ADRENERGIC MODULATION OF THE RELEASE OF 5-HYDROXYTRYPTAMINE (5-HT) FROM THE VASCULARLY PERFUSED GUINEA-PIG ILEUM IN VITRO

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Recent experiments from our laboratory showed that the in vitro perfused guinea-pig ileum is very useful for studying the release of 5-HT from the enterochromaffin cells, the major source of intestinal 5-HT (Schwörer et al., 1987). There is evidence that adrenergic mechanisms may be involved in the regulation of intestinal 5-HT release (see Pettersson, 1979). Therefore, we studied the effect of adrenoceptor agonists and antagonists on the release of 5-HT from the perfused guinea-pig ileum.

Male guinea-pigs were anaesthetized with pentobarbitone (40 mg/kg i.p.) and artificially respirated. An ileal segment with its intact vascular supply was dissected out as described by Holzer & Lembeck (1979). The isolated segment, suspended in 200 ml Tyrode's solution, was perfused via the cranial mesenteric artery at a constant rate of 1 ml/min. 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined by HPLC with electrochemical detection (Schwörer et al., 1987). The perfusate of the first 90 min was discarded, then it was collected during 8 consecutive 5 min periods. Test substances were added to the perfusion medium after 100 min of perfusion.

The mean spontaneous outflow (determined between 90 and 100 min of perfusion) was 176 $\pm$ 9 pmol/g/5 min for 5-HT and 220 $\pm$ 10 pmol/g/min for 5-HIAA (n=46). In control experiments it remained constant during the following 30 min of perfusion.

Isoprenaline (0.1-1 µM) increased the outflow of 5-HT and 5-HIAA by maximally 50 % and this was antagonized by propranolol (0.1 µM). Forskolin (1-30 µM) enhanced the outflow of 5-HT by maximally 130 % and that of 5-HIAA by maximally 60 %. Clonidine (0.1-1 µM) reduced the outflow of 5-HT by maximally 45 %, an effect that was antagonized by tolazoline (1 µM). Propranolol (0.1 µM) or tolazoline (1 µM) alone did not significantly alter the outflow of 5-HT or 5-HIAA.

In the presence of tetrodotoxin (1 µM) the outflow of 5-HT was reduced by 45 % and that of 5-HIAA by 35 %. The effects of isoprenaline, forskolin and clonidine were also observed in the presence of tetrodotoxin excluding an indirect modulation of 5-HT release via the release of neurotransmitter substances.

In conclusion, the release of 5-HT from the enterochromaffin cells is facilitated by activation of ß-adrenoceptors or direct activation of adenylate cyclase and inhibited via α-adrenoceptors.

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Supported by the Deutsche Forschungsgemeinschaft.

NPY POTENTIATION OF α-MEDIATED CONTRACTILE ACTIONS IN PORTAL VEIN - TRUE SYNERGISM OR ADDITIVE EXCITATORY EFFECTS?

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Neuropeptide Y (NPY) is a 36 amino acid peptide that has been reported to coexist with noradrenaline (NA) in many perivascular sympathetic neurones (Uddman *et al*,1986) and to potentiate the effects of α-mediated NA responses post-synaptically (Edvinsson *et al*,1984;Pernow *et al*,1986). This potentiation is not confined to NA alone as histamine's contractile effect is also potentiated in rabbit blood vessels (Edvinsson *et al*,1984). The aim of the present study was to look at the general features of this potentiation and establish whether this is a form of true potentiation or a general subthreshold additive effect.

Male Sprague Dawley rats (250-350g) were killed by stunning and exsanguination. The portal vein was removed and 3-4mm lengths were mounted by means of wire stirrups in 3ml organ baths. The resting tension was 1g and the preparations were allowed to equilibrate for 60 minutes in 2.54mM Ca^{2+} Krebs solution bubbled with $O_2/5\%CO_2$ at 37°C. Non-cumulative dose response curves were constructed using a randomised pattern of drug additions on a 15 minute dose cycle, with a period of 1 minute allowed between doses of the 'potentiating agents' and the spasmogen. Contractions were expressed as a percentage of the maximum response to phenylephrine(PE) established at the beginning of each experiment.

In this preparation NPY, administered 1min before PE, at concentrations of 10nM or less rarely produced contraction *per se* but caused a leftward shift in the dose response curve to PE in a concentration-dependent manner : 1, 10 and 100nM NPY producing shifts of approximately 0.25, 0.5 and 0.75 log units ,respectively. It was also found that 10nM NPY significantly enhanced contractile responses to arginine vasopressin (AVP), bradykinin (BK) and eledoisin (ELE) by approximately half a log dose unit. The potentiation of the various spasmogens by NPY did not appear to be a general subthreshold additive effect because, on replacing 10nM NPY with a subthreshold/threshold dose of AVP, BK or ELE, only AVP was seen to potentiate the PE response.

The leftward shift of the PE dose response curve caused by 10nM NPY was also found to be unaffected by 2μM verapamil, although the responses of both curves with and without NPY were very much reduced. This leftward shift was also found in the presence of 1.25mM Ca^{2+} solutions, and in preliminary matched experiments the potentiation seemed to remain in zero Ca^{2+} solutions (no EGTA), though subsequent control responses to PE were more reduced than those found in matched control tissues. It would appear possible that NPY might be acting by mobilising the intracellular Ca^{2+} stores in some way, as has already been suggested by Edvinsson *et al*,(1987). However, the experiments with BK and ELE administered just prior to PE suggest this is not a mechanism common to all excitatory peptides in this tissue.

D.V.C. is an MRC scholar.

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EFFECT OF HYPOXIA ON THE PHARMACOLOGICAL RESPONSIVENESS OF SHEEP CIRCUMFLEX CORONARY ARTERY RINGS

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During coronary hypoperfusion, coronary artery smooth muscle is subjected to marked alteration in pH, ionic environment and oxygen supply. The aims of the present study were firstly to investigate the direct effects of hypoxia on isometric tension development in sheep coronary artery rings and secondly to determine if hypoxia modified the responsiveness of the arteries to the vasoconstrictor substances K^+, U-46619 a thromboxane mimetic, 5-HT and to the vasodilator, adenosine. Ring segments of the left circumflex coronary artery (O.D. = 3-4 mm) were set up in an organ bath under a resting tension of 1.5 g and isometric tension generated per cross sectional area of the vessel measured. The Kreb's solution was bubbled with 95% O_2 5% CO_2 or with 95% N_2 5% CO_2 to yield a PO_2 in the bath of 520 ± 12 and 45 ± 4 mmHg respectively.

Lowering the PO_2 of the bathing solution caused a sustained contraction which was slightly potentiated by phentolamine 10^{-6}M plus propranolol 10^{-6} M (14.9 ± 2.9 vs 19.1 ± 3.1 gcm^{-2}) markedly reduced by verapamil 10^{-5}M (26.6 ± 3.5 vs 6.8 ± 3.4 gcm^{-2}) and completely abolished by indomethacin, 10^{-6}M (19 ± 2.9 vs -10.2 ± 2.1 gcm^{-2}). These experiments demonstrate that this hypoxic induced contraction in isolated sheep coronary arteries is partly dependent upon extracellular Ca^{++} and is likely to be mediated via the release of vasoconstrictor prostanoids. Hypoxia may also cause the release of noradrenaline which has a vasodilator action.

In order to study the responsiveness of the coronary arterial rings to K^+, U46619, 5-HT and adenosine, cummulative dose response curves to each agent were constructed before and after lowering the PO_2. In the presence of hypoxia, each prepration was reset to the optimum resting tension before reintroduction of the drug. K^+, U46619 and 5-HT caused a dose related vasoconstriction whereas adenosine relaxed arteries precontracted with K^+.

Table 1. EC_{50} values and the maximum contractile or relaxant effect of K^+, U46619, 5-HT and adenosine under normal and hypoxic conditions.

	EC_{50}		Max effect (gcm^{-2})	
	Normal	Hypoxic	Normal	Hypoxic
Potassium, mM	23.9±1.1	23.4±0.9	81.3±5.8	66.4±2.1*
U46619, μM	0.30±0.03	0.19±0.05*	48.6±14.0	129.8±29.9*
5-HT, μM	2.95±0.8	0.78±0.14*	42.6±7.8	48.3±13.8*
Adenosine, mM	0.70±0.1	0.6 ±0.06	86.7±16.4	107.7±10*

Values are mean ± s.e.m. N = 6 - 10. *P<0.05 significantly different from value in normal conditions.

Table 1 shows that hypoxia shifted the dose response curves to U46619 and to 5-HT to the left and markedly increased the maximum tension developed by these agents. In contrast, the dose response curves to K^+ and to adenosine were not shifted by hypoxia. The maximum tension developed by K^+ was reduced in the presence of a low PO_2 but the maximum relaxation induced by adenosine was enhanced. These results indicate that hypoxia modifies the responsiveness of coronary arteries to both vasoconstrictor and vasodilator substances. The mechanism(s) underlying these changes are not known and are currently being investigated.

Y.W. Kwan is supported by an Industrial Studentship from Pfizer Central Research.

THE ANTAGONISTIC EFFECT OF R 56865 ON α_1-ADRENOCEPTOR-MEDIATED CONTRACTION IN ISOLATED RAT AND GUINEA-PIG AORTA

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R 56865 (N-[1-[4-(4-fluorophenoxy)butyl]-4-piperidinyl]-N-methyl-2-benzothiazol-amine) is a compound that displays anti-ischaemic activity and decreases the toxicity of cardiac glycosides in the heart. In this study we examined the effects of R 56865 on contractile responses and ^{45}Ca movements induced by noradrenaline (NA). We compared R 56865 with the calcium-entry blocker nifedipine.

After removal of the endothelium thoracic aortic rings from male Wistar rats (200-300 g) or guinea-pigs (300-400 g) were suspended in Tyrode-solution (0.9 mM Ca^{2+}) gassed with 95% O_2 and 5% CO_2 at 37°C at a resting tension of 1 g. Cumulative dose-response curves were obtained after a pretreatment period of 30 minutes. For the measurement of ^{45}Ca movements we used the method described by Wermelskirchen et al. (in press). In brief, rat isolated aortic strips were equilibrated in Tyrode-solution (37°C) gassed with 95% O_2 and 5% CO_2 at a resting tension of 0.33 g. After pretreatment for 60 minutes, the strips were labelled for 5 minutes in ^{45}Ca (1 µCi/ml) Tyrode-solution in the presence or absence of NA (10^{-5} M). Thereafter, the strips were washed for 45 minutes with a Tyrode-solution of 4°C and the residual ^{45}Ca was measured.

The concentration-response curves for the NA-induced contractions were dose-dependently shifted to the right and their maxima depressed by R 56865 (10^{-8} - 10^{-5} M) in rat aorta. In guinea-pig aorta rightward shifts of the NA-induced concentration-response curve by R 56865 (10^{-7} - 10^{-5} M) were also observed. In both species R 56865 in a concentration of 10^{-6} and 10^{-5} M was equipotent. In rat aorta addition of prazosin (10^{-7} M) completely inhibited the NA-induced contraction remaining after pretreatment with R 56865 (10^{-5} M). Nifedipine (10^{-9} - 10^{-7} M) attenuated the NA-induced contractions in the rat, but not in the guinea-pig aorta. In the rat aorta the effects of R 56865 (10^{-7}- 10^{-5} M) and nifedipine (10^{-7} M) on the concentration-response curve of NA were independent of each other. Nevertheless, R 56865 (10^{-7} - 10^{-5} M) antagonized the NA (10^{-5} M)-induced increase in slowly exchanging ^{45}Ca without affecting the basal uptake of slowly exchanging ^{45}Ca.

In conclusion, two equi-effective concentrations, 10^{-6} and 10^{-5} M, of R 56865 with respect to its non-competitive interaction with NA seems to indicate that it does not directly react with α_1-adrenoceptors in rat and guinea-pig aorta. The lack of interaction between R 56865 and nifedipine on the NA-induced contraction in the rat aorta does not support calcium entry blocking properties of R 56865 (10^{-7} - 10^{-5} M) as far as α_1-adrenoceptor-mediated calcium influx is concerned. The antagonism by R 56865 of the NA-induced increase in slowly exchanging ^{45}Ca may indicate that R 56865 (10^{-6} - 10^{-5} M) decreases the storage of calcium in those slowly exchanging compartments which increase upon NA administration. Therefore it is impossible to measure calcium entry blocking properties of R 56865 by measuring slowly exchanging ^{45}Ca.

Wermelskirchen, D., Wilhelm, D., Wilffert, B., Pegram, B. L., Hunter, J., Nebel, U. & Peters, T. (1988) Pharmacology, in press.

A DOSE-DEPENDENT INHIBITION OF DIGITALIS-INDUCED TOXICITY BY R 56865 IN THE GUINEA-PIG HEART-LUNG PREPARATION

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First observations indicated that R 56865 (N-[1-[4-(4-fluorophenoxy)butyl]-4-piperidinyl]-N-methyl-2-benzothiazolamine) elicits beneficial effects with regard to digitalis - induced changes of action potentials in guinea-pig papillary muscle (Vollmer et al., in press). Therefore the effects of R 56865 on cardiac contractile function and haemodynamic parameters were investigated in blood perfused guinea-pig hearts during cumulative digitoxin application.

The experimental model was an autoperfusing heart-lung-preparation (according to Starling) modified for small rodents. The experiments were performed in guinea-pigs of either sex, weighing 300-350 grams. The animals were respirated with 95% O_2 and 5% CO_2. During the surgical procedure the respiration gas was mixed with halothane (2-3%). The hearts were perfused with whole blood at 37°C. Measured parameters were LVP, $LVdP/dt_{max}$ and cardiac output. The hearts were paced at a constant rate of 320 beats/min.

Control experiments (n= 11) were performed by applying increasing concentrations of digitoxin (10^{-7} - 3 x 10^{-6} M) to the hearts after an equilibration period of 75 min. Each dose of digitoxin was allowed to equilibrate for 20 min. In that way the digitoxin concentration in the circulating blood was augmented until cardiac output was reduced to zero. The pretreated hearts received R 56865 (10^{-9} M, n= 7; 10^{-8} M, n= 8; 10^{-7} M, n= 9 and 10^{-6} M, n= 7) 30 min prior to the addition of digitoxin. Under these conditions pretreatment with R 56865 had no influence on either LVP, $LVdP/dt_{max}$ or cardiac output. The addition of digitoxin up to a concentration of 6 x 10^{-7} M caused a clear positive inotropic effect both in pretreated and in not-pretreated hearts. Pretreatment with R 56865 above a concentration of 10^{-9} M even resulted in a more pronounced positive inotropic effect at 10^{-6} M digitoxin. In control hearts concentrations of digitoxin above 10^{-6} M led to a marked loss of mechanical function whereas pretreated hearts (10^{-7} and 10^{-6} M R 56865) still showed a positive inotropic effect upon digitoxin addition. Digitoxin (3 x 10^{-6} M) completely abolished cardiac function in control hearts. Pretreated hearts nevertheless exhibited a dose-dependent conservation of cardiac mechanical function.

