A SENSITIVE ELECTROCHEMICAL METHOD FOR SIMULTANEOUS DETERMINATION OF HISTAMINE AND NORADRENALINE BY HPLC (HPLC/EC)

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Since histamine (H) and noradrenaline (NA) are important mediators of ischemia/reperfusion-induced cardiac dysfunction (Masini et al., 1987), a simultaneous determination of the two compounds in biological samples would be a useful tool for understanding their role in such cardiac arrhythmias. We therefore describe a simple and sensitive HPLC/EC method based on precolumn derivatization of the compounds with O-phthalaldehyde (OPA) and 2-mercaptoethanol (2-ME) under alkaline conditions (pH=9.5-10.5). This technique of derivatization for producing electrochemically active products of various amino acids has been described by Allison et al (1984).

The HPLC/EC setup consisted of a Waters 501 pump coupled to a Bioanalytical LC-4B detector in oxidation mode and a Nucleosil C-18 reverse-phase column (150 x 4.6 mm; 5 μm particle size). The working electrode potential was kept at +0.70 V vs Ag-AgCl reference electrode. The mobile phase (buffer) consisted of (mM): Na_2EDTA, 0.15; octyl sodium sulfate, 0.43; Sodium Acetate $\cdot 3H_2O$, 100; citric acid, 20; and contained 30% acetonitrile. The buffer was degassed and pumped at 1 ml/min. H and NA were derivatized using the following sequence of additions: 390 μl of buffer, 15 μl NaOH (2N) to bring pH to 9.5-10.5, 15 μl OPA (0.25% in methanol), 15 μl 2-ME (0.25% in methanol) 10-20 μl of standard H and NA or 50 μl tissue extract. More NaOH was required when tissue extracts were assayed. For tissue extracts, they were homogenized in 0.4 M perchloric acid and purified with Amberlite (CG-50). 3-methylhistamine and 3:4 dihydroxybenzylamine were used as internal standards.

The reaction of H and NA with OPA/2-ME at alkaline pH yielded electrochemically active (oxidizable) products. However, no such active products were formed when the reaction was carried out without 2-ME or without OPA. The OPA/2-ME products of H and NA were stable at the buffer pH (4.9) and did not require additional acid to stabilize them. The peak potentials for the OPA/2-ME derivatives of both H and NA were +0.085 V, while +0.7 V was chosen because of low noise and stable baseline.

The relationship between the electrochemical signal of OPA/2-ME derivatives of H and NA and their respective peak heights were found to be linear over the concentration range of 50 pg to 50 ng of H and NA, with the minimum detection being at least 50 pg (signal:noise ratio = 3:1) for each compound.

The capacity factors k' (defined as V_t-V_o/V_o where V_t = retention volume of the compound and V_o=void volume) of various potentially interfering compounds were determined. A comparison of the calculated k' showed that determination of H and NA was not interfered with by 1-, 2-, 3- or 4-methylhistamines, histidine, dopamine, 5HT or adrenaline.

The recoveries of H and NA carried through the procedure ranged between 60 and 75% and the rat heart content of H and NA was determined to be 2.88 ± 0.46 and 0.92 ± 0.10 μg/g (X ± SEM; n=5) respectively. These values of H and NA in the heart are in agreement with those reported by other workers (see Wolff & Levi, 1986; Chaudhry & Vohra, 1984).

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Chaudhry, A. & Vohra, M.M. (1984) Eur. J. Pharmacol. 97, 156-158.
Masini, E. et al. (1987) Agents and Actions 20, 281-283.
Wolff, A.A. and Levi, R. (1986) Cir. Res. 58, 1-16.
Allison, L.A. et al. (1984) Anal. Chem. 56, 1089-1096.

PHARMACOKINETIC AND METABOLIC EVALUATION IN THE RAT OF A NEW PYRROLIDINEDIONE AROMATASE INHIBITOR

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The aromatase inhibitor aminoglutethimide (AG), currently employed as a second-line therapy for advanced hormone-dependent breast cancer in postmenopausal women, has several major side-effects such as inhibition of corticosteroid synthesis and CNS depression. However, a new analogue 3-(4'-aminophenyl)-3-ethyl pyrrolidine-2,5-dione (WSP-1) is as active an aromatase inhibitor as AG (Pourgholami et al. 1987a) but possesses much weaker effects on corticosteroid synthesis (Pourgholami et al. 1987b) and the CNS (Ahmad et al. 1987). The present work compares the pharmacokinetic profile and N-acetylation of WSP-1 and AG in female Wistar rats (200-250g).

The animals were given an oral dose (25mg/kg) of either AG or WSP-1 after withholding food (but not water) for 16h. At various times (up to 24h) thereafter, blood was collected by cardiac puncture under terminal ether anaesthesia from groups (n=8) of the dosed animals. In separate groups of treated rats, urine was collected for 72h after dosing. AG, WSP-1 and their respective N-acetyl metabolites were determined in plasma by reversed phase hplc (Pourgholami et al. 1988).

Table: Pharmacokinetic parameters* of WSP-1 and AG (both 25mg/kg po) in female rats

Compound	Tmax (h)	plasma Cmax (µg/ml)	plasma $t_{0.5}$ (h)	Total clearance (ml/min)	Vd (ml)
WSP-1	0.5	16.6	2.3	1.6	316
AG	2.0	10.2	2.8	1.3	317

*Values are means (n=8); in each case sem <10% of mean.

The results presented in the Table show that WSP-1 rapidly attains its peak level in plasma and that this is considerably higher than in the case of AG. This may indicate that WSP-1 is absorbed more quickly than AG from the gastrointestinal tract. However, the former compound has a slightly shorter $t_{0.5}$ and the total clearance and apparent volume of distribution (Vd) do not significantly ($P>0.05$) differ for the two compounds. Analysis of the 72h urines revealed that very little of the dose of either compound is eliminated unchanged (2.4±0.2% and 2.9±0.1% for WSP-1 and AG respectively). The N-acetyl metabolites of the two compounds were detected in both plasma and urine. For N-acetyl AG, a peak plasma level (5.7µg/ml) was attained at 3h; for N-acetyl WSP-1, the peak level (55.5µg/ml) occurred at 2h. This suggestion of a greater importance of the acetylation pathway for WSP-1 than for AG is supported by the quantitative data for urine (73.2 ± 9.4% and 29.0 ± 1.9% of the administered dose as acetylated WSP-1 and AG respectively in the 72h urine). In man, AG undergoes polymorphic acetylation (Adam et al. 1984) but because of the minor importance of this pathway the phenomenon is not clinically significant. However, if WSP-1 is ever evaluated in man such a pattern of metabolic control may be important in influencing the therapeutic efficiency of this new compound.

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Adam, A.M. et al. (1984) Br. J. clin. Pharmac. 18, 495-505.
Ahmad, B. et al. (1987) J. Pharm. Pharmac. 39, 96P.
Pourgholami, M.H. et al. (1987a) J. Steroid Biochem. 26, 309-312.
Pourgholami, M.H. et al. (1987b) J. Steroid Biochem. 28, 148S.
Pourgholami, M.H. et al. (1988) J. Chromatogr. 424, 163-169.

FPL 63547 - AN INHIBITOR OF ANGIOTENSIN -CONVERTING ENZYME WHICH DEMONSTRATES PREFERENTIAL BILIARY EXCRETION

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FPL 63547 is a novel inhibitor of angiotensin-converting enzyme (ACE) which shows prominent antihypertensive properties in the spontaneously hypertensive rat (Carr et al, 1988). We have investigated the route of elimination of the active diacid form of FPL 63547 in comparison with other ACE inhibitors, enalapril (active form - enalapril diacid) and lisinopril.

Male Sprague-Dawley rats (250g) were anaesthetised with pentobarbitone (54 mg kg^{-1} i.p. initially, followed by 0.25 mg kg^{-1} min^{-1} i.v.), the bile duct was cannulated and the bladder exposed. Each rat then received a single i.v. dose (2 µmol kg^{-1}) of either FPL 63547 (n=5), enalapril (n=3) or lisinopril (n=3), and bile and urine samples were collected for three hours after dosing. Samples were diluted and assayed for ACE inhibitory activity using rabbit lung ACE. The amount of the active form of compound present in each sample was calculated by comparison with a standard curve.

Biliary excretion of FPL 63547 diacid was rapid and consistent, reaching a peak in the first hour after dosing during which 45% of total drug was eliminated by this route, with 9% and 2.7% in the second and third hours respectively. By comparison, only 2.5% was excreted in the urine over the total sampling period. Thus, the elimination of FPL 63547 diacid showed a strong preference for the biliary route over the urinary route resulting in a bile : urine ratio of 22.5 : 1. These results are summarised in Table 1, which also gives comparative data for the other ACE inhibitors tested.

Table 1 Percentage of total dose excreted in 3h (means ± s.e.)

	Bile	Urine	Bile : Urine Ratio
FPL 63547 diacid	56.9 ± 4.8%	2.5 ± 0.2%	22.5 : 1
Enalapril diacid	24.3 ± 1.5%	28.7 ± 3.7%	0.85: 1
Lisinopril	4.2 ± 1.3%	63.4 ± 12.0%	0.07: 1

The preference of FPL 63547 diacid for biliary elimination was in marked contrast to enalapril diacid which showed little selectivity for either route and, particularly, to lisinopril which was excreted almost exclusively in the urine.

The biliary route of elimination will be favoured in patients whose renal function is impaired as a result of disease or age. In such patients the elimination of renally excreted ACE inhibitors will be compromised, necessitating closer monitoring and dose titration/reduction. For example, studies of enalapril (Kelly et al, 1986) and lisinopril (van Schaik et al, 1987) in man with renal impairment have shown elevated plasma levels. Therefore, the elimination profile of FPL 63547, if confirmed in man, may prove to be clinically advantageous.

Carr, R.D. et al (1988) This meeting
Kelly, J.G. et al (1986) Br. J. clin. Pharmac. 21, 63-69
van Schaik, B.A.M (1987) Eur. J. Clin. Pharmac. 32, 11-16

ENHANCED VASODILATOR AND VASOCONSTRICTOR EFFECT OF DRUGS IN PERFUSED RAT KIDNEY OF STREPTOZOTOCIN DIABETIC RATS

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The vasodilator effect of acetylcholine (ACh) in isolated arteries and resistance blood vessels of perfused organs is mediated by endothelium derived relaxing factor (Furchgott, 1983). Oyama and colleagues (1986) have reported decreased aortic EDRF biosynthesis in streptozotocin-diabetic rats. Thus, a deficient vascular EDRF formation or activity may contribute to the cardiovascular pathology of diabetes in man. An important clinical feature of human diabetes is renal nephropathy. For this reason we considered it of interest to examine the response of resistance blood vessels of the perfused rat kidney taken from streptozotocin-diabetic rats.

Male rats (Wistar, 250-300 g) were injected intraperitoneally with streptozotocin (80 mg/kg) or an appropriate volume of acidified saline (pH 4.2). Animals were sacrificed either before or 12 days after treatment. Blood samples (0.5 ml) were collected from ether-anaesthetised animals by cardiac puncture. Plasma glucose was assayed spectrophotometrically. Kidney perfusion was performed as described by Armstrong et al., (1976). Briefly, the left kidney was cannulated, removed to a heated, water-jacketed chamber and perfused (6 ml/min) with warmed (37°C), oxygenated (95% O_2:5% CO_2) Krebs solution containing indomethacin (8 μM). Perfusion pressure was monitored by means of a Bell & Howell transducer connected to a Devices pen recorder.

A four fold increase in plasma glucose concentration (16.7±1.1 mmol/l c.f. 4.0±0.6 mmol/l, n=15-17, $P<0.01$) was observed in rats injected with streptozotocin. Bolus injection (1-20 μl) of noradrenaline resulted in transient, dose related increases in perfusion pressure in kidneys from both control and diabetic animals. Noradrenaline exhibited more potent vasoconstrictor activity in diabetic (EC_{50}, 0.35±0.09 nmol, maximal effect, 231±21 mm Hg, n=10) than in control (EC_{50}, 0.82±0.08 nmol, maximal effect 176±16 mm Hg, n=10) rats. In contrast, bolus injection of ACh or nitroprusside caused dose related and transient falls in perfusion pressure in noradrenaline-preconstricted kidneys. The vasodilator effect of ACh was enhanced in diabetic rats compared with control, normoglycaemic animals. For example, ACh (0.2 nmol) produced a fall in renal perfusion pressure of 41.5±3.4 mm Hg (n=10) in diabetic animals and 24.3±2.3 mm Hg ($P<0.05$) in control rats. The maximal response attainable was also increased in diabetic rats (68±5.6 mm Hg c.f. 33.2±3.2 mm Hg, n=10, $P<0.01$). The response to nitroprusside was unchanged in streptozotocin injected rats. Responses to ACh (but not nitroprusside) in kidneys from both control and diabetic rats were substantially reduced or abolished by mepacrine (10 μM), methylene blue (10 μM) or metyrapone (30 μM) demonstrating the involvement of EDRF in renal vasodilatation due to ACh.

The present results demonstrate increased sensitivity to ACh of renal blood vessels from streptozotocin-diabetic rats. Whether this supersensitivity results from increased endothelial cell turnover, proliferation of endothelial cell cholinoceptors, elevated EDRF biosynthesis or enhanced responsiveness to released EDRF is not known.

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Armstrong, J.M. et al., (1976) Nature, 260, 582-584
Furchgott, R.F. (1983) Circ. Res., 53, 557-573
Oyama, Y. et al., (1986) Eur. J. Pharm. 132, 75-78.

EFFECT OF MEPTAZINOL ON RESPONSES TO SYMPATHETIC NERVE STIMULATION IN RAT VAS DEFERENS

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In contrast to many other opioid analgesics meptazinol potentiates responses to electrical stimulation of cholinergic and sympathetic neurones in isolated tissues (Duchesne et al., 1984). The effect on cholinergic neurones is probably due to inhibition of cholinesterase (Hetherington et al., 1987) but the mechanism responsible for the effect on sympathetic responses is unclear.

All experiments were performed on isolated vas deferens taken from Wistar rats (250-335 g), suspended in Mg^{++}-free Krebs' physiological saline at 38°C, gassed with 5% CO_2 in O_2 and in the presence of naloxone (2×10^{-8}M) to eliminate activation by meptazinol of opioid receptors present in rat vas deferens. Electrical stimulation (1 ms, 300 mA, every 5 min) applied through field electrodes above and below the tissue induces a biphasic response from the isolated whole vas. The two components can be largely resolved by using the bisected vas where the prostatic and epididymal portions show mainly non-adrenergic non-cholinergic (NANC) and noradrenergically mediated responses respectively (McGrath, 1978). Meptazinol (5×10^{-8} to 2×10^{-6}M) produced a reversible and concentration-dependent potentiation of the electrically induced responses in both portions of the vas; responses were increased by at least 100% at the most effective concentration. The prostatic portion of the vas did not respond to phenylephrine but the epididymal portion responded with reproducible contractions which were reversibly antagonised by meptazinol at a concentration which produced potentiation of the electrically induced responses. The cumulative concentration response curves to phenylephrine were shifted to the right by meptazinol (1×10^{-7}M), in a parallel manner giving a pA_2 value of 8.1 $\pm$ 0.4 (mean $\pm$ s.e.m.; n = 5) assuming that the antagonism was competitive. In the presence of yohimbine (5×10^{-8}M) the ability of meptazinol to potentiate the effect of electrical stimulation was not reduced but in the presence of cocaine (1×10^{-5}M) potentiation by meptazinol of the noradrenergically mediated response from the epididymal portion was reduced. The effect of meptazinol on the response mediated by the NANC transmitter was little altered by the presence of cocaine.

It is concluded that meptazinol potentiates the effects of both noradrenergic and NANC transmitters in the rat vas deferens and that only the effects on the noradrenergic component may be mediated through an action involving or requiring the integrity of the $uptake_1$ mechanism.

Duchesne, R.J. et al., (1984). J. Pharm. Pharmac., 36, 560-562.
Hetherington, M.S. et al., (1987). J. Pharm. Pharmac., 39, 185-189.
McGrath, J.C. (1978). J. Physiol., 283, 23-39.

