during both the ischaemic challenge and the recovery phase (Figure 1). Both compounds enhanced the return to baseline values at all doses studied. ECG improvement also preceded overt haemodynamic effects with these drugs indicating that a direct myocardial O_2 balance improvement can occur independently of BP effects. This has already been shown with nicardipine which protects against pacing-induced tachycardia in angina patients in the absence of coronary blood flow changes (Rousseau et al, 1986). This property should prove most beneficial in chronic angina where one would expect the presence of an already-maximal reflex coronary vasodilation to be resistant to this property of an anti-anginal drug.

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COMPARISON OF HEMODYNAMIC EFFECTS OF FENOLDOPAM AND QUINPIROLE IN ANAESTHETIZED RATS

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Dopamine receptors characterized in the peripheral cardiovascular system appear to be of at least two distinct subtypes: the postjunctional DA-1 dopamine receptor found in the vascular smooth muscle (renal and mesenteric beds) and the prejunctional DA-2 dopamine receptor located on the postganglionic nerve neurons. Their stimulation provokes direct vasodilation and inhibition of noradrenaline release, respectively. The aim of this communication is to compare the hemodynamic profile of fenoldopam (SK&F 82526-J), a relatively selective DA-1 dopamine receptor agonist (Hahn et al., 1982; Cavero et al., 1986) and quinpirole (LY171555), a highly selective DA-2 dopamine receptor agonist (Hahn et al., 1983; Cavero et al., 1985).

Male normotensive Sprague Dawley rats (230-260 g) were anaesthetized with pentobarbitone and prepared to measure carotid blood pressure and the upper abdominal aorta (cardiac output), the mesenteric and renal artery and the terminal aorta (hindquarter) blood flows by using a pulsed Doppler technique (Hartley et al., 1974). Fenoldopam (20 µg/kg/min) and quinpirole (10 µg/kg/min) were infused over 15 min to rats pretreated with either i.v. saline, SCH 23390 (5 µg/kg/min) or S-sulpiride (30 µg/kg/min), which were given as infusions started 10 min before and continued throughout the administration of fenoldopam or quinpirole.

Fenoldopam and quinpirole decreased blood pressure significantly. The maximum of quinpirole effect (-35%) was reached at the end of the infusion and persisted for the subsequent 30 min. In contrast, the maximal hypotensive effect (-24%) of fenoldopam occured within the first 5 min but waned by 75% at the end of its infusion. Heart rate was increased (36%) by fenoldopam but decreased (-21%) by quinpirole.
Quinpirole did not change cardiac output (CO), increased (26%) briefly hindquarter blood flow (HQF), elevated (38%) renal blood flow (RF) and decreased (-17%) mesenteric blood flow (MF). Consequently, at the end of the infusion, total peripheral (TPVR), renal (RVR) and hindquarter vascular resistances (HQVR) were decreased more than the mesenteric resistances (MVR). Fenoldopam modified neither CO nor HQF but increased MF (23%) and RF (52%). Thus, MVR and RVR were decreased more than TPVR and HQVR.
S- sulpiride, a selective DA-2 dopamine receptor antagonist, inhibited significantly the hemodynamic effects of quinpirole. However, the increases in renal and hindquarter blood flows were in part reduced by SCH 23390, a selective DA-1 dopamine receptor antagonist. The latter compound blocked all the hemodynamic effects of fenoldopam which were not modified by S-sulpiride.

In conclusion, both the DA-1 and DA-2 dopamine receptor agonists studied were potent renal vasodilators. However, only fenoldopam increased mesenteric blood flow. Furthermore, the hypotensive effect of these compounds was only due to a fall in the systemic resistance. Finally, the inhibition of certain hemodynamic effects of quinpirole by SCH 23390 and S-sulpiride may indicate that the dopamine receptors in the renal and hindquarter vasculature share the properties of either subtype of dopamine receptors.

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DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNELS IN RAT BRAIN ARE INCREASED IN ETHANOL PHYSICAL DEPENDENCE

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Based on neurotransmitter release studies, we have previously suggested (Lynch & Littleton, 1983) that ethanol physical dependence is associated with increased sensitivity to Ca^{2+} of central neurones. Recently we showed (Hudspith & Littleton, 1986) that brain slice preparations from ethanol-dependent rats show an increased inositol lipid response to the dihydropyridine (DHP) Ca^{2+} channel activator BAY K 8644 and that Ca^{2+} "antagonists" of the DHP type are very effective inhibitors of the ethanol withdrawal syndrome (Little et al, 1986). These findings suggest that an increase in the number and/or function of DHP-sensitive Ca^{2+} channels on central neurones might play a role in alcohol physical dependence.

Male Sprague-Dawley rats (250-300 g) were treated chronically with ethanol by inhalation, as described previously (Lynch & Littleton, 1983). Rats were killed by decapitation while still intoxicated and cerebral cortices taken either for estimation of [^{3}H]-noradrenaline (NA) release in the presence of desipramine (superfusion system, release induced by alternating electrical pulses -1 Hz, 100 mA, 2 ms, for two periods S_1 and S_2 of -15 min each) or for [^{3}H]-nimodipine binding using the method of Glossman et al (1983) with minor modifications. In all experiments brain preparations from ethanol-dependent animals were compared direclty with those from controls kept under similar conditions.