Toxic concentrations of cardiac glycosides diminish Na-K-transport due to an inhibition of the Na-K-ATPase. Cellular loss of potassium and gain of sodium as well as shortening of the action potential duration and a decrease of the resting potential are the result. Additionally a net calcium uptake can be observed. In consequence, electrical and mechanical function of the heart is lost. R 56865 diminished toxic effects of digitalis in a dose-dependent manner. R 56865 did not change contractile parameters under control conditions but increased digitoxin's positive inotropic effect. The loss of mechanical function caused by intoxication with digitoxin was significantly reduced by pretreatment with R 56865.

Vollmer, B., Meuter, C. & Janssen, P.A.J. (1988) Eur. J. Pharmac., in press.

THE ANTIARRHYTHMIC EFFECTS OF R56865 IN CARDIAC GYLCOSIDE TOXICITY ARE NOT CAUSED BY INHIBITION OF RECEPTOR BINDING

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R 56865 (N-[-1[4-(4-fluorophenoxy)butyl]-4-piperidinyl]-N-methyl-2-benzothiazol-amine) exerts strong antiarrhythmic effects in case of cardiac glycoside (c. g.) intoxication of the guinea-pig papillary muscle (Vollmer et al., 1988) and in the guinea-pig heart-lung preparation (Schneider et al., 1988). In the effective concentration range (3×10^{-10} to 1×10^{-6} M) R 56865 neither influences the resting membrane potential nor the shape of the action potential nor cardiac contractile force and frequency under control conditions but it prevents c. g. induced extreme shortening of the action potential, decrease of action potential amplitude, lowering of the resting membrane potential, and the occurrence of delayed afterdepolarizations and allows a proper development of the inotropic response. The present investigation was conducted in order to evaluate whether R 56865 interfers with the properties of the binding of c. g. to the Na-K-ATPase.

Na-K-ATPase was prepared from pig-hearts according to the method of Matsui and Schwartz (1966). Under control conditions the specific activity was 45 µmoles ATP/mg protein x h. R 56865 had no influence on enzyme activity over a wide concentration range (10^{-9} to 10^{-6} M). The inhibition by ouabain of the enzyme activity was identical in the absence and presence of R 56865.
Rate of binding and binding equilibria of ^{3}H-ouabain to a crude membrane preparation of guinea-pig heart were not influenced by R 56865 (1×10^{-6} M). K_D-values were 1.39 ± 0.18 and $1.36 \pm 0.25 \times 10^{-7}$ M and B_{max} 9.15 ± 0.29 and 9.35 ± 0.42 pmoles/mg protein in the control and the treated group, respectively.
Isolated atria from guinea-pigs of either sex were incubated in normal Tyrode solution containing 0.9 mM Calcium, at 32°C. Atria were paced and equilibrated for 60 min. R 56865 was added during this period to allow 30 min equilibration. Then ^{3}H-ouabain was added and tissue radioactivity was determined after different time periods up to 4 h. The binding process of ^{3}H-ouabain at a mechanically subthreshold concentration of 1×10^{-8} M remained completely unaffected by R 56865 (3×10^{-7} M). At a concentration of 5×10^{-7} M and a frequency of 2 Hz ouabain acted transitorily positive inotropic (control tension: 7.12 ± 0.5 mN; maximum after 30 min: 16.1 ± 3.4 mN; mean ± S.D., n= 5), followed by a negative inotropic phase. In contrast, a concentration of 1×10^{-6} M R 56865 allowed the development of a monophasic fully pronounced positive inotropic effect throughout the period of observation. In the respective control binding experiment the maximum was attained after 120 min (0.65 ± 0.06 µmoles/kg w.w) when toxicity had already progressed. In the presence of R 56865 the binding process reached final equilibrium after 60 min (0.43 ± 0.03 µmoles/kg w.w.) the curve characteristics being very similar to those observed for the development of contractile force under identical conditions.
It appears that R 56865 does not antagonize toxic effects of c. g. directly at the receptor level. Binding of ouabain has been shown to be a positive cooperative process (Herzig et al., 1985). Binding of a c. g. favours further binding due to an increase of intracellular Na which activates the pump, thus increasing the number of actually available receptor sites. R 56865 inhibits excessive binding under toxic c. g. concentrations. This would be in keeping with the speculation that the compound inhibits c. g. induced Na- or Na-Ca-overload.

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PERTUSSIS TOXIN AND PREJUNCTIONAL α_2-ADRENOCEPTORS IN RAT HEART AND VAS DEFERENS

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Pertussis toxin attenuates α_2-adrenoceptor mediated inhibition of adenylate cyclase in vascular smooth muscle (Boyer et al., 1983). The present study seeks to examine whether the use of pertussis toxin can provide evidence that stimulation of prejunctional α_2-adrenoceptors results in inhibition of adenylate cyclase.

Male wistar rats (250-300 g) were injected intraperitoneally with pertussis toxin (50-200 $\mu g\ kg^{-1}$) or with an equivalent volume of vehicle (50% glycerol, 50% phosphate 0.05M-NaCl 0.5M, pH 7.2), and 18 hours later, rats were either pithed under ether anaesthesia or epididymal portions of vas deferens were obtained.

Resting heart rate (HR) was $333\pm12.6\ min^{-1}$ (n=7) and $267\pm13.4\ min^{-1}$ (n=6) and resting DBP was 25.4±2.3 mmHg and 34.5±3.5 mmHg in pithed rats pretreated with pertussis toxin or vehicle, respectively. Resting HR was significantly higher and resting DBP significantly lower in pertussis toxin treated rats (Student's t-test, $P<0.05$). Pretreatment with pertussis toxin (50-135 $\mu g\ kg^{-1}$) markedly reduced the pressor response to the α_2- adrenoceptor agonist xylazine but did not significantly alter the cardioacceleration to a single stimulus, nor the potency of xylazine at inhibiting this cardioacceleration (xylazine cardio-inhibitory ID_{50}: vehicle 3.16 $\mu g\ kg^{-1}$; pertussis toxin 3.63 $\mu g\ kg^{-1}$; n=4 each).

In epididymal portions of rat vas deferens in the presence of nifedipine (10 μM) to eliminate postjunctional actions of agonists, there was no significant difference in the isometric contraction to a single electrical stimulus, nor in the potency of xylazine at inhibiting this stimulation- evoked contractions, between animals pretreated with pertussis toxin (100- 200 $\mu g\ kg^{-1}$) or vehicle, with IC_{50} values of 32.4 nM (95% confidence limits of 17.8-58.9 nM, n=6) and 20.4 nM (7.6-55.0 nM, n=6), respectively.

In conclusion, the present results with pertussis toxin lend no support for the view that α_2-adrenoceptor mediated inhibition of neurotransmission in the periphery involves inhibition of adenylate cyclase. This is in agreement with the results of Musgrave et al.,(1986) who found that pertussis toxin did not alter the prejunctional inhibitory action of clonidine in mouse atria.

Supported by the Royal College of Surgeons in Ireland.

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NEUROPEPTIDE Y-INDUCED CONTRACTION IN HUMAN SAPHENOUS VEIN: EFFECT OF CALCIUM ANTAGONISTS

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Neuropeptide Y (NPY) is a potent constrictor of some mammalian blood vessels. However, it is usually ineffective in large arteries. We have recently reported that NPY is an effective constrictor of human resistance arteries and saphenous vein (Schachter et al., 1987). We have used the saphenous vein as a model to investigate aspects of the vascular effects of this peptide, in particular its interaction with calcium channel blockers and its dependence on the presence of extracellular calcium.

Human saphenous vein was obtained from patients undergoing coronary by-pass grafting. Rings of vessel were suspended in Krebs-Ringer buffer aerated with O_2 95%/CO_2 5% and attached to an isometric transducer. If the rings contracted in response to depolarisation (120 mM KCl) they were used for further study. After re-equilibration segments were challenged with 100 nM NPY, in the presence or absence of one of the antagonists listed below. All drugs were used at 1 μM concentration, with pre-incubation for 20 min. In some cases normal buffer, containing 2.5 mM $CaCl_2$ was replaced by calcium-free Krebs buffer. Results are expressed as the percentage contraction compared to the control response (median and range), and statistical analysis was performed by the Mann-Whitney test.

Antagonist (no.)		Median (Range)	
Nifedipine	(6)	0	(0-100) *
Nimodipine	(6)	59	(22-206)*
Nisoldipine	(5)	46	(0-60) **
Nitrendipine	(5)	86	(32-131)
Diltiazem	(7)	119	(46-258)
Flunarizine	(9)	93	(0-182)
Verapamil	(8)	140	(46-258)
Ca-free buffer	(5)	0	(all) **

* : inhibition significant at $P<0.05$; ** : at $P<0.01$

These results indicate that 3 calcium channel blockers of the dihydropyridine class are effective antagonists of NPY-induced contractions in human saphenous vein. Other types of calcium antagonists appear to be much less active in this system. It is unclear whether this is related to vessel type, since antagonists other than dihydropyridines may be less potent in veins than arteries (Sjoberg et al., 1987). In contrast to the results of Pernow and Lundberg (1986), it also shows that contractile responses to NPY may be absolutely dependent on the presence of extracellular calcium. Finally, the study confirms that the saphenous vein is a useful model for the pharmacological study of isolated human blood vessels (Docherty, 1987).

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BRADYKININ BUT NOT CAPSAICIN STIMULATION OF AFFERENT FIBERS IN RAT TAIL INVOLVES PROTEIN KINASE C ACTIVATION

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Phorbol esters which activate protein kinase C, depolarize sensory neurones (Lindsay & Rang, unpublished), non-myelinated vagal C-fibers (Rang & Ritchie, 1987) and stimulate capsaicin-sensitive nociceptive afferents in vitro (Bettaney et al, 1987). Bradykinin produces pain by stimulating peripheral nociceptors and probably activates intracellular protein kinase C in a number of cell types (Miller, 1987) including sensory neurones (Burgess unpublished). We have examined the possible involvement of protein kinase C in the action of bradykinin on peripheral nociceptors.

The spinal cord with tail attached was isolated from 1-2 day old rat pups following decapitation and the surface of the skin was removed from the tail. The spinal cord and tail were superfused separately with a physiological solution (composition mM: NaCl 138.6, KCl 3.35, $CaCl_2$ 1.26, $MgCl_2$ 1.16, $NaHCO_3$ 21.0, NaH_2PO_4 0.58, glucose 30.0) at 24°C and equilibrated with 95% O_2, 5% CO_2. Activation of peripheral fibres was assessed by recoring lumbar ventral root (L_3-L_5) depolarization via an extracellular glass micropipette.

When calcium was removed from the tail superfusate, reproducible ventral root depolarizations were obtained to submaximal doses of 4 B-phorbol 12,13 dibutyrate (PDBu, 1μM, 60sec), bradykinin (0.5-0.8μM, 10sec) and capsaicin (0.5-0.8μM, 10sec) applied to the tail. Staurosporine (50-100nM), a protein kinase C inhibitor, perfused on the tail attenuated or abolished the responses to bradykinin and PDBu, but not capsaicin. PDBu and bradykinin were ineffective following functional impairment of Aδ and C fibres by capsaicin (20μM). Conversely, during desensitization to PDBu (1μM), bradykinin was ineffective but capsaicin responses were unchanged. Desensitization with bradykinin (20μM) however, did not change responsiveness to PDBu or capsaicin.

These data confirm that PDBu and bradykinin stimulate capsaicin sensitive fibers. Bradykinin, but not capsaicin, activation involves PDBu sensitive protein kinase C. Furthermore bradykinin desensitization does not appear to involve inactivation of protein kinase C.

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CROSS-DESENSITIZATION OF CARDIAC β-ADRENOCEPTORS AND ITS APPLICATION TO THE CALCULATION OF AGONIST AFFINITIES

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Cardiac β_1-adrenoceptors have been shown to undergo desensitization after prolonged exposure of isolated atria to isoprenaline (Kaumann & Birnbaumer, 1976; Broadley & Herepath, 1987). Other β-agonists, such as terbutaline, also induce desensitization in tissues such as the spleen (Hasegawa & Townley, 1983). The present study examines whether in vitro exposure of atria to isoprenaline desensitizes the β-adrenoceptor-mediated responses to orciprenaline and terbutaline.

Guinea-pig paced left atria (2Hz, 5ms, threshold voltage + 50%) were set up in Krebs-bicarbonate solution containing ascorbic acid (1mM) at 37.5°C gassed with 5% CO_2 in O_2. Increases in isometric tension in response to cumulative addition of (-)-isoprenaline, (±)-terbutaline or (±)-orciprenaline were recorded. At the maximum effect of isoprenaline (10^{-6}M), it was left in contact for 4h and pacing was stopped. Pacing was then resumed and the tissue washed (x5) for 1h before constructing a second isoprenaline curve. In cross-desensitization experiments, orciprenaline or terbutaline were added and at the maximum (1.4 M and 0.27mM), the stimulator was turned off and the agonist replaced by isoprenaline (10^{-6}M) for 4h. The isoprenaline was then washed out (x5) for 1h before constructing a second curve to the agonist. Pre-incubation curves were corrected from time-matched controls and n>4.

Desensitization of atrial β_1-adrenoceptors was exhibited as a significant rightwards shift of the concentration-response curve to isoprenaline following 4h exposure to isoprenaline. The EC50 values (measured at the 50% response on the first curve) were 8.9 and 31.0nM. The maximum response (96.5±6.3%) was not significantly reduced. The left atrial tension responses to orciprenaline were also desensitized by the 4h exposure to isoprenaline, the EC50 increasing significantly from 77 to 150nM. There was a small non-significant depression of the maximum response to 90.4±2.0%. The maximum tension response to terbutaline, however, was significantly depressed to only 34.3±2.5% by incubation with isoprenaline. The rightwards shift of the curve, measured at the EC20, was significantly increased from 7.4 to 41.0μM. Terbutaline was a partial agonist (60.2±2.6% of the isoprenaline maximum), which explains why removal of receptors by desensitization had a more marked effect than on isoprenaline or orciprenaline maxima.