α_1- BUT NOT α_2-SELECTIVE AGONISTS CAUSE PHOSPHOINOSITIDE HYDROLYSIS IN THE FEMORAL VEIN OF THE RAT

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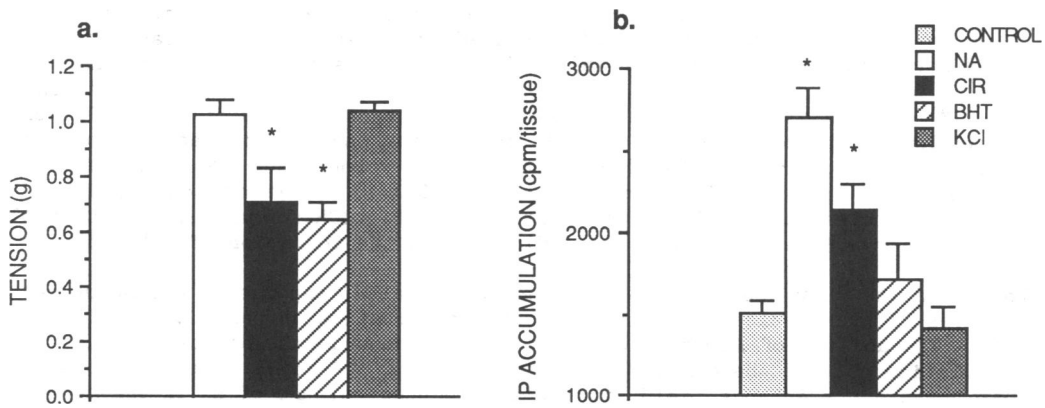
We have previously reported that noradrenaline (NA) induces stimulation of phosphoinositide hydrolysis in the femoral vein of the rat and that this hydrolysis is mediated via α_1-adrenoceptors (Stubbs *et al*, 1988). We now report the effect of selective α-adrenoceptor agonists and KCl on inositol phosphate levels in this vessel.

Femoral veins were removed from male Wistar rats (200-300g), cleaned of adherent tissue and cut open to form flat sheets. The vessels were incubated in 2ml of physiological salt solution (PSS) containing myo-^{3}H-inositol (30μCi/ml) at 37°C gassed with 5% CO_2 in O_2 for 3 hours. Tissues were then washed and placed in 1ml PSS containing 10mM LiCl, and 10^{-5}M noradrenaline (NA), cirazoline (CIR) or B-HT920 (BHT) or 60mM KCl was added. Tissues were incubated for a further 1hr and then 3ml $CHCl_3$:CH_3OH:HCl (200:100:1) was added. After 20 min, the mixture was centrifuged at 500 x g for 5 min, the upper aqueous phase was removed, added to 3ml H_2O, and applied to a Dowex chromatography column. Inositol phosphates were eluted as described by Berridge (1983) and tritiated inositol phosphate content was assessed by liquid scintillation counting. To study contractions, 5mm ring segments of femoral veins were mounted under 0.5g tension between 2 fine steel wires in PSS at 37°C gassed as above. Responses were determined to agonist after a 60 min equilibration period . The results are summarised in figure 1.

Figure 1. The effects of agonists on a) contractile responses and (b) inositol phosphate accumulation in the femoral vein of the rat.



* significantly different from controls, $p<0.05$. Values are mean ± sem, $n\geq6$

These observations support our previous findings (Stubbs *et al*, 1988) that stimulation of α_1- and α_2-adrenoceptors causes contraction of the femoral vein of the rat but it is only the α_1-mediated response that is associated with hydrolysis of phosphoinositides.

Berridge, M.J. (1983). Biochem. J., 212, 849-858.
Stubbs, D. *et al* (1988). Br. J Pharmac. in press.

EFFECTS OF DRUGS AND FIELD STIMULATION ON INOSITOL PHOSPHOLIPID HYDROLYSIS IN MOUSE VAS DEFERENS

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There is evidence that morphine and clonidine inhibit field stimulation-induced release of the non-adrenergic, non-cholinergic (NANC) transmitter but potentiate the release of noradrenaline (NA) in the mouse vas deferens (Forsyth & Pollock, 1986). Thus, morphine and clonidine inhibited the initial NANC component of the motor response to field stimulation but potentiated the secondary NA-mediated component and potentiated ^{3}H-NA overflow. This study re-examined the actions of morphine and clonidine in the mouse vas deferens by comparing the effects of these drugs on field stimulation-induced and NA-induced enhancement of inositol phospholipid (PI) hydrolysis. If morphine and clonidine potentiate neural release of NA, then they should potentiate field stimulation-induced enhancement of PI hydrolysis but have no effect on NA-induced increases in PI hydrolysis.

Vasa from male Porton mice (25-30 g) were incubated in ^{32}P-orthophosphate (2 µCi per vas in 3 ml Hepes buffer, pH 7.2, 37°C, 2 hr), then transferred to Krebs buffer (37°C) gassed with 95% O_2/5% CO_2. Vasa were then field stimulated (FS) (20 Hz, 0.5 ms pulse width, 100 pulses, supramaximal voltage) and/or exposed to drugs. Next, vasa were frozen in liquid nitrogen, homogenized and PI hydrolysis was monitored by measuring the tissue levels of phosphatidic acid (ptdOH) using the lipid extraction method of Lloyd et al (1972). NA (10^{-5} M) and field stimulation increased formation of ^{32}P-ptdOH (NA: 74 ± 15 mean % increase above control ± s.e. mean, n = 5, $P < 0.05$; FS: 71 ± 9 mean % increase above control ± s.e. mean, n = 5, $P < 0.01$). These responses were inhibited by prazosin (10^{-6} M). Morphine (10^{-5} M) did not alter the basal levels of ^{32}P-ptdOH levels but enhanced the ability of field stimulation to increase ^{32}P-ptdOH formation (FS + morphine: 182 ± 30 mean % increase above control ± s.e. mean, n = 5, $p < 0.001$). This effect of morphine was antagonized by naloxone (10^{-5} M). Clonidine (10^{-7} M) did not alter the basal levels of ^{32}P-ptdOH nor the NA-induced increase in ^{32}P-ptdOH formation but enhanced the ability of field stimulation to increase ^{32}P-ptdOH formation (FS + clonidine: 180 ± 18 mean % increase above control ± s.e. mean, n = 5, $P < 0.001$). Tetrodotoxin (10^{-6} M) abolished the effects of field stimulation on ^{32}P-ptdOH formation. Neither ATP (10^{-3} M) nor α,β-methylene ATP (10^{-6} M) affected the formation of ^{32}P-ptdOH.

These results show that the α_1-adrenoceptor mediated response in the mouse vas involves PI hydrolysis but the response to the putative cotransmitter, ATP does not. The ability of morphine and clonidine to enhance the effects of field stimulation but not the effects of exogenous NA on ^{32}P-ptdOH formation, suggests that these drugs enhance field stimulation-induced release of NA in the mouse vas deferens.

Forsyth, K.M. & Pollock, D. (1986) J. Physiol. 381, 109P
Lloyd, J.V. et al (1972) Br. J. Haematol. 23, 571-585

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α_2-ADRENOCEPTOR AND κ-OPIATE RECEPTOR MEDIATED EFFECTS ON INTRASYNAPTOSOMAL FREE $[Ca^{2+}]_i$ INVOLVE BLOCKADE OF AN N-TYPE Ca^{2+}-CHANNEL

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Synaptosomes were isolated from cerebral cortices of 2-4 Male Wistar rats on 4 step discontinuous percoll density gradients (Dunkley et al, 1986) and intrasynaptosomal free $[Ca^{2+}]_i$ measured using the fluorescent Ca^{2+}-indicator fura-2 (Grynkiewicz et al, 1985): basal calcium $[Ca^{2+}]_i$ was found to be 292 nM (median value, n = 128).

Incubation of fura 2-loaded synaptosomes for 1 minute with either U50,488H(1μM) (a specific K-opiate agonist) or clonidine (1μM) reduced $[Ca^{2+}]_i$. These effects were dose dependent; with U50,488H, the maximum decrease (23.3%) occurred at 100μM and the EC_{50} = 0.08μM ($p < 0.003$, paired randomization test) and with clonidine the maximum decrease (30.6%) occurred at 100μM and the EC_{50} = 0.27μM ($p < 0.009$, paired randomization test). These changes in $[Ca^{2+}]_i$ were reversed by inclusion of either naloxone (20μM) or idazoxan (RX781094) (2μM) which produced net increases in $[Ca^{2+}]_i$ of 10.75% and 0.75% respectively.

Studies in which synaptosomes were treated with w-conotoxin fraction GVIA from Conus geographus (w-CgTX) (93nM, 1 min, 37°C) resulted in a 15.7% reduction in $[Ca^{2+}]_i$ ($p < 0.004$, paired randomization test). When either U50,488H(1μM) or clonidine (1μM) plus w-CgTX (93nM) were co-incubated with synaptosomes for 1 min, the reductions in $[Ca^{2+}]_i$ were 11.4% and 18.3% respectively i.e. there were no additive effects. The decrease in $[Ca^{2+}]_i$ in both cases was not significantly different from that after incubation of synaptosomes with w-CgTX (93nM) alone ($p < 0.219$, unpaired randomization test).

As w-CgTX fraction GVIA is reported to be a blocker of both N and L-type Ca^{2+}-channels, this data suggests that clonidine and U50,488H elicit their effects via a blockade of a N- or L-type Ca^{2+}-channel.

Subsequent sutdies in which U50,488H(1μM) or clonidine (1μM) were co-incubated with the L-type Ca^{2+}-channel blocker nifedipine (1μM) (Janis and Triggle, 1987) for 1 minute showed that the action of both U50,488H and clonidine were independent of the L-type Ca^{2+}-channel. This conclusion was based on the observation that co-incubation of synaptosomes with nifedipine alone, significantly reduced $[Ca^{2+}]_i$ by 17.0% ($p < 0.008$ paired randomization test), but when clonidine (1μM) or U50,488H(1μM) were co-incubated with nifedipine (1μM) there was a reduction of 48.1% and 35% in $[Ca^{2+}]_i$ respectively. These values obtained with the α_2- and K-opiate agonists were significantly different from both control ($p < 0.015$, $p < 0.008$ respectively, paired randomization test) and those values obtained with nifedipine alone ($p < 0.001$, $p < 0.001$, respectively, unpaired randomization test). In conclusion we have showed that α_2 -adrenoceptor and K-opiate receptor mediated effects on intrasynaptosomal free $[Ca^{2+}]_i$ involve blockade on an N-type Ca^{2+}-channel.

Dunkley, P. R., Jarvie, P. E., Heath, J. W., Kidd, G. J. and Rostas, J. A. P. (1986) Brain Res. 372, 115-129.

Grynkiewicz, G., Poenie, M. and Tsien, R. Y. (1985) J.Biol.Chem. 260, 3440-3450.

Janis, R. A. and Triggle D. J. (1987) Ann.Rev.Pharmacol.Toxicol. 27, 346-369.

HISTAMINE AND CARBACHOL STIMULATE [^{3}H]-INOSITOL PHOSPHATE ACCUMULATION IN BOVINE LUNG PARENCHYMA TISSUE

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Many smooth muscle spasmogens, such as histamine (HA) and carbachol (CCh), stimulate the breakdown of inositol phospholipids in tracheal smooth muscle (Barnes et al., 1986, Hall & Hill, 1988). In the case of histamine, the stimulation of inositol phosphate accumulation in this tissue can be inhibited by β-adrenoceptor stimulation (Hall & Hill, 1988). Studies of contractile activity in tracheal smooth muscle and parenchymal strips have indicated that the relative sensitivities to CCh and HA varies between the two tissues (Drazen & Schneider, 1978). In this communication we report the effect of HA and CCh on [^{3}H]-inositol phosphate accumulation in bovine parenchymal tissue.

Slices of bovine parenchymal tissue, obtained from freshly slaughtered young cattle, were prelabelled with [^{3}H]-myo-inositol and agonist-induced accumulation of total [^{3}H]-inositol phosphates (after 45 min incubation with agonist) was measured in the presence of 5mM LiCl as described previously for tracheal slices (Hall & Hill, 1988). Receptor antagonists and disodium cromoglycate (DSCG) were added 20 min prior to agonist administration. The incorporation of [^{3}H]-inositol into inositol phospholipids was measured in the chloroform phase following extraction of phospholipids with chloroform\methanol\10M HCl (100:200:1 v\v\v).

HA and CCh produced concentration-related increases in [^{3}H]-inositol phosphate accumulation of similar magnitude (7.5 ± 1.3 fold [n=14] and 6.0 ± 1.2 fold [n=13] above basal for 1mM HA and 1mM CCh respectively) in slices of bovine parenchyma yielding EC_{50} values of 24 ± 5 μM, n=9 (CCh, n=9) and 2.5 ± 0.5 μM (HA, n=9). These values contrast with the EC_{50} values obtained for CCh (3.5μM) and HA (38μM) in bovine tracheal smooth muscle (Hall & Hill, 1988). The inositol phosphate responses to these agonists in bovine parenchyma were antagonised by mepyramine (1μM) or atropine (50nM) indicating the involvement of histamine H_1- and muscarinic receptors respectively. As in tracheal smooth muscle (Hall & Hill, 1988), addition of the β_2-agonist salbutamol (1μM) reduced the response to 0.1mM HA (50 ± 6% decrease, n=3) in bovine parenchyma. A smaller effect of salbutamol (36 ± 7% decrease, n=4) was also observed on the response to 1mM CCh in parenchymal slices. Neither HA (0.1mM) nor CCh (0.1mM) increased the incorporation of [^{3}H]-inositol into membrane inositol phospholipids (HA = 88 ± 10 %, n=3: CCh = 101 ± 8%, n=3: control incorporation = 100%). Incubation of parenchymal slices with DSCG (0.1mM), reduced the maximal response to CCh (38 ± 5% decrease, n=11, $p < 0.001$, Wilcoxon signed rank test), but not that to HA. DSCG had no significant effect on the inositol phosphate response to either CCh or HA in slices of tracheal smooth muscle (n=3).

This study demonstrates that HA and CCh can stimulate [^{3}H]-inositol phosphate accumulation in slices of bovine parenchymal tissue with apparently different relative potencies to those obtained in bovine tracheal smooth muscle. However, the data obtained with DSCG raises the possibility that a portion of the inositol phosphate response to CCh in parenchymal tissue may be secondary to the release of other inflammatory mediators.

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Barnes, P.J. et al. (1986) Br. J. Pharmac. 87, 65P.
Drazen, J.M. & Schneider, M.W. (1978) J. Clin Invest. 61, 1441-1447.
Hall I.P. & Hill, S.J. (1988) Abstract C84 Liverpool BPS meeting

THE CALCIUM POOL MOBILISED BY OXYTOCIN IN THE MOUSE ANOCOCCYGEUS

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Oxytocin (OT) produces contraction of the mouse anococcygeus by acting on receptors similar to those found in the uterus (Gibson, 1986). However, it is not known whether the OT receptors in the two tissues are linked to the same cellular activation processes. In the uterus, the role of calcium ions (Ca) in the contractile response to OT is both complex and dependent upon the component of contraction : phasic contractions involve movement of extracellular Ca through voltage operated channels (VOC ; Edwards et al., 1986) ; tonic contractions may involve movement of extracellular Ca through receptor operated channels (ROC) or release of intracellular Ca (Edwards et al., 1986) ; finally, there seems to be a Ca-independent component amounting to about 10% of the total contraction (Sakai et al., 1981 ; Ashoori et al., 1985). The object of this study was to determine which of these mechanisms are operative during OT-induced contraction of the mouse anococcygeus.

Anococcygeus muscles from male mice (LACA strain ; 25 - 35 g) were set up for the recording of isometric tension responses to OT (Gibson, 1986) ; 4 nM OT (contact time 4 min, 20 min between doses) caused reproducible, submaximal (80 ± 3%, n = 8) contractions and was used in all subsequent studies. ^{45}Ca accumulation was estimated by the lanthanum method (Godfraind, 1976).