The number of [^{3}H]-nimodipine binding sites on cerebral cortical membranes was increased 50% by chronic ethanol administration for 7 days (control B_{max} = 131 ± 14 fmol mg^{-1} protein; ethanol-dependent B_{max} = 196 ± 13 fmol mg^{-1} protein, means ± s.e.m., n = 5). This change was significant at the P = 0.02 level in a Mann-Whitney 'U' test. There was no significant alteration in the binding affinity.

The effect of dihydropyridines on the electrically induced release of [^{3}H]-NA was studied by their incorporation into the superfusate during the second (S_2) period of stimulation. Tetrodotoxin (5 x 10-8M) was present throughout in order to reduce the effects of Na^+ flux and Na^+/Ca^{2+} exchange on neurotransmitter release. Under these conditions the Ca^{2+} agonist BAY K 8644 (5 x 10^{-7}M) enhanced [^{3}H]-NA release from control preparations by 46 ± 8.8% whereas the equivalent value in preparations from ethanol-dependent rats was 99 ± 11.3% (means s.e.m., n = 5). The presence of nitrendipine (5 x 10^{-6}M), a concentration which had no effect alone, significantly inhibited the enhanced release induced by BAY K 8644.

These results are consistent with the hypothesis that an increased number and functional importance of DHP-sensitive Ca^{2+} channels on central neurones is associated with the development of physical dependence on ethanol. It is not yet possible to establish whether the increase in these channels is located presynaptically or post-synaptically, or whether it has a causal relationship to the altered Ca^{2+} sensitivity of neurotransmitter release previously noted (Lynch & Littleton, 1983). However, the ability of DHP Ca^{2+} antagonists to block the ethanol withdrawal syndrome at doses with no overt sedative effects in control animals (Little et al, 1986) suggests strongly that the change may be responsible for the latent neuronal hyperexcitability which underlies ethnaol physical dependence.

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EFFECTS OF THE CALCIUM CHANNEL ANTAGONIST, NITRENDIPINE, ON NITROUS OXIDE ANAESTHESIA, TOLERANCE AND WITHDRAWAL

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Administration of nitrous oxide (N_2O) at greater than atmospheric pressure causes the development of tolerance to its anaesthetic effects. Following the removal of the drug a withdrawal syndrome develops rapidly, characterised by squeaking, crossed forepaws and generalised seizures. Calcium channel antagonists have been shown to inhibit the ethanol (Little et al, 1986) and morphine (Ramakumar & El Fakahany, 1986) withdrawal syndromes. We have now investigated the effects of nitrendipine, a dihydropyridine calcium channel antagonist, on the withdrawal syndrome to nitrous oxide and the development of tolerance to this drug.

Male TO mice (25 - 32g) were injected (i.p.) with nitrendipine, 10, 50 or 100 mg kg^{-1}, suspended in Tween 80 (0.5% in distilled water). Concurrently tested controls received vehicle injections. Body temperatures were maintained at 37°C ± 0.5 for 2h following the injections. Animals were then placed in groups of 4 in a rotating cage inside a 20L pressure chamber, and the O_2 concentrations raised to 0.6%. Four N_2O concentrations were used, between 0.8 and 1.6 atm; each mouse was used once only. Exposure time to N_2O was 1h. Loss of righting reflex, i.e. failure to regain the upright posture within 60 secs of being rolled upside down, was assessed at intervals. Dose response curves, eight mice at each treatment, were constructed and ED_{50} values calculated, using probit analysis. Rectal temperatures were monitored in separate mice, in the chamber but not used for the tests, and maintained as above. At the end of the exposure mice were decompressed at 1 atm min^{-1}, removed from the chamber and suspended by their tails for 10 sec to precipitate the withdrawal syndrome. Occurrence of the withdrawal state, defined as a clonic seizure with crossed forepaws, was assessed by an observer who did not know the prior drug treatment.

Table 1: No. of mice showing withdrawal after N_2O, 1.2 atm, 60 min:

Vehicle:	8/8	Nitrendipine, 50 mg kg^{-1}:	2/8 **
Nitrendipine, 10 mg kg^{-1}	6/8	Nitrendipine, 100 mg kg^{-1}:	0/8 **

*P<0.05, **P<0.01 (cf.controls) Fisher's Exact Test

Table 2: N_2O ED_{50} values (atm), 95% confidence limits, Nitr=Nitrendipine (mg kg^{-1})

Drug	5 min	60 min	% change(5min - 60min)
Vehicle	1.35 (1.25 - 1.46)	1.51 (1.38 - 1.72)	+ 12 *
Nitr.(10)	1.37 (1.27 - 1.45)	1.31 (1.16 - 1.41)	- 5
Nitr.(50)	0.97 (0.89 - 1.12)+	0.91 (0.88 - 1.03)+	- 3
Nitr.(100)	0.83 (0.67 - 0.91)+	0.85 (0.76 - 0.92)+	+ 2

*P<0.05 (cf.5 min data) + P<0.001 (cf.controls) Chi squared analysis

Nitrendipine, at 50 and 100 mg kg^{-1}, significantly decreased withdrawal incidence and potentiated the anaesthetic effects of N_2O. After vehicle treatment there was a significant development of tolerance to N_2O over the 1h exposure, but this was not seen after any of the doses of nitrendipine. Similar results were obtained with different pretreatment times for nitrendipine. The results support our previous suggestions (Little et al, 1986; Dolin et al, 1986) that dihydropyridine-sensitive calcium channels may be involved in mechanisms of tolerance and dependence.

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