The depression of maximum response was utilized to calculate the dissociation constant (K_A) of terbutaline by the method of Furchgott (1966). Equiactive concentrations of terbutaline on pre- (A) and post-desensitization (A') curves of individual experiments were plotted as their reciprocals and K_A calculated as (slope-1)/intercept. The geometric mean K_A value was 34.0(7.8-140)μM which, as expected for a partial agonist, exceeded the EC50 value (7.3(2.5-21)μM) by a factor of only 4.6. At the 46.8% response, this K_A value yielded a receptor occupancy value of 28.8%. This study therefore demonstrates cross-desensitization of β-adrenoceptor-mediated responses of isolated atria between isoprenaline and orciprenaline or terbutaline. A novel method for calculating affinity of β-adrenoceptor agonists is provided when desensitization induces a depression of the maximum response.

Supported by the British Heart Foundation.

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A BRONCHOCONSTRICTION RESPONSE TO ADENOSINE OF GUINEA-PIG AIRWAY PERFUSED LUNGS

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For adenosine to be considered as a possible mediator in bronchial asthma, it would be expected to possess bronchoconstrictor properties. Yet, the predominant response of isolated tracheal preparations is a relaxation (Jones et al. 1980; Darmani & Broadley, 1986). Although administration of adenosine to human subjects has little effect on the airways, in asthmatic patients a bronchoconstrictor effect can be induced (Cushley et al. 1983). The present study therefore examines possible differences between the response to adenosine of guinea-pig isolated trachea and perfused lungs and the effects of sensitization.

Isolated tracheal spirals (3-4cm) from guinea-pigs (400-550g) were set up in Krebs-bicarbonate solution gassed with CO_2 (5%) in oxygen at 37.5°C under a resting tension of 10mN. After 60 min equilibration, tissues developed intrinsic tone and cumulative concentration-response curves for adenosine were constructed. Lungs were perfused at constant flow (5.0ml min^{-1}) with Krebs-bicarbonate solution via the tracheal stump. Perfusion pressure was recorded by means of a pressure transducer at the tracheal cannula, at which point a mercury manometer was also included. Drugs were introduced by bolus into the perfusion solution. Sensitization of guinea-pigs was achieved by giving i.p. injections of ovalbumin at 14 (5mg in 0.1 ml water for injection) and 12 days (10mg) prior to use. Adenosine produced concentration-dependent relaxation of tracheal spirals from untreated guinea-pigs. After sensitization with ovalbumin no contractile component was apparent and there was no evidence of any opposing contractile activity. Indeed, the magnitude of the relaxation in sensitized preparations was greater; the response to the maximum concentration of adenosine (2.5mM) after sensitization (4.7±0.32mN) was significantly greater ($P<0.05$) than in untreated controls (3.6±0.26mN).

The relaxation response to adenosine could only be demonstrated in perfused lungs after raising the perfusion pressure by constant perfusion with carbachol (1.1μM). Adenosine then produced dose-related falls in perfusion pressure which reached a maximum of 11.7±1.2mmHg at the 10mg dose. In normally perfused lungs, adenosine produced a small constrictor response. However, dose-related constrictions could not be demonstrated since usually only one response could be elicited in each preparation. The possibility was considered that tachyphylaxis to this response may occur. Therefore the following protocol of drug administration was adopted for lungs from untreated, sensitized and sham-injected guinea-pigs: carbachol (10μg), adenosine (300μg), adenosine (300μg), ovalbumin (200μg), ovalbumin (200μg). The bronchoconstrictor responses to carbachol were not significantly different between the untreated (21.7±3.2mmHg) and sensitized (23.5±4.6mmHg) lungs. The constriction induced by adenosine, however, was significantly greater in the sensitized preparations (16.2±3.8mmHg) than either sham injected (2.0±0.9mmHg) or untreated controls (2.9±1.0mmHg). The response to the second exposure to adenosine was significantly less in each case, being 4.1±1.3mmHg in sensitized lungs. Sensitization was confirmed by a large constrictor response to challenge with ovalbumin (58.7±3.7mmHg) which was substantially less on second exposure (2.7±1.1mmHg).

This study has therefore demonstrated a bronchoconstrictor response to adenosine in perfused lungs which is enhanced by prior sensitization with ovalbumin. This, like the challenge with antigen, displays tachyphylaxis.

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HOW MUCH CURRENT TRAVERSES ELECTRICALLY-STIMULATED TISSUES?

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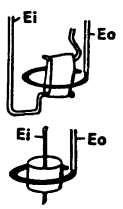
Electrical Field Stimulation (EFS) of isolated tissues attempts to elicit neurogenic responses by the administration of a series of electrical pulses. Experimental conditions to reproduce and compare such responses in a given tissue and between different tissues must be defined. Pulse waveform, duration and frequency and length of the pulse train are reproducible objective measurements as is the magnitude of the current delivered to the tissue bath. However the fraction of this current which traverses the tissue and elicits the response has not to our knowledge been measured previously. We found it necessary to define this fraction in order to design a power source capable of delivering a constant current bipolar stimulus to ring preparations of human cerebral arteries and to determine an appropriate electrode configuration. We report a method whereby the relative electrical impedances of tissue and bathing solution are defined and the fraction of delivered current traversing an isolated tissue preparation determined. A Wayne Kerr Admittance Bridge (WKB) operating at 1.589kHz was used to measure the electrical admittance of tissue baths containing Krebs solution (k) and either a tissue sample (s) or a high-impedance polythene ring of similar dimensions (p). Admittance (unit = Siemen) = G + jBc where G is conductance, Bc is capacitive susceptance and j is a constant. In order to determine the circuit equivalent of k,s resistance was measured separately using a direct current digital multimeter operating as an ohmmeter. Resistance measured in this way was high and differed from 1/G, indicating that k,s could best be described by a series circuit comprising a resistance and capacitance. The impedance (unit = Ohm) of such a circuit is represented by $R_t - jXc$, where Xc is capacitive reactance and R_t is the combined resistance of Krebs solution and tissue. From the WKB output R_t was calculated as $G/(G^2+Bc^2)$ and Xc as $Bc/(G^2+Bc^2)$. Assuming that Xc in both k,s and k,p is the same, current flow in the tissue sample is therefore equal to: $Is = It(R_p - R_s)/R_p$, where It is the current delivered to the bath and R_s,R_p are the calculated resistances of bath containing tissue and polythene substitute respectively.

We have examined a number of organ bath/electrode designs. In two of these a ring segment of basilar artery was encircled by one electrode (E_o), the indifferent electrode (E_i) lying within the vessel lumen. In the first (in a 10ml glass bath) a mounting hook served as E_i; in the second (in a 2mm deep plastic bath) the vessel ring was threaded onto E_i. Is/It was 0.14 in the first and three times higher in the second (Table 1).

To optimize experimental design and allow reproducibility in EFS studies tissue current flow should be measured and reported.

Table 1 Determination of tissue current flow (Is) as a fraction of total current delivered (It). Mean of 4 observations (s.d.)



	Bc (mS)	G (ms)	Xc (Ohms)	R (Ohms)	Is/It
Tissue	4.37(0.3)	3.36(0.2)	144(7)	111(7)	0.14
Polythene	3.77(0.2)	2.86(0.1)	169(7)	128(7)	
Tissue	0.39(0.04)	0.37(0.04)	1350(141)	1276(126)	0.42
Polythene	0.20(0.02)	0.34(0.02)	1282(66)	2216(226)	

280P

XENOPUS EMBRYO TERATOGENESIS WITH CALCIUM ION CHANNEL BLOCKING DRUGS

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Papaverine and ionophore A23187 were shown to inhibit neurulation in Xenopus laevis embryos by Moran and Rice (1976) and by Grunz (1985). Calcium ion chelation has similar effects in rats (Smedley & Stanistreet, 1986). Salmar calcitonin was shown to be embryopathic in Xenopus by Burgess (1982 and 1985), the main lesion being failure of forebrain development. This is easily measured by interocular distance, using animal length as the control measure.

Embryos of Xenopus laevis were treated at the late cleavage stages (7 to 8) with 3% cysteine at pH 7.2 to remove most of the jelly coat. They were then transferred to 800 ml. of dechlorinated water in beakers containing solutions of calcium antagonist drugs. The solutions were changed daily for three days, and then replaced by water until the seventh day (stage 45) when embryos were fixed in Smith's fixative and measured.

Drug concentrations were taken over two log decades from the human mean therapeutic plasma levels. The lowest concentrations used were verapamil, 50 nanomolar, diltiazem, 1.8 micromolar, and nifedipine (dissolved in 90% ethanol and added to 600 ml water as stock), 100 nanomolar.
In increasing drug concentrations, larger numbers of embryos developed with malformations, and often oedema. The abnormalities were incomplete development of forebrain or mandible, and malrotation or even virtual absence of the gut. Using animal length as a control measure, and transoptic distance to measure failure of forebrain development, log dose response curves were constructed and the ED_{50} found to be 0.116 micromolar for nifedipine, 3.5 micromolar for diltiazem and 0.96 micromolar for verapamil. These values correlate with the aqueous and liposolubilities of the drugs.

In interaction experiments embryos reared in nifedipine, at its ED_{50}, showed a movement in the dose-response curve for diltiazem, against controls without nifedipine, by an amount equivalent to the addition of 3.5 micromolar diltiazem. Thus the interaction seems to be simply summative on a molar basis, consistant with a calcium ion channel blocking mechanism for both drugs. Xenopus embryos provide an experimental model for calcium antagonist activity, and for its vertebrate teratogenesis.

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THE EFFECT OF VARIOUS DRUGS USED IN THE TREATMENT OF EQUINE DEGENERATIVE JOINT DISEASE ON EQUINE STROMELYSIN (PROTEOGLYCANASE)

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Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids have been widely used for the treatment of degenerative joint disease in the horse. However, although they produce symptomatic relief, neither group of drugs prevents cartilage degeneration in vivo and there is evidence that corticosteroids may even accelerate cartilage breakdown (Hackett 1982). The neutral metalloproteinase "stromelysin"(previously known as proteoglycanase) has been implicated in the breakdown of cartilage in osteoarthrosis (Martel-Pelletier et al 1984), and the effect that anti-inflammatory drugs at therapeutic dose levels would have on equine stromelysin is therefore of considerable interest. Phenylbutazone, flunixin, betamethasone, sodium hyaluronate and polysulphated glycosaminoglycan (PSGAG) were tested in an in vitro system for assessing stromelysin activity to establish if the lack of efficacy of NSAIDs and corticosteroids and the apparent efficacy of sodium hyaluronate and PSGAG could be explained by an effect on this enzyme.

Stromelysin-containing medium, of equine synovial cell origin, was tested against labelled casein substrate (Cawston et al 1984), with or without the addition of the various drugs under investigation, APMA, OPHE, trypsin and trypsin inhibitor. All the enzyme was present in a latent form, activated by APMA or trypsin, and completely inhibited by OPHE. Trypsin activation produced about 25% of the enzyme activity produced by APMA. When drugs were added to the reaction mixtures, significant inhibition of the enzyme was noted only in the case of PSGAG for which a dose response relationship was established between 2.5 and 20 mg/ml. The possibility that this inhibition related to the activation process rather than enzyme activity per se was considered, but similar results were obtained following prior activation of the enzyme by pre-incubation with APMA followed by dialysis to remove the APMA.

The treatment of osteoarthrosis in man and animals has been confused by the erroneous belief that NSAIDs may be disease modifying, as well as providing symptomatic relief, at therapeutically achieved concentrations. This has been at variance with clinical experience of these agents in the treatment of degenerative joint disease in the horse. Newer evidence that the metalloproteinases may be the important enzymes in producing cartilage erosions led us to investigate two NSAIDs, betamethasone, sodium hyaluronate and PSGAG as inhibitors of the proteoglycan degrading enzyme stromelysin. Since this enzyme also shows activity for casein as a substrate, metalloproteinase-mediated casein degradation can be taken as a measure of the concentration of stromelysin present (Cawston et al 1984). Only PSGAG showed any inhibition of stromelysin activity in vitro at concentrations readily achieved in equine joints. Stromelysin inhibition by this agent may account for at least part of its beneficial effect in degenerative joint disease.

This work was supported by a Welfare Grant from the Home of Rest for Horses.

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κ-OPIOID-INDUCED DIURESIS: INVOLVEMENT OF AN α-ADRENOCEPTOR MECHANISM

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Kappa-opioid receptor agonists have been shown to increase urine output in the rat (Leander, 1983). The mechanism of the kappa-mediated diuretic effect is not fully understood, although Blackburn et al. (1986) reported that a peripheral site of action at the adrenal medulla may be involved. The latter authors concluded that adrenal catecholamines were not involved, as adrenergic antagonists did not alter the kappa-mediated diuresis. However, alpha-adrenoceptor agonists are known to modulate urine output in the rat (Gellai & Ruffolo, 1987), and the present study was designed to investigate further the possibility that kappa opioid and adrenergic mechanisms interact.

Rats (male PVG hooded, 100-130g) were starved overnight and then water-loaded (25ml/kg) immediately prior to s.c. injection of opioid or adrenergic drugs. Urine output was recorded hourly for 5 hours after injection. The kappa agonists U50488H (0.6-10mg/kg) and tifluadom (0.3-6mg/kg) produced dose-dependent diuretic effects. Co-administration of the non-selective alpha-adrenoceptor antagonist phentolamine (1mg/kg) or the selective $alpha_2$-adrenoceptor antagonist idazoxan (1mg/kg) decreased the diuretic effects of U50488H and tifluadom and unmasked a large antidiuretic effect. Neither phentolamine nor idazoxan had any consistent effect when administered on their own. The mu agonist fentanyl (0.03-0.3mg/kg) produced a marked antidiuretic effect. Interestingly, in the presence of alpha-adrenoceptor antagonists the antidiuretic effect of fentanyl was potentiated (see Table). Co-administration of propranolol (1mg/kg) had no effect on the actions of U50488H or fentanyl.