Contractile responses to OT were rapidly and completely lost on changing to Ca-free Krebs solution containing 2 mM EGTA ; there was no evidence of any transient contraction even on first exposure to OT after the change-over. Normal contractile responses recovered on reverting to Ca-containing Krebs solution.

In Ca-free solution, without EGTA, there was a more gradual loss of contractile response to OT, but even here contractions disappeared after 100 min. Under such conditions, in the presence of OT, contractions could be initiated by readdition of Ca (0.1 - 2.5 mM) to the medium ; these experiments were carried out in the presence of 1 μM phentolamine to offset any effect of released noradrenaline, and in the absence of OT readdition of Ca did not cause contraction. Ca-induced contractions, in the presence of OT, were unaffected by nitrendipine (0.001 - 1μM), but were reduced in a concentration-dependent manner by Mn (0.01 - 0.5 mM) ; both nitrendipine and Mn reduced contractions of the mouse anococcygeus elicited by 80 mM K.

In the presence of OT, ^{45}Ca accumulation by the anococcygeus was increased from 4.5 ± 0.6 μmol/g tissue/2 min to 8.6 ± 1.2 μmol/g tissue/2 min ($P < 0.05$).

The calmodulin antagonists trifluoperazine (50 μM) and W-7 (75 μM) produced a slow decline in responses to OT ; in both cases complete inhibition was observed after 120 min. The effect of trifluoperazine could not be reversed, but that of W-7 showed partial recovery (about 50%) after 60 min.

It is concluded that OT causes contraction of the mouse anococcygeus via the mobilisation of extracellular Ca, through channels that are not susceptible to block by nitrendipine , and are therefore likely to be of the ROC type. There was no evidence for a Ca-independent component of the response to OT.

Ashoori, F., Takai, A. & Tomita, T. (1985) Br. J. Pharmac. 84, 175 - 183.
Edwards, D., Good, D.M., Granger, S.E., Hollingsworth, M., Robson, A., Small, R.C. & Weston A.H. (1986) Br. J. Pharmac. 88, 899 - 908.
Gibson, A. (1986) Br. J. Pharmac. 88, 155 - 160.
Godfraind, T. (1976) J. Physiol. 260, 21 - 35.
Sakai,K., Yamaguchi,T. & Uchida,M. (1981) Arch. int. Pharmacodyn. Ther. 250, 40-54.

THE EFFECTS OF CALCIUM CHANNEL BLOCKERS ON GENTAMICIN INDUCED RENAL DAMAGE IN SPONTANEOUSLY HYPERTENSIVE WISTAR RATS

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Nephrotoxicity is a major dose-limiting feature to the long term use of the aminoglycoside antibiotics. The nephrotoxic effects of gentamicin are attributed to a selective accumulation within renal proximal tubule cells (Williams et al., 1986) and the work of Godson & Ryan (1987) suggests that gentamicin decreases calcium binding to high affinity binding sites on the brush border membrane. There have been contradictory reports in the literature that Ca channel blockers may or may not have a favourable effect on acute gentamicin induced renal damage (Lee & Michael, 1985; Heidemann et al. 1986; Watson et al., 1987). These reports have prompted us to study the effects of the calcium channel blockers on urinary levels of calcium, leucine aminopeptidase (LAP) and N-acetyl-ß-D-glucosaminidase (NAG) which are early indicators of nephrotoxicity and to compare the results with conventional creatinine clearance studies of acute renal damage.

A rat model of acute renal damage was developed through the repeated s.c. administration of gentamicin sulphate (G) 50 mg/kg/bd for a period of 12 days. Experimental groups were orally dosed with either nitrendipine (N), verapamil (V) or diltiazem (D) 12.5 mg/kg/bd while control groups received equivalent volumes of Bayer placebo (B) vehicle 3 days before and throughout gentamicin treatment. Damage to renal function was assessed every 4 days by measuring creatinine clearance (Cre-Cl), output volume, calcium and the enzymes (LAP) and (NAG) from 24-hour urine samples.

Table 1: Urinary excretion rates

Treatments	Calcium (mg/kg/24h)	NAG (mU/mg Crea./24h)	LAP	Cre-Cl (min/ml)	Volume (ml)
B	2.60±0.38	17.58± 1.33	15.83±2.23	1.76±0.12	22±4
G+B	11.56±1.16	174.58±12.66	6.86±1.33	1.62±0.25	22±4
G+N	14.30±1.41	118.12±23.13	0.62±0.38	0.40±0.10	47±7
G+V	9.00±0.41	138.79±21.69	1.69±0.86	0.31±0.11	33±3
G+D	8.61±0.89	222.11±21.52	4.96±1.50	0.73±0.13	32±3

The results are the means ± s.e.mean for 6 animals. The urinary excretion values shown for Calcium: day 4; NAG: day 8; LAP, Cre-Cl and Volume: day 12.

The results indicate that changes in urinary calcium, NAG and LAP are more sensitive indicators of gentamicin induced nephrotoxicity than creatinine clearance. The calcium channel blockers do not protect against this toxicity and the LAP and creatinine clearance values indicate that calcium channel blockers potentiate the nephrotoxicity of gentamicin in spontaneously hypertensive Wistar rats.

Godson, C. & Ryan, M.P. (1987), Br. J. Pharmac. 91, 490P.
Heidemann, H. et al. (1986), Arch. Pharmac. (Supp.) 322, R42.
Lee, S.M. & Michael, U.F. (1985), Exp. Mol. Path. 43, 107-114.
Watson, A.J. et al. (1987), J. Clin. Pharmac. 27, 625-7.
Williams, P.D. et al. (1986), J. Pharmac. Exp. Ther. 237, 919-925.

EFFECT OF BILE ACID ON THE RELEASE OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) FROM THE RAT GASTROINTESTINAL TRACT

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VIP is present in the gut wall along the length of the mammalian gastrointestinal tract (Bloom et al, 1983). Amongst its many actions on the gastrointestinal tract, it increases secretion of water and electrolytes from the small intestine and the colon and has been proposed as a mediator of diarrhoea (Krejs, 1987). The diarrhoeagenic effects of bile acids are thought to be mediated through the activation of the adenylate cyclase system (Conley et al, 1976). VIP is one of the most potent activators of the adenylate cyclase system (Dupont et al, 1980). We have studied the effects of sodium cholate on the release of immunoreactive VIP from the rat colonic tissues and compared it with that from the stomach.

Blood-free gastric and colonic tissues were obtained from male Sprague Dawley rats. The tissues were chopped (4mm X 4-6mm) and incubated in Kreb's solution alone or containing different concentrations of sodium cholate at 37^{o}C for 15 minutes. The incubation was stopped by adding acetic acid (0.5M) and immediately spinning (1,000g, 15 minutes). The supernatants were then boiled for 5 minutes and cooled. The VIP concentrations of the incubates were determined by radioimmunoassay (Amersham International plc) after making appropriate dilutions in the assay buffer.

Incubation of chopped colonic tissues in the presence of increasing concentrations of sodium cholate, up to 5mM, caused a concentration-related increase in the release of immunoreactive VIP. At 1mM sodium cholate, there was 100% ($P<0.01$), at 2mM, 457% and at 5mM, 650% increase in the release of immunoreactive VIP when compared with the control incubates in Kreb's only. Above 5mM and up to 15mM bile acid there was a decrease in the VIP release. Similar concentrations of the bile acid caused a smaller increase in the release of VIP from rat gastric tissues. At 5mM bile acid, there was 170% increase ($P<0.01$) in the release of VIP from the gastric tissues. The results show that the bile acid is a potent releaser of colonic VIP. The diarrhoeagenic effects of bile acids may thus be mediated via the release of VIP from the intestinal wall.

Bloom, S. R. and Bryant, M. G. (1973) Gut 14, 823.

Conley, D., Coyne, M., Chung, A., Bonorris, G. and Schoenfeld, L. (1976) Gastroenterology 71, 72-75.

Dupont, C., Laburthe, M., Broyart, J. P., Bataille, D. and Rosselin, G. (1980) Eur J Clin Invest 10, 67-76.

Krejs, G. J. (1987) Am J Med 82 (suppl 5B), 37-48.

TIME DEPENDENT INHIBITION OF OEDEMA FORMATION IN RABBIT SKIN BY DEXAMETHASONE

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We have previously shown (Peers & Flower, 1987) that dexamethasone pretreatment inhibits the development of oedema in rabbit skin in response to the direct-acting stimuli histamine (H), bradykinin (BK) and PAF. We now report the effect of dexamethasone pretreatment upon responses to neutrophil-dependent stimuli LTB4, fMLP and zymosan-activated serum (ZAS), a source of C5a desArg (Wedmore & Williams, 1981). We compare the time-course of the development of the effects of steroid upon responses to both groups of stimuli.

Californian rabbits of either sex, weight 2-3kg were used. Vascular permeability was measured by the extravasation of [^{125}I] - human serum albumin injected i.v. prior to intradermal injections (0.1ml) of stimuli; 30 min later, the animals were killed, the injection sites removed, and plasma exudation calculated as described by Wedmore & Williams (1981). Dexamethasone (1mg/kg) was given i.v. at the indicated interval before intradermal injections.

The responses to intradermal H (3×10^{-9} moles), BK (3×10^{-8} moles), PAF (5×10^{-9} moles), LTB4 (3×10^{-10} moles), fMLP (10^{-10} moles) and ZAS (0.05ml) all coinjected with 3×10^{-10} moles PGE_2 are shown in the Table. Saline injection caused response of <10µl; this value has been deducted. The effects of dexamethasone pretreatment are shown in the Table.

Table 1: Time-dependent inhibition of responses by dexamethasone

Mediator	Control (µl oedema) (n=11)	Inhibition (%) by dexamethasone given at indicated pretreatment time 1h (n=4)	2h (n=6)	5h (n=5)
H + PGE_2	87 ± 14	12.6 ± 23.0	57.5 ± 8.0*	81.6 ± 4.6*
BK + PGE_2	175 ± 20	9.1 ± 10.9	44.0 ± 6.9*	62.9 ± 6.3*
PAF + PGE_2	57 ± 9	12.3 ± 22.8	56.1 ± 12.2*	73.7 ± 3.5*
LTB4 + PGE_2	45 ± 6	-20.0 ± 22.2	40.0 ± 8.9	53.3 ± 8.9*
fMLP + PGE_2	63 ± 12	3.2 ± 14.3	58.7 ± 11.1*	68.3 ± 9.5*
ZAS + PGE_2	62 + 7	8.1 + 17.7	46.8 + 11.3*	72.6 + 4.8*

* $P < 0.05$ — Values shown $\bar{x} \pm$ s.e.m. for (n) rabbits.

Dexamethasone inhibits the responses to both direct-acting and neutrophil-dependent stimuli with a similar time-course of action. Increased vascular permeability in response to direct-acting stimuli occurs when gaps are formed between endothelial cells following their contraction (Grega 1986). It is unclear how neutrophils stimulate increased permeability, but it may occur following release of some substance or enzyme from the neutrophil. The similar time-course of action of dexamethasone against responses to both groups of stimuli suggests a similar mechanism of action. This is not subsequent to inhibition of prostaglandin synthesis, since indomethacin is without effect (Peers & Flower 1987; Wedmore & Williams 1981). Dexamethasone may act upon vascular endothelial cells to prevent gap formation.

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Grega G J (1986) TIPS 7 452-457
Peers S H & Flower R J (1987) Br. J. Pharmac. 92 628P
Wedmore C V & Williams T J (1981) Nature 289 646-650

TOPICAL AND SYSTEMIC ADMINISTRATION OF PREDNISOLONE AND 5-AMINOSALICYLIC ACID IN AN IMMUNE-COMPLEX MODEL OF COLITIS

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We have previously described a model of acute colitis which exhibited many features of human clinical colitis (Walsh et al, 1987), and responded to the actions of some anti-colitic drugs (Walsh and Zeitlin, 1987). In the present study we report the actions of topically-applied prednisolone and 5-aminosalicylic acid (5-ASA) on this model and compare the anti-inflammatory effect with that of systemic application.

Colitis was induced in male BKA mice (20-30g) fed on normal laboratory diet (Oxoid Ltd.) and given water ad libitum. Pre-formed human serum albumin (HSA)-anti HSA immune complex was injected i.v., following intra-rectal formalin (1%) instillation (Walsh et al, 1987). Topical application of drug (prednisolone; 5-ASA) or vehicle alone was administered daily for three days prior to induction of colitis and then for a further five days. The animals were killed and samples of distal colon were taken for histological assessment and measurement of tissue water content. Symptomatic colitis was graded according to an arbitrary scoring system.

Prednisolone at 1.11, 8.3 and 28 x 10^{-5}M and 5-ASA at 1.31, 11.1 and 20 x 10^{-5}M, caused a statistically significant ($P<0.05$) suppression of the increase in colonic tissue water content caused by the induction of colitis. The dose-response curve for prednisolone was shifted to the left by a factor of 10, compared with systemically applied drug. The maximum response produced by systemic application of prednisolone was 82.4%, while that produced by topical application was 97.3% The maximum response achieved using 5-ASA was no more than 34.4%. Topical application of 5-ASA produced no significant shift in the dose response curve when compared with systemic application.

This model thus responds to topical and systemic application of clinically effective anti-colitic drugs in a consistent way and is suitable for the screening of novel therapeutic agents administered by these routes.

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Walsh, L.P., Zeitlin, I.J., Blackham, A., Norris, A.A. & Jarrett, F. (1987) Br. J. Pharmacol. 91 : 294P.
Walsh, L.P. & Zeitlin, I.J. (1987) Br. J. Pharmacol. 92 : 741P.

THE IN VITRO EFFECTS OF MEVINOLIN AND METFORMIN ON CHOLESTEROGENESIS IN RAT INTESTINAL CELLS

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Hypercholesterolaemia is frequently encountered in human diabetic subjects. Mevinolin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase is under investigation as a hypocholesterolaemic agent. Metformin (N'N'-dimethyl biguanide) is widely used in the management of Type 2 diabetes mellitus. The potential of this antihyperglycaemic drug as a cholesterol-lowering agent has not been investigated, therefore this may be of interest in view of its reported antiatherogenic properties (Marquie, G. et al., 1970). This study investigates the effects of mevinolin and metformin on cholesterol biosynthesis *in vitro* in isolated rat intestinal cells.

Crypt and villous cells were isolated from the small intestine using a dual buffer technique (Hegazy et al., 1983) and incubated either in the presence of mevinolin (0 - 0.5μM) or metformin (1μM) in Krebs' bicarbonate buffer pH 7.4 to assess their effect on cholesterol biosynthesis. Incorporation of [^{14}C]-acetate into digitonin precipitated sterols (DPS) was the index used to quantify the relative rates of *de novo* cholesterogenesis. The key regulatory enzyme of cholesterol biosynthesis, HMGCoA reductase, was assayed using the method of Shapiro (Shapiro et al., 1974). The expressed (dephosphorylated) and total enzyme activities were estimated separately by incubation of enzyme reactants in the presence and absence of 5 x 10^{-2}M sodium fluoride, an inhibitor of cellular phosphatases.

Table 1: The effect of mevinolin on [^{14}C]-acetate incorporation

	Mevinolin concentration (Molar)						
	0	2.5×10^{-11}	2.5×10^{-10}	2.5×10^{-9}	2.5×10^{-8}	2.5×10^{-7}	5×10^{-7}
pmole ^{14}C -acetate incorporation	184	170	153	103	35	20	7
% Inhibition	0	8%	17%	43%	81%	89%	96%

The incorporation of [^{14}C]-acetate into DPS by cultured rat enterocytes is highly sensitive to mevinolin (Table 1) and the process is more than 90% inhibited at a drug concentration of 5×10^{-7}M. The process is therefore highly dependent on the activity of HMGCoA reductase. Cells cultured in the presence of metformin showed no change in total HMGCoA reductase activity over the 2hr culture period but the percentage expressed activity increased by 40% in this time. [^{14}C]-acetate incorporation into DPS by cells cultured with metformin under the same conditions was increased 20% over control cells.

It would appear therefore that while mevinolin is a potent inhibitor of intestinal cholesterogenesis in rats, the antihyperglycaemic agent metformin may, in short term culture at least, promote cholesterol formation possibly by promoting dephosphorylation of pre-existing HMGCoA reductase.