AGONIST	DOSE mg/kg	MEAN URINE OUTPUT (ml±s.e.) at 3hr CONTROL	AGONIST ALONE	+ IDAZOXAN	+ PHENTOLAMINE	n VALUE
U50488H	10	3.6±0.2	6.6±0.4	2.6±0.9	1.0±0.3	5
TIFLUADOM	6	3.5±0.1	6.7±0.5	3.5±0.9	1.5±0.4	5
FENTANYL	0.3	3.0±0.2	1.9±0.2	0.6±0.3	0.0±0.0	5
UK14304	0.6	2.6±0.1	5.4±0.3	3.7±0.2	2.9±0.2	5
ST587	16	3.2±0.2	0.7±0.2	0.9±0.3	-	5

The selective $alpha_2$-adrenoceptor agonist UK14304 (0.03-0.6) produced a diuretic effect which was antagonised by co-administration of idazoxan (1mg/kg). The selective $alpha_1$-adrenoceptor agonist ST587 (1-16mg/kg) produced an antidiuretic effect, which was not antagonised by idazoxan (1mg/kg) (see Table), but was partially reversed by the $alpha_1$-antagonist WB4101 (3mg/kg); urine output (ml ± s.e.): ST587, 0.7±0.4; ST587 + WB4101, 1.9±0.3; n=5.

The results suggest that the kappa-opioid-induced diuresis involves an adrenergic component. One possibility is that kappa agonists increase the release of catecholamines from the adrenal medulla and that the circulating catecholamines cause an $alpha_2$-adrenoceptor-mediated diuresis.

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EVIDENCE FOR DIFFERING BOMBESIN RECEPTOR SUBTYPES IN GUINEA-PIG VAS DEFERENS AND RAT URINARY BLADDER

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Rather little is known of the characteristics or subtypes of the receptors in mammalian systems that recognise members of the family of peptides showing sequence homology with bombesin (BB), so in the present study we have compared them in two preparations of contrasting properties.

The guinea-pig vas deferens has recently been shown (Fox *et al*,1987) to respond to members of the BB family with potentiation of the contractile response to nerve stimulation through an action predominantly at a prejunctional locus. Members of the BB family were assayed at four dose levels and, since slopes and maxima of log concentration-response lines increased with potency, the Relative Activity estimates shown in Table 1 were taken at the arbitrary level of 100% potentiation of the twitch response, but the rank order of potency is unchanged however expressed:the maximum responses shown are to 10μM peptide.

The rat urinary bladder, in contrast, responds postjunctionally to members of the BB family giving contractions with essentially parallel log concentration-response lines, and a two dose level assay yielded the Relative Activity estimates shown in Table 1.

Table 1 Relative Activities of bombesin-related peptides

ANALOGUE	GUINEA-PIG VAS DEFERENS		RAT URINARY BLADDER
	Max Response	Relative Activity	Relative Activity
Bombesin	1.00	1.00 (n=24)	1.00 (n=16)
GRP_{18-27}	0.52	0.21	0.2
Alytesin	0.46	0.21	0.7
Litorin	0.37	0.21	1.1
Ranatensin	0.26	<0.01	0.9
Neuromedin B	0.19	<0.01	0.8

The differences in rank orders of potency suggested different BB receptor subtypes in the two preparations and this point was pursued further using putative antagonists based on the tachykinin sequence since the two families share some sequence homology and there have recently been reports describing antagonism of responses to BB by tachykinin antagonists. One such antagonist, [D-Pro^4,Lys^6,D-Trp^{7,9,10},Phe^{11}]-SP(4-11) has previously been reported to have a greater pA_2 against BB than SP(6.36 v 5.33) in the bladder preparation (Mizrahi *et al*, 1985), but this antagonist was found inactive at 10μM in the vas deferens preparation, as were [D-Pro^4,D-Trp^{7,9,10}]-SP(4-11), [D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11}]-SP(1-11) and [D-Arg^1,D-Trp^{7,9},Leu^{11}]SP(1-11) which have been reported active against BB in certain other systems. Antagonists effective in the vas deferens are still being sought, but some recently developed analogues of BB such as [D-Phe^{12},Leu^{14}]-BB(1-14) offer encouragement in this respect (Jensen *et al*,1986).

In summary, both agonist and antagonist data point to differences in the receptors in the bladder and vas deferens which presumably serve to recognise the mammalian neuropeptide counterparts of BB, ie, neuromedin B and GRP_{18-27} (=neuromedin C) and these subtypes seem generally to correspond with the preliminary subdivision into BB_1 and BB_2 respectively (Regoli, 1985).

We acknowledge a Wellcome Trust Vacation Scholarship to AJF and an MRC Scholarship to JMH.
(All peptides were from Peninsula Labs Europe.)

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MULTIPLE BRADYKININ B_2 RECEPTOR SUBTYPES IN SMOOTH MUSCLE PREPARATIONS?

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Bradykinin (BK) receptors are currently classified into B_1 or B_2 subclasses. Under this scheme, B_2 receptors are defined by exclusion as those that are less sensitive to the agonist [des-Arg^9]-BK than to BK itself and are not antagonised by [des-Arg^9,Leu^8]-BK (Regoli & Barabé, 1980). However, though present in a variety of systems with diverse actions, it is not yet clear if the B_2 group represents a homogeneous subclass of BK receptors.

To investigate this point further, in the present study we have examined a series of BK analogues with systematic variations in the 3,5,7 and 8 positions. Results are presented showing their activities on a number of putative B_2 pharmacological test systems including contraction of the guinea-pig ileum, the biphasic response of the guinea-pig taenia caeci, relaxation of the rat duodenum, contraction of the rabbit iris sphincter, and the neurogenic and musculotropic responses of the field-stimulated rat vas deferens.

The pattern of activity of the analogues tested suggests the possibility of heterogeneity of B_2 receptors between these preparations. Thus, the analogue D-Arg[Hyp^3,Thi^{5,8},D-Phe^7]-BK (B3824=B4162=B4881) had low antagonistic activity ($pA_2 \approx 5$) on the biphasic response of the guinea-pig taenia caeci (Carter *et al*, 1987) and on responses to BK in the rat vas deferens. In contrast, however, this analogue was found to be a moderately potent antagonist ($pA_2 \approx 6.4$) of BK-induced contraction in the guinea-pig ileum, and has previously been found to be active in such systems as BK-induced vascular permeability in the rabbit (Longridge *et al*, 1985), and the algesic action of BK on the human blister base (Clegg & Whalley, 1986). Likewise, the analogue Lys-Lys-[Hyp^3,Thi^{5,8},D-Phe^7]-BK (B4310) was relatively inactive against BK in the taenia caeci and vas deferens, though reported to be very active ($pA_2 \approx 7.5$) in some other systems (Lembeck & Griesbacher, 1987), of which we confirm antagonism of contraction of rabbit iris sphincter and relaxation of the rat duodenum. On the latter preparation, other structurally similar members, including Lys,Lys-[Hyp^{2,3},Thi^5,D-Phe^7]-BK (B4308), but also [D-Pal^7]-BK (B3632), were very active antagonists ($pA_2 \approx 7.5$), although rather inactive in most of our other test systems. Further analogues, in particular [D-Thi^7]-BK (B3618) and [Hyp^3,D-Phe^7]-BK (B4416), showed instead weak agonist activity on a number of these systems, with the latter compound appearing to show a higher ratio of neurogenic to musculotropic activity on the rat vas deferens as compared to BK itself, suggesting differing receptors pre- and postjunctionally in this tissue.

Overall these data confirm a number of observations by others, while also suggesting there are two or more subclasses of B_2 receptor. The structural modifications of the linear BK molecule that produce active antagonists seem now somewhat clearer, and there is consequently hope of developing even more selective agents of high activity that may be used to classify BK receptor subtypes with confidence and could also be of useful therapeutic potential.

We acknowledge an MRC Research Scholarship for JMH, and the Wellcome Trust for Vacation Studentships to JLF and AJF and support to IKMM. We thank Dr RJ Vavrek and Prof JM Stewart for synthesis of the analogues tested which are designated by their laboratory numbers, and Prof M Schachter for helpful discussion and advice.

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EX VIVO ACTIVATION OF SIALYL TRANSFERASE BY CARBOCYSTEINE IN SULFUR-DIOXIDE-INDUCED BRONCHITIS IN THE RAT

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Carbocysteine (S-carboxy-methylcysteine, Rhinathiol® CBC) is a mucolytic agent which improves the visco-elastic properties of bronchial mucus in vivo (Puchelle et al, 1980). In bronchitic rats and humans, CBC increases the relative proportions of sialomucins in bronchial mucus which could explain its mucoregulatory actions (Huyen, 1973; Havez et al, 1970). We therefore studied the effects of CBC in vitro and ex vivo in both normal and bronchitic rats on sialyl transferase, which is the enzyme responsible for the addition of sialic acid to mucus glycoproteins.

Male Sprague-Dawley rats (340-360g, 13-19 per group) were made bronchitic by repeated exposure to SO_2 (300-400ppm, 3-4 hours per day, 4-5 days week 1, and 2-3 hours per day 1-2 days week 2, according to the state of the animals) in stainless-steel inhalation chambers (Quevauviller et al, 1970). During this time, they received 500mg.kg^{-1}.day^{-1} CBC or its vehicle p.o. for 15 days. Rats not being exposed to SO_2 received the same treatment. The animals were then killed by decapitation and the lungs were removed. Sialyl transferase was assayed in sub-cellular fractions prepared by differential centrifugation of lung homogenates and using CMP ^{14}C-sialic acid as substrate and desialysed fetuin as exogenous acceptor (Levrat, 1981). The results are expressed as pmoles radioactivity incorporated per mg protein per 2 hour incubation at 37°C.

Sialyl transferase activity was located in both the 10,000g (mitochondria and golgi) and 100,000g (microsomes) pellets with only minor activity in the cytosolic supernatants. Double reciprocal analysis gave Km values of 0.76 and 0.37μM for 10,000g and 100,000g pellets respectively. When tested in vitro between 10^{-6} and 10^{-3}M, CBC had no effect on sialyl transferase in microsomes from lungs taken from healthy or bronchitic rats.

In the mitochondria- and golgi-containing fractions, after repeated administration of CBC, the sialyl transferase activities were as follows: (mean ± S.E.M.) 13.4 ± 1.1, 14.7 ± 1.6, 17.3 ± 1.7 and 20.0 ± 1.2 pmoles/mg protein for the controls, controls plus CBC, bronchitic and bronchitic plus CBC respectively. The increase in activity of the CBC-vs vehicle-treated bronchitic rats is significant ($p<0.05$, Kruskall-Wallis test). There was no difference in the activity of the microsomal enzyme compared to vehicle-treated controls in either healthy or bronchitic animals.

As CBC levels are increased in lung tissue in bronchitic rats (Servin et al, 1976), it is possible that a selective increase in sialyl transferase activity in a golgi-containing fraction of bronchitic lungs could explain the relative increase in sialomucins after oral treatment in bronchitic subjects.

We thank Mme J. Levrier, Mme A. Coste and Mr. P. Ferrari for providing us with bronchitic rats.

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THE EFFECT OF NEUROPEPTIDE Y (NPY) ON THE ADRENERGIC NEUROTRANSMISSION IN ISOLATED RAT SEMINAL VESICLE

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Neuropeptide Y (NPY), a 36 amino acid peptide has been shown to be widely distributed in the mammalian peripheral sympathetic nerves, including those supplying the male genitourinary organs (Lundberg et al. 1982; Sundler et al. 1986). The precise functional role of NPY in sympathetic neurotransmission remains yet to be determined. Rodent seminal vesicle is known to be richly supplied with sympathetic adrenergic nerves (Sjostrand, 1965; Bouquet and Noach, 1971). We report here the in vitro effects of NPY on the adrenergic transmission in the isolated rat seminal vesicle. Seminal vesicles were obtained from freshly killed albino Wistar rats, 200-250g.; the distal portion of each horn approximately 1 cm long was cut, the seminal content gently squeezed out and the preparation was suspended between parallel platinum electrodes in a 1 ml organ bath. The bathing medium was Krebs-Henseleit solution, bubbled with 95% O_2 + 5% CO_2 mixture at 37°C. After allowing equilibration period of 60 min. the preparation was subjected to electrical field stimulation (trains of 3-90 pulses, 0.1 ms pulse duration, 10 Hz, supramaximal voltage). The responses were readily extinguished by tetrodotoxin, 0.5 μM or phentolamine, 1 μM, and were therefore neurogenic and adrenergic. Cumulative administration of NPY, 0.75-250 nM, produced inhibition of the electrically evoked contractions to trains of 3 pulses in a concentration-dependent manner (mean % inhibition ± S.E. of the mean, 11.3 ± 5.9% to 70 + 3% respectively, n=5). The inhibitory effect of NPY was inversely proportional to the number of pulses in the train, being most intense at 3-pulse trains (mean % inhibition ± S.E. of the mean produced by NPY, 75 nM : 68 ± 7% with 3 pulses; 28 ± 8% with 30 pulses; and 8 ± 5% with 90 pulses). NPY, 75 nM, had no inhibitory effect on the contractions evoked by exogenously applied noradrenaline, indicating a presynaptic mechanism of action for the inhibition of the electrically evoked contractions of the rat seminal vesicles by NPY.

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EFFECT OF VALPROIC ACID AND SOME OF ITS METABOLITES ON THE BINDING OF DANSYLSARCOSINE AND WARFARIN TO HUMAN ALBUMIN

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Experiments with fluorescent probes such as dansylsarcosine and warfarin have established the presence of two structurally selective binding sites on human albumin (HA). Warfarin interacts with site I whereas dansylsarcosine binds to site II (Sudlow et al, 1975). The aim of this work was to determine whether valproic acid and its major metabolites bind to either site I or II.

Fluorescence was used to determine binding parameters for the interaction of warfarin and dansylsarcosine with HA (Maes et al, 1981). All solutions were prepared with 0.1M sodium phosphate buffer, pH7.4 and binding was measured at 30°C. Aliquots (2µl) of a solution containing warfarin or dansylsarcosine (100µM) and 2µM HA were titrated with 2ml of 2µM HA. Resultant fluorescence was measured at 380 and 475nm with excitation at 320 and 350nm for warfarin and dansylsarcosine, respectively. Valproate and its metabolites were added to the HA solution to give a final concentration of 10µM.

Scatchard plots for both probes were linear and the values of n, number of binding sites, and k, apparent association constant, estimated from such plots are listed in Table 1. In the presence of valproate there was little change in n but k for warfarin and dansylsarcosine was decreased by about 40%. Of the five metabolites tested three : 2-propyl glutaric acid, 2-propyl-4-methyl butyrolactone and 5-hydroxy-2-propyl pentanoic acid had no effect on probe binding. By contrast, 2-propyl-2-en and 2-propyl-4-en-pentanoic acid inhibited the binding of both probes. The 2-en metabolite was more potent than its 4-en analogue and each of these metabolites displaced warfarin and dansylsarcosine to a greater extent than valproate. (Table 1).