Hegazy, E. et al (1983) Eur. J. Cell Biol. 90, 132 - 136.
Marquie, G. et al (1970) C.R. Seanc, Soc. Biol. 164, 1128 - 1133.
Shapiro, D. J. et al (1974) Biochem. Biophys. Acta. 370, 369 - 377

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COMPARATIVE EFFECTS OF LOPERAMIDE AND VERAPAMIL ON CONTRACTIONS OF GUINEA-PIG ILEUM

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Loperamide is an effective, widely used antidiarrhoeal drug. Reynolds et al. (1984) suggested that calcium channel blockade accounts for at least part of the drug's action at antidiarrhoeal doses. In the present work we compared the actions of loperamide and the calcium channel blocker verapamil, on contractions of guinea-pig ileum induced by various stimuli.

Loperamide antagonised calcium-induced isometric contractions in segments of guinea-pig distal ileum suspended in calcium-free, depolarising (40mM KCl) Tyrode solution at 37°C, gassed with 5% CO_2 in O_2. Incubation for 20 minutes with loperamide induced dextral shifts (eg dose ratio 12.6±1.5 with 10μM loperamide, n=6) in the cumulative concentration-response curve to $CaCl_2$ (0.01-100mM) compared with control, the maximal response to $CaCl_2$ being unaffected. Application of loperamide (10-100μM) during the plateau of an established submaximal contraction to $CaCl_2$ (3mM) caused a rapid, full relaxation; loperamide was readily removed by washing. These characteristics are similar to those reported for the calcium antagonists diltiazem, nifedipine and verapamil (Alyami and Wood, 1988).

A comparison was made of the ability of loperamide (10nM-50μM) and verapamil (10nM-10μM) to suppress agonist responses in segments of ileum suspended in Krebs-Henseleit solution at 32°C. Concentration-response curves for the phasic component of histamine (H, 0.01-10μM) and carbachol (C, 0.01-10μM) contractions and both phasic and tonic components of KCl contractions (KP and KT, 0.6-100mM) were compared before and after 30 minutes equilibration with either loperamide or verapamil. Only one agonist and one antagonist was tested in any tissue, n>5. Both antagonists caused significant concentration-related suppression of agonist responses, verapamil being consistently more potent than loperamide. IC_{50} values (antagonist concentration required to reduce maximal agonist responses to 50% of control value) against each agonist were (μM): H 0.44±0.08, 2.11±0.37; C 1.88±0.37, 8.90±3.43; KP 0.24±0.06, 3.75±1.01; KT 0.02±0.004, 0.33±0.05 for verapamil and loperamide respectively.

More detailed examination of the effects of each antagonist was made by assessing the ability of low concentrations of verapamil (0.03-0.3μM) and loperamide (0.1-1μM) to reduce maximal and submaximal (approximately 50% control maximum) phasic responses to H and K (n>7). Submaximal KP responses were significantly ($P<0.05$) more sensitive to inhibition by both antagonists than were maximal KP responses, whereas submaximal and maximal H responses were equally inhibited. For example verapamil (0.1μM) and loperamide (0.3μM) reduced both maximal and submaximal H responses to 65-70% of respective control values but although maximal KP contraction was reduced to 70-75%, the submaximal contraction was reduced to 30-40% of control.

The results suggest that loperamide has a similar profile of activity to the calcium channel blocker verapamil, although it is less potent. The concentrations of loperamide used are within the range reported to show significant antidiarrhoeal activity in the guinea-pig (Reynolds et al, 1984).

Alyami, A.M. and Wood, D. (1988) Br. J. Pharmac. 94, 467P
Reynolds, I.J., Gould, J. and Snyder, S.H. (1984) J. Pharm. Exp. Ther. 231, 628-632

606P

CARDIAC AND RESPIRATORY EFFECTS OF BENZODIAZEPINE DERIVATIVES IN RATS

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Acute toxicity of benzodiazepines is low in clinical dosage. Cardiovascular and respiratory depression may occur after intravenous administration, particularly if other central depressants are administered (Goodman et al., 1985).

Four benzodiazepines, diazepam, chlordiazepoxide and the anti-epileptic clonazepam, and the 1,5-benzodiazepine clobazam, were compared as to their effects on some cardiac and respiratory parameters. Three doses of each drug were used: 5, 10 and 15 mg/kg, administered by i.p. injection. The parameters concerned were respiratory rate, heart rate, diastolic and systolic blood pressure and the electrocardiogram (ECG). Female rats (Cpb Wu:Wi), 170-180 g, were used. They were anaesthetized with pentobarbitone (2 times 12,5 mg/kg) i.p. Control registrations and measurements were performed after saline injection. Effects were measured 5, 10, 15, 20, 30, 45, 60 and 90 min after administration. Temperature and humidity were kept constant (34°C[1] and 55% respectively), using a climate chamber.

Respiratory rate
Effects of diazepam, chlordiazepoxide and clonazepam are very similar. They produce a rapid dose-dependent decrease of more than 20%, at the highest doses, with a maximum effect at 20 min, after which partial recovery takes place slowly. Clobazam 10 and 15 mg/kg depresses the respiratory rate during the first twenty minutes with more than 30%, after which the rate returns to normal.

Heart rate
Diazepam is the only benzodiazepine which induces - rapid - dose-dependent depression of the heart rate (about 25% at the highest dose level, maximum effect at 10 min). Recovery to normal takes place.

Diastolic blood pressure
Diazepam, chlordiazepoxide and clobazam produce a rapid dose-dependent fall of the diastolic blood pressure, (maximum effect 20%, at 15 mg/kg, within five min). The pressure subsequently remains at this level. In the case of clonazepam, diastolic blood pressure fall is also dose-dependent, but more gradual.

Systolic blood pressure
Within five min after injection of diazepam (all three doses), a decline of about 10% is followed readily by a rapid return to normal. Chlordiazepoxide has no noticeable effect on the systolic blood pressure. This is also true for clobazam, although a slight dip seems to be evoked between 0 and 5 min after injection. Clonazepam on the other hand produces elcits a marked dose-dependent fall of the systolic blood pressure, comparable to its effect on the diastolic pressure.

Electrocardiogram
Effects on the ECG are negligible.

In conclusion, either of the four drugs investigated may have evident cardiac and respiratory effects. Differences only exist in the quality and quantity of these effects.

Goodman Gilman, A., L.S. Goodman, Th.W. Rall, F. Murad, eds., The Pharmacological Basis of Therapeutics, 7th ed., 1985, p. 466.

EFFECTS OF TWO BENZODIAZEPINES ON NEUROMUSCULAR TRANSMISSION AFTER TETANIC STIMULATION OF THE RAT PHRENIC NERVE

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Myorelaxation produced by benzodiazepines is thought to be of central origin. Direct effects have been described as absent, potentiating and inhibitory. A biphasic action on the twitch tension of the phrenic nerve-diaphragm in vitro was found [1]).
Chlordiazepoxide displays no such effect. In high concentrations it induces depression. Van Wilgenburg and Leeuwin showed that diazepam in low concentrations reduces the decay phase of miniature endplate currents whereas no effect on the growth phase was seen[2]). In higher concentrations the rising time of the growth phase was increased significantly by a factor 2 or more to values higher than 1 msec. No such effect was observed using chlordiazepoxide. This report describes effects of diazepam and chlordiazepoxide on the tetanic stimulated phrenic nerve-diaphragm preparation of the female rat. Diazepam yielded effect in concentrations between 0.02 mM and 0.31 mM, whereas chlordiazepoxide was effective in concentrations between 0.05 mM and 0.87 mM. Duration of the stimulation was 0.5 msec. Diazepam displays a marked biphasic effect (figure 1): at low concentrations the tetanus is enhanced, whereas at higher doses the tetanic effect cannot be maintained any longer. At the highest concentration depression is complete.

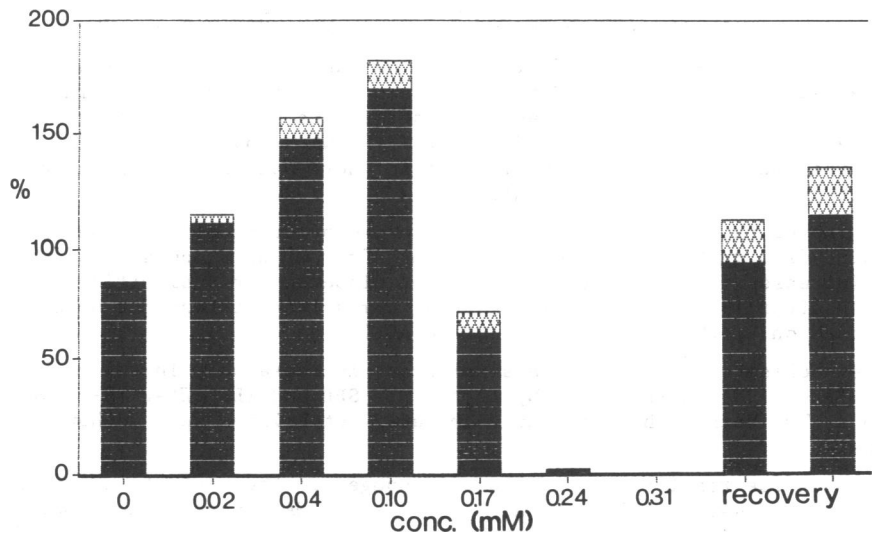

Figure 1. Effect of diazepam at 30 Hz.

Effects are dose-dependent and are virtually the same at 30 and 40 Hz stimulation. Chlordiazepoxide also produces this biphasic pattern, although much weaker than diazepam and in relatively high con- centrations. We tentatively conclude that diazepam and chlordiazepoxide interfere directly with events at the neuromuscular junction. The differences between the two seem to be rather quantitative than qualitative.

[1]) Leeuwin, R.S., B.F.M. Werdmuller and H. van Wilgenburg (1986), Neurosc.L., suppl. 26, S179.
[2]) Van Wilgenburg, H. and R.S. Leeuwin (1986), Neurosc.L., suppl. 26, S180.

EXPERIMENTAL JUVENILE-ONSET DIABETES AND THE SYMPATHETIC NERVOUS SYSTEM

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Autonomic neuropathy is a common neurological complication of diabetes mellitus which can affect the cardiovascular, genitourinary and gastrointestinal systems (Hosking et al, 1978; Watkins and Edmonds, 1983). The pathogenesis of these dysfunctions remains unclear. In the present study, we assessed possible functional abnormalities in the adrenergic nerves of rats modelling juvenile-onset diabetes for 6-9 wk and 19-22 wk by comparing the endogenous noradrenaline (NA) stores, synthesis rate and metabolism of labelled NA (^{3}HNA) in their heart (HT), spleen (SPL), ileum (ILE), kidney (KID) and vasa deferentia (VD) with those from age-matched controls. The NA content of the tissues was determined with an HPLC coupled to an EC detector and various metabolites of ^{3}HNA were separated by using double column (Alumina and Dowex 50 x 4) chromatography as described previously (Chaudhry and Vohra, 1985). Diabetes was induced by streptozotocin (80 mg/kg i.p.) in 5 wk old male Sprague-Dawley rats weighing 115-120 g. Control rats were injected with an equal volume of citrate buffer. Both groups were fed rat chow.

At both periods the body weights of diabetic rats were significantly ($P<0.05$) lower than those of the corresponding controls. The tissue wet weight was decreased in the HT, SPL and VD but not KID after both periods of diabetes. However, the ratio of tissue wet weight to body weight was increased after 6-9 wk of diabetes in KID and VD and after 19-22 wk in HT, KID and VD.

The NA content (per organ) of all organs was unchanged after 6-9 wk of diabetes but was significantly reduced in the HT and SPL after 19-22 wk. In contrast, the NA concentration (per g) was increased in the HT after both periods of diabetes, SPL after 6-9 wk and VD after 19-22 wk. However, when the tissue NA concentration was expressed per mg protein there was no significant change in these tissues of the diabetic animals.

The turnover rate (synthesis) of NA, assessed by using alpha methyl-para-tyrosine (250 mg/kg i.p.) and sacrificing after 4 and 8 hours, showed a significant increase in the ILE after 6-9 wk of diabetes but a significant decrease in the KID after 19-22 wk. The statistical comparison (t-test for unpaired data) was based on k, the rate constant of NA efflux.

The metabolism of ^{3}HNA showed a significant increase only in the methylated metabolites (VMA, MOPEG and NMN) in the HT, SPL and ILE after 19-22 wk of diabetes but both methylated and deaminated metabolites were unchanged after 6-9 wk.

Although these results indicate subtle changes in the content, synthesis and metabolism of NA, we believe that the increased NA concentration (per g) that we observed in various tissues is the result of a decreased tissue wet weight in the diabetic animals. Some of our findings are contrary to those reported by others (Ganguly et al, 1986; Yoshida et al, 1985) and may be attributed to the age and weight of the animals, and the dose of streptozotocin (i.e. the model) we used.

We thank MRC of Canada and N.B. Heart Foundation for their financial support.

Chaudhry, A. & Vohra, M.M. (1985) J Pharmacol Exp Therap 232: 850-856.
Ganguly, P.K. et al (1986) Cir Res 59: 684-693.
Hosking, D.T. et al (1978) Diabetes 27: 1043-1055.
Watkins, P.J. & Edmonds, M.E. (1983) Diabetologia 25: 73-77.
Yoshida, T. et al (1985) Diabetologia 28: 692-696.

EFFECTS OF EMULSIFIED PERFUOROCHEMICALS ON CIRCULATING ENZYME CONCENTRATIONS

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Emulsified perfluorochemicals (PFCs) are undergoing evaluation as potential physiological gas-transport fluids and have been tested for oxygenation of ischaemic tissues, solid tumours and isolated organs (Lowe, 1987). PFC emulsion particles can accumulate in lymphoid tissues and this depends on several variables, including emulsion composition and dose, species used and tissue concerned (Lowe and Bollands, 1988). However, little information is available describing the effects of emulsified PFCs on tissue biochemistry. We have therefore examined changes in circulating enzyme concentrations following injection of low doses of PFC emulsions in rats.

Female Wistar rats (body weight (b.w.): 140-160g; n = 82) were used. They were injected intravenously (i.v.) via a tail vein with 10 ml/kg b.w. of either the proprietary emulsion, Fluosol-DA 20% (F-DA; Green Cross, Japan) or a novel emulsion consisting of 20% (w/v) perfluorodecalin (FDC) and 1% (w/v) of a C-16 oil additive, perfluoroperhydrofluoranthrene, emulsified in an isotonic aqueous phase with 4% (w/v) Pluronic F-68 (Sharma et al., 1987); control rats received sterile saline (0.9% NaCl). Blood was removed from the retro-orbital plexus at the following times after injection of emulsion: 3, 6, 12, 24, 48 hr, 4 and 8 days. Plasma alkaline phosphatase (ALP) and lactate dehydrogenase concentrations were measured by standard assays (Lowe & McNaughton, 1986).

Mean (± s.e.m.) plasma ALP and LDH concentrations in controls at 6hr after injection were 193 ± 12 U/l (n = 14) and 277 ± 30 U/l (n = 8) respectively. Mean plasma LDH concentrations (U/l) following injection of either F-DA or novel emulsion were as follows (no. observations in parentheses):

Time of sampling	F-DA	Novel emulsion
3h	283 ± 81 (6)	291 ± 73 (3)
6h	550 ± 103 (5)**	378 ± 151 (4)
12h	280 ± 15 (3)	228 ± 17 (4)
24h	156 ± 29 (4)*	177 ± 36 (4)
48h	308 ± 96 (3)	312 ± 40 (4)
4 days	387 ± 143 (4)	348 ± 67 (5)
8 days	227 ± 11 (3)	283 ± 71 (4)

*P < 0.05, **P < 0.01 compared to corresponding control mean value.

Plasma ALP concentrations were similar to control following injection of either F-DA or novel emulsion; overall mean ALP concentrations following F-DA or novel emulsion injection were 185 ± 13 U/l and 186 ± 5 U/l respectively.