Table 1 Binding parameters of warfarin and dansylsarcosine

Inhibitor	Warfarin		Dansylsarcosine	
	n	k x 10^{-5} (M^{-1})	n	k x 10^{-5} (M^{-1})
None	1.08	3.45	0.84	9.47
Valproate	0.91	2.05	0.81	6.35
4-En-metabolite	0.65	1.50	0.76	4.89
2-En-metabolite	0.49	0.85	0.66	2.45

Valproate seems to interact with both site I and II. Similarly its 2-en and 4-en metabolites also inhibited binding to both sites, but displacement by these was greater than that by the parent drug. It would seem that introduction of a double bond into the carbon skeleton of valproate increases affinity for HA. This may be due to increased hydrophobicity of the 2 and 4-en metabolites since hydrophobic interactions provide much of the binding energy when ligands interact with HA. (Perrin, 1986).

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IN VIVO AND IN VITRO STUDIES OF THE SITE OF INHIBITORY ACTION OF OMEPRAZOLE ON ADRENOCORTICAL STEROIDOGENESIS

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Omeprazole, a benzimidazole which is a potent inhibitor of gastric acid secretion reduces the peak cortisol response to ACTH on normal man and at high concentrations inhibits basal and ACTH-stimulated cortisol synthesis by adrenocortical cells (Howden et al.1986). We identified the site of omeprazole action in cortisol's biosynthetic pathway both clinically and in vitro. For the clinical study 8 male subjects received 60 mg omeprazole or placebo daily for 7 days in a random-ised,cross-over study. On day 6 of each treatment urine was collected for 24h for determination of free cortisol and of excretion rates of 14 steroid metabolites by gas chromatography. Although the excretion rates of all steroids, including cortisol,showed a tendency to decrease with omeprazole only the effect on dehydroepiandrosterone sulphate was statistically significant ($p<0.05$). These data suggest a weak action of the drug at the level of cholesterol cleavage.

For in vitro studies, cortical cells from the zona fasciculata/reticularis region of several bovine adrenal glands were isolated by collagenase digestion. In one experiment cortisol and deoxycortisol were measured in the supernatant of cell suspensions which had been incubated ± 10^{-8}M ACTH plus various doses of omeprazole. Basal and ACTH-stimulated cortisol and 11-deoxycortisol production were all inhibited ($p<0.001$) with IC50 values <20µg omeprazole/ml. Table 1 shows the effects of 100µg omeprazole/ml on the conversion of precursors to cortisol. Steroids were added to the incubations at a concentration of 5×10^{-6}M (n=5, mean ±SE; *$p<0.05$; **$p<0.01$; ***$p<0.001$, compared with controls). Note that the degree of inhibition is greatest for the earlier precursors in the cortisol pathway and that deoxycortisol to cortisol conversion is unaffected.

Table 1. Effect of omeprazole on cortisol synthesis from various precursors

Precursor	Cortisol production (ng.10^6 cells $^{-1}h^{-1}$) Control	Omeprazole	% Inhibition
Basal	1.4 ± 0.3	0.1 ± 0.1**	93%
20αOH cholesterol	10.0 ± 1.2	1.7 ± 0.4***	83%
Pregnenolone	136.0 ±11.0	85.0 ± 9.0*	38%
17αOH pregnenolone	113.0 ±27.0	65.0 ±10.0	42%
Progesterone	116.0 ±10.0	90.0 ±12.0	22%
17αOH progesterone	272.0 ±33.0	156.9 ±25.0*	43%
11-Deoxycortisol	255.0 ±58.0	278.0 ±34.0	0%

In conclusion, the in vitro data indicate that omeprazole acts predominantly at the level of cholesterol side-chain cleavage - the first and rate limiting step in steroidogenesis. Unlike other drugs containing the imidazole ring (etomidate, ketoconazole, cimetidine), omeprazole has no significant effect on 11β-hydroxylation of 11-deoxycorticosteroids. Although high concentrations of omeprazole were required to produce in vitro steroidogenic effects, in vivo evidence of a similar weak inhibition of cholesterol cleavage rather than 11β-hydroxylation was apparent from an analysis of urinary steroid metabolites.

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POTENTIATION OF THE DIURETIC AND NATRIURETIC EFFECTS OF ATRIAL NATRIURETIC FACTOR (ANF) BY M&B 22948

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The action of atrial natriuretic factor (ANF) in a number of tissues is associated with an increase in intracellular cGMP (Ballermann & Brenner, 1985). The selective cGMP phosphodiesterase inhibitor, M&B 22,948, has been shown to potentiate the biological actions of ANF (Martin et al, 1986: Kurtz et al, 1986) which is consistent with cGMP acting as a second messenger for the peptide. In the present study the effect of M&B 22948 on ANF-induced diuresis, natriuresis and kaliuresis was studied in the anaesthetized rat.

Male Wistar rats (350-460g) were anaesthetised with pentobarbitone sodium and artificial ventilation applied. Both external jugular veins were cannulated, one for constant infusion of saline (0.2 ml/min), the other for the drug or vehicle control solution which was infused at 0.032 ml/min for 30 min after a steady basal urine flow rate had been achieved. Urine was collected from catheterised ureters for seven consecutive 10 min periods after the start of drug infusion. Na^+ and K^+ concentrations were determined by flame photometry and cGMP by radioimmunoassay. BP was recorded from the cannulated left carotid artery and HR derived from the BP pulse.

In this model both rat ANF and M&B 22948 had diuretic and natriuretic actions at doses lower than those which reduced BP. The dose of each agent was titrated to obtain a threshold causing a minimal effect, compared to vehicle control, on urine and electrolyte excretion (0.03µg/kg/min, ANF; 3µg/kg/min, M&B 22948). Combination of these dose levels of the 2 agents resulted in a diuresis and natriuresis (but not kaliuresis) that were significantly greater than an additive effect of the 2 agents i.e. there was a positive interaction (Table 1). BP and HR were unaffected. Variable increases in urine cGMP were obtained in response to threshold doses of ANF and M&B 22948, alone or in combination.

Table 1 Change (± s.e.m.) in excretion from pre-treatment levels at 20 min after infusion.

Treatment	urine volume (µl/100g/10min)	Na^+ (µEq/100g/10min)	K^+ (µEq/100g/10min)
Vehicle control	3.8 ± 5.0	1.5 ± 0.6	1.1 ± 0.9 (n=5)
ANF(0.03µg/kg/min)	8.5 ± 1.5	3.2 ± 0.9	4.5 ± 2.6 (n=4)
M&B 22948(3µg/kg/min)	16.2 ± 7.4	5.0 ± 1.9	3.9 ± 0.9 (n=5)
ANF + M&B 22948	75.3 ± 22.7*	22.1 ± 6.0*	8.6 ± 1.1 (n=4)

*$P<0.05$ for positive interaction of the two substances (ANOVA).

These preliminary results suggest that M&B 22948 potentiates the diuretic activity of ANF and the most reasonable explanation is that this is due to inhibition of the breakdown of elevated tissue cGMP.

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COMPARISON OF HUMAN α CGRP, ACETYLCHOLINE, SODIUM NITROPRUSSIDE, FORSKOLIN AND M&B 22948 ON RAT MESENTERIC VASCULATURE

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Many vasodilators including acetylcholine and sodium nitroprusside cause vascular relaxation associated with an increase in cyclic GMP. The calcitonin gene-related peptide (hαCGRP) appears not to increase cyclic GMP but may increase cyclic AMP in vascular smooth muscle (Grace et al, 1987; Edvinsson et al, 1985). The present experiments have compared the vascular effect of hαCGRP with acetylcholine, sodium nitroprusside, forskolin (which activates adenylate cyclase; Seamon & Daly, 1981) and M&B 22948 (a selective inhibitor of cyclic GMP phosphodiesterase; Gruetter et al, 1981).

Rat mesenteric arterial rings were set up in Krebs solution at 37°C and tone induced with noradrenaline (10^{-7} M). These rings were relaxed by acetylcholine (10^{-9} - 10^{-6} M), sodium nitroprusside (10^{-9} - 10^{-6} M), hαCGRP (10^{-9} - $3x10^{-7}$ M), forskolin (10^{-9} - 10^{-7} M) and M&B 22948 (10^{-8} - $3x10^{-6}$ M). Rubbing of the intimal surface of some rings was used to remove the endothelium. This procedure significantly reduced the relaxation evoked by acetylcholine and hαCGRP. In intact mesenteric rings the presence of methylene blue (10^{-5} M) reduced the relaxant effect of acetylcholine, hαCGRP, forskolin and M&B 22948, but not that of sodium nitroprusside.

Rat isolated mesenteric vasculature was perfused at a constant flow (5.8 ml min^{-1}) with Krebs solution at 37°C and the perfusion pressure was increased by the vasoconstrictor noradrenaline (10^{-5} M). Vasodilatation was evoked by acetylcholine (10^{-11} - 10^{-6} mol), sodium nitroprusside (10^{-10} - $3x10^{-8}$ mol) hαCGRP ($3x10^{-11}$ - $3x10^{-9}$ mol), forskolin ($3x10^{-11}$ - 10^{-8} mol) and M&B 22948 ($3x10^{-9}$ - 10^{-6} mol). Sodium deoxycholate (2.4 x 10^{-3} M for 60 sec) was used to chemically remove the endothelium (Byfield et al, 1986) and abolished the effect of acetylcholine but not that of sodium nitroprusside. Of the other vasodilators M&B 22948 was reduced by deoxycholate treatment although hαCGRP and forskolin were unaltered. In the intact perfused mesenteric vasculature the effects of acetylcholine and M&B 22948 were shifted to the right 30 and 5 fold respectively by methylene blue (10^{-5} M) although this treatment did not alter responses to hαCGRP and forskolin. In another set of experiments isolated mesenteric vasculature was perfused with magnesium-free Krebs solution. This reduced the vasodilatation evoked by acetylcholine, hαCGRP, forskolin and M&B 22948 but not that of sodium nitroprusside.

In mesenteric arterial rings the relaxant effect of hαCGRP appeared similar to that of acetylcholine and was not the same as either forskolin or M&B 22948. However, in the rat perfused mesenteric vasculature hαCGRP was similar in three ways to forskolin and not to any of the other vasodilators (endothelial dependent, reduced by methylene blue and by magnesium-free Krebs solution). Therefore, while the mechanism of action of forskolin and hαCGRP may be quite separate, these findings are consistent with the hypothesis that, in the mesenteric arterioles, the peptide might act directly on the smooth muscle and stimulate the production of cyclic AMP.

We thank Celltech Ltd. for support.

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HETEROLOGOUS REGULATION OF ANF RECEPTORS IN RAT CULTURED VASCULAR SMOOTH MUSCLE CELLS

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The regulation of the receptors for one hormone by a second hormone has been only documented in a few instances (HOLLENBERG, 1985). Studying the regulation of atrial natriuretic factor (ANF) receptors in rat vascular smooth muscle cells, we found a new example of heterospecific regulation between the two antagonistic hormone systems ANF and Angiotensin II (ANG II). As shown in table 1, ANG II was able to down regulate ANF receptors to the same extent than ANF itself although it affected slightly the K_d value that ANF did not modify. Moreover, the ANG II antagonist, Sar_1-Ile_8-ANG II completely inhibited the action of ANG II indicating a receptor mediation of this effect. Since ANF increases cyclic GMP in smooth muscle cells, we compared the consequence of ANF and ANG II pretreatment on ANF induced cyclic GMP response. Contrasting results were obtained with ANF and ANG II which respectively decreases or increases the production of cyclic GMP induced by ANF (10-7M) although the number of binding sites was diminished in the two cases (table 1).

Table 1 Regulatory effects of ANF [rat ANF (1-28)] and ANG II on ANF receptors in cultured rat aortic smooth muscle cells (n = 5).

	Preincubation conditions (24 h treatment)		
	Control	ANF 10^{-8}M	ANG II 10^{-8}M
Kd ($x10^{+10} xM^{-1}$)	2.81 ± 0.33	2.49 ± 0.59	1.73 ± 0.26
Bmax (fmol/10^6 Cells)	208 ± 24	82 ± 20	90 ± 9
% diminution over Bmax Control	-	61	57
cGMP Production (fmol/10^6Cells) induced by ANF 10^{-7}M	480 ± 31	299 ± 32	619 ± 110

The present data demonstrate a specific receptor interaction between the two antagonistic peptidic hormones. (ANF and ANG II). They also show that the regulation of ANF receptors is heterogenous providing a new evidence of multiple classes of ANF receptors. Further studies are undertaken to determine the mechanism (s) involved.

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EFFECTS OF A NOVEL PERFLUOROCHEMICAL EMULSION ON LYMPHOID TISSUES AND IMMUNOCOMPETENCE IN RATS

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We have recently reported physico-chemical characteristics of novel perfluorochemical (PFC) emulsions with enhanced stability (Davis et al., 1986) and have now studied the effects of one such emulsion on lymphoid tissues and immunocompetence in rats previously or subsequently injected with sheep red blood cells (SRBC).

Female Wistar rats (body weight (b.w.): 140-160g; n = 69) were used. They were immunized by intravenous (i.v.) injection of ca. 5 x 10^8 double-washed SRBC suspended in 1.0 ml Hank's saline; the day of immunization = day 0. Groups of animals also received single i.v. injections of 10 ml/kg b.w. of a novel FDC emulsion (Davis et al., 1986) via the same route on one of the following days relative to immunization: -7, -4, -1, +1, +4, and either 1h before or simultaneously at immunization; control animals received either SRBC or saline (0.9% w/v NaCl) followed by SRBC 24hr later. Rats were sacrificed on day +7 and weights of liver, spleen, thymus and mesenteric lymph nodes (MLN) recorded. Plasma antibody (Ab) titres to SRBC were measured by haemagglutination assay.

Mean organ weights (expressed as % b.w.) and day +7 $\log_2$ Ab titre in SRBC-injected control animals (n = 15) were: liver 4.63 ± 0.05; spleen 0.25 ± 0.01; thymus 0.28 ± 0.01; MLN 0.08 ± 0.01; Ab titre 7.1 ± 0.4. Comparable measurements in saline-injected control rats receiving SRBC on day 0 (n = 19) were similar. Overall changes in mean organ weights and day +7 Ab titres in experimental groups injected with the novel emulsion were:

Time	Liver	Spleen	Thymus	MLN	Ab titre
-7	↑	↑↑↑	↓↓	Unc	↓
-4	↑	↑↑↑	↓	Unc	Unc
-1	↑	↑↑↑	↓↓	↑	Unc
-1h	↑↑↑	↑↑↑	↓↓↓	Unc	↑
0	↑↑↑	↑↑↑	↓↓	Unc	Unc
+1	↑↑↑	↑↑↑	↓↓↓	Unc	Unc
+4	Unc	↑↑↑	↓↓↓	Unc	Unc

↑ = increased ($P < 0.05$), ↑↑ ($P < 0.01$), ↑↑↑ ($P < 0.001$); ↓ = decreased ($P < 0.05$), ↓↓ ($P < 0.01$), ↓↓↓ ($P < 0.001$); Unc = unchanged compared to controls.