The increase in plasma LDH at 6h following F-DA injection was in agreement with previous observations following exchange-transfusion of rats with this emulsion (Lowe & McNaughton, 1986). The absence of corresponding changes in ALP concentrations contrasted with previous results (Lowe & McNaughton, 1986) but this may reflect either differences in dose of F-DA administered or different enzyme sensitivities to emulsified PFCs. The present results also show that composition is an important determinant of enzymic responses to emulsified PFCs.

Lowe, K.C. (1987) Comp. Biochem. Physiol. 87A, 825-838.
Lowe, K.C. & McNaughton, D.C. (1986) Experientia 42, 1228-1231.
Lowe, K.C. & Bollands, A.D. (1988) Biomat. Art. Cells Art. Org. (in press).
Sharma, S.K. et al (1987) In Oxygen Transport to Tissue, Vol. IX (eds Silver, I.A. & Silver, A.), pp. 97-108. London, Plenum.

EFFECTS OF PERFLUOROCHEMICAL EMULSION COMPONENTS ON RAT LIVER CYTOCHROMES P-450

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Perfluorochemical (PFC) emulsion particles can accumulate in lymphoid tissues and recent interest has focussed on the consequences of this for immunological responsiveness (Lowe & Bollands, 1988) and reticuloendothelial system clearance function (Cuming et al., 1988). However, the extent to which cellular biochemistry may be altered by injection of emulsions or their components has been little studied. Since previous work showed that perfluorodecalin (FDC) oil can induce hepatic microsomal cytochromes P-450 (P-450) in both rats (Obraztsov et al., 1985) and mice (Khlopushina et al., 1986), the present experiments have examined the effects of low doses of different PFC emulsions or their surfactant component on this enzyme system in rats.

Male or female Wistar rats (body weight (b.w.): 175-292g; n = 47) were used. They were injected intravenously (i.v.) via a tail vein with 10 ml/kg b.w. of one of the following: (i) Fluosol-DA 20% (F-DA; Green Cross, Japan); (ii) Oxypherol (FC-43; Green Cross); (iii) a novel 20% (w/v) FDC emulsion containing 1% of a C-16 higher boiling point oil (HBPO) additive, perfluoroperhydrofluoranthrene, emulsified with 4% Pluronic F-68 (Sharma et al., 1987); (iv) commercial grade Pluronic F-68 (I.C.I.); (v) Pluronic F-68 following ion-exchange and charcoal purification; or (vi) saline (0.9% w/v NaCl). Animals were sacrificed at 7 days after injection and both liver and spleen were removed and weighed. Livers were homogenized in 1.15% (w/v) KCl buffered with tris/HCl and hepatic microsomal total protein and P-450 concentrations measured by Lowry assay and differential centrifugation/spectrophotometry respectively (Omura & Sato, 1964).

Mean spleen weight was increased significantly to a maximum of 55% in both male and female rats injected with F-DA ($P<0.002$), FC-43 ($P<0.001$) or novel emulsion ($P<0.01$). Spleen weight also increased by 29% ($P<0.02$) in female rats injected with commercial grade Pluronic. Mean liver weight increased by up to 32% following injection of F-DA or novel emulsion in male rats ($P<0.001$) but was unchanged in all other cases. No significant changes in liver microsomal P-450 concentrations were noted in female rats injected with PFC emulsion or Pluronic. In contrast, mean P-450 concentrations were increased to a maximum of 65% ($P<0.01$) in male rats injected with novel emulsion but were similar to the mean control value (0.96 ± 0.12 nmol/mg protein; n = 4) in all other cases.

These results show that the novel emulsion is an effective inducer of hepatic microsomal P-450 in male rats. It is tempting to speculate that the FDC component was the active principle involved since it can increase liver P-450 concentrations in rats and mice (Obraztsov et al., 1985; Khlopushina et al., 1986). However, any contribution that the C-16 HBPO component of the novel emulsion may make to enzyme activation remains to be determined.

Cuming, R., Bollands, A.D., Sharma, S.K. & Lowe, K.C. (1988) Br. J. Pharmac. 93, 293P.

Khlopushina, T.G., Kovalev, I.E. & Lysenkova, E.M. (1986) Biochem. (USSR), 51, 574-577.

Lowe, K.C. & Bollands, A.D. (1988) Biomat. Art. Cells Art. Org. (in press).

Obraztsov, V.V., Shekhtma, D.G., Sologub, G.R. & Beloyartsev, F.F. (1985) Biochem. (USSR), 50, 1041-1048.

Omura, T. & Sato, R. (1964) J. Biol. Chem., 239, 2370-2385.

Sharma, S.K., Bollands, A.D., Davis, S.S. & Lowe, K.C. (1987) In Oxygen Transport to Tissue, Vol. IX (eds. Silver, I.A. and Silver, A.), pp. 97-108. London, Plenum.

TOLERANCE TO THE SEDATIVE EFFECT OF AMINOGLUTETHIMIDE IN RATS

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Common side-effects observed in breast cancer patients receiving aminoglutethimide (AG) are ataxia, dizziness, drowsiness and depression. However, these signs of CNS depression diminish within 2-6 weeks of commencing therapy (Harris, 1985). Tolerance to the CNS effects of AG on chronic dosing also develops in mice (Abusrewill et al. 1986) and this appears to involve dispositional and functional mechanisms (Ahmad et al. 1987). In the rat AG is a marked hepatic enzyme inducer of the phenobarbitone type (Damanhouri et al. 1987) and it was of interest to examine the development of tolerance to the CNS effects of AG in this species.

Groups (n of at least 5) of male Wistar rats (80-150g) were used in one of three assessments of CNS-depressant activity:- spontaneous locomotor activity measured 20 min after AG (50mg/kg ip), rectal temperature 1h after AG (100mg/kg po) and duration of absence of righting reflex after AG (200mg/kg ip). Initially, AG (50mg/kg ip) caused a 58% reduction in locomotor activity. However, after 10 daily doses at this level, animals had become completely tolerant to the effect. AG (100mg/kg po) caused a max. hypothermic response (35.4±0.3°C compared with 37.5± 0.2°C for controls) 1h after dosing. This effect was also abolished after 10 daily doses of AG (100mg/kg po). The sleeping-time response to AG (200mg/kg ip) was significantly ($P<0.05$) changed from 122±11 min to 91±7 min by daily treatment of rats with (AG 100mg/kg po) for 2 weeks. The latter dose regimen also significantly ($P<0.01$) altered the pharmacokinetic profile of AG (50mg/kg po; Table), the plasma $t_{0.5}$ being reduced and total clearance being increased without significant change in apparent volume of distribution (V_d).

Table: Pharmacokinetic parameters† (mean±s.e.m.) of AG (50mg/kg po) in rats receiving either vehicle (carboxymethylcellulose 0.75%, po; control) or AG (100mg/kg po) daily for 2 weeks.

Treatment	Plasma $t_{0.5}$ (h)	V_d (ml)	Total clearance (ml/min)
Vehicle (n=5)	6.3 ± 0.2	406 ± 30	0.7 ± 0.1
AG (n=5)	2.7 ± 0.3*	450 ± 74	1.90± 0.1*

†Calculated by computer program assuming a single compartment (Johnston & Woollard, 1983). *$P<0.01$ compared with control values.

The results thus demonstrate the development of a marked tolerance to the CNS actions of AG when rats are repeatedly dosed with the drug and this appears to involve a significant dispositional component.

Acknowledgement is made to Ciba-Geigy, Horsham, for financial assistance.

Abusrewill, M., Ahmad, B. & Nicholls, P.J. (1986) Br. J. Pharmac. 89, 832P.
Ahmad, B., Abusrewill, M., Brain, K.R. & Nicholls, P.J. (1987) Br. J. Pharmac. 92, 738P.
Damanhouri, Z., Herbert, S.A. & Nicholls, P.J. (1987) Pharmac. Ther. 33, 145-152.
Harris, A.L. (1985) Exp. Cell Biol. 53, 1-8.
Johnston, A. & Woollard, R.C. (1983) J. Pharmac. Meth. 9, 193-199.

DETERMINATION OF DIFFERENTIAL EFFECTS OF ANAESTHETICS ON BRAIN HAEMODYNAMICS USING MICROSPHERES IN THE RAT

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In the majority of brain areas, blood flow is quantitatively coupled to the local metabolic rate for glucose though agents such as lignocaine may uncouple this relationship (Concezione et al. 1986). The actions of some anaesthetics however on brain haemodynamics are largely unknown. It was the purpose of this study therefore to investigate the effects that a range of parenterally administered general anaesthetic agents might possess on brain blood perfusion in the rat.

The study was performed using male Wistar rats (270±19g) and the following anaesthetic regimens: (H) - Fentanyl & fluanisone (0.26 & 8.3mg/kg, Hypnorm) in combination with midazolam (4.16mg/kg, Hypnovel) given i.p.; (U) - i.p. urethane (1.75mg/kg); (P) - i.p. pentobarbitone (67mg/kg); (K) - i.p. ketamine (80mg/kg) in combination with i.p. midazolam (5mg/kg); (S) - i.v. alphaxalone & alphadolone (9 & 3mg/kg, Saffan). After the animals had reached a sufficient depth of anaesthesia the right carotid artery was catheterised, the tip of the catheter was manipulated into the left ventricle and 60000-80000 Sn-113 labelled 15um microspheres were injected. Simultaneously blood was withdrawn from the left femoral artery at a constant rate of 0.43ml/min for 90 sec after microsphere injection. Cardiac output and brain haemodynamics were calculated as described by McDevitt and Nies (1976).

Table 1. Anaesthetics and brain haemodynamics (mean ± s.d., n=6)

	K	H	S	P	U	DMRT
Cardiac output (ml/min/100g body weight)	19.27 ±3.74	32.36 ±6.50	24.36 ±2.52	22.82 ±2.93	17.42 ±1.43	H S P K U
Brain blood flow (ml/min/g tissue)	0.74 ±0.20	0.64 ±0.15	0.55 ±0.05	0.55 ±0.06	0.40 ±0.09	K H S P U

DMRT - analysis of variance and Duncan's Multiple Range test. The groups are ranked left to right in decreasing magnitude and those jointly underlined are not significantly different (P>0.05) from each other.

A brain blood flow rate of 1.0ml/min/g tissue has been reported in chronically catheterised conscious rats using microspheres (Idvall et al. 1980; Idvall et al. 1981). In relation to this finding, the present results suggest that all of the anaesthetics studied depressed brain blood perfusion to differing degrees and also modified cardiac output differentially. There was no significant correlation (P>0.05) between anaesthetic-induced changes in cardiac output and brain blood flow (r=0.15). It was noteworthy, however, that U and K displayed the most marked effect on cardiac output whilst brain blood flow with K was almost twice that for U. The brain perfusion values for H, S and P on the other hand were comparable to one another but all remained significantly greater (P<0.05) than U. It is a possibility that these central ischaemic actions might influence neuronal homeostasis (Maekawa et al. 1986) so it becomes an important consideration which anaesthetics are most active in this respect.

Concezione, T. et al. (1986) Anesthesiology, 64, 771.
Idvall, J. et al. (1980) Acta Anaesthiol. Scand., 24, 257-263.
Idvall, J. et al. (1981) Anesthesiology, 55, 297-304.
Maekawa, T. et al. (1986) Anesthesiology, 65, 144-151.
McDevitt, D.G. and Nies, A.S. (1976) Cardiovas. Res., 10. 494-498.

PLACENTAL TRANSFER AND FETAL UPTAKE OF BUPIVACAINE IN THE RABBIT: EFFECT OF ADRENALINE

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Adrenaline may reduce utero-placental blood flow in sheep (Hood, 1986) and humans (Jouppila, 1978), and its epidural use has been associated with increased fetal/maternal bupivacaine concentration ratios (Reynolds and Taylor, 1971). We have therefore studied its effect on bupivacaine distribution in near term, pregnant rabbits who were infused intravenously with bupivacaine (1.25mg/ml) alone (n=8) or with adrenaline (1.25µg/ml: n=8). Fetuses were removed at 15 minute intervals, and simultaneous samples of maternal arterial blood, mixed fetal blood, amniotic fluid, placenta and fetal brain were obtained for bupivacaine estimation. Bupivacaine was measured in maternal brain, removed at the end of the experiment. Statistical analysis was performed using a nested analysis of covariance. No significant differences were found in fetal/maternal plasma bupivacaine ratios, as reported previously (Laishley et al, 1988). There was significant ($p<0.001$) accumulation of bupivacaine with time in placenta, amniotic fluid, fetal plasma, and fetal brain, with no significant differences occurring between the two groups. Mean data on the combined groups are presented in figure 1. Fetal/maternal plasma ratios rose significantly with time ($p<0.05$), but fetal brain/plasma ratios decreased ($p<0.05$). Adrenaline has no significant effect on fetal uptake and distribution in the rabbit of bupivacaine, which accumulated in the fetal compartment with a half time of about 30 minutes. There was less bupivacaine accumulation in brain than plasma possibly because of efficient buffering in the brain.

Hood, D.D., Dewan, D.M. & James, F.M. (1986) Anesthesiology 64, 610P.
Jouppila, R., Jouppila, P., Kuikka, J. & Hollmen, A. (1978) Br.J.Anaesth 50, 275P.
Laishley, R.S., Carson, R.J. & Reynolds, F. (1988) Br.J.Anaesth 60, 330P.
Reynolds, F. & Taylor, G. (1971) Br.J.Anaesth 43, 436P.

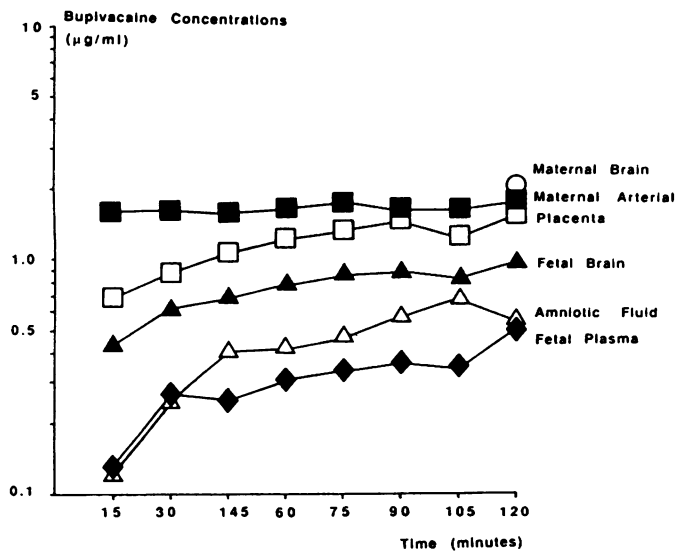

Figure 1 Mean bupivacaine concentrations (n=16)

AZIDOTHYMIDINE (AZT) AND HEPATIC DRUG METABOLISM

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Azidothymidine (3-azido 3'-deoxythymidine; zidorudine or AZT) is a thymidine analogue that inhibits human immunodeficiency virus (H.I.V.) replication in vitro. AZT is used in acquired immunodeficiency syndrome (AIDS) which is considered to be caused by infection with a human retrovirus - H.I.V.

Recent studies suggest that AZT administration can decrease mortality and the frequency of opportunistic infections in a selected group of patients with AIDS (Fischl et al., 1987). Patients in whom AZT has been beneficial are likely to require other treatments, including multiple and prolonged drug regimens. Such patients show a marked propensity to adverse drug reactions such as rash with co-trimoxazole and the potential for possible interactions with AZT is largely unknown. In this study we investigated the effect of AZT on hepatic oxidative drug metabolism in male Wistar rats using an in vivo aminopyrine breath test (ABT) as an index of drug metabolizing ability.

Six rats received a single dose of AZT orally at three dose levels; 17 mg/kg/day (equivalent to human dose); 170 mg/kg/day and 1700 mg/kg/day. Each dose level was separated by five days. The aminopyrine breath test was performed pretreatment (control) and 1 hour after each of the three administrations of AZT. ^{14}C aminopyrine 1 μCi/kg was injected into the peritoneal cavity of unanaesthetised rats. The rats were housed in individual metabolic cages and the expired gas was passed through concentrated sulphuric acid and then through a scintillation vial containing 10 ml of a 1:4 (v/v) ethanolamine/methanol mixture to trap all exhaled $^{14}CO_2$. Samples were collected for 20 minute periods over 240 min. Trapped radioactivity ($^{14}CO_2$) decreased exponentially during the collection period. The elimination rate constant (kel) was calculated by least square regression analysis of the logarithm of the $^{14}CO_2$ production with respect to time and the elimination half life was calculated.

Treatment with AZT at the lower dose levels did not produce any significant change. However dosing at 1700 mg/kg AZT did result in a significant 14% increase in the elimination half-life (Table 1).

Table Aminopyrine-$^{14}CO_2$ elimination half-life (min, mean±S.D.; n = 6, *p <0.05 Student's t test significantly different from control

Control	38.4 ± 0.8
AZT 17 mg/kg	39.2 ± 0.9
AZT 170 mg/kg	37.8 ± 1.5
AZT 1700 mg/kg	43.9 ± 4.5 *

While AZT treatment resulted in a small prolongation in the elimination half life suggesting an inhibitory effect on hepatic n-demethylation the dose required was approximately one hundred times greater than those used in clinical practice. While it seems unlikely therefore that AZT will produce significant drug interactions as a result of inhibition of hepatic drug metabolism, further studies in patients will be required to determine the effect of AZT on drug metabolism in man.

Fischl, M.A. et al. (1987) New Eng. J. Med. 317, 4, 185-191.

CHOLINERGIC ACTION OF TRIMETHYLAMINE AND ITS METABOLITES

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Trimethylamine (TMA), both in salt form and liberated from precursors by the action of gut bacteria, is ingested in large amounts in the normal diet, especially from fish and dairy products. The compound is usually metabolised extensively to its N-oxide (TMAO), although this reaction displays a genetic polymorphism where certain individuals have a relatively deficient N-oxidation capacity ('Fish Odour Syndrome'). Demethylation to dimethylamine (DMA) has been shown to be a relatively minor route of metabolism in man and the possible methylation to the quaternary compound, tetramethylammonium (TetMA), has not yet been investigated (Al-Waiz *et al.*, 1987).

Owing to the known pharmacological activity of TetMA and related compounds (Ing 1956) it was decided to investigate the metabolites of TMA for their potential pharmacological effects, especially those possibly related to the gastrointestinal tract.

In isolated tissue experiments segments of guinea pig ileum were subjected to co-axial stimulation (0.5ms duration, 64v, 0.1Hz), to avoid non-uniformity of spontaneous contractions, after the method of Paton (1957). Mepyramine (125nM) and hexamethonium (10μM) were added to the Krebs medium for this procedure.

The maximum contraction obtained in the presence of carbachol (postganglionic parasympathetic stimulant) was seen at 0.7μM, the tissue developing 2.4g of tension. Construction of cumulative dose-response curves permitted the calculation of an EC_{50} for carbachol of 0.1μM, for TetMA of 80μM and for TMA of 2000μM, all developing the same maximum response. TMAO and DMA were without effect.

The effect of carbachol was competitively antagonised by atropine as expected, but the antagonism observed with atropine against TetMA and TMA was not of a simple competitive nature, although a shift to the right of the normal dose-response curve was seen the maximum response could not be obtained with increasing dose.

These experiments suggest that TetMA, and to a lesser extent TMA, have an action at postganglionic cholinergic sites. In individuals where N-oxidation of this abundant dietary amine is restricted, the possible evocation of an alternative methylation pathway with its potential pharmacological sequelae, and that of TMA itself, must be considered.

Al-Waiz, M., Mitchell, S.C., Idle, J.R. & Smith, R.L. (1987) Xenobiotica 17, 551-558.

Ing, H.R. (1956) In 'Hypotensive Drugs': M. Harrington (ed.), pp. 7-22. Oxford, Pergamon.

Paton, W.D.M. (1957) Br. J. Pharmacol., 12, 119-127.

THE EVALUATION OF EXTUBE™ EXTRACTION COLUMNS FOR URINE SCREENING OF BASIC AND ACIDIC DRUGS AT pH7

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Extubes are a family of disposable extraction columns designed for rapid sample preparation. In the analysis of drugs from biological samples a major emphasis is placed on the isolation of the drug of interest from the biological matrix. Classical liquid-liquid extraction methods can provide the analyst with good to excellent drug recoveries (Stewart et al, 1984). The methodology can however, be time consuming or large volumes of organic solvents may be necessary, emulsion formation with some organic solvents may hinder phase separation.

Extube columns are supplied in two main forms CHEM ELUT and TOX ELUT. The columns contain a specially modified form of diatomaceous earth which absorbs and distributes an aqueous sample over a large surface area thus allowing for the maximum contact with the extracting solvent. No emulsions are formed.

A range of representative acidic and basic drugs were added to "drug free" horse urine and their extraction from urine at pH7 was performed. The method and techniques used have been previously described (Power et al 1988). The majority of drugs were detected down to the 5 ug/20 ml level. Table 1 outlines some typical results obtained however, a total of 30 drugs were investigated via TOX ELUT.

The CHEM ELUT columns provided a cleaner extract for GC/MS and this aided confirmatory analysis especially if the drug was present at low concentration. In general, using TOX ELUT columns, the basic drugs were extracted well at pH7, whereas the acidic drugs were extracted with some loss of sensitivity when compared with their pH2 extraction.

Table 1 Representative drugs

	TLC	GC		TLC	GC
Caffeine	+	+	Amylobarb	-	+
Nefopam	+	+	Meclofenamic acid	+	+
Promazine	+	+	Diclofenac	+	+

All data for 5 ug/20 ml level.

Extube column extraction gave rise to cleaner extracts for TLC, GC and GC/MS and it had the added advantage of being much quicker than the traditional liquid-liquid extraction procedure. The ease of operation and reduction in solvent required coupled with good sensitivity and good reproducibility indicate that Extubes are a viable alternative to conventional liquid-liquid extraction methods.

We are grateful to the Irish Turf Club for support.

Stewart, J.T., Reeves, T.S. & Honiberg, I.L. (1984) Anal. Lett., 17(816):1811-1826
Power, J.D., McKenna, B. & Lambert, M.B. (1988) Presented at the Seventh International Conference of Racing Analysts & Veterinarians, Kentucky.

DISPOSITION OF VITAMIN K_1 2,3-EPOXIDE IN THE RAT

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Although the disposition and metabolism of vitamin K_1 has been studied extensively in man (McBurney et al, 1980) and animals (Watanabe et al, 1974), there is little data on the fate of vitamin K_1 2,3-epoxide, the major metabolite of vitamin K_1, during coumarin anticoagulation. We have therefore attempted to determine the disposition and metabolic fate of vitamin K_1 2,3-epoxide.

Male Wistar rats (200-300g; n = 5) were used. In the first group, an indwelling jugular catheter was implanted under anaesthesia (sodium methohexitone; 60 mg kg^{-1} i.p.). At the same time, the potent and long-acting coumarin, brodifacoum (10 mg kg^{-1} i.p.) was given. Five hours later, when clotting factor synthesis had ceased and the animals were fully conscious, each rat received a dose of tritiated vitamin K_1 (2-12μCi kg^{-1}) or vitamin K_1 2,3-epoxide (2-12 μCi kg^{-1}; prepared by the method of Thierry-Palmer, 1984) *via* the indwelling catheter. Urine and faeces were collected over the next 48h. The major organs and tissues were then removed and analysed for the presence of radioactivity.

In a further experiment we attempted to isolate and characterise the urinary and biliary metabolites of vitamin K_1 2,3-epoxide. After dosing the animals as described above, urine was collected in 6 hourly aliquots and stored frozen (-70°C) until analysed. Bile was collected from rats anaesthetized with urethane (14%; 1ml $100g^{-1}$, i.p.) *via* a catheter inserted into the common bile duct for a total of 5h after the administration of a dose of [^{3}H]-vitamin K_1 2,3-epoxide (i.v.) and was frozen (-70°C) until analysed. To determine the metabolic profile of vitamin K_1 2,3-epoxide, samples of urine or bile were treated with acid (perchloric acid in tetrahydrofuran; pH < 2), followed by extraction into diethyl ether. This extract was purified by reverse-phase gradient high performance liquid chromatography (HPLC) and analysed *via* liquid scintillation spectrometry and plasma spray mass spectrometry (MS).

Total recovery of radioactivity from rats was 55% of the dose. Of this, 15.6 ± 1.8% and 9.7 ± 1.1% were excreted in the faeces and urine, respectively. The largest concentrations of radioactivity were found in the liver (27.9 ± 0.3%) and the lung (1.1 ± 0.12%). Radiolabelled metabolites of vitamin K_1 2,3-epoxide were extracted from urine and bile most efficiently when the extraction medium was maintained below pH2, suggesting that the metabolites are carboxylic acids. The elution profile of urine was complex, whereas bile appeared to contain a single metabolite not found in either urine or bile from animals given tritiated vitamin K_1. Analysis of the biliary metabolite by plasma spray mass spectrometry gave a strong ion at m/z 387 (M + 1 ion for the metabolite), the molecular weight of vitamin K_1 2,3-epoxide is 466. These data suggest that vitamin K_1 2,3-epoxide like vitamin K_1 (McBurney et al, 1980) may undergo biotransformations which involve ω and β-oxidation of the isoprenoid side-chain.

McBurney A., et al. (1980). Biochem. Med. 24 250-267.
Thierry-Palmer M. (1984). J. Chem. Ed 61 179-180.
Watanabe M., et al. (1974). Chem. Pharm. Bull. 22 176-182.

THE EFFECTS OF EXOGENOUS STEROID HORMONES ON DEFENCE AGAINST UTERINE INFECTION

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The mammalian uterus is sterile despite the fact that the vagina is colonised by large numbers of microorganisms of many different species. Mucolytic drugs have been shown to increase the risk of uterine infection indicating that the cervical mucus plug is a major protective barrier against bacterial invasion (Malhi et al., 1987), although prednisolone-induced immunosuppression has shown that bacteria may normally breach the cervix, but are eliminated by the immune system (Malhi et al. in prep). Oestrogens are thought to decrease cervical mucus tenacity in addition to an immunosuppressant effect and may therefore predispose to uterine infection. Progestagens are also immunosuppressant but are thought to increase cervical mucus tenacity. This study investigates the effects of combinations of a mucolytic, an oestrogen and a progestagen on uterine microbial status.

Female Dunkin-Hartley guinea-pigs (300-500g) received vehicle control; norethisterone acetate (NA) 1mg/kg/day; ethinyloestradiol (EO) 50mcg/kg/day; bromhexine hydrochloride (BHC) 30mg/kg/day or combinations of these drugs i.p. for 21 days at the end of which the uteri were examined for bacterial presence. Total leucocyte determinations were made regularly throughout the study and used as an index of immunosuppression.

The results (table 1) show that both steroids produce a reduced leucocyte count, indicating some degree of immuosuppression. All uteri from control and NA-treated animals were sterile, however bacteria were isolated from the uteri of all animals treated with EO and BHC, NA appears to reduce these effects.

Table 1 The effects of exogenous steroid hormones and a mucolytic drug on uterine microbial status and total leucocyte count

TREATMENT	NUMBER OF SAMPLES	MEAN MICROBIAL COUNT/ml UTERINE WASH SOLN ± SEM	MEAN FINAL LEUCOCYTE COUNT (wbc/l) ±SEM $x10^9$
CONTROL	8	0	11.00 ± 2.60
BHC	7	86.1 ± 15.4**	6.90 ± 2.10
NA	8	0	0.64 ± 0.75
EO	8	38.8 ± 5.4**	0.39 ± 0.11
NA+EO	8	7.8 ± 5.3	0.45 ± 0.07
NA+BHC	8	55.3 ± 21.7*	0.56 ± 0.05
EO+BHC	8	110.3 ± 19.1**	0.55 ± 0.08

Statistical differences from control: * $p<0.05$; ** $p<0.001$ Mann-Whitney U-test

The results suggest that BHC compromises the cervical mucus plug allowing uterine colonisation by bacteria beyond the host's defence capabilities. EO produces uterine infection probably due to a combination of immunosuppression and mucolysis. However, NA causes immunosuppression without uterine infection suggesting an enhanced protective effect possibly via a thickening of cervical mucus. This is supported by the fact that NA reduces the effectss of BHC and EO presumably by thickening of cervical mucus. Hence NA may be of use in the prevention of uterine infections despite its immunosuppressant effects.

Malhi, J.S., Gard, P.R., Hanlon, G.W., Marriott, C. (1987) J. Pharm. Pharmac. 39. 1025-1028.
Malhi, J.S., Gard, P.R., Hanlon, G.W., Marriott, C. (in prep) Accepted for the International Symposium on Mucus and Related Topics, Manchester, UK.

PURIFICATION AND SOME PROPERTIES OF HUMAN LIVER AMINOBUTYRATE AMINOTRANSFERASE

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Aminobutyrate aminotransferase (GABA-T;E.C. 2.6.1.19) is the target enzyme for a number of suicide enzyme inhibitors which are anticonvulsants. One such compound, 4-aminohex-5-enoate has been shown to be an effective antiepileptic drug in man in controlled clinical trials. GABA-T has been purified from several sources including rabbit brain (John & Fowler, 1976). We have used an adaptation of this method to purify the enzyme from human liver.

The purification procedure involved a combination of heat treatment, acetone precipitation, ion-exchange chromatography and gel filtration. An overall purification of 200-fold was achieved (Table 1).The enzyme was a dimer with a subunit molecular weight of 49,900 (PAGE/SDS).

Sample	Volume (ml)	Activity (Units)	Protein (mg)	Spec Activity (Units/mg)	Yield (%)
Homogenate	800	91.1	7608	0.012	100
Heat	610	77.5	1635	0.047	85
Acetone	140	57.4	1236	0.046	63
DEAE-cellulose	517	51.8	352	0.147	57
CM-cellulose	125	33.9	29	1.178	37
TSK-gel	5	9.5	3.9	2.410	10

Table 1 : Stages in the purification of human liver GABA-T. Units are umol/min.

The absorption spectrum of human liver GABA-T showed characteristic absorption maxima at 412nm and 330 nm, the former corresponding to coenzyme bound in the active aldimine form. Upon reaction with 4-aminohex-5-enoate a characteristic shift in the absorption spectrum was observed, from 412 to 330nm. The reaction was followed continuously at 412nm and was found to be first order throughout its course. Kinetic data calculated from the spectral transition were Ki = 10.8 mM and ki = 0.5 s^{-1} .

The nature of the adduct formed during the reaction of 4-aminohex-5-enoate with GABA-T was investigated by heating the inactivated enzyme to 100°C. A compound was released which was identified as pyridoxamine-5'-phosphate, indicating that the 4'-C of the coenzyme remains bonded to the inhibitor nitrogen throughout the inactivation process. This contrasts with the mechanism found to occur when some other pyridoxal phosphate-dependent enzymes are inactivated by "suicide" inhibitors (Likos et al.,1982; Ueno et al.,1982).

John R A & Fowler L J (1976).Biochem J,155,645_651.
Likos J J, Ueno H, Feldhaus R W & Metzler (1982). Biochemistry, 21,4377_4386.
Ueno H, Likos J J & Metzler D E (1982). Biochemistry,21,4387_4393.

OUABAIN SENSITIVE POTASSIUM UPTAKE BY INTACT RAT PERITONEAL MAST CELLS

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Digitalis glycosides inhibit the plasma membrane Na/K pump, and treatment of various secretory tissues with these drugs is known to enhance their secretory response. However, there are conflicting results in the literature concerning the role of the Na/K pump in the mechanism of histamine secretion from rat peritoneal mast cells (Okazaki et al., 1976; Frossard et al., 1983). In addition, previous investigations on the ATPase activity of membrane preparations of rat mast cells or intact cells have demonstrated a calcium-magnesium-dependent activity and no convincing evidence of the presence of a Na/K-dependent ATPase activity (Magro, 1977; Cooper & Stanworth, 1976).