The changes in organ weights were generally consistent with those in rats injected with F-DA but otherwise treated in an identical manner to the present study (Bollands & Lowe, 1987). Liver weight increase was greater than following F-DA injection of SRBC-immunized animals and this may reflect the use of more Pluronic F-68 in the novel emulsion, since it has been claimed that this surfactant can promote hepatomegaly in rats (Goodman et al., 1984). These results did not substantiate the immunopotentiating effects of an identical emulsion when injected intraperitoneally in rats 24 h prior to SRBC (Bollands et al., 1987), except when emulsion and SRBC injections were separated by only 1h. This further demonstrates the importance of route and timing of PFC emulsion injection relative to immunization.

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EFFECTS OF EMULSIFIED PERFLUOROCHEMICALS ON RETICULOENDOTHELIAL SYSTEM CLEARANCE FUNCTION IN RATS

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There is growing interest in the potential applications of emulsified perfluorochemicals (PFCs) for physiological gas transport and we have reported physicochemical properties of novel perfluorodecalin (FDC) emulsion stabilized by perfluorinated higher boiling point oil (HBPO) additives (Davis et al., 1986; Sharma et al., 1987). Since recent attention has focussed on the uptake of PFCs into lymphoid tissues and its consequences for immune system function (Bollands & Lowe, 1986, 1987; Bollands et al., 1987), the effects of one such emulsion on reticuloendothelial system (RES) clearance function in rats have been studied. An objective was to compare the responses with those produced by the commercial emulsion, Fluosol-DA 20% (F-DA; Green Cross, Japan).

FDC (ISC Chemicals Ltd, Avonmouth) was emulsified by sonication with 3.1% Pluronic F-68 in an isotonic aqueous phase to give a final 20% (w/v) preparation. The emulsion contained 1% of a C-16 HBPO additive, perfluoroperhydrofluoranthrene, to enhance stability (Davis et al., 1986). Female Wistar rats (body weight (b.w.): 160-190g; n = 52) were injected intravenously (i.v.) via a tail vein with 10 ml/kg b.w. of F-DC emulsion or F-DA; control animals received sterile saline (0.9% NaCl). Groups of animals were then injected with 10 ml/kg of a 1:4 dilution of colloidal carbon (Indian Ink; Windsor & Newton Ltd, Harrow) at either 3h, 6h, 12h, 24h, 48h, 4 days and 8 days following injection of emulsion or saline. Blood samples (20 µl) were collected from a tail vein at 0.5, 2, 5, 10, 15, 20, 30, 45, 60 and 90 mins after colloidal carbon injection; blood was then lysed in 4 ml 0.1% acetic acid and optical density at 695 nm recorded. The elimination constant (K) was determined from linear regression analysis of absorbance against time (Hudson & Hay, 1980).

Injection of F-DA produced a biphasic inhibition of carbon elimination with values of K decreased to 52% of the mean control value (0.054 ± 0.060; n = 3) at 6 h and 70% of control at 4 days ($P < 0.05$). Mean K values at 6h and 48h in animals receiving the novel emulsion fell to 64% and 79% of control respectively, although these changes were not significant.

These results show that pre-treatment of rats with F-DA produces a transient depression of RES phagocytic function, as determined by colloidal carbon clearance. The biphasic inhibition of carbon clearance produced by F-DA was consistent with that produced by greater doses of the emulsion, although the timing of the second decrease in K occurred 48 h later (Lutz & Metzenauer, 1980). The variable effects of F-DA and the novel emulsion on RES clearance function probably reflects differences in composition since their mean particle diameters, as measured by Photon Correlation Spectroscopy, were similar (F-DA: 266 nm; FDC: 237 nm). The 6% perfluorotripropylamine component of F-DA, which has a tissue half-life in rats almost 9 times that of FDC (Naito & Yokoyama, 1978), would be expected to make a significant contribution to RES blockade. However, corresponding tissue retention data for the C-16 HBPO additive in the novel emulsion are not presently available.

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THE EFFECT OF LITHIUM ON INOSITOL PHOSPHATE METABOLISM IN HL60 CELLS

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Inhibition of inositol monophosphate phosphatase resulting in an alteration in the inositol lipid cycle has been suggested to be the mechanism by which lithium exerts its effect in the treatment of manic depression (Berridge et al., 1982). Studies in vitro have demonstrated that lithium amplifies inositol monophosphate (Ins P_1) levels in receptor activated tissues. Thus lithium has become a useful tool in analyses of inositol phosphate production in stimulated cells. However, it is becoming apparent that lithium exerts effects on the metabolism of several inositol phosphates and that its action is not simply restricted to the monosphosphate phosphatase (Hansen et al., 1986). We have therefore investigated the effect of lithium on inositol phosphate metabolism in formylmethionylleucylphenylalanine (FMLP) stimulated human promyelocytic leukaemia HL60 cells and in HL60 cell homogenates.

In HL60 cells stimulated with FMLP over a time course of 3 minutes 30mM LiCl caused a marked increase in Ins P_1 levels, as previously described (Carey et al., 1987). In addition there was an increase in inositol bisphosphate (Ins P_2) and a smaller increase in inositol tris + tetrakisphosphate ("Ins P_3"). Since this latter fraction contained Ins 1,3,4,5 P_4, Ins 1,4,5, P_3 and Ins 1,3,4, P_3 this effect of lithium was further investigated by studying the metabolism of [^{3}H] Ins 1,4,5, P_3 and [^{3}H] Ins 1,3,4,5 P_4 by HL60 cell homogenates.

Metabolism of Ins 1,4,5, P_3 by HL60 cell homogenates incubated in the absence of ATP was rapid, with 50% hydrolysis to Ins 1,4 P_2 occurring within 3 minutes. 30mM LiCl did not affect the degradation of Ins 1,4,5 P_3, but did cause accumulation of Ins P_1 produced after longer incubation times. Ins 1,3,4,5 P_4 metabolism was also rapid, with 50% hydrolysis occuring at about 3 minutes. 30mM LiCl did not affect the hydrolysis of Ins P_4 but did have an inhibitory effect on the metabolism of the Ins P_3 produced from Ins P_4. HPLC anaylsis, using a modification of the method of Irvine et al., 1985, showed this to be an effect on Ins 1,3,4 P_3.

These data suggest that in HL60 cells lithium not only inhibits inositol monophosphatase causing accumulation of Ins P_1, but also inhibits the enzyme(s) involved in the metabolism of Ins P_2 and Ins 1,3,4 P_3. Inhorn et al (1987) have recently described a lithium sensitive inositol polyphosphate 1-phosphatase which degrades Ins 1,4,P_2 and Ins 1,3,4, P_3 via removal of the phosphate from the 1 position. Thus the effects of lithium on Ins P_2 and Ins 1,3,4P_3 metabolism in HL60 cells may occur as a result of inhibition of a single enzyme activity.

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PHOSPHOINOSITIDE (PI) METABOLISM IN PRIMARY CULTURES OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Cultured human endothelial cells have been shown to synthesize prostacyclin in response to histamine, bradykinin, ATP and thrombin (McIntyre et al., 1985). The mechanism of prostacyclin release involves an increase in intracellular calcium and such an increase has been seen in response to histamine (Rotrosen & Gallin, 1986), and thrombin (Hallam & Pearson, 1986). In many cell types Ca^{++}-linked events are associated with increased PI metabolism, and such a change has recently been reported after stimulation of cultured human endothelial cells with histamine (Resink et al., 1987). We have used a simple technique for the investigation of the role of increased PI turnover in the action of a range of vasoactive agents in human endothelial cells.

Human umbilical vein endothelial cells were grown to confluence in 24-well plates and studied 3-8 days after initial seeding. Myo-(3H)-inositol (10μCi/ml) was added to the medium 48h before use. The cells were then washed with buffered saline and incubated with 20mM LiCl for 30min at 37°, followed by incubation for 1h with agonist, still in the presence of lithium. Inositol-1-phosphate (IP) was extracted and separated from free inositol and other water-soluble products (Conn & Sanders-Bush, 1985). Under these conditions IP accumulation was taken as an index of PI turnover.

Histamine (100μM) increased IP production by 263 ± 45% over basal levels (mean ± SEM, n=10). The response was dose-related, with a median EC50 of 1.35 μM (range 0.68 - 1.75 μM, n=5). The response to 1μM histamine was abolished by 10μM mepyramine, but was unaffected by 10μM cimetidine, indicating that the action of histamine is mediated via the H1 receptor. Thrombin (1 U/ml) stimulated PI turnover by 185 ± 39% (n=9). This effect was dose-dependent, but a maximal response had not been reached at thrombin (10 U/ml). The response to thrombin 1 U/ml was only partially blocked by hirudin 1 U/ml, possibly because hirudin may be less stable than thrombin during prolonged incubation. The response to ATP (100μM) was highly variable, with no effect in 6 out of 12 experiments, giving an overall increase of PI turnover of 64 ± 31%. It is likely that most of the ATP is degraded by cellular ectonucleotidases. There was no response to 100μM adenosine. The reason for the variability of the effect of ATP remains unclear. There was no detectable response to bradykinin (1μM), noradrenaline (10μM), 5-hydroxytryptamine (100μM), angiotensin II (100nM) or arginine vasopressin (100nM). For all the above, n=3-6.

In conclusion, the stimulation of PI turnover has been observed in human umbilical vein endothelial cells in response to histamine, thrombin and ATP but, unexpectedly not to bradykinin. This pathway is likely to be linked to the release of prostacyclin and possibly other vasoactive substances.

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INHIBITION OF THE ATP-K^+ CHANNEL BY GLIBENCLAMIDE ANALOGUES IN A RAT INSULINOMA CELL LINE

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Recently it has been proposed that the site of action of hypoglycaemic sulphonylureas is the ATP-K^+ channel or a closely associated protein (Sturgess et al., 1985). Inhibition of this channel has been suggested as the link between glucose metabolism and the initiation of insulin secretion (Cook and Hales, 1984). To date the only sulphonylureas tested at the single channel level have been tolbutamide and glibenclamide. We now report the effects of a range of glibenclamide analogues upon the ATP-K^+ channel present in the plasma membranes of CRI-G1 insulin secreting cells.

Single channel currents were recorded using the outside-out membrane patch configuration. Membrane patches were exposed to symmetrical 140mM K^+ solutions. The intracellular membrane surface was bathed with low Ca^{++} solution ($<10^{-8}$M). Glibenclamide and four related compounds, HB 180, Hoe 036, HB 985, HB 699, all caused varying degrees of ATP-K^+ inhibition as shown in Table 1.

Table 1: Percent ATP-K^+ channel inhibition induced by different concentrations of glibenclamide and analogues

Compound	Concentration		
	1uM	10uM	100uM
Glibenclamide	70.3±5.00 (9)	81.5±5.00 (8)	-
HB 180	70.3±5.69 (5)	78.6±4.87 (5)	-
Hoe 036	73.9±10.02 (3)	84.3±6.04 (4)	-
HB 699	38.9±11.73 (5)	80.7±4.65 (5)	-
HB 985	-	61.1±6.80 (5)	85.9±8.37 (3)

These results provide further evidence that the action of the sulphonylureas is mediated through inhibition of the ATP-K^+ channel. The inhibition caused by Hoe 036 and HB 180 suggests that slight structural modifications of the ring structure belonging to the non-sulphonylurea moiety of glibenclamide do not greatly affect the ability of these compounds to inhibit the ATP-K^+ channel. Furthermore, data obtained with HB 699, the benzoic acid derivative of the non-sulphonylurea moiety of glibenclamide, suggests that the sulphonylurea structure is not a prerequisite for inhibition of the channel. HB 699 has been reported to possess an insulinotropic action and decrease $^{86}Rb^+$ efflux in isolated islets (Garrino et al., 1985). Additionally the analogue HB 985, which only differs from glibenclamide by the substitution of a cyclohexane ring with a methyl group was active, although less effective than HB 699, even though it has been reported to have no hypoglycaemic properties (Geisen et al., 1985). These data support the proposition that the second generation hypoglycaemic agents possess two active sites (Rufer et al., 1974), both of which can interact with the ATP-K^+ channel.

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GLUCOCORTICOIDS INHIBIT ACETYLATED LOW DENSITY LIPOPROTEIN UPTAKE BY MOUSE PERITONEAL MACROPHAGES

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Glucocorticoids effectively inhibit atherosclerosis in certain animal models of the disease (Bailey & Butler, 1985), but their mechanism of action is unknown. Macrophage-derived foam cells containing large amounts of esterified cholesterol are a major feature of atherosclerosis. The cholesterol is probably derived, at least in humans, from the excessive uptake of low density lipoproteins (LDL). LDL may be modified in the arterial wall so that it becomes recognised by the abundant acetyl-LDL receptors on macrophages and is internalised rapidly.

We have recently found that low concentrations of glucocorticoids decrease the uptake of acetyl-LDL by macrophages. Mouse resident peritoneal macrophages were isolated and cultured for 4-7 days in Dulbecco's modified Eagle's medium containing 10% (v/v) foetal calf serum and gentamicin (50μg/ml) with or without a glucocorticoid. Human ^{125}I-labelled acetylated LDL (10μg protein/ml) was then added and, after an overnight incubation, its non-iodide trichloroacetic acid-soluble degradation products in the medium were measured (Drevon *et al.*, 1981) as a measure of its uptake by the cells. Pre-incubation (but not co-incubation) with glucocorticoids decreased the uptake of acetyl-LDL by up to 50%. Half the maximum observed inhibition was obtained with about 0.1μM hydrocortisone and about 0.1-1nM dexamethasone. Hydrocortisone did not cause acetyl LDL to accumulate in the cells, thus eliminating the possibility that it may have acted by inhibiting the lysosomal degradation of internalised acetyl-LDL. Pre-incubation with oestradiol, testosterone or progesterone (all at 1μM) did not inhibit acetyl-LDL uptake, whereas 1μM aldosterone, which has a slight degree of glucocorticoid activity, consistently gave a small inhibition. The presence of the glucocorticoid antagonist, RU 38486, during pre-incubation with hydrocortisone largely prevented the inhibition of acetyl-LDL uptake, suggesting a receptor-mediated effect rather than a non-specific membrane effect.