The aim of the present investigation was to characterize the mast cell Na/K pump mechanism and to attempt to estimate quantitatively the ion transport activity. Pure populations of peritoneal mast cells were obtained by differential centrifugation of mixed peritoneal cells in a gradient of Percoll. The cells were washed in order to remove Percoll and then incubated in presence or absence of calcium. The ouabain-sensitive and ouabain-resistant uptake of potassium into intact cells were measured by use of the radioactive potassium analog, ^{86}Rubidium (^{86}Rb), as a tracer for potassium.

In the absence, but not in the presence of calcium there was a very active cellular uptake of potassium of 650 pmol $(10^6 \text{ cells})^{-1}$ min^{-1} by a ouabain-sensitive mechanism. In presence of calcium, 1.2 mmol/l, the uptake was only 20 percent of that value. The ouabain-resistant potassium uptake was 200 pmol $(10^6 \text{ cells})^{-1}$ min^{-1}, and it was not influenced by changes in the calcium concentration of the medium. The ouabain-sensitive uptake of potassium was temperature-dependent. The maximum uptake occurred at 30°C and the temperature coefficient was 2. It was dependent on pH of the incubation medium, maximum uptake was observed at pH 7.6, and it was inhibited when the mast cells were treated with inhibitors of the energy metabolism. The Lineweaver-Burk plot of the relation between the potassium concentration of the medium and the rate of potassium uptake was used to estimate the maximum rate of potassium uptake, and it was 1.7 nmol $(10^6 \text{ cells})^{-1}$ min^{-1}. K_m was 1.8 mmol l^{-1}.

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Cooper, P.H. & Stanworth, D.R. (1976) Biochem. J. 156, 691.

Frossard, N., Amellal, M., Landry, Y. (1983) Biochem. Pharmacol., 32, 3259-3262.

Magro, A.M. (1977) Clin. exp. Immunol. 30, 160-167.

Okazaki, T., Ilea, V.S., Okazaki, A., Miche, K., Reisman, R.E. & Arbesman, C.E. (1976) J. Allergy clin. Immunol. 57, 454.

DIAPHRAGMATIC FATIGUE INDUCED BY PROLONGED INTERMITTENT ELECTRICAL STIMULATION OF RAT ISOLATED PHRENIC NERVE-DIAPHRAGM PREPARATION

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The importance of peripheral muscle fatigue was first demonstrated by Merton (1954), and it was shown that force generation was impaired by applying supramaximal stimulation to the motor nerve supplying the muscle. Furthermore, fatigue was related to the frequency of nerve stimulation, number of stimuli delivered to the muscle, and to metabolite accumulation within the muscle (Marsden,Meadows & Merton,1983; Garland,Garner & McComas,1987; Dawson,Gadian & Wilkie,1980). In the present study, we investigated respiratory muscle fatigue , induced by prolonged intermittent nerve stimulation,at 50 Hz for 0.5-1.5 s duration, in the rat diaphragm preparation, to see how fatigue was influenced by the type of stimulation and by increased tetanic duration.

The phrenic nerve-hemidiaphragm preparation was isolated from male,Sprague-Dawley rats,weighing 200-300 g. The preparation was set up in 80 ml organ bath containing Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% CO_2 in oxygen. The phrenic nerve was stimulated,at 50 Hz,with 2-5 V (supramaximal), for 0.5,1.0 and 1.5 s durations. Stimulation was continued,intermittently,i.e., in a 3s cycle of contraction/relaxation pattern, for several minutes,until a complete loss of tension (fatigue) was obtained, at each set of tetanic durations. The time,fatigue rate and the number of stimuli delivered to fatigue the muscle were recorded. In addition, the tension remaining at the end of a fixed number of stimuli (e.g., 1200) was measured and this was expressed as a percentage of the control value. Fatigue cost,measured as tension x time to fatigue units, was also calculated for each set of experiments.

The results are shown in Table 1.

Table 1. Tetanic fatigue in the rat diaphragm preparation,stimulated indirectly at 50 Hz for various durations.*

(1) Tet. duration(s)	(2) Control(C) Max. tension (g)	(3) Time to Fatigue (min)	(4) Fatigue rate (2/3)	(5) Total No. stimuli delivered	(6) Tension (g) at 1200 stimuli	(7) Tension as % of C	(8) Fatigue cost (2 x 3)
0.5	5.0±0.2	9.0±1.0	0.6	27×10^3	3.8±0.3	76±4.0	45.0
1.0	5.5±0.5	7.0±0.2	0.8	21×10^3	3.3±0.1	60±3.0	38.5
1.5	6.0±0.2	4.5±0.1	1.3	13.5×10^3	3.0±0.1	50±2.0	27.0

(*): The results are means±s.e.,n=8. $P < 0.001$ with respect to the control value.

It was concluded that the rat diaphragm was fatigued,by electrical intermittent nerve stimulation,and that the fatigue was dependent on the pattern of stimulation. The time taken to fatigue the muscle decreased with increasing tetanic duration. Furthermore,fewer stimuli were needed to fatigue the muscle when stimulated at longer than shorter tetanic duration.

Dawson,M.J.,Gadian,D.G.& Wilkie,D.R.(1980). J.Physiol., 299, 465-484
Garland,J.,Garner,S.H.& McComas,A.J.(1987). J.Physiol., 384, 33 P
Marsden,C.D.,Meadows,J.C.& Merton,P.A.(1983). In: Motor Control Mechanisms in Health and Disease, J.E.Desmdt,ed., Raven Press,New York,pp. 169-211
Merton,P.A.(1954). J. Physiol., 123, 553-564

ACTIONS AND INTERACTIONS OF VERAPAMIL AND D-600 WITH POTASSIUM IN THE SMOOTH MUSCLE OF RAT ISOLATED SEMINAL VESICLE

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Verapamil and D-600 are class four of antidysrhythmic drugs, calcium channel blockers known to inhibit transport of calcium across the cell membrane, thereby impairing the contractility in smooth and other types of muscle. Other therapeutic activities include anti-anginal and vasodilatation (for a review see Singh, Ellrodt &Peter,1978). High potassium depolarizes the membrane and produces a marked contraction in the smooth muscle human saphenous vein (Greenidge,Suer, Tugwell & Wali,1987). In the present investigation, the effects of two calcium channel blockers,namely verapamil and D-600, on the voltage-dependent contractions produced by potassium chloride (KCl) were studied in the smooth muscle of rat seminal vesicle, and the results were compared with those reported previously in other types of smooth muscle.

Seminal vesicle from adult,male,Sprague-Dawley rats,weighing 200-300 g, were used. The preparation was cut spirally (2-3 cm long and 2 mm width) and set up in separate organ baths,containing 20 ml of Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% CO_2 in oxygen. The contractions produced by KCl were recorded isometrically.
The Results are shown in Table 1.

Table 1. Effects of verapamil and D-600 on KCl-induced contractions in rat isolated seminal vesicle.*

	(1) KCl	(2) KCl+Ver	(3) KCl+D600	P_f(Col. 1 v 2 & 3)	P_f(Col. 2 v 3)
Control(C) maximum contraction (g)	3.6±0.3	1.2±0.1	1.5±0.2	0.001	0.05
EC_{50} value (mM)	14.2±0.1	52.7±0.5	37.1±0.2	0.001	0.05

(*): The results are means±s.e.,n=6. Ver:Verapamil(1 μM), D-600 (10 μM).
KCl (1.34-268.4 mM) produced concentration-dependent contractions in seminal vesicle. The mean EC_{50} values of KCl,alone,and KCl+verapamil or KCl+D600 are shown in Table 1. The mean EC_{50} values of verapamil and D-600,in producing a shift in KCl curve,were 1.0±0.1 and 9.5±0.3 μM, respectively.

It was concluded that KCl produced large contractions in rat isolated seminal vesicle,similar to those reported in vascular smooth muscle (Greenidge,et.al., 1987). Both Verapamil and D-600 significantly($P < 0.001$) reduced these contractions indicating blockage of voltage-dependent calcium channels.Verapamil was more potent than D-600 in reducing KCl-induced contractions. The smooth muscle of the seminal vesicle is innervated by cholinergic as well as adrenergic postganglionic neurones (Hucovic,1961; Bouquet & Noach,1971; Fedan,Besse,Carpenter & Teague, 1977). Experiments are in progress to further analyse the effects of calcium channel blockers on mediators and neurotransmitters in rat seminal vesicle preparation.

Bouquet,J.& Noach,E.L.(1971). Arch.Int.Pharmacodyn.Ther.,189, 397-398
Fedan,J.S.,Besse,J.C.,Carpenter,F.G.& Teague,R.S.(1977). J.Pharmacol.Exp.Ther., 201, 285-297
Greenidge,E.,Suer,A.H.,Tugwell,A.C.& Wali,F.A.(1987). Br.J.Pharmac.,90, 221 P
Hucovic,S.(1961). Br.J.Pharmac., 16, 188-194
Singh,B.N.,Ellrodt,G.& Peter,C.T.(1978). Drugs, 15, 169-197

SMOOTH MUSCLE RELAXATION AND MEMBRANE HYPERPOLARISATION IN THE GUINEA PIG INTERNAL ANAL SPHINCTER

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Smooth muscle relaxation occurs in response to inhibitory nerve stimulation and drugs. The contribution of the membrane potential to the relaxation varies among different tissues (Bolton & Large, 1986). Relaxation to inhibitory nerve stimulation in the rat anococcygeus, for example, is accompanied by only small electrical changes (Creed et al., 1975), but in the circular muscle of the guinea pig internal anal sphincter (gpIAS) by large membrane hyperpolarisations (Lim & Muir, 1985). A number of drugs, which relax smooth muscle by different mechanisms, have now been used to investigate the relationship between the mechanical event and changes in membrane potential. The gpIAS was chosen and measurements made with conventional microelectrode and simultaneous mechanical techniques (Lim & Muir, 1985; Muir & Stirrat, 1987). The results were obtained in the presence of phentolamine and atropine (each 10^{-6}M) to abolish, respectively, α-adrenoceptor-mediated responses of the gpIAS and cholinergic responses from any residual longitudinal muscle. They fell into two broad categories.

Sodium nitroprusside (SNP 10^{-9}-10^{-6}M) and 2-O-propoxyphenyl-8-azapurin-6-one (M&B 22948 10^{-9}-10^{-5}M) relaxed the gpIAS without any significant change in membrane potential. Small hyperpolarisations (8±3mV, SD, n=15) were observed but only at concentrations (10^{-5}M and 10^{-4}M respectively) which reduced the tone by over 80% and may be non-specific. On the other hand, the relaxations produced by nerve stimulation (single pulse and 5 pulses at 5, 10 and 20 Hz, 0.5ms, supramaximal voltage) adenosine triphosphate (ATP 10^{-6}M), cromakalim (BRL34915 10^{-9}-10^{-5}M) and isoprenaline (10^{-9}-10^{-5}M) were each accompanied by a significant membrane hyperpolarisation; no relaxation occurred without a change in membrane potential. Hyperpolarisation of the membrane by passage of large current pulses (5s) also relaxed the muscle.

The hyperpolarisations produced by nerve stimulation and ATP had a rapid rate of decline (41±20mVs, SD, n=69 to maximum) a short duration (2.7±0.8s, SD, n=69 to recovery at the membrane potential 44±3mV, SD, n=29) and could reach 33mV (5 pulses at 10Hz and ATP 10^{-4}M) mean 16±9mV, SD, N=12 (5 @ 10Hz). They were inhibited by apamin (5×10^{-6}M) and tetraethylammonium (TEA 8×10^{-2}M). These findings support the proposal that ATP may be the inhibitory transmitter in this tissue (Lim & Muir, 1985; Muir & Stirrat, 1987). On the other hand, the cromakalim-induced membrane hyperpolarisation was slow to reach a maximum (0.6 ±0.2mVs^{-1}, SD, n=12) and prolonged (596±238s, SD, n=12) with an amplitude of 19±6 mV, SD, n=12. It was inhibited by TEA (8×10^{-2}M) but not by apamin. The hyperpolarisation produced by isoprenaline was also slow to reach a maximum (0.5 ±0.3mVs^{-1}, SD, n=6) and prolonged (40±4s, SD, n=6) but much smaller in amplitude (14±6mV, SD, n=6). It was antagonised by propranolol (10^{-6}M) and TEA (8×10^{-2}M).

The results suggest that significant hyperpolarisation is a common but not a necessary accompanyment to smooth muscle relaxation in this tissue. Differences in the relationship between the relaxation and the electrical response may reflect the involvement of different ion channels and/or transduction mechanisms.

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Bolton, T.B. & Large, W.A. (1986) Quart. J. exp. Physiol. 71, 1-28.
Creed, K.E., Gillespie, J.S. & Muir, T.C. (1975) J. Physiol. 245, 33-47.
Lim, S.P. & Muir, T.C. (1985) Br. J. Pharmac. 86, 427-437.
Muir, T.C. & Stirrat, A.A. (1987) Digestive Dis. Sci. 32, 922.

IN VIVO EVIDENCE FOR CHOLINERGIC AND SEROTONERGIC COMPONENTS OF THE EFFECTS OF CISAPRIDE ON GASTRIC MOTILITY

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Cisapride (CIS) is a novel prokinetic agent which in clinical trials has shown efficacy in the treatment of a number of gastrointestinal motility disorders. Previous studies of its mechanism of action have largely been performed in vitro using a variety of preparations (Schuurkes et al 1985). Therefore this study utilised the ferret, an established model of human gastric function to investigate the mechanism of action of CIS in vivo. Ferrets were anaesthetised with urethane (1.5g/Kg) and prepared for recording of corpus and antral pressure as described previously (Andrews et al. 1987).

CIS enhanced the response to submaximal levels of vagal stimulation (10sec 3Hz 20V 0.5msec) especially in the antrum ($p<0.05$). In the corpus neither vagally mediated non adrenergic non cholinergic (n.a.n.c.) relaxation or that induced by splanchnic nerve stimulation was affected by CIS. The inhibition observed in the antrum after these manoeuvres was also not affected. CIS 2mg/Kg s.c. increased the amplitude of spontaneous contractions in both the corpus and antrum in intact and acutely abdominally vagotomised (VX) animals. The duration of its effect was $>$ 2 hours. Gastric acid secretion was not changed. In acute and chronic (3week) VX animals in the presence of atropine (1mg/Kg i.v) and guanethidine (5mg/Kg i.v.) corpus and antral motility was stimulated by CIS. The response to CIS was greater in chronic VX animals than in acute VX ($p<0.05$). In chronic VX animals in the presence of atropine and guanethidine the 5-HT_3 receptor antagonist BRL43694 (1mg/Kg i.v.) reduced but did not abolish the effects of CIS in the antrum ($p<0.05$).

In conclusion CIS acts to increase gastric motility in the ferret in vivo via an intramural cholinergic mechanism that can be activated by the vagus. There is no evidence for an action on extrinsically driven inhibitory processes. In addition there is an intrinsic non cholinergic component to its action which can be sensitised by vagal denervation. Part of this non cholinergic effect is mediated through 5-HT_3 receptors.

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Andrews, P.L.R., Bingham, S. & Wood, K.L. (1987) J. Physiol. 388, pp 25-39.
Schuurkes, J.A.J., Van Neuten, J.M., Van Daele, P.G.H., Reyntjens, A.J. & Janssen, P.A.J. (1985) J. Pharmac. Exp. Ther. 234, pp 775-783.

RABBIT ISOLATED SPONTANEOUSLY BEATING ATRIA AS A PREPARATION FOR THE STUDY OF 5-HT_3 RECEPTOR MEDIATED RESPONSES

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5-Hydroxytryptamine (5-HT) is known to stimulate the isolated perfused (Langendorff) heart preparation of the rabbit by releasing noradrenaline from sympathetic nerve terminals, a response blocked by the 5-HT_3 antagonist MDL 72222 (Fozard, 1984). Perfused preparations of this type are difficult to use for quantitative study of drug receptor interactions, particularly when bolus injections into perfusion streams may not achieve equilibrium of agonists with receptors. We describe a simple organ bath technique using spontaneously beating right atria of the rabbit for the study of 5-HT_3 receptor activity and the potency of some 5-HT_3 antagonists (MDL 72222; GR 38032F, Brittain et al., 1987; ICS 205930, Donatsch et al., 1984; BRL 24924A, Cooper et al., 1987). Male New Zealand White rabbits (1.5 - 3.0 Kg) were killed by pentobarbitone-Sodium overdose (60 mg/Kg i.v.). Right atria were placed in 6 ml organ baths containing Ringer-Locke solution at 32°C. A tension of 2 g was used for isometric recording of spontaneous beating. Rate was derived using a Grass tachograph. Atropine (10^{-6}M) was present to prevent muscarinic effects of released acetylcholine. Responses (positive chronotropy) were obtained to non-cumulative additions of 5-HT. Antagonist was allowed 45 min to equilibrate with the tissues. Responses were again obtained to 5-HT. At least three concentrations of each antagonist were assessed. Each tissue was exposed to one antagonist concentration only. pA_2 values and Schild plot slopes derived by the method of Arunlakshana & Schild (1959) are shown in Table 1. Separate experiments were performed to compare 5-HT and the selective 5-HT_3 agonist 2-methyl-5-HT. EC_{50} values are shown in Table 1.

Table 1. Agonist and antagonist actions at atrial 5-HT receptors.

	pA_2 (95% limits)	Slope (95% limits)	n*
MDL 72222	9.1 (8.9-9.9)	0.8 (0.4-1.1)	5
GR 38032F	10.8 (10.5-11.6)	0.8 (0.4-1.2)	6
ICS 205930	10.6 (10.4-10.8)	1.3 (0.9-1.6)	6
BRL 24924A	9.2 (8.9-10.3)	1.0 (0.4-1.6)	7
	agonist EC_{50} μM (95% limits)		
5-HT	5.8 (2.9-7.7)		6
2-methyl-5-HT	7.5 (3.4-12.5)		6

* No. of tissues per concentration.

Antagonist potencies, together with the agonist action of 2-methyl-5-HT are consistent with a 5-HT_3 mediated response. The nicotinic agonist dimethyl phenyl piperazinium (DMPP) which increases atrial rate by release of endogenous catecholamines, was tested in the absence and presence of 10^{-5}M GR 38032F. At this concentration, there was no antagonism of the effect of DMPP, demonstrating lack of action of GR 38032F at nicotinic receptors and B-adrenoceptors. In contrast, propranolol (10^{-7}M) reduced maximum responses to 5-HT and DMPP by 52 (±2)% and 65 (±7)% respectively. Metergoline (10^{-6}M) had no effect on responses to 5-HT, indicating lack of involvement of 5-$HT_{1A, B, C}$ and 5-HT_2 receptors. The results suggest that the chronotropic action of 5-HT in rabbit atria involves receptors belonging to the 5-HT_3 subtype. The results also suggest the usefulness of this preparation as an in vitro assay for the investigation of drug effects at 5-HT_3 receptors.

Arunlakshana, O., & Schild, H.O. (1959). Br. J. Pharmac. Chemother. 14, 48-58.
Brittain, R.T. et al., (1987). Br. J. Pharmac. 90 87P.
Cooper, S.M. et al., (1987). Br. J. Pharmac. 88, 383P.
Donatsch, P. et al., (1984). Br. J. Pharmac. 81, 34P.
Fozard, J.R. (1984). Naunyn-Schmiedeberg's Arch. Pharmac. 326, 36-44.

THREE TYPES OF 5-HT_1-LIKE RECEPTOR RECOGNISED BY TRYPTAMINE AFFINITY AND EFFICACY 'FINGERPRINTS'

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Functional 5-hydroxytryptamine (5-HT) receptors classified as 5-HT_1-like are a heterogeneous class (Bradley et al., 1986). However, attempts to identify each receptor type positively, by estimating antagonist dissociation constants, have been frustrated by the absence of suitably selective ligands and by the failure of available antagonists to behave competitively. Recently, we used a single set of tryptamine analogues to differentiate quantitatively between two types of 5-HT_1-like receptor mediating relaxation of endothelium-intact (RbJV+E) and denuded (RbJV-E) rings of rabbit jugular vein (Martin et al., 1987). In this study we now extend these observations by demonstrating that the same set of tryptamines also distinguishes a third 5-HT_1-like receptor mediating contraction of the rabbit saphenous vein (RbSV).

Changes in tissue isometric force were recorded from vascular ring preparations (Leff and Martin, 1986; Martin et al., 1987). Treatment of RbSV with phenoxybenzamine (0.3μM, 30min) prevented the uptake of 5-HT by sympathetic neurones. In each assay, estimates of agonist affinity (pK_A) and efficacy (τ) were obtained in 4-8 experiments for 5-HT, 5-carboxamidotryptamine (5-CT), 5-methyltryptamine (5-MeT), (±)α-methyl-5-HT (α-Me-5-HT) and N,N-dimethyltryptamine (NN-DMT) by operational model-fitting of concentration-effect curve data (Black et al., 1985).

In RbSV, results obtained with selective agonists and antagonists were consistent with the activation by 5-HT of a 5-HT_1-like receptor similar to that described in dog saphenous vein (Feniuk et al., 1985). Tryptamine affinity and relative efficacy estimates (shown below) provided quantitative evidence that this receptor was different from the two types of 5-HT_1-like receptor in RbJV.

Tissue		5-HT	5-CT	5-MeT	α-Me-5-HT	NN-DMT
RbSV	pK_A	7.12	7.53	6.43	5.68	5.82
	τ	1.00	0.98	1.00	0.93	0.65
RbJV+E*	pK_A	8.36	7.51	7.17	8.14	6.57
	τ	1.00	0.95	1.14	1.04	0.50
RbJV-E*	pK_A	6.20	6.66	5.67	<4.5	5.94+
	τ	1.00	4.70	0.53	-	-

* from Martin et al., (1987). + pK_B by Schild analysis.

These results show that unlike the conventional probes, tryptamines permit a rigorous analysis of 5-HT receptors which provides a reliable, quantitative basis for their classification.

Black,J.W. et al. (1985) Br. J. Pharmac., 84, 561-571.
Bradley,P.B. et al. (1986) Neuropharmac., 25, 563-576.
Feniuk,W. et al. (1985) Br. J. Pharmac., 86, 697-704.
Martin,G.R. et al. (1987) Naunyn-Schmiedeberg's Arch. Pharmac., 336, 365-373.
Leff,P & Martin,G.R. (1986) Br. J. Pharmac., 88, 585-593.

COMPARISON OF DISSOCIATION CONSTANTS ESTIMATED BY β-ADRENOCEPTOR INACTIVATION AND DESENSITISATION IN VITRO

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Receptor inactivation or desensitisation reduce the responses to β-agonists and result in a dextral shift and depression in the maximum of the concentration-response curve. Furchgott (1966) showed that receptor inactivation can be used to calculate the dissociation constant (K_A) of an agonist. Acute and chronic β-adrenoceptor desensitisation (Herepath and Broadley, 1988; Kenakin and Ferris, 1983, respectively) has been also used to estimate the K_A value. In the present study we have compared the K_A values for prenalterol calculated using both receptor inactivation and acute desensitisation of the β-adrenoceptor.

Left, paced (2 Hz, threshold voltage + 20%, 5 msec) atria were isolated from rats pretreated with reserpine (5 mg kg^{-1}, i.p., 18 hours previously). In separate experiments, atria were exposed to either BAAM (bromoacetylalprenolol methane; 60 min) or isoprenaline (120 min), at varying concentrations, in order to inactivate or desensitise the β-adrenoceptors, respectively. Control studies were undertaken under identical conditions, except that BAAM or isoprenaline were not added.

Cumulative concentration response curves to isoprenaline and prenalterol were then constructed. The K_A values (expressed as the -log K_A = pK_A) were estimated using the methods of Furchgott (1966) and Herepath and Broadley (1988), in which equiactive concentrations from control and post inactivation or desensitisation curves were plotted in a double reciprocal manner. The K_A value was then calculated from the plot using the relationship K_A = (slope-1)/intercept and the fraction of receptors remaining (q) was given by the reciprocal of the slope. All values quoted are mean, SEM <5%, n = 6-8.

In control experiments for the receptor inactivation studies, the potency, which is expressed as the -log EC_{50} (and maximal response) of isoprenaline and prenalterol were 8.8, (814 mg) and 7.7 (908 mg), respectively. In control experiments for the desensitisation studies, these values for isoprenaline and prenalterol were 8.6 (856 mg) and 7.5 (686 mg), respectively.

In the inactivation studies, the pK_A values calculated after exposure to BAAM (32 nM, 100 nM, 0.32 μM, 1 μM and 3.2 μM) were 6.7, 7.0, 6.5, 6.2 and 6.2, respectively. The corresponding q values were 0.37, 0.15, 0.05, 0.03, and 0.004, respectively. In the desensitisation studies, the pK_A values calculated after exposure to isoprenaline (3.2 nM, 32 nM, 320 nM and 560 nM) were 7.1, 6.9, 6.9 and 6.8, respectively. The corresponding q values were 0.52, 0.34, 0.15 and 0.08, respectively. In separate studies, prenalterol was employed as an antagonist of responses to isoprenaline and the dissociation constant (pK_A), calculated by the method of Stephenson (1956), was 7.1. This is in good agreement with the value of 7.1 reported by Kenakin and Ferris (1983).

In summary, receptor inactivation by BAAM provides estimates of the K_A value which are not independent of the level of receptor inactivation. Desensitisation, in contrast, provides estimates of the K_A value which are independent of the level of inactivation. We conclude that desensitisation provides a more reliable estimate of the dissociation constant of partial β-agonists than receptor inactivation by BAAM.

Furchgott, R.F. (1966) Adv. Drug Res., 3, 21-55.
Herepath, M.L. and Broadley, K.J. (1988), Br. J. Pharmac., 93, 277P.
Kenakin, T.P. and Ferris, R.M. (1983), J. Cardiovas. Res, 5, 90-97.
Stephenson, R.P. (1956), Br. J. Pharmacol., 11, 379-393.

DESENSITISATION OF ILEAL MUSCARINIC RECEPTORS IN VITRO

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Short-term exposure to carbachol results in heterologous desensitisation to muscarinic and histaminergic agonists in the guinea-pig ileum (Siegel et al., 1984; Eglen and Whiting, 1987). These workers suggested that desensitisation is not due to a reduction in the number of functional muscarinic receptors alone but may also involve post-receptor events. This hypothesis has been investigated using functional and competition radioligand binding methods previously described by Eglen and Whiting (1987) and Michel and Whiting (1987), respectively.

In functional studies, acute desensitisation of guinea-pig ileal longitudinal strips resulted in a reduction in the maximum response and dextral shift in the concentration-response curve to carbachol. The degree of desensitisation induced by exposure (30 min) to carbachol was concentration dependent (0.1 μM-10 μM) and was greatest after exposure to 10 μM carbachol (control EC_{50} = 0.22 μM; max (% KCl) = 94; desensitised EC_{50} = 0.65 μM, max (% KCl) = 69; mean, SEM <10%, n=8). There was no further significant ($P<0.05$) desensitisation following 240 min of exposure to 10 μM carbachol. In contrast, at any time point studied responses to KCl (50 mM) were not desensitised after exposure to carbachol (0.1-10 μM).

In radioligand binding studies, guinea-pig ileal longitudinal strips were exposed to carbachol (10 μM) for varying periods (60-240 mins). Control studies were undertaken in parallel. EDTA-washed membranes were then prepared and radioligand binding experiments were carried out at 32°C using [^{3}H]NMS. Three hours was allowed for equilibration. Atropine (10 μM) was used to define non-specific binding.

[^{3}H]NMS bound to a homogeneous population in both control and desensitised tissues. The K_d value for [^{3}H]NMS was not significantly ($P<0.05$) different from control tissue at any of the time points studied (240 min exposure: control K_d = 0.219 ± 0.022 nM; desensitised K_d = 0.278 ± 0.037, mean ± SEM, n=3). This was also true for the total number of binding sites (240 min exposure: control B_{max}= 3.53 ± 0.42 pmol/mg protein; desensitisation B_{max}= 3.71 ± 0.52 pmol/mg protein). In the presence of 30 μM Gpp(NH)p, membranes obtained from tissues exposed to 10 μM carbachol for 240 min exhibited a significantly ($P<0.01$) higher K_i values at both the low and the high affinity binding sites for carbachol (240 min exposure; K_H-control = 0.51 ± 0.25 μM; desensitised = 8.82 ± 1.94 μM; K_L-control = 37.93 ± 12.9 μM; desensitised = 234 ± 46.6 μM, mean ± SEM, n =3). Desensitisation also increased significantly ($P<0.05$) the relative proportion of the high affinity sites (240 min exposure; control = 24.75 ± 9.34% R_H; desensitised = 74.33 ± 9.28% R_H; mean ± SEM; n=3).

The decrease in the functional ileal responses to carbachol following short-term desensitisation, therefore, may be due to changes in the efficiency of the stimulus-response coupling mechanisms and not to receptor down-regulation.

Eglen, R.M., and Whiting, R.L. (1987) J. Pharm. exp. Ther., 240, 404-409.
Michel, A.D. and Whiting, R.L. (1987) Br. J. Pharmac., 92, 755-767.
Siegel et al., (1984) J. Auton. Pharmac. 4, 109-126.

INTERACTION OF TACRINE WITH MUSCARINIC RECEPTORS

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Tetrahydroaminoacridine (Tacrine or THA) is a cholinesterase inhibitor which is currently being evaluated for its effects in treatment of SDAT (Summers et al., 1986). Recently, it has become apparent that the compound is also a potassium channel blocker (Drukarich et al., (1986). The results of the present study indicate that the compound also binds to muscarinic receptors.

Ligand binding studies on the M1, M2 and M3 muscarinic receptors were conducted as described by Michel & Whiting (1988). Briefly, the M2 and M3 receptors of cardiac and submaxillary gland membranes were labelled by incubation with [^{3}H]N-methylscopolamine (0.1 nM) in 3 ml of Tris-krebs for 2 h at 37°C. M1 receptors or rat cortex were labelled using [^{3}H]pir (0.3 nM). Incubations were terminated by vacuum filtration using a Brandel cell harvester. Non specific binding was determined using 1 uM atropine. Data were analysed using iterative curve fitting techniques. Functional assays on phosphatidylinositol (PI) turnover were conducted as decribed by Kunysz et al., (1988).

In ligand binding studies, the pKI value and Hill slope (nH) for THA at the M1, M2 and M3 receptors were respectively, 5.5 (2.1), 5.2 (1.6) and 5.5 (2.1). The high nH values may have been due to allosteric interactions of THA since at a concentration of 10 to 100 uM THA decreased the dissociation rate of [^{3}H]NMS from the M2 receptor of cardiac membranes.

In functional studies of PI turnover THA at concentrations of 10 to 100 uM blocked the ability of carbachol to stimulate IP accumulation in both cortical slices and in the human astrocytoma cell line 1321 N1. In the 1321 N1 cells the compound produced a dose dependent inhibition of carbachol-stimulated PI turnover at concentrations of 10 - 100 uM (pA_2 = 5 $\pm$ 0.1; As slope = 1.15 $\pm$ 0.06; n=4. At low concentrations (30 uM), THA appeared as a competitive inhibitor and its effects were additive with atropine (Dose ratios:- THA (30 uM) = 4: Atropine (6 nM) = 10: Atropine (6nM) + THA (30 uM) = 14). At concentrations greater than 100 uM, curvature of the AS plot occured.

The results of the present study provide evidence that THA functions as a muscarinic receptor antagonist at low concentrations (3 - 100 uM) and also as an allosteric regulator of muscarinic receptor binding at high concentrations (>100 µM). Since the compound displays relatively low potency (Kd = 1 uM) in inhibiting cholinesterase (Kaul, 1962) THA is clearly a non-specific cholinesterase inhibitor.

Drukarch et al., (1987) Eur J. Pharm. 141, 153-157.
Kaul, P.N. (1962). J. Pharm. Pharmacol., 14, 243
Kunysz et al., (1988). Br. J. Pharmac. 93, 491-500.
Michel, A.D. & Whiting, R.L. (1988). Eur. J. Pharmac., 145, 61-66
Summers et al., (1986). New Engl. J. Med., 315, 1241.