Table 1 Effect of RU 38486 on inhibition of acetyl-LDL uptake by hydrocortisone

Steroids	Degradation of ^{125}I-labelled acetyl-LDL (μg protein/mg cell protein in 24h; mean±s.e.mean, n=4)
None	55.3 ± 2.5
Hydrocortisone (1μM)	24.4 ± 2.0
Hydrocortisone (1μM) plus RU 38486 (0.1μM)	32.5 ± 1.7
Hydrocortisone (1μM) plus RU 38486 (1μM)	43.0 ± 1.4
Hydrocortisone (1μM) plus RU 38486 (10 μM)	46.5 ± 1.8
RU 38486 (0.1μM)	61.6 ± 0.9
RU 38486 (1μM)	51.7 ± 1.7
RU 38486 (10μM)	54.3 ± 3.4

Pre-incubation for 7 days with cyclooxygenase inhibitors (5μM indomethacin, 25 μM acetylsalicylic acid) or lipoxygenase inhibitors (5 and 25μM nordihydroguaiaretic acid, 10 and 100μM BW755C) did not inhibit acetyl-LDL uptake, suggesting that the inhibition by glucocorticoids was not due to their effects on arachidonic acid metabolism.

These findings raise the possibility that glucocorticoids may inhibit experimental atherosclerosis in part by decreasing the uptake of modified LDL by macrophages in the arterial wall.

C.V. de W. was supported by an SERC CASE award in collaboration with Smith Kline & French Research Ltd.

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ELEVATION OF GUINEA-PIG PLASMA CORTISOL LEVELS INDUCED BY TRAVEL STRESS

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In a previous study we have demonstrated an increase in the sensitivity of isolated left atria to β-adrenoceptor stimulation by isoprenaline after guinea-pigs had been stressed by transportation (Broadley & Herepath, 1987). Enhanced β-adrenoceptor sensitivity can be brought about by corticosteroids (Samuelson & Davies, 1984) and it is known that plasma corticosteroids are raised by various forms of stress (Axelrod & Reisine, 1984). The present study was therefore undertaken to determine the plasma cortisol levels in guinea-pigs immediately after travel-induced stress. Since the method employed was a radioimmunoassay intended for human plasma, it was first necessary to validate this for use with guinea-pig plasma.

Plasma cortisol concentrations were measured with a commercial radioimmunoassay kit (Coat-a-Count; Diagnostic Products Ltd.). The reliability of the assay in guinea-pig plasma was tested by assaying serial dilutions of one of the kit standards and a plasma sample from a guinea-pig which had been stressed with ether in order to elevate plasma cortisol. There was no significant difference in the slopes of the resulting regression lines, indicating that the use of guinea-pig plasma does not cause a loss of specificity. Recovery of standard added to guinea-pig plasma was between 80 and 112% (mean 98%), and the intra-assay coefficient of variation was 11.4% at a cortisol concentration of approximately 30 nmol L^{-1}.

To determine the effects of stress on cortisol levels, we compared guinea-pigs immediately after the stress of transportation by British Rail for approximately 18h (approx. 150 miles) with those kept for at least a week in the animal house and used after the relative quiet of a weekend. Blood samples were collected into heparinized tubes either by stunning and decapitation or by cannulation of the carotid artery under urethane anaesthesia (1.5gkg^{-1} ip). Tubes were centrifuged (4,000g) and plasma removed for determination of cortisol levels.

In unstressed guinea-pigs, the mean (±s.d., n=7) cortisol levels after urethane anaesthesia (1688±193 nmol L^{-1}) were significantly ($P<0.05$) greater than after decapitation (677±194 nmol L^{-1}, n=6), confirming previous observations that anaesthesia elevates guinea-pig plasma cortisol levels (Manin et al. 1983). In travel-stressed animals, the cortisol levels were significantly ($P<0.05$) raised above the corresponding unstressed value, whether urethane (2045±85 nmol L^{-1}, n=5) or decapitation (1990±91 nmol L^{-1}, n=6) was used for blood collection. However, the effect of travel-stress was more marked without the background effect of urethane.

This study has therefore demonstrated that plasma cortisol levels are raised by stress induced by travel and that this is a likely explanation for the raised cardiac β-adrenoceptor sensitivity previously observed in guinea-pigs after this form of stress.

Materials for radioimmunoassay were kindly provided by Diagnostic Products (UK) Ltd. Supported in part by British Heart Foundation.

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AN ELECTROPHYSIOLOGICAL STUDY OF MUSCARINIC INHIBITION OF ACETYLCHOLINE RELEASE IN THE Mg^{2+}-BLOCKED FROG SARTORIUS MUSCLE

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The release of acetylcholine (ACh) from some central and peripheral neurones is inhibited by activation of presynaptic muscarinic receptors (Molenaar & Polak, 1980). However at the neuromuscular junction neurochemical evidence is equivocal (Gunderson & Jenden, 1980; Kilbinger & Wessler, 1987), perhaps because of differing methodology. This report concerns the results of a study undertaken to investigate the effects of two muscarinic ligands, oxotremorine and atropine, on electrophysiological correlates of ACh release.

Sartorius muscles were equilibrated in Ringer solution containing 0.5 mM Ca^{2+} and 3 mM Mg^{2+} before impalement with 3M KCl-filled microelectrodes. During continuous superfusion samples of mepps (usually > 100) and epps (60 or 120) evoked at 0.33 or 20 Hz were collected from fibres with a large signal to noise ratio for calculation of quantal content (M) from the amplitude quotient mean epp/mean mepp. M values were determined 10 min before (control), 10 min (oxotremorine) or 15 min (atropine) after addition of a drug and then subsequently during the wash phase. When the effects of oxotremorine were tested in the presence of atropine the latter was present throughout the experiment. Results, mepp frequency (F) or M, are expressed (mean $\pm$ s.e. mean) as the ratio test/control and the values compared with those from an untreated group of fibres.

At a stimulation rate of 0.33 Hz the ratio M test/M control was 1.04 $\pm$ 0.05 (n=8) in the untreated group but decreased ($P < 0.01$) to 0.53 $\pm$ 0.06 (n=7) in fibres exposed to oxotremorine (100 μM). At 20 Hz the ratios were 0.69 $\pm$ 0.06 (n=7) and 0.43 $\pm$ 0.03 (n=7) for oxotremorine 30 and 100 μM respectively: values significantly less ($P < 0.05$) than that of 1.13 $\pm$ 0.06 (n=7) determined for the untreated group. Recovery of M after removal of the drug required approximately 1 h. Atropine (100 nM) reduced ($P < 0.01$) the effects of oxotremorine (100 μM) by about 60% but itself did not affect the M-ratio (1.07 $\pm$ 0.06, n=8, 0.33 Hz; 1.00 $\pm$ 0.04, n=7, 20 Hz).

In fibres treated with oxotremorine (30 and 100 μM) the F-ratios were 0.72 $\pm$ 0.09 (n=6) and 0.58 $\pm$ 0.10 (n=7) respectively and were significantly less ($P \leq 0.05$) than that of 1.12 $\pm$ 0.10 (n=8) recorded for the untreated fibres. Oxotremorine-induced reductions in mepp frequency were prevented by atropine (100 nM) which alone had no effect.

Depolarization (9 $\pm$ 1 mV, n=21) of endplates, which could be blocked by d-tubocurarine (1 μM), was evoked by oxotremorine (100 μM). The amplitude of intracellularly-recorded mepps and iontophoretically-evoked ACh potentials were reduced only by the highest concentration (100 μM) of oxotremorine whereas the time course of focally recorded mepps was unchanged.

These data support the concept of muscarinic modulation of ACh release at motor nerve terminals.

Supported by the Joint Research Board.

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A COMPARISON OF RELAXATIONS INDUCED BY NANC STIMULATION AND BY NITROVASODILATORS IN THE MOUSE ANOCOCCYGEUS

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Non-adrenergic, non-cholinergic (NANC) relaxations of the mouse anococcygeus (Gibson, 1988), like those of bovine retractor penis (Bowman & Drummond, 1984), are blocked by the guanylate cyclase inhibitor N-methyl-hydroxylamine (NMH), suggesting that activation of guanylate cyclase by the NANC transmitter is a vital step in the neurotransmission process. Nitrovasodilator drugs also activate guanylate cyclase (Ignarro & Kadowitz, 1985) ; indeed, it has been suggested that the inhibitory factor from bovine retractor penis, a putative NANC transmitter (Gillespie & Martin, 1980), might be nitric oxide (NO) generated by acidification of extracted nitrite (Furchgott, 1986). Thus, it is possible that investigation of the interactions between NANC stimulation and nitrovasodilator drugs might lead to the production of useful new drugs which could modify the NANC neuro-transmission system. In this study, some of these interactions have been examined using the mouse anococcygeus.

Anococcygeus muscles were isolated from male mice (LACA strain) and set up for the recording of isometric tension responses ; biphasic NANC relaxations to 60 s trains of field stimulation (10 Hz) were obtained as described previously (Gibson & Yu, 1983).

Sodium nitroprusside (SNP ; 0.01 - 1 μM) and glyceryl trinitrate (GTN ; 0.2 - 50 μM) caused dose-related relaxations of carbachol (50 μM)-induced tone ; SNP was 10 - 100 times more potent than GTN and had a much steeper dose-response curve. Freshly prepared sodium nitrite (non-acidified ; 0.05 - 1 mM) also caused dose-related relaxations ; after acidification (pH = 2 for 2 min followed by neutralisation) there was a 10-fold leftward shift of the nitrite dose-response curve, and the relaxations became more rapid and transient. The effect of NMH was studied on concentrations of the nitrovasodilators which reduced tone by 40 - 60%. 2 mM NMH significantly reduced relaxations to 0.1 μM SNP (by 69 ± 6% ; n = 8), 5 μM GTN (by 39 ± 6% ; n = 6), and 50 μM acidified nitrite (by 64 ± 12% ; n = 5) ; however, relaxations to 400 μM non-acidified nitrite were unaffected by 2 mM NMH. As found previously (Gibson, 1988), 2 mM NMH also reduced both phases of NANC relaxation (first phase by 49 ± 5% ; second phase by 60 ± 7%).

Anococcygeus muscles could be made tolerant to GTN by previous exposure to 20 μM GTN for 1 h ; this caused a significant (80 ± 2%) reduction in response to 5 μM GTN, but had no effect on NANC relaxations. Responses to 0.1 μM SNP were totally blocked by 150 μM potassium ferricyanide, and those to 50 μM acidified nitrite were totally blocked by 40 μM hydroquinone ; neither procedure had any effect on NANC relaxations.

It is concluded that NANC stimulation, SNP, GTN, and acidified nitrite produce relaxations of the mouse anococcygeus via a common mechanism, probably activation of guanylate cyclase. Non-acidified nitrite relaxes the anococcygeus by a different mechanism, not susceptible to block by NMH. Although NANC stimulation and the nitrovasodilators share a common mechanism each drug could be antagonised by a procedure which had no effect on NANC-induced relaxation.

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AUTORADIOGRAPHIC LOCALIZATION OF CALCITONIN GENE-RELATED PEPTIDES BINDING SITES IN GUINEA-PIG AND HUMAN LUNG

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Calcitonin gene-related peptide(CGRP) is a 37 amino acid peptide which is widely distributed in sensory neurones and nerve fibres in the respiratory tract of several species including man. Pharmacological studies indicate that CGRP is a potent vasodilator and also constricts human airways in vitro (Palmer et al.,1987). Using [^{125}I]human CGRP(hCGRP), high affinity binding sites have been demonstrated in membrane preparations from rat visceral organs including lung (Nakamuta et al.,1986). Using a method developed previously to study neuropeptide receptors (Carstairs & Barnes,1986), we have now studied the binding of [^{125}I]hCGRP to guinea-pig and human lung sections in order to localize binding sites. Slide-mounted guinea-pig and human lung sections (16μm thick) were incubated with [^{125}I]hCGRP(0.25nM) at 4^{0}C in the presence of 50mM TRIS-HCl buffer(pH 7.4) containing 5mM $MgCl_2$, 2% polypeptide, 500 KI units ml^{-1} aprotinin and 0.5mg ml^{-1} bacitracin. Under these conditions, binding was stable and there was no degradation of labelled peptide. Maximum binding was found at 60 min at 4^{0}C and was stable for 2h. [^{125}I]hCGRP binding was displaced by native hCGRP with an IC_{50} of 5.0nM. Salmon calcitonin was less potent (IC_{50} 175nM) whilst substance P(1μM) was ineffective, consistent with previous studies in rat lung membranes. Computer analysis of the competition curve for hCGRP was consistent with the presence of a single, high affinity binding site. Non-specific binding in the presence of 1μM hCGRP made up only 10% of total binding for guinea-pig lung and 35% for human lung.

For autoradiographic studies, cryostat sections (8μm thick) of lung from guinea-pig and human (obtained at lobectomy for bronchial carcinoma and microscopically normal) were thaw-mounted onto microscopic slides coated with poly-l-lysine. The slides were incubated under conditions described above. Autoradiograms showed specific labelling over discrete structures in both guinea-pig and human lung. Labelling was particularly dense on bronchial blood vessels in both guinea-pig and human lung. There was no labelling over epithelium and relatively little over smooth muscle of any airways in either species. Pulmonary arteries and veins were very densely labelled in both guinea-pig and human lung and labelling was over endothelial and smooth muscle cells. In both species, there was also uniform labelling over the alveolar walls.

CGRP is a potent vasodilator and our findings of localization of CGRP binding sites on pulmonary and bronchial blood vessels suggest that this peptide may be important in mediating pulmonary and airway blood flow in humans.

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LOPERAMIDE REDUCES CHOLINERGIC NERVE ACTIVITY IN HUMAN ISOLATED COLON BY OPIOID AND NON-OPIOID MECHANISMS

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Calcium channel antagonism by loperamide has been reported to contribute to the compound's anti-diarrhoeal properties (Reynolds et al., 1984).

The purpose of the present experiments was to determine if loperamide, like morphine (Burleigh & Trout, 1986) reduced cholinergic nerve activity in human isolated taenia coli muscle (taenia) and whether such an action was due solely to opioid receptor stimulation.

Taenia were incubated in [^{3}H]-choline to radiolabel neuronal stores of ACh. Preparations were then stimulated twice (1 Hz, 1 ms, 480 pulses at 200 mA) and the superfusate sampled for tritium content. Radioactive release was expressed in terms of a fractional release ratio of the two stimuli so that control ratios could be compared to ratios where drugs were introduced between the stimuli. For calcium antagonism loperamide was tested against calcium induced contractions in a potassium rich calcium free Krebs.

Loperamide (1.9 - 97.3 μM) depressed ACh release in a dose-dependent manner - the maximum effect being greater than the maximum effect of 0·42 achieved by morphine - naloxone (2.7 μM) reversed this effect although the depression remained significant at the higher doses (Fig. 1). Loperamide also caused a dose-dependent reduction (59% at 7.8 μM, 84% at 19.5 μM) of submaximal contractions to calcium (2.6 mM).

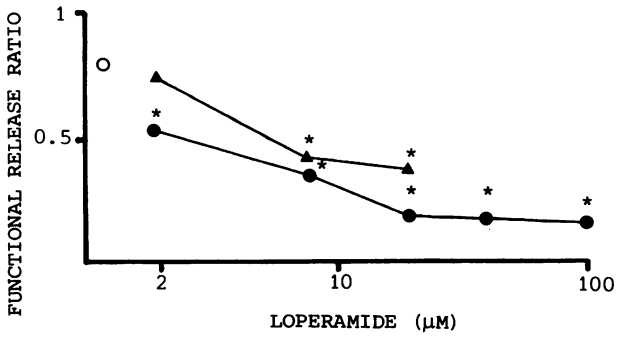

FIG. 1. : Effect of loperamide (●—●) and loperamide + naloxone (2.7 μM, ▲—▲) on evoked release of tritiated material from human taenia muscle strips. Open symbol is control. Data expressed as medians, * P > 0.05 significantly different from control value.

It appears that reduction of human colonic cholinergic nerve activity by loperamide is only partially due to opioid receptor stimulation. The additional action may be due to calcium antagonism as loperamide antagonised calcium contractions in concentrations causing submaximal reduction of ACh released.

Supported by The Wellcome Trust and Smith Kline French Foundation.

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THE EFFECT OF CALCIUM, LITHIUM AND SODIUM IONS ON THE CONTRACTILITY OF MYOFIBROBLASTS

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Knowledge of the contractile mechanism possessed by myofibroblasts is still rudimentary. It has been suggested that myofibroblasts possess a similar contractile mechanism to smooth muscle (Majno et al. 1973). However, such a comparison is at variance with in vitro findings which include the lack of a response of myofibroblasts to barium ions (Gabbiani et al. 1973). This study investigates the importance of calcium and sodium ions and the effect of lithium ions on the responses induced by barium ions, potassium ions, mepyramine, angiotensin and vasopressin on myofibroblasts and on vascular smooth muscle.

The rat testicular capsule (n = 8) was used as a model for myofibroblasts (Lal & Naylor 1985) and the rat aortic strip (n = 6) as a model for vascular smooth muscle. A superfusion system was used (37°C, 2ml min^{-1}) with the physiological solutions; (a) Krebs, (b) Tyrode, (c) Krebs + 10-100mM lithium, (d) Tyrode + 50mM lithium (e) Sodium free Tyrode and (f) Calcium-Sodium free Tyrode. The response to barium and potassium chloride (1-8mg), mepyramine (40-160µg), vasopressin (8-32ng), and angiotensin (16-64ng) were studied. They were measured as contractile force (mg) and duration of response (secs). Statistical analyses were carried out using Wilcoxon's Rank Sum test and the Mann Whitney U test.

In either normal Krebs or Tyrode barium and potassium ions were 100 and 20 times respectively more potent in causing a contraction in vascular smooth muscle than in the myofibroblast tissue. In addition the onset time (<5 secs) and duration of the response (120-180 secs) for potassium ions, and the onset (<10 secs) and prolonged duration (>480 secs) to barium ions in the vascular muscle were faster and more prolonged ($P<0.05$) than for myofibroblasts. Responses to vasopressin and angiotensin were also greater in the vascular tissue ($P<0.05$). Mepyramine (40-640µg) caused a contractile response in the testicular capsule but was ineffective on the aortic strip.

In Krebs containing 10mM lithium ions mepyramine induced contractions in the testicular capsule preparation were unaffected either in terms of the contractile force or the duration of the response. At 50mM lithium they were potentiated in duration (1.5 times) but not in magnitude and at 100mM lithium the contractions were abolished. In Tyrode containing 50mM lithium the duration of the contractile response to vasopressin, angiotensin and mepyramine was potentiated in both preparations ($P<0.05$), whereas the contractile force was not affected. In sodium free Tyrode both the magnitude and the duration of the response to all three agonists was reduced. In the testicular capsule, for example, the response was reduced by a median % value of 28 for vasopressin, 26 for angiotensin and 61 for mepyramine ($P<0.05$). In calcium and sodium free Tyrode, the effects induced by vasopressin, angiotensin and mepyramine were not significantly different from the results obtained in the sodium free solution.

The quantitative and qualitative differences in contractile responses caused by barium and potassium ions, mepyramine, vasopressin and angiotensin in the rat testicular capsule and aortic strip indicate that the contractile mechanisms in vascular smooth muscle differ from those in the myofibroblast. The contractile responses in the myofibroblast are indicated to be mediated via a lithium/sodium sensitive mechanism the significance of which is being further investigated.

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EMCA - INDUCED CHOLINERGIC LESIONS IN SERUM-FREE BRAIN REAGGREGATE CULTURES: STUDIES WITH NEUROTROPHIC FACTORS

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ECMA has been proposed as a selective cholinergic neurotoxin in vivo (Fisher & Hanin, 1986). We have recently shown that at low concentrations in vitro (12.5 μM) ECMA appears also to produce selective cholinergic lesions in rat brain reaggregate cultures in a serum-supplemented medium (Pillar et al, 1987).

Reactive synaptogenesis in both developing and aging brain may involve the action of neurotrophic factors (NTF) and the production of such factors in conditions such as Alzheimers Disease has been of interest (see Atterwill & Bowen, 1986). Injured or denervated brain in vivo secretes increased of NTF's in an age-dependent manner (Needels et al, 1985) and NTF's such as TRH (Kanamoto et al, 1986) and NGF (Atterwill & Bowen, 1986) stimulate cholinergic neuronal regeneration. We have now tested ECMA in serum-free reaggregate cultures with a view to examining the production and action of NTF's.

Foetal rat whole brain reaggregate cultures were prepared as previously described (Pillar et al, 1987) in a serum-supplemented (S+) or serum-free medium (S-) (Atterwill & Bowen, 1986). ECMA was added to the cultures at 9 DIV in concentrations of 12.5, 25 or 50μM. ChAT activity was measured at +2, +48 and +96h following treatment. 12.5 μM ECMA appears to produce selective cholinergic lesioning following 48h exposure, whereas 50μM produces a more generalised cytotoxicity in reaggregate cultures. In certain experiments TRH (50μg/ml, daily from 9 DIV) or NGF (7S subunit, 5ng/ml, 0 and 48h) were added during ECMA treatment.

ECMA (12.5μM) causes a rapid, HC_3 sensitive loss of ChAT activity in S+ reaggregates (38%, +2h, Pillar et al, 1987). In contrast, S- reaggregates showed minimal or no immediate loss of ChAT activity but a 40-50% inhibition was achievied by 48h and was maintained until 96h. Although this was also less than that achieved in S+ reaggregates it may correspond to the HC_3 - insensitive phase and represent cholinergic neuronal loss. At higher ECMA concentrations (25-50μM) up to 85% ChAT activity was lost in the S- reaggregates. In both S+ and S- brain reaggregates NGF produced increased ChAT activity with more marked effects in S+ (45% increase, +48-96 h) than in S- medium (20-25% increase, + 2-96 h).

TRH had no effect on ChAT in the S+ cultures but produced small increases in S- conditions (approx 20%, +2-48h). Despite a residual 'ECMA-resistant' pool of ChAT in the cultures, neither NTF was found to cause a reversal of the lesion.

In conclusion, it has been shown that ECMA can produce a cholinergic deficit in both S+ and S- reaggregates. This should enable studies not only of NTF lesion-reversal but lesion-induced NTF secretion. Further studies will include NGF added together with GM_1 ganglioside (Doherty et al, 1985).

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EFFECT OF GENTAMICIN ON Ca^{2+}-ACTIVATED K^+ TRANSPORT INTO ONE STEP INSIDE-OUT MEMBRANE VESICLES PREPARED FROM HUMAN RED BLOOD CELLS

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Despite extensive investigation, the mechanisms by which aminoglycoside antibiotics (AGs) exert their toxicities are not understood. It has been speculated that interference with K^+ movements may contribute to the development of toxicity, at least in the sensory cells of the inner ear (Lee & Harpur, 1985). Since Ca^{2+}-activated K^+ channels are present in at least two target tissues for AGs, the inner ear (Ashmore & Meech, 1986) and the motor nerve terminal (Mallart, 1985), and since AGs are known to compete with divalent cations for binding sites in a number of biological systems, it was considered that a study of the effect of AGs on Ca^{2+}-activated K^+ movements was merited.

As a model to investigate the interaction between Ca^{2+}-activated K^+ channels and the AG, gentamicin (GEN), we have employed one step inside-out red blood cell vesicles (IOVs) prepared as described by Alvarez et al. (1984). The activity of the Ca^{2+}-activated K^+ channels was assessed from measurement of the uptake of ^{86}Rb, which is congeneric with K^+ in this transport system. The system was first characterised by establishing a dose response to Ca^{2+}.

GEN alone (in the presence of 4×10^{-4}M EGTA) increased ^{86}Rb flux into the IOVs. The maximal effect (at t = 10 min and GEN concentrations $\leq 10^{-4}$M) was about 30% of that seen with 10^{-5}M Ca^{2+}. However, there was marked inter-individual variability depending on the source of the blood from which the IOVs were prepared. Maximal GEN-stimulated ^{86}Rb uptake into IOVs prepared from the blood of one donor was markedly greater than all others, such that in this case 10^{-4}M GEN produced >100% of the activation caused by 10^{-5}M Ca^{2+}. IOVs from this donor showed normal Ca^{2+}-activated ^{86}Rb uptake. This GEN-dependent ^{86}Rb uptake was not antagonised by 1mM quinine (which reduced the Ca^{2+}-dependent uptake by 74% at 10^{-6}M Ca^{2+}) or by 1mM ouabain, or by 1mM furosemide, in all cases when added to the external incubation medium.

The rate of Ca^{2+}-activated ^{86}Rb uptake was unaltered by GEN; half time to equilibrium (10 min) was 1.8 min for 10^{-6}M Ca^{2+} alone and 1.9 min for 10^{-6}M Ca^{2+} in the presence of 2×10^{-4}M GEN. However, the extent of activation of K^+ channels by Ca^{2+} was decreased by GEN. GEN (2×10^{-4}M) reduced Ca^{2+}-activated ^{86}Rb uptake to 19% of that seen with 10^{-6}M Ca^{2+} alone after 10 min (paired 't'-test $p < 0.02$). This residual activity was comparable to that produced by 2×10^{-4}M GEN alone. At very high concentrations of Ca^{2+} (>10^{-4}M) the inhibitory effect of 2×10^{-4}M GEN was overcome, although non-specific mechanisms of Ca^{2+}-activation of ^{86}Rb uptake are likely to be involved at these concentrations. These results exclude data obtained using IOVs from the one donor where a particularly marked GEN-activated ^{86}Rb uptake was seen.

Thus GEN appears to exert two effects on ^{86}Rb uptake into IOVs. Firstly, it produces a concentration-related stimulation of uptake, the mechanism of which remains to be elucidated. Secondly, it antagonises the effect of Ca^{2+} on ^{86}Rb uptake. The possible relevance of either of these effects to the toxicity of GEN requires further investigation.

We thank Roussel Laboratories Ltd. for a gift of gentamicin.

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THE EFFECT OF SUPEROXIDE DISMUTASE (SOD) ON THE BREAKDOWN OF NITRIC OXIDE (NO)

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Endothelium-derived relaxing factor (EDRF) has been recently identified as nitric oxide (NO) (Palmer et al., 1987). EDRF is inactivated by superoxide anion (O_2^-) in the effluent from superfused endothelial cells (EC) (Gryglewski et al., 1986a). The O_2^- could be already present in the Krebs' solution or additionally released by EC or both. Here, we tested the effect of SOD on NO survival in Krebs' solution and in the effluent from perfused bovine aortic EC (BAEC).

NO solution was prepared by injecting 1ml of NO gas at atmospheric pressure into 40ml of helium deoxygenated water held in a rubber sealed Wheaton flask. Aliquots of the NO solution (1-20 μl) were withdrawn with a Hamilton microsyringe. The NO was bioassayed in a cascade (Vane, 1964) of four-de-endothelialized strips of rabbit aorta (RbA). The bioassay tissues were contracted by either U46619 (30-60 nM) or noradrenaline (100-500 nM). The NO was given either over the tissues (O.T.) or through a delay coil (20-70 s) in order to measure the inactivation of NO. EC were cultured on microcarrier beads. The cell column and the bioassay cascade were assembled as described by Gryglewski et al (1986b).

SOD destroys superoxide anion and enhances the amount of EDRF bioassayed after release from EC by bradykinin, ADP or AA ($n>30$). The "t1/2" of NO in the delay coil was 25 ± 3 s ($n=8$). SOD given either immediately over the tissues (O.T.) or into the delay coil did not affect the responses of the 1st tissue to NO given O.T. However, when NO was given through the delay coil, SOD enhanced its survival as detected by the first tissue ("t1/2" = 61 ± 13 s $n=8$; $p< 0.025$). Both EDRF and NO had a much shorter half-life when measured over the cascade ("t1/2" = 4-6 s, $n>20$). Removal of the first three tissues of the cascade did not affect the disappearance of NO when given O.T. ($n=4$). SOD given either O.T. or through the delay coil increased only slightly the survival of NO in the cascade, suggesting that here the disappearance of NO is mainly due to escape of the NO gas from the solution.

Thus, there is O_2^- in the Krebs' solution, but the amount does not account for the strong protection by SOD of EDRF when it is released from EC by bradykinin. In this situation, EDRF is in Krebs' solution for < 10 s before being assayed. Therefore, the amounts of EDRF that would be destroyed by the O_2^- present in the Krebs' solution cannot account for the potentiation given by SOD. These results point to the simultaneous release from EC of EDRF and O_2^-.

The William Harvey Research Institute is supported by a grant from Glaxo Group Research Limited.

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